# DIETARY REGULATION OF HIGHLYPOLYUNSATURATED FATTY ACIDS CONTENT IN FARMED NILE TILAPIA (*ORIOCHROMIS NILOTICUS*) AND AFRICAN CATFISH (*CLARIUS GARIEPINUS)*

**KEVIN MBOGO OMOLO**

**DOCTOR OF PHILOSOPHY**

**(Biochemistry)**

**JOMO KENYATTA UNIVERSITY OF**

**AGRICULTURE AND TECHNOLOGY**

**2018**

# Dietary Regulation of Highly Polyunsaturated Fatty Acids Content in Farmed Nile Tilapia (*Oriochromis niloticus*) and African Catfish (*Clarius gariepinus)*

**Kevin Mbogo Omolo**

**A thesis submitted in fulfillment for the Degree of Doctor of Philosophy in Biochemistry of Jomo Kenyatta University of Agriculture and Technology**

**2018**

## DECLARATION

This thesis is my original work and has not been presented for a degree award in an any other institution

Signature……..… Date…….

**Kevin Mbogo Omolo**

This thesis has been submitted for examination with our approval as university supervisors.

Signature……. Date……..

**Prof. Arnold Onyango, PhD**

**JKUAT, Kenya**

Signature:……. Date……

**Prof. Gabriel Magoma, PhD**

**JKUAT, Kenya**

Signature……..…… Date…….

**Dr. Kenneth Ogila, PhD**

**JKUAT, Kenya**

## DEDICATION

I dedicate this thesis to family members Judith Awuor (wife) David Peters (son), Delina Abby (daughter) and parents Beatrice Mbogo and Ernest Omolo

## ACKNOWLEGEMENTS

I am grateful to the almighty God for giving me life and strength to overcome challenges. I appreciate the cordial assistance rendered by my supervisors, Prof. Arnold Onyango (Food Science Department, JKUAT), Prof. Gabriel Magoma (Biochemistry Department, JKUAT) and Dr. Kenneth Ogila (Zoology department, JKUAT) for their keen guidance, supervision and persistent support throughout the course of research and writing up of thesis.

I would also like to thank the Deutscher Akademischer Austauschdienst (DAAD), JICA-Kenya and Training Programmes, Jomo Kenyatta University of Agriculture and Technology for their financial support, KEMFRI-Sagana for helping in the setup of experimental tanks and provision of fingerlings, Food Biochemistry laboratory, JKUAT, for bench space where fatty acid analyses were done, Department of Biochemistryand PAUSTIlaboratories where molecular works was done.

I am grateful to David Rasugu of Food Science department, JKUAT, Dr. Steven Ger of Biochemistry Department, JKUAT, Dr. Jonathan Munguti and Dr. Paul Orina of KEMFRI-Sagana for your support towards this research. I would also like to thank fish farmers in Kiambu, Muranga and Kirinyaga counties who made this dream possible; Michael (Unique farm-Kagio), Chris (Samaki Tu farm-Mangu) Jane (Jasa farm-Thika) Maggy (Jambo farm-Kiambu), Macharia (Thika West district fisheries department) among others.

Thank you Judith Ochanda and the family. Many a time I would spend time away from the house in an attempt to realize this dream. You supported me both psychologically and spiritually reminding me that am capable. To Uncle George Mbogo and brother Elly Omolo, thank you for believing in me and giving me the opportunity to education. This would have remained just a dream if you did not support me.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **AA** | Arachidic acid |
| **ALA** | Alpha-linolenic acid |
| **AOAC** | Association of official Analytical Chemists |
| **ARA** | Arachidonic acid |
| **cDNA** | Complimentary DNA |
| **CHD** | Coronary Heart Diseases |
| **CT** | Threshold cycle number |
| **CVD** | Cardiovascular Diseases |
| **DHA** | Docosahexaenoic Acid |
| **ELOVL** | Elongase gene |
| **EPA** | Eicosapentaenoic Acid |
| **FA** | Fatty Acid |
| **FADS2** | Fatty Acyl desaturase gene |
| **FAME** | Fatty Acid Methyl Esters |
| **FAO** | Food and Agriculture Organization |
| **FM** | Fish Meal |
| **FO** | Fish Oil |
| **HUFA** | Highly Polyunsaturated Fatty Acids |
| **LA** | Linoleic acid |
| **LA** | Linoleic acid |
| **LC-PUFA** | Long Chain Polyunsaturated Fatty Acids |
| **LO** | Linseed oil |
| **MUFA** | Monounsaturated fatty acid |
| **n-3** | Omega 3 |
| **n-6** | Omega 6 |
| **PCR** | Polymerase chain Reaction |
| **PUFA** | Polyunsaturated Fatty Acid |

| | |
|---|---|
| **SFA** | Saturated fatty acids |
| **SFO** | Sunflower oil |
| **SGR** | Specific Growth Rate |
| **VO** | Vegetable oil |
| **WHO** | World Health Organization |

## ABSTRACT

Vertebrates including fish, cannot produce PUFA *de novo* as they lack the $\Delta^{12}$ and $\Delta^{15}$ desaturases required to desaturate oleic acid (C18:1 n-9) to linoleic acid (C18:2 n-6) and then to α- linolenic acid (C18:3n-3). Therefore, biosynthesis of long chain fatty acid occurs from dietary precursors, α-linolenic acid (C18:3n-3) and linoleic acid, C18:2 n-6 through a series of microsomal fatty acid elongase (ELOVL) and desaturase (FADS2) mediated reactions. As a result, dietary composition of fish feeds has direct influence on fish fillet fatty acids composition including the n-3 long chain highly polyunsaturated fatty acids such as eicosapentaenoic acid, (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3). Traditionally, fish feeds have been formulated using fish oil (FO) as lipid supplement to provide the essential fatty acids needed for optimum fish growth. However, the ever growing aquaculture sector can not be supported by the current world production of fish oil, prompting research on the possible use of vegetable oil as a replacement to fish oil in fish feed. The current study investigated the use of linseed oil as a dietary lipid source of the precursor, α-linolenic acid (C18:3n-3) for EPA and DHA synthesis in Nile tilapia (*Oriochromis niloticus*) and African catfish (*Clarius gariepinus*) feeds. In addition, the effect of dietary linseed oil on the fatty acid profile of Nile tilapia and African catfish was also investigated. Six iso-nitrogenious diets were formulated with linseed: sunflower oil ratios as follows; diet 1(100: 0), diet 2 (75:25), diet 3(50:50), diet 4 (25:75), diet 5 (0:100) and washout diet (100% olive oil).

Thirty tilapia and thirty catfish fingerlings with an average body weight of 2.6 g and 9.1g respectively, were set in triplicate tanks (1000-liter capacity) for each diet treatment and fed twice a day to apparent satiation, with commercial diet used as a control diet. Growth parameters were measured in terms of changes in body weight and length. Tissue fatty acid composition was determined using gas chromatography. Real time PCR with CYBR green as fluorescent dye was used to determine the expression of ELOVL and FADS2 genes in each feeding experiment.

Survival rate and specific growth rate were significantly ($P<0.05$) lower with >50% dietary linseed oil composition. Influence of dietary fatty acid composition on tissue fatty acid composition was observed as tissue fatty acid content varied with dietary inclusion of vegetable oil. Significantly higher total n-3 fatty acids were observed with diet 1 (100% linseed oil) in tissues of both *O. niloticus* and *C. gariepinus*. Tissue composition of n-3 fatty acids decreased with decreased dietary composition of linseed oil in both fish species suggesting a correlation between dietary lipid and tissue fatty acids.Tissue arachidonic acid, ARA C20: 4 n-6, content was low in both

the fish tissues relative to the dietary precursor, linoleic acid, (LA, C18: 2 n-6) suggesting a possible utilization of ARA especially in intensive fish culture. Polyunsaturated fatty acids, especially DHA and EPA, deposition in the tissues increased with the feeding period with the third feeding month recording significantly ($P<0.05$) higher DHA, total n-3 and n-3/n-6 ratio in both fish species compared to first feeding month suggesting a greater accumulation of n-3 HUFA with age. There was significantly ($p<0.05$) higher gene expression in fish groups fed >50% of dietary linseed oil in both species compared to fish fed diet containing 0% linseed oil. The esression of both ELOLV and FADS2 genes followed same pattern.Data on the fatty acid profiles for fish sampled from selected fish farms showed that differences in feeds used by different fish farmers contributed to differences in the fatty acid.Some of the experimental diets in the present study resulted into higher DHA and EPA than both wild fish and fish sampled from different fish farms thus its possible to have farmed fish with even better fatty acid profiles than wild fish. For wild fish sampled from different beaches along Lake Victoria, heavier fish had significantly higher n-3 HUFAs compared to lighter suggesting the effect of weight or age on tissue fatty acids profiles.

In conclusion, dietary linseed oil >50% reduced growth and survival rate in tilapia and catfish, however, it increased tissue accumulation of long chain n-3 fatty acids which also increased with the length of feeding period. Further research will be necessary to determine other dietary components whose co-supplimentation with linseed will result into composition optimal survival rate, growth and at the same time enhance good levels of long chain fatty acids such as DHA and EPA.

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND INFORMATION

Fish is a highly nutritious and health promoting food. Fish's protein, which accounts for approximately 16% of animal protein consumed worldwide, has a good balance of essential amino acids (Aguiar *et al.,* 2011). Fish is also a good source of essential minerals and vitamins such as vitamin A, calcium, iron, zinc and iodine (Guerin *et al.,* 2011). In addition, fish contains highly unsaturated n-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). A lot of health benefits associated with fish consumption is attributed to these fatty acids (Ruxton *et al*., 2004; Torrejon *et al*., 2007). High n-3:n-6 ratio is associated with reduced risks for non-communicable diseases such as diabetes, cardiovascular, neural development and neurological pathologies (Brouwer *et al*., 2006; Calder, 2006; Calder and Yaqoob, 2009; Eilander *et al.*, 2007). The best sources of long chain n-3 fatty acids are sea fish however, fresh water fish have higher ability to synthesize highly polyunsarturated fatty acids (HUFAS) from dietary α-linolenic acids (Sahena *et al.*, 2009). Therefore, freshwater's fish fatty acid profile can be modified to contain larger proportions of EPA (C20:5 n-3) and DHA (C22:6 n-3**) (**Biró, 2013).The lipid composition of farmed fish is more constant and less affected by seasonal variations than that of wild fish (Cahu *et al.,* 2004), as its flesh fatty acid profile directly reflects the fatty acid composition of the diet (Ng *et al.,* 2003) and culturing usually occurs under controlled environments. With the growing knowledge on the health benefits of fish consumption across the world, there has been an increasing demand for fish and fish products (Kris-Etherton *et al.,* 2003). This demand, coupled with increased population, has contributed to the decline of natural fish supply.

National Economic Survey-Kenya (2016) reported that the freshwater fish production has been declining and that production dropped by 12.3% from 136.4 thousand tonnes in 2015 to 119.6 thousand tonnes in 2016. Aquaculture has since expanded to fill the supply gap created by dwindling natural fish supply not only in Kenya but across the world. The advent of government-funded Economic Stimulus Program (ESP) coupled with several aquaculture facilities in various parts of the country to serve as research centers, training facilities, and sources of fingerlings and feed for fish farmers have led to the recent expansion of national aquaculture production in Kenya (Nyonje *et al*., 2011). However, a gap exists as this expansion has been hit by unavailability of efficient and inexpensive fish feeds for different stages of fish development (Munguti *et al.,* 2012). Fagbenro (1999) argues that the success of fish farming heavily depend on the provision of suitable and economical fish feeds formulated from locally available feedstuff especially agricultural by-products to reduce the price of complete feeds. This is because aquafeeds take up between 40-60% of the fish farm's production costs and is a major constraint to fish farming in resource poor regions (Ali *et al.,* 2009). Fish diets have been traditionally formulated using fishmeal and fish oil (FO) (Sargent and Tacon, 1999; Pike, 2005). However, global fish oil production has reached a plateau and is not expected to rise much beyond the current level of production. Also, it is predicted that within a decade or so, fish oil production may not be sufficient to meet the demand of aqua-culture. Therefore, it is necessary to introduce alternative lipid sources to guarantee the sustainable development of aquaculture (Jordal *et al.,* 2007; Bouraoui *et al.,* 2011). Studies have shown that it is possible to replace fish oil by plant seed oils (soybean oil, linseed oil, rapeseed oil, olive oil, palm oil and corn oil) in aquafeeds (Mourente *et al.*, 2005).

Several studies involving warm water freshwater species like catfish *Heterobranchus longifilis* (Ng *et al.,* 2003) and tilapia *Oreochromis* sp. (Bahurmiz, 2007) have demonstrated the possibility of inclusion of high levels of plant oils such as sunflower and canola oil (CO) (Bell *et al.,* 2003b), linseed oil (Kowalska *et*

*al*.,2010a, Molnar *et al*.,2012) without compromising growth or feed conversion. There is also an established knowledge that dietary lipid influence fish fillet fatty acid especially EPA and DHA composition (Ng *et al.,* 2003; Ji *et al.,* 2011). Moreover, there is evidence that farmed fish may have low concentrations of essential n-3 LC-PUFAS compared to the wild which is attributed to aquafeeds (Trattner, 2009; Weaver *et al*., 2008). The present study exploited the possibility of supplementation of aquafeeds with linseed oil and sunflower oil to improve the fillet fatty acid composition of commonly cultured fish; African catfish and Nile tilapia. These fresh water fish have been shown to bio-convert dietary linolenic acid (C18:3 n-3) into long chain n-3 polyunsaturated fatty acids. In addition, this research focused on the effect of different composition of dietary linseed oil supplementations on growth performance, survival and n-3 PUFAs in cultured tilapia and catfish.

**1.2 Statement of problem**

Although increased fish farming and consumption may potentially help to lower incidence of cardiovascular diseases in Kenya, this is unlikely to happen if the farmed fish have inferior fatty acid profiles. The mostly farmed fish species in Kenya namely African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*), have only low to moderate amounts of beneficial n-3 highly polyunsaturated fatty acids (HUFAs). Prior to this study, the effect of fish farming on fatty acid profiles in Kenya had not been determined while studies elsewhere showed that fish farming may greatly increase the n-6: n-3 ratios thus making farmed fish detrimental to health (Hossain, 2011).

Therefore, there is a need to investigate the effect of fish farming on fish fatty acid profile and develop feed formulation with more dietary n-3: n-6 ratios. This will ensure high quality fish feeds, which are inexpensive, to support production of fish with desirable fatty acid composition. There is need to compare fatty acid profile between wild and farmed catfish and tilapia as well as to investigate the role of dietary vegetable oil in regulation of highly polyunsaturated fatty acids content in their tissues.

## 1.3 Justification of the study

The knowledge of the dietary benefits of fish has gained ground in Kenya resulting into rise in fish consumption even among communities which were not traditionally dependent on fish. This has seen natural fish supply reduce. While aquaculture may increase fish supply, availability of high quality fish feeds remains a major limitation to this expansion. Aqua feeds take up between 40-60% of the fish farm's production costs and is a major constraint to fish farming in resource poor regions (Ali *et al.*, 2009). Better fish growth and high quality fish can be achieved through formulation and processing of diets with all the nutritional requirements (Al – Ruqaie, 2007).

Fish diets have been traditionally formulated using fishmeal and fish oil (FO) (Sargent and Tacon, 1999; Pike, 2005). However, with aquaculture expanding worldwide at over 10% per year, the increasing demand for FO is outstripping global supplies (Barlow 2000; Tacon 2004). The only sustainable alternative to FOs is vegetable oils (VO), which are rich in C18 polyunsaturated fatty acids (PUFA) but devoid of the n–3 HUFA mainly eicosapentanoic acids (EPA) and docosahexaenoic acid (DHA) (Sargent *et al.,* 2002). Studies have reported possibilities of replacing dietary fish oil with vegetable oil in various fish feeds without affecting fish growth (Turchini *et al*., 2009, 2010).

Linseed oil (LO) is distinguished by the highest content of α-linolenic acid (18:3 n-3, ALA) (Popa *et al*., 2012), which is the metabolic precursor of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA, 20:5 n-3 and DHA, 22:6 n-3.

Recent studies have shown that linseed can be produced in Kenya in diverse agro-ecological zones (Lilian *et al.,* 2016) and with the ability of tilapia and catfish to convert ALA to EPA and DHA; linseed oil would be a locally available fish feed ingredients.

### 1.4 Null hypothesis

Dietary linseed oil supplementations do not influence tissue fatty acid profiles in Nile tilapia (*Oriochromis niloticus*) and African catfish (*Clarias gariepinus*).

### 1.5 Objectives

#### 1.5.1 Broad objective

To investigate the effect of different dietary vegetable oil supplementations on tissue polyunsaturated fatty acid profiles in farmed Nile tilapia and African catfish

#### 1.5.2 Specific objectives

1. To determine fatty acids profiles of wild and farmed African catfish and Nile tilapia sampled from selected regions in Kenya

2. To investigate the effect of formulated experimental diets on growth performance and survival of farmed African catfish and Nile tilapia

3. To investigate the effect of formulated experimental diets on tissue fatty acid profiles of farmed African catfish and Nile tilapia

4. To investigate the effect of fish feeding period on polyunsaturated fatty acid profiles of farmed African catfish and Nile tilapia

5. To determine the effect of formulated experimental diets on the relative expression of hepatic fatty acyl desaturase (FADS2) and fatty acyl elongase (ELOVL) genes in farmed African catfish and Nile tilapia

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Nutritional profile of fish

Fish is an important source of food with good nutritive value; it provides high quality protein, essential fatty acids, vitamins and a variety of minerals such as calcium, potassium, phosphorus, iron, copper and iodide (Sarvenaz and Sampels, 2017). Fish protein has since long been considered having a high nutritional value (Sargent, 1997). In addition, aquatic protein is highly digestible and rich in several peptides and essential amino acids that are limited in terrestrial meat proteins, for example methionine and lysine as suggested by Tacon and Metian (2013). In recent past, research has focused on the beneficial health effects of fish protein in human nutrition (Rudkowska *et al.,* 2010; Pilon *et al.,* 2011). These studies have indicated the relationship between fish protein, peptides or hydrolysates and inflammation, metabolic syndrome, osteoporosis, insulin resistance, obesity-related comorbidity and development of cancer. For example, a sardine protein diet has been shown to lower insulin resistance, leptin and TNFa, improved hyperglycemia and decreased adipose tissue oxidative stress in rats with induced metabolic syndrome (Madani *et al.*, 2012).

Proteins from variousfish as bonito, salmon, mackerel, herring and cod have shown anti-inflammatory properties while salmon and cod protein in addition improved insulin sensitivity in rats (Ouellet *et al.,* 2007; Pilon *et al.,* 2011). Dort *et al.* (2012) found cod protein to better promote growth and regeneration of skeletal muscle after trauma compared to peanut protein and casein and suggested this also to be partly because of the improved resolution of inflammation by cod protein.

Salmon calcitonin, a 32-amino acid peptide with blood calcium lowering functions has been used for medical purposes for more than 30 years (Chesnut *et al.,* 2008). Decreased risk of metabolic syndrome in adults has been attributed to the

consumption of lean fish (Torris *et al.,* 2016). Drotningsvik *et al.* (2015) indicated that already a low dietary intake of cod protein (25%) compared to a casein only diet, improved lipid metabolism and glucose regulation in obese rats. In addition to its valuable lipid and protein composition, fish is also a significant source of vitamin D (Holick, 2008b).

Furthermore, a significant correlation between higher fish intake and a lower risk of hip fractures was found in Chinese elderly (Fan *et al.,* 2013). Selenium is toxic in large doses; but it is essential as a micronutrient in animals and humans. In humans, selenium functions in the form of sialoproteins as cofactor for reduction of diverse antioxidant enzymes, such as glutathione peroxidases and is also responsible for the function of the thyroid gland as a cofactor for the three of the four known types of thyroid hormone deiodinases (Holben and Smith, 1999). Selenium has also shown to decrease the toxicity of methyl mercury (Ralston and Raymond, 2010). Seafood is a good source of selenium and was ranked on place 17 of 25 by the USDA National Nutrient Database according to (Ralston, 2008). In addition, it was found that selenium and selenite from fish was highly bioavailable and had a higher bioavailability than selenium from yeast (Fox *et al*., 2004).

Calcium is another important mineral in human nutrition being important for bone density. Calcium salts provide rigidity to the skeleton and calcium ions play a role in many if not most metabolic processes (FAO Agriculture and Consumer Protection department, 2002). Fish and other aquatic animal food products are rich source of calcium (Martinez-Valverde *et. al*., 2000). An average of 68 to 26 mg/100 g of calcium in crustaceans, molluscs and fish, was documented compared to around 14 mg/100 g in terrestrial meats (Tacon and Metian, 2013).

In addition, also salmon and cod bones were evaluated as a good source for well absorbable calcium (Malde *et al*., 2010). The authors suggested these fish bones as a valuable by-product to be used as a natural calcium source in functional foods or food supplements.

In various publications fish and seafood are suggested to be a better source of phosphorus with an average between 204 and 230 mg/100g phosphorus in fish, mollusks and crustaceans, compared to176 mg/100 g in terrestrial meats (Martınez-Valverde *et al.*,2000; Tacon and Metian,2013)

**2.2 Fish as dietary sources of highly polyunsaturated fatty acids**

Consumption of fish meal has been shown to be associated with reduced risks of sudden cardiac death. Fish contain large quantities of the very-long-chain and highly polyunsaturated fatty acids with 20 and 22 carbons and 5 and 6 double bonds, eicosapentaenoic acid (EPA; 20:5n23) and DHA which confer various health benefits to humans. They prevent heart diseases through variety of actions which include the prevention of arrhythmias (ventricular tachycardia and fibrillation), they are prostaglandin and leukotriene precursors, they have anti-inflammatory properties, inhibition of the synthesis of cytokines and mitogens, stimulation of endothelial-derived nitric oxide, being antithrombotic as well as having hypolipidemic properties with effects on triacylglycerols and VLDLs. Omega-3 fatty acids are a family of polyunsaturatedfatty acids (PUFA) which have in common a carbon-carbon double bond in the ω-3 position.

Important omega-3 fatty acids in nutrition are: α-linolenic acid (18:3, ALA), eicosapentaenoic acid (20:5, EPA), and docosahexaenoic acid (22:6, DHA). The three polyunsaturates have 3, 5 and 6 double bonds in a carbon chain of 18, 20 and 22 carbon atoms, respectively (Trattner, 2009). The human body cannot synthesize omega-3 fatty acids *de novo*, but can synthesize all the other necessary omega-3 fatty acids from the simpler omega-3 fatty acid α-linolenic acid (Vance and Vance, 2008,). Generally, sea fish, especially those in cold waters have higher levels of DHA and EPA than fresh water fishes (Sahena *et al.*, 2009). The oil from marine fish has a profile of around seven times as much omega-3 as omega-6 but farmed salmon, being grain fed, have a higher proportion of omega-6 than wild salmon

(Weaver *et al.*, 2008). Other oily fish such as tuna also contain omega-3 in somewhat lesser amounts (Weaver *et al.*, 2008).

Although sea fish is a dietary source of omega-3 fatty acids, they do not necessarily always synthesize them; they obtain them from the algae in their diet. For this reason, there is often a significant difference in EPA and DHA concentrations in farmed versus wild caught fish (Trattner, 2009). Research show that farm raised fish tend to have less EPA and DHA compared to wild type fish which may be attributed to the farming practices adopted by fish farmers. In one such study, the fatty acid profiles of farmed tilapia and channel catfish in the American marketwas found to be so high in linoleic and arachidonic acids and so low in DHA and EPA, that it was concluded that consumption of these fishes is in fact harmful rather than beneficial to cardiovascular health (Weaver *et al.*, 2008).

### 2.3 Effect of fatty acids and fish consumption on human health

Palmitic acid (16:0, PA) is the most common saturated fatty acid which can be provided in the diet or synthesized endogenously from other fatty acids, carbohydrates and amino acids. Palmitic acid represents 20–30% of total fatty acids (FA) in membrane phospholipids (PL), and adipose triacylglycerol (TAG) (Carta *et al*., 2015). According to the World Health Organization, consumption of palmitic acid increases the risk of developing cardiovascular disease by increasing Low density lipoproteins levels in the blood (WHO, 2003). However, retinyl palmitate is an antioxidant and a source of vitamin A in human body.

Palmitic acid, regardless of obesity, impairs leptin and insulin's ability to regulate food intake and body weight and decreases activation of phosphatidylinositide 3-kinases (PI3K) (Benoit *et al.*, 2009). Phosphatidylinositide 3-kinases (PI3K) are a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer.

Palmitic acid may also decrease oxidation of fatty acids and glucose in muscle cells, which elevates these levels in tissues and blood, and decreases adiponectin production, which may both promote insulin resistance (Kennedy *et al.*, 2009).

Stearic acid, compared with other SFA, has been shown to lower plasma LDL cholesterol levels, and have no effect on HDL cholesterol (Hunter et al., 2010). Therefore, even though stearic acid is a SFA, it does not appear to adversely affect cardiovascular diseases (CVD) risk, possibly because it is desaturated in part to oleate (18:1 n-9) during metabolism (Sampath and Ntambi, 2005). Although animals are able to derive the required saturated fatty acids *de novo*, these fatty acids are common components of diet especially in animal derived foods. Unfortunately, high dietary levels of these acids are capable of raising the levels of low density lipoproteins (LDL) in the circulatory blood which is linked to the development of diabetes, atherosclerosis and related cardiovascular problems such as heart attack, stroke and hypertension (Ginsberg *et al*., 1998).

Oleic acid is the dominant monounsaturated fatty acid in oils of both plant and animal origin. Studies state that oleic acid is effective in prevention of ischemic heart diseases (Cocchi *et al.*, 2009). It has been shown that oleic acid inhibits platelet aggregation induced by platelet aggregation factor (PAF) as well as the secretion of serotonin. A study carried out by Mendez has revealed that oleic acid blocked the action of HER2/neu an oncogene causing cancer (Kudo *et al.*, 2011). Oleic acid reduces cardiovascular diseases such as hypertension, aneurysm, thrombosis (Cocchi *et al.*, 2009). n-6 fatty acids potentiate inflammatory processes and consequently predispose to or exacerbate many inflammatory diseases such as alzheimers disease, rheumatoid arthritis, cardiovascular diseases and atherosclerosis (Patterson *et al*., 2012).

In the body, LA and ALA are partly converted to other non-essential fatty acids by elongation and further desaturation.This results into formation of n-6 series fatty acids such as gamma linolenic acid, dihomo-gamma linolenic acid and arachidonic

acid.GLA has been demonstrated to have efficacy in the treatment of rheumatoid arthritis (DeLuca *et al*., 1995). Mechanisms for the effect of GLA against rheumatoid arthritis and atherosclerosis have been reviewed by Fan and Chapkin (1998). Dietary GLA, thus, increases the content of delta-aminolaevulinic acid (DALA) in cell membranes without concomitant rise in the levels of Arachidonic acids.Dihomo Gama Linolenic Acid(DGLA) can be converted to an anti-inflamatory type of prostaglandins in contrast to pro-inflamatory prostaglandins derived from arachidonic acids (Monrig *et al*., 2011).

There has been increased scientific and public interest in the role of omega 3 fatty acids found in fish and fish oils in prevention and management of cardiovascular diseases (Morimoto *et al*., 2006;Connor,2000). The basis of this heightened interest in dietary intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) comes partly from epidemiological and population studies indicating that increased consumption of fish as a source of omega-3 fatty acids are often associated with decreased mortality (as well as morbidity) from cardiovascular disease (CVD) (Connor,2000).

This reduction in coronary risk has been related to the capacity of the omega-3 fatty acids such as EPA and DHA of marine lipids to lower serum triglyceride levels and decrease platelet aggregation (Harris and Jacobson, 2009; Harris, 2008) and blood pressure (Bonaa, *et al*., 1990). The main form of CVD is coronary heart disease (CHD), which makes up more than one-half of all CVD events (World Health Organization, 2005). Dietary intervention with marine lipids on patients with cardiovascular risk has been stimulated by epidemiological observation showing that population groups consuming large amounts of seal and fish experienced low mortality from coronary heart disease (Harris and Jacobson, 2009; Harris, 2008).

Coronary heart disease (CHD) is caused by arteriosclerosis, which is triggered by an unbalanced fatty acid profile in the body (World Health Organization, 2005). In particular, saturated fatty acids and polyunsaturated n-6 fatty acids have a close relationship with CHD (Simopoulos, 2004).

Clinical and epidemiological studies have indicated that dietary n-3 fatty acids, including α-linolenic (an essential fatty acid) and its metabolites eicosapentaenoic and docosahexaenoic acid reduce the risk of suffering CHD (de Lorgeril *et al.*, 1994, 1996).These compounds are derived from fish oil, and epidemiologic evidence suggests some relationships between ingestion of fish oil and both cardiac disease and depressive disorder in different cultures (Nemets *et al.*, 2008). Therefore, fish could be the food with the best potential for combating both malnutrition and diet related chronic diseases because of its high quality protein, water-soluble and fat-soluble vitamins, variety of minerals as well as the presence of highly polyunsaturated fatty acids (Stansby 1973).

Recent reports have revealed that dietary DHA-rich fish oils influences brain development, learning, memory and visual function (Salem *et al*., 1986; Suzuki *et al*., 1998a) increasing the uncorrected visual acuity in young people with myopia (Suzuki, *et al*., 1998b), and even improvement of intelligence and visual acuity in the elderly (Suzuki *et al*., 2001). The n-3 fatty acids reduce risks of inflammatory diseases and possibly behavioral disorders (Connor, 2000). The n-3 fatty acids have also been associated with decreased total mortality and positive effects on early neurodevelopment (Mozaffarian and Rimm, 2006).

### 2.4 African Catfish

African Catfish, *Clarias gariepinus* (Burchell, 1822) is a benthopelagic, dioecious, omnivorous fish which is widely tolerant to extreme environmental conditions. They are usually dark gray or black coloration on the back, fading to a white belly (Yalçin*et al.*, 2002). They are recognised by their long dorsal and anal fins which give them rather eel-like appearance.

Cat fish has a slender body; a flat bony head and a broad, terminal mouth with four pairs of barbells. Its prominent barbells give it the image of cat-like whiskers. They also have a large, accessory breathing organ composed of modified gill arches. Also, only the pectoral fins have spines.

**Figure 1: African catfish (*Clarius gariepinus*)**

They have an average adult length of 1-1.5 meters reaching a maximum length of 170 cm (67.0 inches.*C. gariepinus* is appreciated by consumers for the quality of its meat (Pruszyriski, 2003). Mostly they are smoked and used in soups. The fish is mostly cultured in earth ponds. African catfish is an excellent species for aquaculture because it is omnivorous, grows fast and tolerates relatively poor water quality (Rad *et. al*., 2003).

Their nutritional requirements in fish pond are highly variable and are influenced by factors such as management practices, stocking densities, availability of natural foods, temperature, fish size daily feed ration and feeding frequency. The optimal temperature for growth appears to be $30^0$C. However, temperature in the range of 26-$33^0$C are known to yield acceptable growth performance.

## 2.5 Nile tilapia

Nile tilapia, *Oreochromis niloticus*, (Linnaeus, 1758) is one of the first fish species cultured due to their aquacultural characteristics which include among others tolerance to poor water quality and the fact that they eat a wide range of natural food organisms (Popma and Masser, 1999). Tilapia is a generic name of a group of cichlids native to Africa.

The groups consist of three aquaculturally important genera; *Oreochromis*, *Sarotherodon* and *Tilapia* (Popma and Masser, 1999). Reproductive behavior is the most notable characteristic which distinguishes these genera with tilapia being nest builders while the other genera being mouth breeders. More than 90% of commercially farmed fish are Nile tilapia. Less commonly farmed species include blue tilapia (*O.aureus*), Mozambique tilapia (*O.mossambicus*) and the Zanzibar tilapia (*O.urolepis hornorum*). Nile tilapia has strong vertical bands, blue tilapia has interrupted bands and the Mozambique tilapia has weak or no bands. The scientific names for tilapia has been revised a lot in the last 30 years creating some confusion in naming tilapia with names given as *Tilapia niloticus*, *Sarotherodon niloticus* and currently *Oreochromis niloticus* (Popma and Masser, 1999).

**Figure 2: Nile tilapia (*Oriochromis niloticus*)**

Tilapia can easily be identified by interrupted lateral lines, a characteristic of cichid family of fishes. They are laterally pressed and deep bodied with long dorsal fins. The forward portion of the dorsal fin is heavily spined. Spines are also found in the pelvic and anal fins. The cultured species is distinguished by different banding patterns on the caudal fin (Popma and Masser, 1999). Tilapias grow best in waters with temperature range of 20-35$^0$C attaining a weight of 500g in eight months if breeding is controlled and food supply is adequate (Popma and Masser, 1999). Tilapia is omnivorus feeding on phytoplankton, zooplanktons, macrophytes and detritus.

Higher yield has been realized in semi-intensive system of fish farming, which require much greater investment in terms of management and stocking (Popma and Masser, 1999). For commercial significance, male tilapia is known to grow almost twice faster as females thus it is preferable to stock only males (monosex culture) to achieve the fastest growth and reach market size in the shortest period of time. Tilapia has also been known to do well in polyculture ponds with cat fish and other predatory fish (Popma and Masser, 1999)

**2.6 World aquaculture**

Aquaculture is the world‘s fastest growing agro-food sector, growing at an annual rate of 8.8% over the past 30 years (Toufique and Belton, 2014). With the growing benefits in quality, efficiency, technology, and knowledge, farmers are developing freshwater aquaculture farms. While global wild capture fishing remains under threat to overexploitation especially in certain regions where catches are concentrated, the global aquaculture contribution has increased from about 13% to 42% (FAO, 2014).

The rise in global aquaculture production will hopefully bring down the wild capture production to safer more sustainable levels. According to the (FAO, 2012a) the increase in fish consumption is largely driven by population growth, urbanization, change in food preference, rising income levels and efficiency in the aquaculture

industry. About 58.3 million people were directly involved in the global aquaculture industry (FAO, 2014).

The international aquaculture statistics of the FAO (2014) states that America (mainly South America) and especially Africa has much lower per capita fish consumption levels and supply per capita than the global average. Africa and South America therefore have much more potential to increase its production and per capita consumption of fish. In Africa, this will close food and nutrition gaps in many regions as well as contributing to the socio economic development.

African aquaculture development has seen the greatest growth per continent at 11.7% in the past 12 years (FAO, 2014). Despite the wealth of natural and human resources, Africa ‘s contribution to global farmed fish production is very low, at 2.23% of global production (FAO, 2014) with North Africa making up 69% of the total African production. Egypt is the leading producer in Africa, producing 630 000 tonnes in 2007 (Rana, 2011).

Across Africa many policy makers have placed aquaculture development high on their development agendas and strategic frameworks. International assistance has been requested to help develop the sector. Challenges hindering development of aquaculture is mainly tied to challenges and restraints namely: Water, land, financial, and support mechanisms needed for aquaculture production have become difficult to access as competition for these resources becomes tighter. These challenges are a threat to the sustainability to the aquaculture sector in Africa (FAO, 2014). Brummett *et al* (2008) suggested that a pragmatic business approach supporting the development of SMEs (small and medium-scale private enterprises) would improve and provide more benefits to aquaculture development in Africa in relation to centrally planned subsidized projects. Such programs have seen increase in aquaculture activities in Kenya through economic stimulus programme, 2010 (Nyonje *et al.,* 2014)

## 2.7 Aquaculture in Kenya

Fish farming was initiated by colonialist in 1950s through the introduction of trout in rivers for sport fishing before culture of species such as tilapia, common carp, and catfish came into place (Ngugi *et al*., 2007). The "eat more fish campaigns" promoted by the government in the late 1960s accelerated the interest in rural fish farming (Ngugi and Manyala, 2004; Ngugi *et al*., 2007). By 2006, the mean yield from fish farming was approximately 5.84 MT $yr^{-1}$valued at $10.78 million, accounting for only 3% of the total fish production (National Economic Survey, 2006).

With the advent of government-funded Economic Stimulus Program (ESP) coupled with several aquaculture facilities in various parts of the country to serve as research centers, training facilities, and sources of fingerlings and feed for fish farmers, the national aquaculture production was estimated at 12,000 MT/y, equivalent to 7% of the total production and valued at $21 million by 2011 (Nyonje *et al*., 2011). In addition, there has been a growing knowledge on the health benefits of fish consumption across the world, increasing demand for fish and fish products (Kris-Etherton *et al.,* 2003). The freshwater fish production has also been declining with current production dropping by 12.3 per cent from 136.4 thousand tonnes in 2015 to 119.6 thousand tonnes in 2016 (National Economic Survey, 2016). These factors have propelled the steady growth of aquaculture in Kenya.

## 2.8 Aqua feed industry in Kenya

The performance of the aquaculture sector in Kenya has been hit by a number of constraints including unavailability of efficient and inexpensive fish feeds for different stages of fish development (Munguti *et al.,* 2012). Much of the aquafeeds used in Kenya are either produced on-farm or by small-scale semi-commercial feed manufacturers which require improvements in terms of the quality and preparation to bring about improved productivity and cost savings (Munguti *et al.,* 2014). The most

commonly used feed ingredients include: Ochonga (*Caridina nilotica*), Omena (*Rastrineobola argentea)*, Wheat or rice bran, Sunflower or cotton seed cake and cassava for binding. These ingredients are ground separately and later mixed to ratios before pelletizing to produce semi floating pellets, which are then sun, dried and stored in gunny bags (Munguti and Charo, 2011). Due to the increased demand for fish feed, unscrupulous dealers took advantage to compromise the quality of fish feed, prompting the government to initiate efforts to establish fish feed standards. The standards have helped manufacturers to improve quality of their products (Munguti *et al.*, 2014). However, the cost of these feeds is usually the limiting factor to most farmers thus they prefer locally mixed feeds (Munguti *et al.*, 2014).

The paradox of fish meal (FM) and fish oil (FO) use in aquaculture has also sparked major research into alternative feed ingredients to replace FM and FO over the past two decades across the world. The focus of the recent and current research has been on terrestrial plant meals, concentrates and oils; and many of these studies have led to an impressive reduction in the average inclusion of FM and FO in commercial feeds (Olsen and Hasan, 2012). The nutritive composition of fish depends on dietary composition thus dietary supplementation is important for improved fish quality.

**2.9 Dietary supplementation of aqua feeds with vegetable oil**

Recent studies have shown considerable success in partially or totally replacing FO with VO in many fish species (Ng, 2003; Turchini *et al*., 2009, 2010). Different fish species have shown varied sensitivity to VO supplementation levels. In addition, studies have shown that replacement of high-quality FO with VO sources affect the growth performance, fatty acid profile or health parameters of fishes, such as gilthead seabream (*Sparus aurata*) (Menoyo *et al.*,2004) and European sea bass (*Dicentrarchuslabrax L*.) (Mourente *et al*., 2005). The extent of such effects depends on the vegetable oil source and level of replacement. In comparison with other vegetable oils, linseed oil (LO) is distinguished by the highest content of α-linolenic acid (18:3n-3, ALA) (Popa *et al*.,2012), which is the metabolic precursor of n-3

long-chain polyunsaturatedfatty acids (LC-PUFA) such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). Other vegetable oil sources such as sunflower (SFO), soy (SO), peanut (PO), and rapeseed (RO) oils have been used as the main lipid source in feeds do not lower growth rates in Atlantic salmon (Salmo salar) if appropriate supplies of eicosapentaenoic (C20:5 n-3, EPA) and docosahexaenoic (DHA, C22:3 n-3) acids are provided (Torstensen *et al.,* 2005). Feeding pikeperch (*Sander lucioperca*) feeds supplemented with vegetable oils (LO, PO) comprising 80% of the overall quantity of lipids in high-fat feeds (approximately 190 g fat/kg feed) does not influence fish growth, but it does alter the histological structure of internal organs and the blood biochemical parameters (Kowalska *et al.,* 2010a,). Increased contents of both linolenic acid (C18:3 n-3, ALA) and linoleic acid (C18:2 n-6, LA) in pikeperch diets result in lowered vacuolization in hepatocytes and pyknotic nuclei as well as in increased ceruloplasmin activity in fish blood (Kowalska *et al.,* 2010a). Rainbow trout fed diets supplemented with VO with an excess supply of n-3 PUFA result had reduced immune response (Wang *et al.*, 2006). According to Wang *et al.,* (2006) and Lin and Shiau (2007), dietary deficits of essential fatty acids (EFA) suppress immune cell function.

While altering the n3/n6 ratio in feeds by varied supplementation with corn oil or LO, does not reduce tilapia growth rates (Karapanagiotidis *et al.*, 2007). Feeding fish feeds supplemented with VO, with dominating LA and ALA, might alter the proximate composition of their bodies (Turchini *et al.,* 2009). While some fish species can bio convert LA and ALA into highly unsaturated fatty acids (HUFA), physiological accumulation occurs when the diets supply excessive quantities of these fatty acids (Stubhaug *et al.,* 2005). Oils rich in LA (e.g., SFO) increase the share of n-6 PUFA in salmon fillets (Ruyter *et al.,* 2006), while the addition of oils rich in ALA (LO) can improve the retention in fillets of desirable FA such as EPA and DHA while simultaneously lowering the accumulation of n-6 PUFA (Menoyo *et*

*al.,* 2007). The content of FA from the n-3 and n-6 series in the diet can be modulated using a mixture of VO with high contents of ALA or LA.

### 2.10 Fatty acid profiles of selected Vegetable oils

In recent past, production of vegetable oil (VO) has increased considerably and exceeded one hundred fold that of FO (Bimbo, 2007; Turner *et al*., 2008). This make oils extracted from oilseeds or other vegetable products an inexpensive and sustainable alternative to FO. Vegetable oils are generally rich in n-6 and n-9 fatty acids, mainly linoleic acid (LA, 18:2n-6) and oleic acid (OA, 18:1n-9), with moderate or low levels of n-3 PUFA, mainly α-linolenic acid (ALA, 18:3n-3) (Guil-Guerrero *et al.,* 2001; Aberoumand, 2009). Seed oils from some borage plants can contain relatively high content of stearidonic (SDA, 18:4n-3) at ~15% and γ-linoleic (GLA, 18:3n-6) at ~12% by weight in the Patterson'sCurse (*Echium plantagineum*) shrub (Erdemoglu *et al.,* 2004; Whelan and Rust, 2006b). Such differences in n-3 PUFA content may have numerous effects on the biology of cultured fish (Sargent *et al.,* 2002; Miller *et al.,* 2008a). Feeding fish on oil rich in SDA/GLA may allow farmed fish to bypass one of the rate limiting steps and biosynthesize LC-PUFA. The later approach, through the use of *Echium* oil (EO) or SDA-containing oil from other sources, has shown to date contrasting results due to species differences, their varied ecosystems and a changing ability for LC-PUFA biosynthesis during the different life-history stages (Tocher *et al.,* 2006b; Miller *et al.,* 2008a; Bharadwaj *et al.,* 2010; Codabaccus *et al.,* 2012).

**Table 1: World production (million metric tons in 2011) and fatty acid profiles (as percentage of total fatty acids) of selected vegetable oils. (White, 2008; Rincón-Cervera and Guil-Guerrero, 2010).**

| **Source** | **Palm** | **Soybean** | **Canola** | **Sunflower** | **Cottonseed** | **Olive** | **Linseed** | **Echium** |
|---|---|---|---|---|---|---|---|---|
| Production | 50.6 | 42.9 | 22.8 | 13.2 | 5.4 | 3.5 | 0.75 | nd |
| Fatty acids | | | | | | | | |
| 14:00 | 1.1 | 0.1 | 0.1 | 0.2 | 0.9 | 0.1 | 0.0-0.4 | 0.1 |
| 16:00 | 45.1 | 9.7-11.0 | 2.8-3.9 | 6.8 | 24.7 | 7.5-20.0 | 6.3 | 7.7 |
| 18:00 | 4.6-5.2 | 3.5-4.0 | 1.3-2.1 | 4.7 | 2.3 | 0.5-5.0 | 4-6 | 3.9 |
| **Total SFA** | 53.5 | 13.4 | 7.4 | 12 | 27.8 | 14.6-25.0 | 11.4 | 11.2 |
| 16:1n-7 | 0.1 | 0.1-0.7 | 0.2 | 0.1 | 0.7 | 0.3-3.5 | 0.5 | 0.0-0.2 |
| 18:1n-9 | 33.8-38.8 | 21-23.4 | 23.8-63.1 | 18.6-25.3 | 17.6 | 55.0-83.0 | 15-39 | 14.9-17.2 |
| **Total MUFA** | 35.1-39.0 | 25 | 64.7 | 27.2 | 19.2 | 58.5-85.0 | 19.6-40.0 | 15.5-19.9 |
| 18:3n-3 | 0.3 | 7.8 | 7.3-9.2 | 0.5-6.5 | 0.3 | 0.5-1.5 | 35-53 | 30.5-33.2 |
| 18:4n-3 | nd | nd | nd | nd | nd | nd | nd | 12-18 |
| **Total n-3 PUFA** | 0.3 | 8 | 12 | 6.8 | 0.3 | 0.5-1.7 | 56 | 44-47 |
| 18:2n-6 | 9.1 | 53.2 | 14.6-18.7 | 50.1-65.2 | 53.3 | 3.5-21.0 | 15-19 | 14-15.2 |
| 18:3n-6 | nd | nd | nd | nd | nd | nd | nd | 11.8 |
| **Total n-6 PUFA** | 9.4 | 53.5 | 20.1 | 68 | 53.5 | 4.0-22 | 15.2-19.5 | 26-27.5 |
| n-3:n-6 | 0 | 0.2 | 0.6 | 0.1 | 0 | 0.1-2.5 | 2.7-3.8 | 0.9-1.6 |

## 2.11 Synthesis of long chain fatty acids in fresh water fish

Fatty acid synthesis begins with a rate-limiting step where Acyl-CoA is condensed with malonyl-CoA to produce 3-ketoacyl-CoA. In the second step, 3-ketoacyl-CoA is reduced to 3-hydroxyacyl-CoA by a 3-ketoacyl-CoA reductase (Moon and Horton, 2003). Nicotinamide adenine dinucleotide phosphate (NADPH) is used as a reducing agent in this reaction. 3-Hydroxyacyl-CoA is then dehydrated by 3-hydroxyacyl-CoA dehydratase, generating 2, 3-*trans*-enoyl-CoA. Finally, 2, 3- trans enoyl CoA is reduced to an Acyl-CoA having two more carbon chain units than the original Acyl-CoA. A 2, 3-*trans*-enoyl-CoA reductase catalyzes this reaction using NADPH as a reductant. These reactions repeat until palmitic acid, C16:0 is formed (Moon and Horton, 2003).

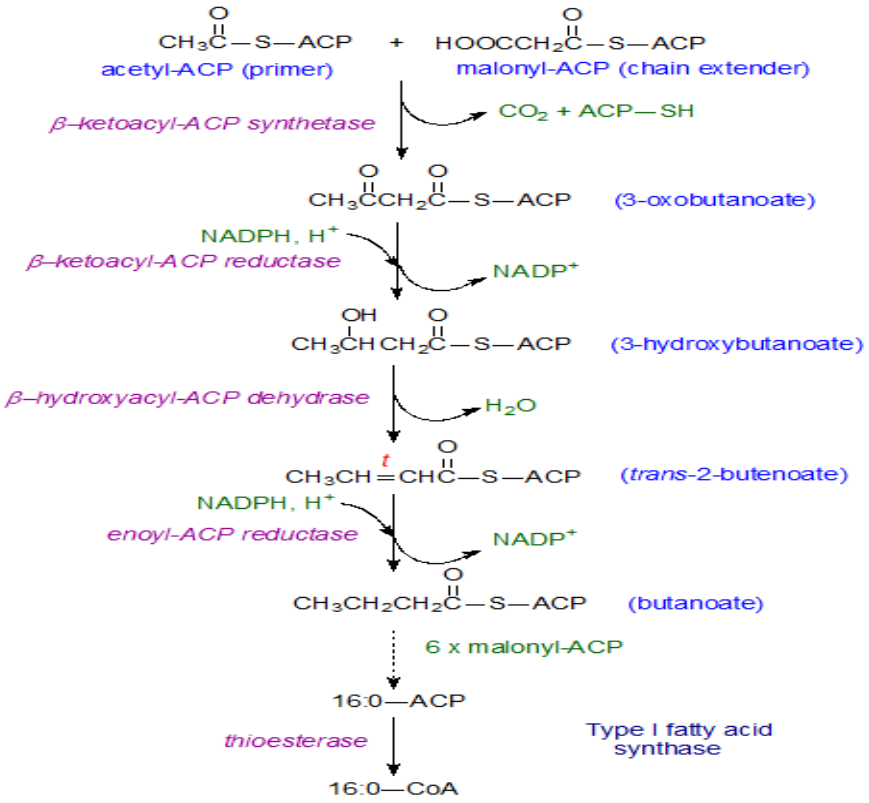



**Figure 3: Pathway for palmitic acid biosynthesis**

Palmitic acid (C16:0) and stearic acid (C18:0) are elongated by endoplasmic reticulum (ER) membrane-embedded enzymes following their conversion to acyl-CoAs. They can be converted to palmitoleic acid (C16:1*n* − 7) and oleic acid (C18:1*n* − 9), respectively, by Δ9 desaturase (stearoyl-CoA desaturase) (Akio, 2012). However, neither Δ12 desaturase, which produces linoleic acid (C18:2*n* − 6) from oleic acid, nor Δ15 desaturase, which converts linoleic acid to α-linolenic acid (C18:3*n* − 3), exists in humans so linoleic acid and α-linolenic acid must be consumed through food (Akio, 2012). Dietary linoleic acid and α-linolenic acid can be converted to other n − 6 and n − 3 FAs, respectively. For example, α-linolenic acid is metabolized to eicosapentaenoic acid (EPA; C20:5 *n* − 3) via desaturation by Δ6 desaturase and subsequent elongation. EPA is further converted to the well-known very long-chain PUFA (VLC-PUFA) docosahexaenoic acid (DHA; C22:6*n* − 3) by two successive elongation cycles, desaturation by Δ6 desaturase and β-oxidation in a peroxisome (Akio, 2012).

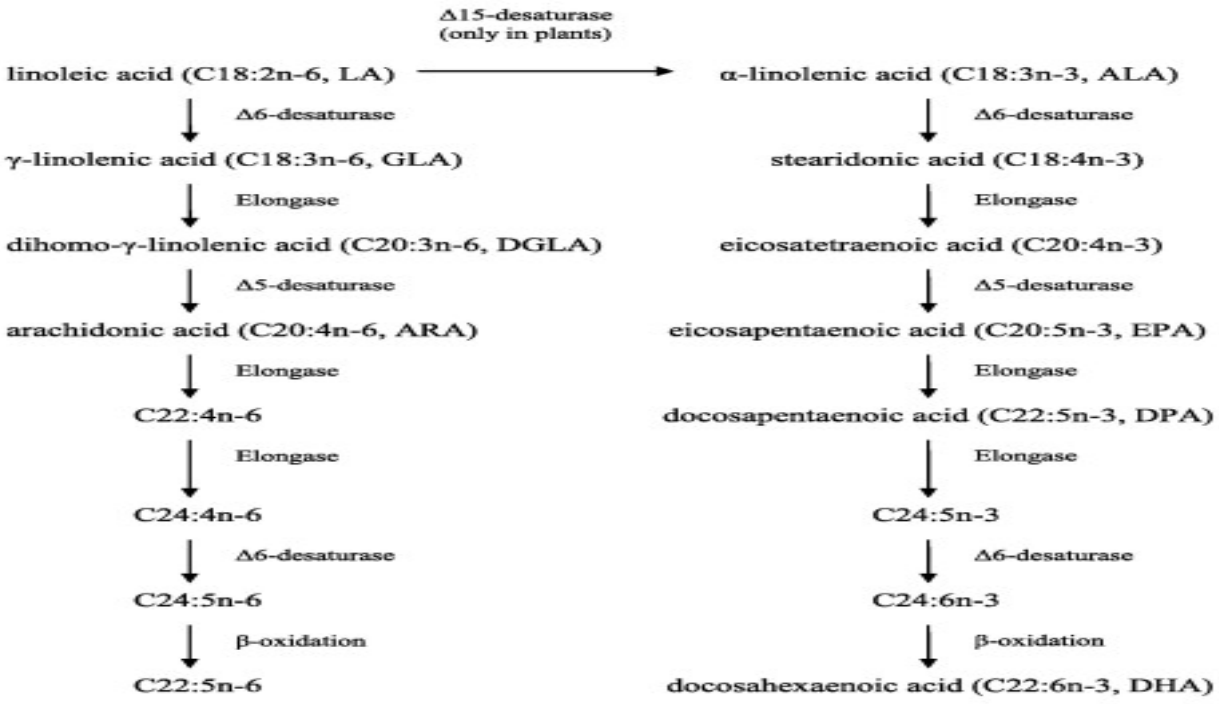



**Figure 4: Elongation and desaturation of ALA and LA to long chain PUFA (Tanghe and Smet, 2013)**

## 2.12 Metabolism of dietary n-3 polyunsaturated fatty acids

ALA is the dietary source for n-3 PUFA synthesis. From the diet, ALA is absorbed by the gastrointestinal tract and then secreted into the blood stream for delivery to the liver (Burge and Calder, 2005). Bioavailability of ALA depends on the efficiency of uptake and the degree of partitioning towards degradation as well as incorporation into structural organs and storage reservoirs such as adipose tissues (Burge and Calder, 2005). The reservoirs avail lipids for mobilization during periods of increased body demand (Flachs *et al*., 2009). In addition to assimilation, ALA is used for energy production through beta oxidation resulting into carbon cycling (Cunnane *et al*., 2003) as well as being a substrate for the synthesis of EPA and DHA via series of desaturation,elongation and peroxisomal beta oxidation steps (Spretcher,1995). Desaturation and elongation processes occur in the endoplasmic reticulum of the liver with the final beta oxidation step (oxidation of tetracosahexaenoic acid (C24:6) to DHA C22: 6) occurring in the peroxisomes.

## 2.13 Fatty acyl Desaturases

Fatty acyl-coenzyme A (CoA) desaturases are enzymes responsible for the introduction of a double bond at a specific position of the acyl chain of a long-chain fatty acid (Guillou *et al.*, 2010). The CoA desaturases can be divided into two broad families including the stearoyl-CoA desaturases (SCD) and the fatty acyl desaturases (Fad) (Marquardt *et al.*, 2000). SCD exhibits Δ9-desaturation activity and is the enzyme alluded to above that is responsible for the desaturation of 16:0 and 18:0 to produce 16:1 n-7 and 18:1 n-9, respectively. On the other hand, Fad enzymes constitute a family of genes in vertebrates with three members; FADS1, FADS2 and FADS3. The FADS1 gene product has been demonstrated to have Δ5 desaturation activity, whereas FADS2 encodes a protein with prominent Δ6 activity. Despite sequence similarities with other FADS family members, no functional activity has been yet attributed to FADS3 (Guillou *et al.*, 2010).

Several FAD cDNAs have been isolated and functionally characterised from a relatively wide range of fish species of freshwater, marine and diadromous fish including gilthead sea bream (Seiliez *et al.*, 2003; Zheng *et al.*, 2004a), Atlantic salmon (Hastings *et al.*, 2005; Zheng *et al.*, 2005a; Monroig *et al.*, 2010a), Nile tilapia (Zheng *et al.*, 2004a), carp (Zheng *et al.*, 2004a), turbot (Zheng *et al.*, 2004a), Atlantic cod (Tocher *et al.*, 2006), cherry salmon (Alimuddin *et al.*, 2005, 2007), rabbitfish (Li *et al.*, 2008, 2010), cobia (Zheng *et al.*, 2009a), European sea bass (Gonzalez-Rovira *et al.*, 2009; Santigosa *et al.*, 2011), barramundi (Mohd-Yusof *et al.*, 2010), nibe croaker (Yamamoto *et al.*, 2010), striped snakehead fish (Jaya-Ram *et al.*, 2011), Northern bluefin tuna (Morais *et al.*, 2011) and red sea bream (Sarker *et al.*, 2011). Phylogenetic analyses performed on the deduced amino acid sequences of the Fad genes revealed that all the desaturases so far isolated from fish are FADS2 orthologues (Monroig *et al.*, 2011b). Whereas mammalian FADS2 has predominantly Δ6-desaturase activity, the fish Fad enzymes show a large variety of functions (Monroig *et al.*, 2011b).

Thus, fish Fad with Δ6-desaturation functionality have been isolated from a variety of both marine (gilthead sea bream, turbot, Atlantic cod, cobia, European sea bass, barramundi, and Northern bluefin tuna) and freshwater fish (common carp and rainbow trout). Interestingly, the diadromous species Atlantic salmon, probably the fish species whose LC-PUFA metabolism has been more investigated, possesses four distinct genes encoding FAD proteins with high homology to mammalian FADS2 (Monroig *et al.*, 2011b).

Whereas three desaturases, termed Δ6Fad_a, Δ6Fad_b, and Δ6Fad_c were found to be strict Δ6-like desaturases (Zheng *et al.*, 2005a; Monroig *et al.*, 2010a), a fourth desaturase gene was determined to have a Δ5-desaturase capability despite being phylogenetically closer to FADS2 (mammalian Δ6) than FADS1 (mammalian Δ5) (Hastings *et al.*, 2005). Salmon Δ5Fad is the only monofunctional Δ5-desaturase so far discovered, and enables this species to perform all desaturation reactions required

in the LC-PUFA biosynthesis pathways, i.e. Δ6 and Δ5 desaturations. It is therefore important to emphasise that Δ5 desaturases are apparently missing in marine teleosts, and this has been hypothesised to be a limiting step explaining the low ability of marine fish to biosynthesize DHA (Monroig *et al.*, 2011b).

### 2.14 Elongases of very long-chain fatty acids

Elongases of very long-chain fatty acids (ELOVL) are the initial and rate-limiting enzymes responsible for the condensation of activated fatty acids with malonyl-CoA required for biosynthesis of long-chain fatty acids (Nugteren, 1965). Seven members of the Elovl family, termed ELOVL 1-7, have been identified in mammals that differ from each other in their substrate specificity (Jakobsson *et al.*, 2006). Generally, ELOVL1, ELOVL3, ELOVL6 and ELOVL7 have been determined to participate in elongation of saturated and monounsaturated fatty acids. Contrarily, Elovl family members involved in the biosynthesis of LC-PUFA, namely ELOVL2, ELOVL4 and ELOVL5, have been extensively investigated in a range of fish, especially farmed species (Monroig *et al.*, 2011b). Elovl5-encoding cDNAs have been isolated from many species, with zebrafish Elovl5 being the first cloned and functionally characterised (Agaba *et al.*, 2004). Similar to mammalian ELOVL5, zebrafish Elovl5 efficiently elongated PUFA including C18 (18:4n-3 and 18:3n-6) and C20 (20:5n-3 and 20:4n-6), with only low conversion shown towards C22 substrates (22:5n-3 and 22:4n-6) (Monroig *et al.*, 2011b).

Later, ELOVL5s were cloned and characterised in Atlantic salmon (Hastings *et al.*, 2005) and in catfish, tilapia, turbot, gilthead sea bream and Atlantic cod (Agaba *et al.*, 2005). Further publications on cobia (Zheng *et al.*, 2009a), barramundi (Mohd-Yusof *et al.*, 2010), Southern (Gregory *et al.*, 2010) and Northern (Morais *et al.*, 2011) confirmed that fish ELOVL5s all demonstrated the ability to elongate C18 and C20 PUFA with n-3 substrates generally being preferred as substrates over n-6 series fatty acids (Monroig *et al.*, 2011b).

Other potential substrates for ELOVL5 are 18:3n-3 and 18:2n-6, which can be converted to 20:3n-3 and 20:2n-6, respectively, suitable substrates for Δ8-desaturation (Guillou *et al.*, 2010). The understanding of fatty acid elongation pathways in fish has considerably advanced over the last few years with the investigation of other Elovl family members involved in the biosynthesis of LC-PUFA. Thus, ELOVL2 enzymes with the ability to elongate C20 (20:5n-3 and 20:4n-6) and C22 (22:5n-3 and 22:4n-6) LC-PUFA substrates have been cloned and functionally characterised in Atlantic salmon (Morais *et al.*, 2009) and zebrafish (Monroig *et al.*, 2009).

Although 20:5 n-3 and 20:4 n-6 are also elongation substrates for ELOVL5, 22:5n-3 and 22:4 n-6 are only efficiently converted by ELOVL2. Importantly, the ability of ELOVL2 to elongate 22:5n-3 to 24:5n-3 has been regarded as key for the production of DHA via the Sprecher pathway. To date, no ELOVL2 cDNA has been isolated from a marine fish species, and this has been hypothesised as another possible factor underlying their very limited ability for DHA biosynthesis (Morais *et al.*, 2009). Recent studies, however, have revealed that other enzymes may effectively elongate C22 PUFA in marine fish, which could compensate for the lack of ELOVL2.

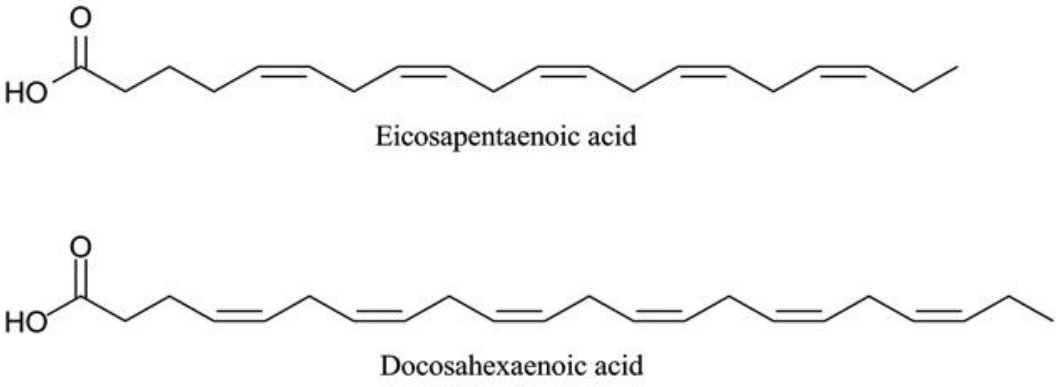



**Figure 5: Structure of EPA and DHA**

## 2.15 Dietary regulation of desaturase and elongase expression

Nutritional regulation of gene expression and the activity of the LC-PUFA biosynthesis pathway have also been demonstrated in freshwater and salmonid fish species. For example, LC-PUFA biosynthesis activity was increased by EFA-deficiency and modulated by different C18 PUFA in carp cells (Tocher and Dick, 1999, 2000).

Furthermore, in feeding trials with salmonids, LC-PUFA biosynthesis was increased (Tocher *et al.* 1997, 2001), and expression of LC-PUFA biosynthesis genes, especially Δ6 Fad, was induced in salmon liver and intestine in fish fed diets containing vegetable oils lacking LC-PUFA compared to fish fed diets containing fish oil rich in EPA and DHA (Zheng *et al.*, 2004b, 2005a, b; Leaver *et al.*, 2008b; Taggart *et al.*, 2008). In contrast, liver and intestinal Δ6 Fad expression and activity was generally not significantly affected in Atlantic cod fed diets containing either vegetable or fish oil (Tocher *et al.*, 2006), possibly reflecting differences in the Fad gene promoters in cod and salmon (Zheng *et al.*, 2009b). Regulation of FADS2 and ELOVL genes was also investigated in marine fish larvae, a developmental stage particularly sensitive to suboptimal LC-PUFA supply in the diet (Navarro et al., 1997; Sargent *et al.*, 1997, 1999; Izquierdo, 2000).

Therefore, Δ6 Fad appeared to be up-regulated in larval gilthead sea bream fed on micro diets formulated on rapeseed and soybean oil with low EPA and DHA compared to larvae fed on diet formulated with fish oil (Izquierdo *et al.*, 2008). A recent study on nibe croaker, a marine sciaenid teleost, reported increased expression of a putative Δ6 Fad in response to *Artemia* diets containing low C20-22 LC-PUFA (Yamamoto *et al.*, 2010). The expression of an ELOVL5-like elongase, however, did not show nutritional regulation in this species.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study sites

Proximate composition analysis of experimental diets and fatty acid composition in fish muscles and liver were done in Food Biochemistry laboratory, Food Science department-Jomo Kenyatta University of Agriculture and Technology (JKUAT). Fish culturing was partly done at Kenya Marine and Fisheries research institute (KEMFRI)-Sagana and aquaponics greenhouse, JKUAT. RNA extraction, cDNA synthesis and PCR were done in Molecular laboratory, Biochemistry department, JKUAT. Real-Time PCR was partly done in PAUSTI laboratory, JKUAT

## 3.2 Sampling of wild and farmed catfish and tilapia

Wild fish were randomly sampled from four different beaches along Lake Victoria namely, Ralayo, Luanda Kotieno, Wichlum and Usenge, all in Siaya County. Fish were sampled in two groups based on their weight. Tilapia were grouped as; WTLI (400g-800g) and WTL2 (1kg-2kg) whereas catfish were grouped as WCF1 (600g-1.5kg) and WCF2 (1.8 kg-3kg). Farmed samples were obtained from three different fish farms namely JSF (Muranga county), SMKTU (Kiambu county) and UNIF(Kirinyaga county). Weights for farmed Nile tilapia ranged between 800g and 1300g whereas farmed African catfish ranged between 1kg and 1.5kg. Sampled fish were stored in ice during transportation and later kept under $-80^0$C for later fatty acid analysis.

## 3.3 Ingredients for experimental diets

The experimental diets used in this study contained freshwater shrimps (*Caridina nilotica*) as the main protein source, rice bran, wheat flour, popcorn maize flour, vegetable oil blends and vitamin and mineral premixes.

Fresh water shrimps were obtained from Wich lum beach along Lake Victoria in Siaya County, Kenya. The vegetable oil blends comprised of linseed oil, olive oil and sunflower oil. Popcorn maize, wheat flour, olive oil and sunflower oil were obtained from local supermarkets, Vitamin and mineral premixes were obtained from Tam Feeds and linseed oil was extracted from linseeds using extruder machine at the Biomechanical engineering workshop, Jomo Kenyatta University of Agriculture and Technology (JKUAT)

### 3.4 Formulation of experimental diets

Pearson's square method was used in feed formulation to determine the proper proportions of the feed components to meet the dietary requirements. The powdered components of the experimental diets were carefully weighed and pre-mixed prior to the addition of vegetable oil blends. The powdered ingredients were dry mixed thoroughly for 2 minutes in a bench top food mixer before addition of distilled water. Vegetable oil blend was then added and mixing continued for further 10 minutes. All the experimental diets were made on grade 12 meat mincer as an extruder fitted with a die plate with 2mm diameter holes. The soft feed dough was cold extruded into the 2mm die-size strand, pelleted and dried at ambient temperature for 3 hours. The feeds were then placed on a sieve and overdried at $40^0$C for approximately 24 hours until the moisture content was 10 % ( w/w). The dried feeds were then broken into 2-3mm pellets, sealed in plastic bags and stored at -$20^0$C until commencement of feeding trials. All equipment used for making up feeds were washed and dried before the next diet was produced to avoid cross-contamination.

### 3.5 Experimental setup for feeding experiments

Polyculture of monosex fish, 8 weeks old *O. niloticus* and 4 weeks old *C. gariepinus* which were previously fed on commercial diets were stocked in 1000-liter experimental tanks for a period of 150 days under recirculating water system.

The fingerlings were obtained from Kenya Marine and Fisheries Research Institute, KEMFRI, Sagana fish hatcheries. Before the feeding experiment was initiated, separate tanks were used as holding tanks in which fingerlings were fed on washout diet for a period of two weeks.

Washout diet was supplemented with 100% olive oil to neutralize the omega 3 fatty acids content in fingerlings as well as to acclimatize fingerlings. Three tanks containing 30 Nile tilapia and 30 African catfish were set for each experimental diet. Feeding was done twice/day at 9.00am and 4.00pm up to apparent satiation. Continuous water circulation was maintained using a water pump with water conditions and quality checked and maintained regularly for optimum water quality. This was done by replacing water in cases of water contamination. Sampling was done by obtaining 3 Nile tilapias and 3 African catfish randomly after 24 hours fasting period. For fatty acid analysis, sampling was done monthly while monitoring of growth parameters was done after every two weeks. The fish samples were weighed and length measured for growth parameters analysis. The samples were then anaesthetized and dissected for liver and muscles extraction. The extracted liver and muscles were then kept under $-80^0$C for later fatty acid and total RNA analysis.


**Figure 6: Experimental tanks used for culturing Nile tilapia and African catfish**

### 3.5 Proximate analysis

#### 3.5.1 Moisture

Moisture content, crude protein, crude fat, crude fiber and ash for diet ingredients and experimental diets were determined according to AOAC methods specification 950.46 (AOAC), 1995) (table 1), in brief, moisture content was determined by weighing 2g of sample into a moisture dish and transferred to an oven previously heated to temperatures of $105^0$C and drying done for 1 hour. The final weight of the sample was taken after the drying period and cooling in a desiccator. The flour residue was then reported as total solids and loss in weight as moisture by formulas given below (AOAC, 1995, method 925. 10).

$$\text{Moisture (\%)} = \frac{W1-W2}{W1}x100$$

Where: $W_1$= Weight of sample before drying, $W_2$ = Weight of sample after drying

#### 3.5.2 Crude protein

Crude protein was determined by Semi-Micro Kjeldahl method where about 1g of sample was weighed into a digestion flask together with a catalyst composed of 5g of $K_2SO_4$ and 0.5g of $CUSO_4$ and 15ml of concentrated $H_2SO_4$. The mixture was heated in a fume hood till the digest color turned blue signifying the end of the digestion process.

The digest was cooled, transferred to a 100ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. Ten (10) ml of diluted digest was transferred into a distilling flask and washed with about 2ml distilled water. 15 ml of 40% NaOH was added and this was also washed with about 2ml distilled water. Distillation was done to a volume of about 60ml distillate. The distillate was titrated using 0.02N-HCL to an orange colour of the mixed indicator which signified the end point (AOAC, 1995, Method 20.87-32.1.22).

Calculations were done using the formula below:

$$\text{Nitrogen\%} = (V_1 - V_2) \times N \times f \times 0.014 \times \frac{100}{V} \times \frac{100}{S}$$

Where $V_1$=Titer for the sample (ml); $V_2$= Titer for blank (ml)

N= Normality of standard HCL solution (0.002); F= Factor of standard HCL solution

V= Volume of diluted digest taken for distillation (10ml) S= Weight of sample taken (g)

Crude protein %= Nitrogen × protein factor

### 3.5.3 Crude fat

Crude fat was determined through soxhlet extraction method which gives intermittent extraction of oil with excess of fresh organic solvent used. About 5g of samples were weighed into extraction thimbles and the initial weights of the extraction flasks taken. Fat extraction was done using petroleum ether in soxhlet extraction apparatus for 8 hours.The extraction solvents were evaporated and the extracted fat dried in an oven for about 15 min before the final weights of the flasks with extracted fat were taken (AOAC, 1995, Method 920.85-32.1.13).

Calculations were done using the formula below:

$$\text{Crude fat (\%)} = \frac{W1 - W2}{W1} x\ 100$$

Where: $W_1$= Weight of sample before extraction; $W_2$ = Weight of sample after extraction

### 3.5.4 Crude fibre

Crude fiber was determined by weighing 2g of the sample into a 500ml conical flask. About 200ml of boiling 1.255M $H_2SO_4$ was added and boiling done for 30min under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter and the residue washed to completely remove the acid with boiling water. 200ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30min. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCL and again washed with boiling water to rinse the acid from the residue.

The residue was washed twice with alcohol and thrice with ether. It was then dried in an oven at $105^0$C in a porcelain dish to a constant weight ($W_1$). Incineration was done in a muffle furnace at $550^0$C for 3hrs, the dish was then cooled in a desiccator and the final weight ($W_2$) taken (AOAC, 1995, Method 920.86-32.1.15). Calculations were done as follows

Crude fiber (%) = $\frac{W1-W2}{W} x\ 100$

Where: $W_1$= Weight of acid and alkali digested sample; $W_2$ = Weight of incinerated sample after acid and alkali digestion; W= Weight of sample

Ash was determined from sample weights of between 2-5g weighed in pre-conditioned crucibles. The samples were first charred by flame to eliminate smoking before being incinerated at $550^0$C in a muffle furnace to the point of white ash.

The residues were cooled in desiccators and the weights taken (AOAC, 1995, Method 925.03-32.1.05).

Calculations were done as shown below:

Crude ash (%) = $\frac{\text{Weight of Ash(g)}}{\text{Weight of sample (g)}}$ x 100

### 3.6 Extraction of total Lipids and preparation of fatty acid methyl esters

Lipids extraction was done according to the procedure by Bligh and Dyer (1959). Lipids in experimental diets, tilapia liver and muscles were extracted by homogenization of finely ground 0.5 g of samples in chloroform–methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant and cold isotonic saline, 0.9% sodium chloride. This was mixed vigorously and allowed to stand for 20minutes. The mixture was then centrifuged at 3000rpm for 10minutes and the aqueous layer was then separated from organic layer using a micropipette. The bottom layer, chloroform, was then transferred to 100ml reflux flask, quick fit, and evaporated to dryness under vacuum evaporator.

Fatty acid methyl esters (FAME) were then prepared from vegetable oils and extracted total lipid by acid-catalyzed trans-esterification by adding 5ml of 1% $H_2SO_4$ (v/v) in methanol at $70^0$C, for 3hrs. FAME were then extracted into 750ml of distilled water and 10 ml of hexane, dehydrated using anhydrous sodium sulphate, $Na_2SO_4$ and concentrated to 0.5 ml under vacuum evaporator. The concentrated FAME were then transferred to GC vials for later GC analysis

### 3.7 Gas Chromatography Analysis

FAME were separated and quantified by gas-liquid with on-column injection, equipped with a fused silica capillary column (SUPELCO Column Omegawax$^{tm}$530, 30m x 0.5mm x 0.5µm) with nitrogen as carrier gas and temperature programming from $170^0$C to $220^0$C for 18 $min^{-1}$ and final time of 47minutes totaling to a run time of 75minutes. Injection and detection temperatures were $240^0$C and $260^0$C respectively. The programmer rate for both GC and decoder were set at $5min^{-1}$ with an attenuation of 3. All the GC analyses were done under same conditions. Individual methyl esters in the sample were identified by comparison with known FAME standards obtained from Kobian chemicals.

### 3.8 Growth performance for tilapia and catfish

Growth performance was measured through the following ways;

**Initial body weight** (IBW) (g); was measured before transferring to experimental tanks

**After washout body weight** (g) **(**WBW**)** was measured at the 3-weeks washout period

**Final Body weight (g)** (FBW) was measured at the end of the experimental feeding period

**Length (cm**): was measured from the tip of the snout to the end of the tail

**Weight gain (%)**: was calculated based on [(FBW-WBW)/WBW] x 100

**Specific growth rate (SGR, %)** was calculated based on [(log of FBW-Log of WBW)/ feeding days] x100

**Survival (%)** was calculated based on [(initial number of fish before feeding -dead fish number during feeding/initial number of fish before feeding) x 100] (Wei-Chun tu, 2011)

### 3.9 ELOVL and FADS genes analysis

#### 3.9.1 Extraction and preparation of liver tissues

Fish were first fasted for one day before sampling and weighing was done. Fish were killed with an overdose of anesthetic, chloroform, before dissection and liver extraction. The liver was then placed into RNAlater® for RNA stabilization purposes. Liquid nitrogen was carefully poured into the Eppendorf tube containing up to 100mg of liver sample to crystallize the liver samples, making it easy to crush and homogenize. The samples were then crushed using a sterile Eppendorf micro pestle to a fine powder. 350 µl ice-cold X1 Phosphate Buffer Saline (PBS), pH 7.4, buffer was then added to the sample and mixed into a uniform paste.

The contents were vortexed thoroughly at high speed for 1 minute to homogenize the sample into a uniform mixture. The samples were then centrifuged at 12,000 rpm at 4$^{o}$C for 10 minutes and supernatant used in the next stage of RNA extraction.

### 3.9.2 Extraction of total RNA

Total RNA was extracted from catfish and tilapia liver tissue using TRIzol reagent (Invitrogen, Carlsbad CA, USA) according to the manufacturer's protocol. 250 µl of liver homogenate each from the two fish species was pipetted into a clean sterile microfuge tube containing 750 µl Trizol$^{TM}$ LS reagent. The contents were then mixed by gently inverting the tube and allowed to stand at room temperature for 10 minutes to allow it to completely dissociate the nucleoprotein complexes. 200 µl ice-cold Chloroform was then added to the sample. The tubes were capped securely and contents vortexed thoroughly at high speed for 15 seconds. The samples were then allowed to stand at room temperature for another 10 minutes. The samples were then centrifuged at 12,000 rpm at 4$^{o}$C for 10 minutes. The contents separated into three phases (a colorless upper aqueous phase containing the RNA, an interphase and a lower pink Trizol$^{TM}$ phase). The upper aqueous phase was carefully pipetted out taking care NOT to disturb the interphase.

500 µl ice-cold isopropanol was then added to the sample, followed by 1 µl RNA-grade Glycogen. Contents of the tube were then mixed by slowly inverting the tubes. The contents were then incubated overnight at -20$^{o}$C.The next morning, the tube contents were centrifuged at 12,000 rpm at 4$^{o}$C for 10 minutes. The supernatant was then emptied into a discard container, taking care not to lose the pellet. The pellet was then washed using 200 µl ice-cold 75% Ethanol by inverting the tube and centrifuging briefly at 12,000 rpm at 4$^{o}$C for 1 minute.

The supernatant was emptied into a discard container and the wash step repeated two more times. After the final wash, the contents are centrifuged at 12,000 rpm at 4$^{o}$C for 30 seconds and the excess liquid (approximately 30 µl) pipetted out using a

sterile pipette tip. 18 µl of sterile Nuclease free water was then added to the pellet and allowed to stand at room temperature for 5 minutes. The contents were then tapped gently to re-dissolve the RNA.

### 3.9.3 Complimentary DNA Synthesis

First strand cDNA templates were prepared from purified RNA samples (20ng/µL) using RevertAid First Strand cDNA synthesis kit (Fermentas, Thermoscientific, UK) according to the manufacturer's protocol. 20ng/µl template RNA followed by 0.25 µMOligo $(dT)_{18}$ primer was then put in a micro centrifuge tube, nuclease free water added to bring the volume to 12µl. The content was then mixed gently, spun down and incubated at $65^0$C for 5 minutes.

The tubes were then cooled on ice for 1 minute and final concentrations of the following reagents added; 1 × reaction buffer (250mM Tris-HCL pH 8.3, 250mM KCl, 20mM $MgCl_2$, and 50mM Dithiothreitol), 1U/µl Ribolock RNase inhibitor, 1mM dNTP Mix and 10U/µl RevertAid M-MULV Reverse Transcriptase. The reaction mixture was spun briefly and incubated at 42ºC for 60 minutes, followed by termination at 70ºC for 5 minutes and finally chilling on ice.

### 3.9.4 Preparation and running of agarose gels

Agarose gel was run to observe total RNA, complementary DNA (cDNA) and amplification products for FAD, ELOVL and beta –Actin genes. Agarose gel was prepared by dissolving 0.8g Agarose in 100ml TBE buffer and the contents boiled in a microwave. The contents were then allowed to cool down to around 45ºC before adding 180 µl of 37% formaldehyd**e** and 0.5 µl Ethidium Bromide (EtBr). Combs were set in a casting tray and the gel poured in and allowed to set. 5 µl samples were mixed with 3 µl loading dye and loaded into the gel submerged in X1 TAE buffer. The ladder used was QuickLoad 100kb ladder from BioLabs. The gel was run at 70V, 8mA for 45 minutes.

### 3.9.5 RNA Integrity, Concentration and Quality

RNA integrity was ascertained by observing two bands of RNA on the 1.2% formaldehyde agarose gel electrophoresis. RNA concentration and quality was measured by using a nanodrop (ThermoScientific, USA) on the basis of absorbed Ultra Violet (U.V) light at wavelength of 230nm, 260nm and 280nm and equating the ratio of Optical Density (OD).

A ratio of 1.8-2.0 was taken as pure RNA with values below this denoting contamination with other nucleic acids (foreign DNA in the sample) while values higher than 2.0 indicating contamination with extraction reagents e.g phenol (due to incomplete wash steps during the extraction). The ratios used in this study ranged between 1.8 and 2.0. The samples' concentrations were normalized to 50 ng/µl before proceeding to DNase treatment.

### 3.9.6 DNase Treatment

DNase treatment was done for the RNA sample using a BioLabs DNase kit (New England, UK) following the manufacturer's protocol (Sakurai *et al*., 2016). The following components were added to a sterile PCR tube in the order;

**Table 2: Dnase treatment set up**

| | |
|---|---|
| RNA sample | 12 µl |
| X10 Reaction buffer | 2 µl |
| DNase | 2 µl |
| **Total** | **16 µl** |

The samples were then incubated at 37$^{o}$C for 37 minutes. 1 µl RNA-grade 50mM EDTA was then added to the mixture and the contents mixed by tapping gently on the sides followed by a final incubation step at 65$^{o}$C for 10 minutes.

### 3.9.7 Designing of Primers

FADS and ELOVL genes for both *Oriochromis niloticus* and *Clarius gariepinus* were retrieved from gene bank. The sequences were then taken to Primer3Plus

(http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) to design primers for the expression analysis. Primer design conditions were set at; expected product size (150-200), primer length (18-21) bases, Tm (55-60)$^{o}$C and GC (40-60%). Primer validation and testing was done using primer stats software. The primers were then sent to Inqaba Biotech, South Africa for synthesis.

**Table 3: Primer sequences for *Clarius gariepinus***

| Gene | **Forward Primer** | **Reverse Primer** | **Product size(bps)** | **TM($^{0}$C)** |
|---|---|---|---|---|
| B-Actin | 5'TCCGTGACAT-CAAGGAGAAG-3' | 5'AAGGAAAGAT-GGCTGGAAGAG-3' | 177 | 59.8 |
| FADS | 5'GCCCTATAAC-CATCAGCACAA-3' | 5'AAGCACCAAAG-AACCCAGAAT-3' | 196 | 60 |
| ELOVL | 5'GCAAGGCGGC-TATAATTTCTT-3' | 5'TCACAAACCA-CCAGATGTTCA-3' | 200 | 59.7 |

**Table 4: Primer sequences for *Oriochromis niloticus***

| **Gene** | **Forward Primer** | **Reverse Primer** | **Product size(bps)** | **TM($^{0}$C)** |
|---|---|---|---|---|
| B-Actin | 5'TCCGTGACAT-CAAGGAGAAG-3' | 5'-AAGGAAAGAT GGCTGGAAGAG3' | 177 | 59.8 |
| FADS | 5'-CCGAACTCAAC CAGTGGAATA-3' | 5'AATGAGATGAA CCAAGCCAGA-3' | 180 | 59.7 |
| ELOVL | 5'-TTCTCCAAGCT CATCGAGTTC-3' | 5'CTGTTTAGTG AGGCACCGAAG3' | 170 | 59.8 |

### 3.9.8 Quantitative Real Time Polymerase Chain Reaction (qPCR) and Polymerase Chain Reaction

The expression of FADS2 and ELOVL genes relative to the expression of beta-actin, a housekeeping gene, was used to determine the influence of dietary lipids on the expression of FADS2 and ELOVL genes which code for enzymes fatty acyl desaturases and elongases respectively. These genes are involved in the elongation and desaturation of dietary α-linolenic acid, C18:3 to EPA, C20:5 and DHA, C22:6. Amplification and quantification was done by light cycler (Roche Technologies). Reaction volumes of 10µl containing 1× Maxima SYBR Green/ROX master mix (Fermentas, Thermoscientific, UK), 2ng/µl of cDNA template, 0.3mM of each primer and nuclease free water were prepared. Conventional PCR was also carried out to assess the effectivity of the designed primers. The thermocycling conditions were set at one cycle of 95ºC for 5 minutes, followed by 40 cycles at 94ºC for 30 seconds, 55ºC for 30 seconds and 72ºC for 45 seconds. At least three replicates for each feeding trial was set. Data acquisition performed during the extension step. Beta-actin was used for normalization of the template cDNA.

### 3.10 Data management and analysis

Data on fatty acid composition of fish for each experimental diet, proximate composition and growth parameters were tabulated on spreadsheet and analyzed using one-way analysis of variance (ANOVA) under Genestat and SPSS analytical tools. Individual means were compared and separated using Duncan's multiple range test with significant level, $p<0.05$.

The expression level of ELOVL and FADS2 genes was quantified by determining the Ct values for each of the reaction. Baseline and threshold for Ct calculations were set manually with rotor –gene 6000 series software and was the same for all the experiments. The gene expressions level of target gene relative to the housekeeping gene (beta actin) was determined by delta delta Ct (ddCt) method in which the

discrepancy between the Ct for the test genes and housekeeping gene was first calculated to normalize the variation in the amount of cDNA in each reaction (Livak and Schmittgen, 2001). Descriptive statistics (means and plots of averages) were done in spread sheet.

# CHAPTER FOUR

# RESULTS

## 4.1 Proximate composition of experimental diets and ingredients

Experimental diets were formulated from *Cardina niloticus*, wheat flour, rice bran, popcorn floor and blends of vegetable oils (Table 5). Six diets were formulated with each diet containing same ingredients but different concentrations of olive, sunflower and linseed oils (Table 5). Dietary linseed oil: sunflower oil inclusion was as follows; diet 1 (100:0), diet 2 (75:25), diet 3 (50:50) diet 4 (25:75) diet 5 (0:100) and washout diet (100% olive oil) (Table %)

**Table 5: Composition of experimental diets**

| **Ingredients ( g/100g)** | **Washout** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** |
|---|---|---|---|---|---|---|
| Fish shrimps | 51.7 | 51.7 | 51.7 | 51.7 | 51.7 | 51.7 |
| Rice bran[4] | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 |
| Wheat flour[1] | 10 | 10 | 10 | 10 | 10 | 10 |
| Popcorn flour | 10 | 10 | 10 | 10 | 10 | 10 |
| Yeast[1] | 1 | 1 | 1 | 1 | 1 | 1 |
| Vegetable oil | 14 | 14 | 14 | 14 | 14 | 14 |
| Oil proportions (% w/w in 14% oil) | | | | | | |
| Sunflower[1] | 0 | 0 | 25 | 50 | 75 | 100 |
| Olive oil[1] | 100 | 0 | 0 | 0 | 0 | 0 |
| Linseed oil[2] | 0 | 100 | 75 | 50 | 25 | 0 |
| Premixes[3] | 2 | 2 | 2 | 2 | 2 | 2 |

[1]Obtained from local supermarket
[2]Extracted from linseeds using oil extruder machine, BEED department, JKUAT-Kenya
[3]Obtained from Tam feeds, Nairobi, Kenya
[4]Obtained from mwea rice mill

The proximate composition of experimental diets and ingredients are presented in Table 6. There was no significant difference ($P<0.05$) in proximate composition of the experimental diets except for commercial diet which had significantly lower crude protein (36%), crude fat (8.3%) and fiber (2.2%) (Table 6).Significantly high carbohydrate content was observed in popcorn flour (71.4%) followed by wheat flour 68.9% and 57.9% in rice bran.

Fresh water shrimp, *Caridina niloticus,* which was the main protein source in the experimental diets had significantly ($P<0.05$) higher (61.1%) crude protein value compared to the crude protein content in popcorn (7.8%), wheat flour (8.9%) and rice bran (8.7%). However, significantly lower fiber (1.9%) content was recorded in wheat flour, with high fiber content (11.3%) observed in rice bran (Table 6). Popcorn flour, rice bran and wheat flour recorded significantly low crude fat, 5.5%, 4.8% and 4.8% respectively.

**Table 6: Proximate composition of experimental diets and dietary ingredients**

| | Proximate Analysis | | | | | |
|---|---|---|---|---|---|---|
| | Protein | Crude fat | Ash | Moisture | Fibre | CHO |
| Diet 1 | 45.9$^{c}$ ±0.21 | 23.2$^{ef}$ ±1.93 | 13.6$^{d}$ ±1.2 | 9.1$^{cde}$ ±0.16 | 3.3$^{d}$ ±0.23 | 4.9$^{a}$±0.13 |
| Diet 2 | 45.4$^{c}$ ±0.21 | 25.0$^{f}$±1.46 | 13.2$^{d}$ ±1.02 | 8.9$^{cd}$ ±0.13 | 2.9$^{cd}$ ±0.43 | 4.6$^{a}$±0.21 |
| Diet 3 | 45.1$^{c}$ ±0.14 | 25.1$^{fg}$ ±1.5 | 11.3$^{cd}$ ±0.36 | 10.1$^{cde}$ ±0.22 | 3.34$^{d}$ ±0.25 | 5.1$^{ab}$±0.17 |
| Diet 4 | 45.5$^{c}$ ±0.31 | 25.0 $^{f}$ ±1.3 | 12.7$^{cd}$ ±0.73 | 10.3$^{de}$ ±0.35 | 3.3$^{d}$ ±0.1 | 4.7$^{a}$±0.11 |
| Diet 5 | 44.2$^{c}$±0.4 | 24.9$^{f}$ ±1.2 | 12.8$^{d}$±1.05 | 9.8$^{e}$ ±0.32 | 3.4$^{d}$ ±0.2 | 4.9$^{a}$±0.24 |
| Washout | 45.6$^{c}$ ±0.17 | 25.4$^{g}$ ±1.4 | 13.9$^{cd}$ ±0.33 | 9.4$^{cde}$ ±0.16 | 3.3$^{d}$ ±0.2 | 4.9$^{a}$±0.18 |
| Commercial[1] | 36.0$^{b}$ ±3.97 | 16.3$^{d}$ ±0.4 | 13.5$^{d}$ ±0.06 | 7.7$^{b}$ ±0.12 | 2.2$^{ab}$ ±0.13 | 24.3$^{d}$±2.3 |
| Caridina[2] | 61.1$^{d}$ ±0.14 | 8.3$^{c}$ ±2.67 | 6.3$^{ab}$ ±3.71 | 6.7$^{a}$±0.11 | 4.3$^{e}$ ±0.26 | 13.3$^{c}$±1.4 |
| Popcorn flour[3] | 7.8$^{a}$ ±0.21 | 5.5$^{a}$ ±0.45 | 3.7$^{a}$ ±4.2 | 9.3$^{cde}$ ±0.95 | 2.3$^{b}$ ±0.18 | 71.4$^{g}$±1.8 |
| Wheat flour[3] | 8.9$^{a}$ ±0.31 | 4.9$^{a}$ ±0.98 | 6.7$^{ab}$ ±2.03 | 8.7$^{c}$ ±0.17 | 1.9$^{a}$ ±0.04 | 68.9$^{f}$±1.23 |
| Rice Bran[4] | 8.7$^{a}$ ±0.35 | 4.8$^{a}$ ±0.96 | 8.6$^{bc}$ ±2.1 | 8.7$^{c}$ ±0.69 | 11.3$^{f}$ ±0.17 | 57.9$^{e}$±1.42 |

[1] Skretting, Fontaine-Les-Vervins-France obtained from Jambo fish farm, Kiambu, Kenya
[2] Obtained from Wich lum beach of Lake Victoria, Kenya
[3] Obtained from local supermarket
[4]Obtained from Mwea rice mills

## .4.2 Identification of individual fatty acids

Standard fatty acid methyl esters (C4-C24) were run and the profile obtained as shown (Figure 7). Individual fatty acids in the samples were identified using standard fatty acid methyl esters run under the same conditions as samples. The retention times were used as reference for peaks representing particular fatty acid and the fatty acid composition was calculated from the percentage concentration. (Table 7).

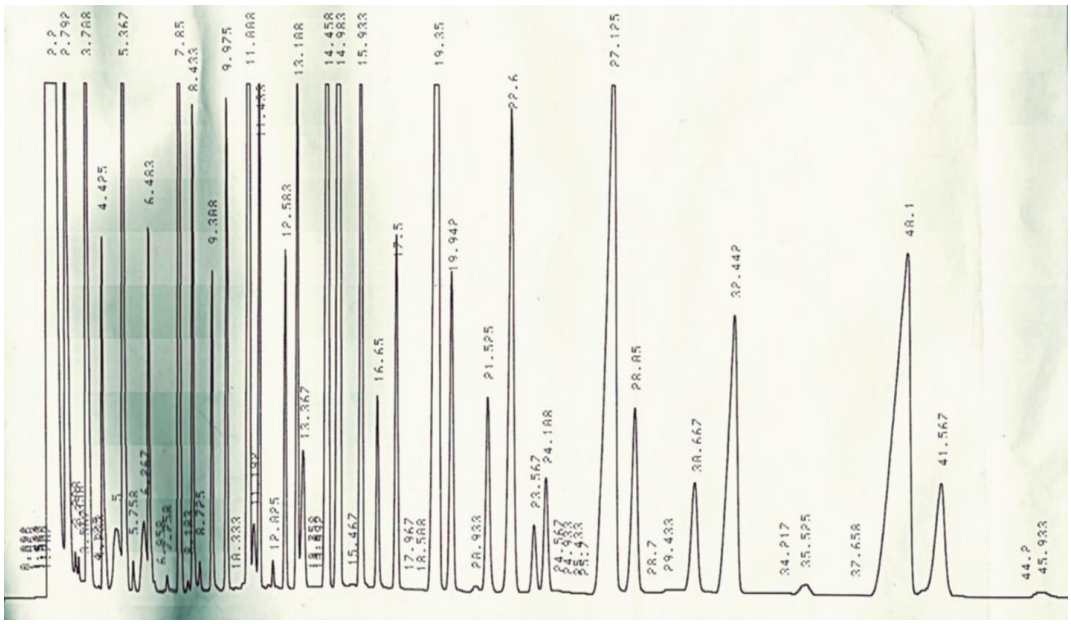



**Figure 7: Chromatogram for standard fatty acid methyl esters**

**Table 7: Retention time for fatty acids in the standard fatty acid methyl esters**

| Retention time | Configuration | Name |
|---|---|---|
| 2.2 | $C_6H_{14}$ | Hexane |
| 2.7 | C4:0 | Butyric acid |
| 3.7 | C8:0 | Caprylic acid |
| 4.4 | C10:0 | Capric acid |
| 5.3 | C11:0 | Undecanoic acid |
| 6.4 | C12:0 | Lauric acid |
| 7.8 | C13:0 | Tridecanoic acid |
| 8.4 | C14:0 | Myristic acid |
| 9.3 | C14:1 | Myristoleic acid |
| 9.9 | C15:0 | Penta decanoic acid |
| 11.0 | C15:1 | Cis10-pentadecanoic acid |
| 11.4 | C16:0 | Palmitic acid |
| 12.5 | C16:1 | Palmitoleic acid |
| 13.1 | C17:0 | Heptadecanoic acid |
| 14.4 | C17:1 | Cis 10-heptadecanoic acid |
| 14.9 | C18:0 | Stearic acid |
| 15.9 | C18:1 | Oleic acid |
| 16.6 | C18:1 | Elaidic acid |
| 17.5 | C18:2 | linoleic acid |
| 15.4 | C18:2 | Linolelaidic acid |
| 19.3 | C18:3 | α-linolenic acid |
| 19.9 | C18:3 | Gamma-linolenic acid |
| 21.5 | C20:0 | Arachidic acid |
| 22.6 | C20:1 | Cis-11-eicosaenoic acid |
| 23.5 | C20:2 | Cis-11,14-eicosadienoic acid |
| 24.1 | C20:3 | Cis-11,14, 17-eicosatrienoic acid |
| 27.1 | C20:4 | Arachidonic acid |
| 28.05 | C20:5 | Cis-5,811,14, 17-eicosapentaenoic acid |
| 30.6 | C21:0 | Heneicosanoic acid |
| 32.4 | C22:0 | Behenic acid |
| 35.5 | C22:1 | Erusic acid |
| 40.1 | C22: 2 | Cis-13,16-docosadienoic acid |
| 41.5 | C22:6 | Cis-4,7,10,13,16,19-Docosahexaenoic acid |
| 45.9 | C24:0 | Lignoceric acid |

### 4.3 Fatty acid composition of experimental diets and ingredients

Fatty acid composition of diets varied significantly ($p<0.05$) among the diets with significantly high α-linolenic acid (C18:3, n-3), oleic acid (C18:1 n-9) and linoleic acid (C18:2, n-6) composition in diet1, washout diet and diet 5 respectively (Table 8). The dietary fatty acid composition variation was attributed to the vegetable oils supplemented at different proportions in the diets (Table 6) and difference in fatty acid composition of linseed oil, olive oil and sunflower oil (Figure 7). Diet 1 had significantly ($p<0.05$) higher linoleic acid (16.6%) with this composition reducing up to 2.5% in diet5 which had no dietary linseed oil inclusion. Dietary n-3 fatty acids also decreased steadily from diet1 to washout diet with significantly ($p<0.05$) high n-3 fatty acids (19.7%) observed in diet 1(Table 8). There were no significant differences in dietary DHA, C22:6, n-3 and EPA C20:5, n-3 in all the formulated diets except commercial diets which had relatively high DHA and EPA values (3.4±0.71 and 1.7±0.25 respectively). Significantly ($p<0.05$) low dietary n-3 fatty acids (6.3%) was observed in wash out diet. However, the n-3 composition in commercial diet and diet 1 were not significantly different (19.7% and 19.4%) respectively. Palmitic acid, C16:0, was the dominant dietary saturated fatty acid (SFA) in all the diets.In addition, oleic acid, C18:1 was the dominant dietary monounsaturated fatty acid (MUFA) in all the diets (Table 8)

**Table 8: Fatty acid composition of experimental diets**

| | Dietary treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| C10:0 | $1.2^{a}\pm0.02$ | $1.4^{a}\pm0.05$ | $1.5^{a}\pm0.9$ | $1.3^{a}\pm0.81$ | $3.4^{b}\pm0.21$ | $3.8^{b}\pm0.6$ | $1.5^{a}\pm0.31$ |
| C12:0 | $1.3^{b}\pm0.04$ | $1.5^{b}\pm0.08$ | $2.5^{c}\pm0.6$ | $2.5^{c}\pm0.02$ | $3.5^{d}\pm0.18$ | $3.2^{d}\pm0.09$ | $0.4^{a}\pm0.06$ |
| C14:0 | $1.2^{a}\pm0.01$ | $1.2^{a}\pm0.04$ | $3.9^{d}\pm1.5$ | $3.3^{c}\pm0.08$ | $2.6^{b}\pm0.08$ | $2.2^{b}\pm0.05$ | $1.05^{a}\pm0.2$ |
| C16:0 | $10.5^{a}\pm0.8$ | $12.2^{b}\pm0.12$ | $14.7^{c}\pm0.27$ | $15.8^{d}\pm0.7$ | $15.1^{c}\pm0.7$ | $16.3^{d}\pm0.51$ | $12.2^{b}\pm0.23$ |
| C18:0 | $2.2^{a}\pm0.3$ | $3.1^{b}\pm0.8$ | $4.2^{c}\pm0.13$ | $4.3^{c}\pm0.14$ | $5.3^{d}\pm0.16$ | $5.1^{d}\pm0.12$ | $3.4^{b}\pm0.18$ |
| C20.0 | $2.8^{b}\pm0.01$ | $2.6^{b}\pm0.09$ | $2.3^{b}\pm0.01$ | $1.3^{a}\pm0.4$ | $1.5^{a}\pm0.03$ | $1.4^{a}\pm0.08$ | $2.4^{b}\pm0.04$ |
| **$^{a}\sum$SFAs** | $19.53^{a}$ | $22.39^{a}$ | $29.42^{bc}$ | $28.73^{b}$ | $31.7^{bc}$ | $32.2^{c}$ | $21.22^{a}$ |
| C16:1 | $3.3^{a}\pm0.03$ | $3.5^{a}\pm0.6$ | $3.7^{ab}\pm0.14$ | $4.2^{bc}\pm0.18$ | $4.7^{c}\pm0.7$ | $4.6^{c}\pm0.21$ | $3.6^{a}\pm0.9$ |
| C18:1 | $22.3^{a}\pm0.61$ | $23.8^{b}\pm0.41$ | $24.6^{c}\pm0.9$ | $25.2^{d}\pm0.27$ | $25.4^{d}\pm0.17$ | $29.8^{e}\pm 0.22$ | $22.6^{a}\pm0.15$ |
| **$^{b}\sum$MUFAS** | $25.7^{a}$ | $27.4^{b}$ | $28.4^{b}$ | $29.5^{c}$ | $30.2^{c}$ | $34.5^{d}$ | $26.3^{a}$ |
| C18:2 | $21.6^{b}\pm0.32$ | $22.1^{bc}\pm0.6$ | $22.5^{cd}\pm0.41$ | $22.9^{d}\pm0.3$ | $28.4^{e}\pm0.8$ | $20.3^{a}\pm0.63$ | $20.6^{a}\pm0.6$ |
| C18:3 | $16.4^{e}\pm0.32$ | $14.8^{d}\pm0.7$ | $13.4^{c}\pm0.5$ | $12.8^{b}\pm0.4$ | $2.7^{a}\pm0.9$ | $2.5^{a}\pm0.9$ | $12.2^{b}\pm0.6$ |
| C20:5 | $1.08^{a}\pm0.81$ | $1.2^{ab}\pm0.3$ | $1.04^{a}\pm0.13$ | $1.1^{a}\pm0.23$ | $1.36^{ab}\pm0.73$ | $1.02^{a}\pm0.51$ | $1.75^{b}\pm0.25$ |
| C22:6 | $2.2^{a}\pm0.31$ | $2.2^{a}\pm0.51$ | $2.1^{a}\pm0.93$ | $2.1^{a}\pm0.84$ | $2.2^{a}\pm0.5$ | $2.3^{a}\pm0.23$ | $3.45^{b}\pm0.71$ |
| **$^{c}\sum$PUFAS** | $41.43^{c}$ | $40.42^{d}$ | $39.15^{c}$ | $38.9^{c}$ | $34.81^{b}$ | $26.2^{a}$ | $40.1^{d}$ |
| **$^{d}\sum$n-3** | $19.7^{d}$ | $18.3^{c}$ | $16.6^{b}$ | $16.02^{b}$ | $6.3^{a}$ | $5.92^{a}$ | $19.4^{d}$ |

Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric Acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid C18:2 Linoleic acids, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid a∑SFAs: Total saturated fatty acids b∑MUFAs: Total monounsaturated fatty acids $^{c}\sum$PUFAs: Total Polyunsaturated fatty acids. $^{d}\sum$n-3: Total omega-3 fatty acids

The experimental vegetable oils used in this study were analyzed and were found to be predominated with specific fatty acid. Sunflower oil, olive oil and linseed oil predominantly contained linoleic acid (C18:2, n-6), oleic acids (C18:1, n-9) and α-linolenic acid (C18:3, n-3) respectively in addition to othe fatty acids (Figure 8). These predominant fatty acids influenced the dietary fatty acid compositions of the experimental diets (Table 8).

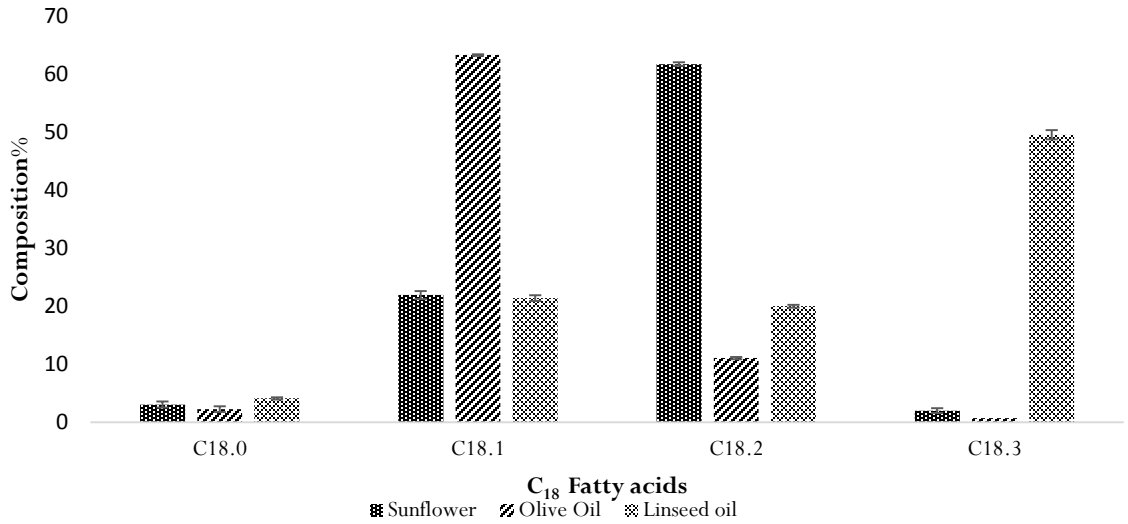



Figure 8: Compositions (%) of C18 fatty acids in vegetable oils used for experimental diets formulation. * Stearic acid, C18:0, Oleic acid, C18:1, Linoleic acid, C18:2, α-Linolenic acid, C18:3

## 4.4 Fatty acid profiles of wild catfish and cultured catfish obtained from selected farms

Data on the fatty acid composition of wild and farmed catfish are presented in Table 9. Palmitic acid, C16:0 was the predominant fatty acid followed by oleic acid, C18:1, in both farmed and wild catfish. Palmitic values ranged between 29.3±0.62% and 33.8±0.2% whereas oleic acid values ranged between 20.8± 0.45% and 26.2± 0.48% (Table 9). There was approximately 1:1 ratio of saturated to unsaturated fatty acids with major saturated fatty acids being palmitic acid, C16:0, stearic acid, C18:0, myristic, C14:0, arachidic, C20:0 lauric, C12:0 and capyrilic acids, C10:8) in the catfish muscles.

Wild fish had a relatively higher content of saturated fatty acids than farmed fish. Monounsaturated fatty acids (MUFAs) were the second predominant group fatty acids attributed by the presence of oleic acid (C18:1) andpalmitoleic acid (C16:1). Omega 3 (n-3) fatty acids in catfish ranged between 3.2± 0.11% and 6.7± 0.33% Farmed catfish (JSF) recorded significantly higher ($p<0.05$) n-3 fatty acids (6.7± 0.33%) among the farmed catfish.

There was no significant difference in n-3 fatty acid values between SMTU and UNIF which recorded n-3 values of 4.4± 0.13% and 4.85± 0.03% respectively (Table 9). However, n-3/n-6 ratios were significantly high in JSF (0.6±0.03%) among the farmed catfish.

**Table 9: Fatty acid profiles for wild and farmed African catfish obtained from different fish farms**

| | Wild Fish | | Farmed Fish | | |
|---|---|---|---|---|---|
| | **WCF1** | **WCF2** | **JSF** | **SMTUF** | **UNIF** |
| C10:0 | $1.2^{c}\pm 0.09$ | $0.8^{b}\pm 0.05$ | $0.72^{b}\pm 0.07$ | $0.3^{a}\pm 0.04$ | $1.2^{c}\pm 0.07$ |
| C12:0 | $1.7^{d}\pm 0.06$ | $1.2^{b}\pm 0.02$ | $1.14^{b}\pm 0.03$ | $0.5^{a}\pm 0.03$ | $1.4^{c}\pm 0.02$ |
| C14:0 | $2.9^{b}\pm 0.12$ | $1.8^{a}\pm 0.07$ | $3.4^{c}\pm 0.12$ | $2.9^{b}\pm 0.09$ | $4.1^{d}\pm 0.09$ |
| C16:0 | $33.8^{c}\pm 0.2$ | $38.6^{d}\pm 0.40$ | $31.5^{b}\pm 0.5$ | $31.0^{b}\pm 0.17$ | $29.3^{a}\pm 0.62$ |
| C18:0 | $11.1^{b}\pm 0.09$ | $9.1^{a}\pm 0.5$ | $8.8^{a}\pm 0.23$ | $15.0^{c}\pm 0.5$ | $11.2^{b}\pm 0.47$ |
| C20:0 | $1.0^{c}\pm 0.09$ | $0.31^{a}\pm 0.09$ | $0.26^{a}\pm 0.06$ | $0.16^{a}\pm 0.03$ | $0.72^{b}\pm 0.06$ |
| **∑SFAs** | $51.9^{d}\pm 0.31$ | $51.9^{d}\pm 0.70$ | $45.9^{a}\pm 0.6$ | $49.9^{c}\pm 0.19$ | $47.9^{b}\pm 0.6$ |
| C16:1 | $6.9^{b}\pm 0.2$ | $5.1^{a}\pm 0.16$ | $7.4^{b}\pm 0.42$ | $5.8^{a}\pm 0.24$ | $8.9^{c}\pm 0.20$ |
| C18:1 | $22.1^{ab}\pm 0.9$ | $26.2^{c}\pm 0.48$ | $21.9^{ab}\pm 0.2$ | $20.8^{a}\pm 0.45$ | $23.1^{b}\pm 0.29$ |
| **∑MUFAS** | $29.0^{b}\pm 1.0$ | $31.3^{bc}\pm 0.6$ | $29.3^{b}\pm 0.30$ | $26.7^{a}\pm 0.7$ | $32.05^{c}\pm 0.18$ |
| C18:2 | $10.3^{b}\pm 0.24$ | $9.1^{a}\pm 0.11$ | $10.2^{b}\pm 0.22$ | $9.6^{ab}\pm 0.31$ | $12.2^{c}\pm 0.42$ |
| C20:4 | $1.01^{b}\pm 0.11$ | $0.53^{a}\pm 0.05$ | $0.7^{a}\pm 0.06$ | $0.5^{a}\pm 0.04$ | $0.9^{b}\pm 0.08$ |
| **∑n-6** | $11.3^{c}\pm 0.14$ | $9.7^{a}\pm 0.13$ | $10.9^{bc}\pm 0.25$ | $10.2^{ab}\pm 0.3$ | $13.2^{d}\pm 0.35$ |
| C18:3 | $1.2^{c}\pm 0.07$ | $0.72^{a}\pm 0.06$ | $1.1^{c}\pm 0.03$ | $0.9^{b}\pm 0.14$ | $1.7^{d}\pm 0.10$ |
| C20:5 | $1.8^{cd}\pm 0.05$ | $0.93^{a}\pm 0.05$ | $2.14^{d}\pm 0.10$ | $1.3^{b}\pm 0.14$ | $1.6^{c}\pm 0.04$ |
| C22:6 | $2.3^{c}\pm 0.32$ | $1.52^{a}\pm 0.05$ | $3.4^{d}\pm 0.24$ | $2.2^{b}\pm 0.13$ | $1.5^{a}\pm 0.11$ |
| **∑n-3** | $5.4^{c}\pm 0.30$ | $3.2^{a}\pm 0.11$ | $6.7^{d}\pm 0.33$ | $4.4^{b}\pm 0.13$ | $4.85^{b}\pm 0.03$ |
| **∑PUFAS** | $16.6^{c}\pm 0.4$ | $14.5^{b}\pm 0.2$ | $17.6^{cd}\pm 0.5$ | $12.8^{a}\pm 0.32$ | $18.41^{d}\pm 0.38$ |
| **n-3/n-6** | $0.48^{c}\pm 0.02$ | $0.32^{a}\pm 0.01$ | $0.61^{d}\pm 0.03$ | $0.43^{bc}\pm 0.02$ | $0.4^{b}\pm 0.01$ |

Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acids, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acids, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acids. [a]∑SFAs: Total saturated fatty acids [b]∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids. [d]∑n-3: Total omega-3 fatty acids, ∑n-6: Total omega 6 fatty acids, WCF1: wild catfish (1.8-3kg) WCF2: wild catfish(0.6-1.5kg), JSF Jasa fish farm, SMTUF: Samaki TU farm, UNIF Unique fish farm

WCF1 were group of catfish with body weight between 1.8-3kg whereas WCF2 were catfish with body weight ranging between 0.6-1.5kg. This difference in body weight resulted into varied body content of fatty acids. For instance, n-3 values were significantly high in WCF1 (5.4± 0.30%) compared to WCF2 (3.2± 0.11%) among the wild catfish. In addition, there was significantly higher n-3/n-6 ratios in WCF1 compared to WCF2; however, MUFAS were relatively low in WCF1 compared to WCF2.

No significant difference in saturated fatty acid content was observed between WCF1 and WCF2. The major n-3 fatty acids present were linolenic acid (C18:3, n-3), eicosapentaenoic acid (C20.5, n-3) and docosahexaenoic acids (C22:6, n-3)

### 4.5 Fatty acid profiles of wild and farmed Nile tilapia obtained from selected farms

Compositions of individual fatty acids were expressed as the percentage of all fatty in the tilapia muscles and data presented in the Table 10. WTL1 were wild tilapia with weight range between 1-2kg whereas WTL2 wild tilapia had their weight ranging between 0.4-0.8 kg. Palmitic acid and oleic acids were the predominant fatty acid in both farmed and wild tilapias. WTL2 recorded significantly higher palmitic acid (44.1±3.2%) compared to WTL1 (36.3±2.5%). However, there was no significant difference in palmitic values in SMTUF and JSF with values 30.5±1.3% and 32.8±1.9% respectively. Like in wild catfish, wild tilapia recorded significantly higher ($P<0.05$) saturated fatty acids compared to the farmed tilapia.

However, there was no significant difference in monounsaturated fatty acids, which were the second most dominating fatty acids after saturated fatty acids, among the wild tilapia (Table 10). Highly polyunsaturated fatty acids like EPA and DHA were significantly higher in WTL1 (1.4± 0.27% and 3.1±0.23%, respectively) compared to WTL2 (0.8±0.053% and 2.0±0.43% respectively). JSF recorded significantly high EPA and DHA values (2.2±0.23% and 4.6±0.54% respectively) among the farmed tilapia (Table 10)

**Table 10: Fatty acid profiles of wild and farmed Nile tilapia obtained from different fish farms**

| | Wild Tilapia | | Farmed Tilapia | | |
|---|---|---|---|---|---|
| | **WTL1** | **WTL2** | **SMTUF** | **JSF** | **UNIF** |
| C10:0 | $0.28^{a}$ ±0.03 | $0.87^{c}$ ±0.07 | $0.8^{c}$ ±0.4 | $0.43^{b}$ ±0.04 | $0.9^{c}$ ±0.1 |
| C12:0 | $1.5^{d}$ ±0.3 | $1.1^{c}$ ±0.2 | $0.3^{a}$ ±0.04 | $0.8^{b}$ ±0.07 | $1.2^{c}$ ±0.3 |
| C14:0 | $2.5^{b}$±0.3 | $2.1^{a}$±0.11 | $2.8^{b}$±0.5 | $3.3^{c}$±0.61 | $3.9^{d}$±0.6 |
| C16:0 | $36.3^{c}$±2.5 | $44.1^{d}$±3.2 | $30.5^{b}$±1.3 | $32.8^{b}$±1.9 | $25.4^{a}$±3.1 |
| C18:0 | $12.5^{c}$ ± 1.2 | $8.0^{a}$±1.1 | $16.6^{d}$±1.6 | $9.3^{ab}$±0.3 | $11.1^{bc}$±0.8 |
| C20:0 | $0.7^{e}$ ±0.01 | $0.33^{c}$ ±0.02 | $0.13^{a}$ ±0.01 | $0.23^{b}$±0.03 | $0.5^{d}$ ±0.02 |
| **∑SFAs** | $54.7^{cd}$ ± 2.1 | $56.6^{d}$±1.7 | $50.7^{bc}$±1.21 | $47.0^{ab}$±1.4 | $43.1^{a}$±2.14 |
| C16:1 | $7.2^{a}$±0.3 | $6.0^{a}$± 0.5 | $5.6^{a}$±0.7 | $7.1^{a}$±0.7 | $9.0^{b}$±0.3 |
| C18:1 | $20.6^{ab}$±0.3 | $22.1^{ab}$±1.4 | $20.3^{a}$±0.53 | $22.7^{b}$±0.2 | $25.8^{c}$±1.8 |
| **∑MUFAS** | $27.8^{a}$ ± 2.4 | $28.1^{a}$±1.5 | $25.9^{a}$ ±2.7 | $29.8^{a}$ ±2.4 | $34.8^{b}$±3.21 |
| C18:2 | $10.1^{b}$±1.32 | $7.9^{a}$±0.9 | $7.8^{a}$±1.4 | $10.7^{b}$±1.2 | $9.7^{b}$±0.5 |
| C20:4 | $0.85^{b}$ ±0.2 | $0.5^{a}$ ±0.01 | $0.5^{a}$ ±0.01 | $0.6^{a}$±0.12 | $0.9^{b}$ ±0.11 |
| **∑n-6** | $10.9^{b}$±1.23 | $8.4^{a}$±0.1 | $8.3^{a}$±0.3 | $11.3^{b}$±0.13 | $10.6^{b}$±1.50 |
| C18:3 | $0.9^{bc}$±0.33 | $0.43^{a}$±0.06 | $0.73^{b}$±0.23 | $1.2^{c}$ ±0.6 | $1.5^{d}$ ±0.6 |
| C20:5 | $1.4^{b}$± 0.27 | $0.8^{a}$ ±0.053 | $1.0^{a}$ ±0.02 | $2.2^{c}$±0.23 | $1.4^{b}$ ±0.6 |
| C22:6 | $3.1^{b}$ ±0.23 | $2.0^{a}$ ±0.43 | $3.0^{b}$ ±0.5 | $4.6^{c}$ ±0.54 | $2.1^{a}$ ±0.33 |
| **∑n-3** | $5.5^{b}$ ± 0.2 | $3.3^{a}$ ±0.13 | $4.8^{b}$ ±0.2 | $8.0^{c}$ ±0.67 | 5.1b ±0.6 |
| **∑PUFAS** | $16.4^{b}$±0.5 | $11.7^{a}$±1.6 | $13.1^{a}$±1.3 | $19.4^{c}$±1.9 | 15.8b ±1.2 |
| n-3/n-6 | $0.5^{b}$±0.02 | $0.39^{a}$±0.06 | $0.57^{c}$±0.08 | $0.71^{d}$±0.05 | $0.5^{b}$±0.05 |

Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acid, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid. [a]∑SFAs: Total saturated fatty acids [b]∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids. [d]∑n-3: Total omega-3 fatty acids, ∑**n**-6: Total omega 6 fatty acids, WTL1: wild tilapia (1-2kg) WTL2: wild tilapia (0.4-0.8kg) JSF Jasa fish farm, SMTUF: Samaki TU farm, UNIF Unique fish farm.

### 4.6 Effect of experimental diets on growth parameters and survival of tilapia and catfish

The weight and body length of both catfish and tilapia fingerlings were measured before and after two weeks of washout period and the values recorded as shown in Table 11. The catfish body weight and length before washout were 9.1±1.3g and 4.5±0.5cm respectively whereas after washout, the weight and length was 14.7±1.0g and 6.1±0.2 respectively.

Catfish body weights and lengths were relatively higher than that of tilapia whose initial weight and length were 2.6±0.2g and 1.6±0.1cm respectively whereas the body measurements after washout were 5.4±0.4 (body weight) and 2.7±0.1 (body length).The body weight and length after washout were significantly higher than before washout body weight and length (Table 11)

**Table 11: Mean body weight and length before and after washout**

| | **Body weight(g)** | | **Body Length(cm)** | |
|---|---|---|---|---|
| Fish type | Before washout | After washout | Before washout | After washout |
| Catfish | 9.1±1.3 | 14.7±1.0 | 4.5±0.5 | 6.1±0.2 |
| Tilapia | 2.6±0.2 | 5.4±0.4 | 1.6±0.1 | 2.7±0.1 |

Increase in body weights and lengths after 150 days of feeding period were measured, recorded and analyzed as shown in Tables 11, 12 and 13. Weight gain and specific growth rate were significantly higher in both tilapia and catfish fed commercial diets.

Significantly higher weight gain (1689%) and specific growth rate (1.3±0.02) in tilapia was recorded with diet 4 (75% SFO: 25% LO) compared to other formulated diets. Weight gain ranged between 750% and 1689% among the experimental diets with commercial diet recording significantly ($p<0.05$) higher weight gain (2625%) in

tilapia. Specific growth rates (SGR) ranged between 0.9 and 1.3 among the experimental diets. Significantly ($p<0.05$) low SGR (0.9) and survival rate (94%) was observed in tilapia fed diet1 (Table 12).

**Table 12: Nile tilapia growth parameters**

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Washout | Commercial |
|---|---|---|---|---|---|---|---|
| **FBW[1]** | $46.8^{a}\pm6.3$ | $55^{ab}\pm4.0$ | $64.4^{b}\pm6.4$ | $97.4^{c}\pm4.9$ | $69.2^{b}\pm6.8$ | $66.4^{b}\pm8.8$ | $147.6^{d}\pm2.9$ |
| **FBL[2]** | $13.5^{a}\pm1.2$ | $14.9^{ab}\pm0.7$ | $16.5^{bc}\pm0.3$ | $17.4^{c}\pm0.3$ | $16.5^{bc}\pm0.4$ | $16.5^{bc}\pm0.3$ | $21.4^{d}4\pm0.4$ |
| **Weight gain (%)** | $750^{a}$ | $937^{ab}$ | $1099^{ab}$ | $1689^{c}$ | $1216^{b}$ | $1175^{ab}$ | $2625^{d}$ |
| **SGR[3] (%)** | $0.9^{a}\pm0.05$ | $1.1^{ab}\pm0.04$ | $1.16^{b}\pm0.01$ | $1.3\pm0.02^{c}$ | $1.2^{bc}\pm0.06$ | $1.2\pm0.08^{b}$ | $1.5^{d}\pm0.01$ |
| **Survival (%)** | $94^{a}$ | $95.67^{a}$ | $96^{b}$ | $97.67^{c}$ | $97^{c}$ | $97.33^{c}$ | $98.33^{d}$ |

* Same row with different superscripts varied significantly ($p<0.05$)
[1]FBW-Final body weight (grams)
[2]FBL-Final body length (cm)
[3]SGR-Specific growth rate

Specific growth rate was significantly high in African catfish fed commercial diet (1.19 ±0.18) compared to catfish fed experimental diets. However, washout diet and diet 3 showed relatively better growth of catfish among the experimental diets though there was no significant ($p<0.05$) differences in specific growth among catfish fed experimental diets. Percentage weight gain was significantly high in catfish fed commercial diet compared to those fed experimental diets; however, there was no significant difference in weight gain among catfish fed experimental diets (Table 13). There were no significant differences in survival rates among catfish fed diet 5, washout and commercial diet however survival rate was relatively low in catfish fed diet 1 (Table 13).

**Table 13: African catfish growth parameters**

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Washout | Commercial |
|---|---|---|---|---|---|---|---|
| **FBW** | $75.8^{a}$ ±1.4 | $84.8^{a}$±3.5 | $131.8^{bcd}$ ±3.7 | $104.4^{abc}$ ±0.4 | $96.8^{ab}$ ±7.03 | $150.8^{cd}$ ±5.8 | $185.8^{e}$±5.7 |
| **FBL** | $23.9^{b}$ ±1.1 | $20.3^{a}$ ±0.7 | $25.2^{b}$ ±0.5 | $24.7^{b}$ ±0.3 | $24.6^{b}$ ±0.7 | $28.38^{c}$ ±1.4 | $31.9^{d}$±2.2 |
| **Weight gain (%)** | $440.1^{a}$ | $492.5^{b}$ | $801.4^{bc}$ | $609.9^{ab}$ | $575.9^{ab}$ | $958.8^{c}$ | $1762.9^{d}$ |
| **SGR (%)** | $0.77^{a}$ ±0.11 | $0.83^{a}$ ±0.1 | $1.03^{ab}$ ±0.01 | $0.92^{ab}$ ±0.01 | $0.89^{ab}$ ±0.06 | $1.1^{ab}$ ±0.07 | $1.19^{b}$ ±0.18 |
| **Survival (%)** | $95^{a}$ | $96.67^{ab}$ | $96.6^{ab}$ | $96.7^{ab}$ | $98.33^{c}$ | $98.33^{c}$ | $98.33^{c}$ |

* Same row with different superscripts varied significantly ($p<0.05$)
1FBW-Final body weight (grams)
2FBL-Final body length (cm)
3SGR-Specific growth rate

### 4.7 Influence of experimental diets on fatty acid profiles of farmed fish

#### 4.7.1 Effect of experimental diets on the fatty acid profiles of tilapia

After 150 days of feeding period, fatty acid profiles of tilapia and catfish were analyzed and data presented in the Tables 14-17. Fatty acid profiles in tilapia fillet and liver followed the same pattern. PUFAs were the most dominant group of fatty acids in both the tilapia tissues. In tilapia muscles, PUFA was highest in diet 1(43.7%) with washout diet recording significantly low PUFA value (22.5%). The PUFA values reduced significantly from diet 1 to washout diet (Tables 14). Long chain n-3 fatty acids such as EPA, C20:5 n-3 and DHA, C22:6 n-3 were significantly higher in diet 1 (8.5±0.2% and12.2±0.8% respectively) with relative reduction in EPA and DHA values from diet 1 to washout. This pattern was also observed in the tilapia fillet n-3/n-6 ratios where in diet 1, n-3/n-6 ratios were significantly high (2.1%) compared to 0.6% in diet 5 (Table 14).

## Table 14: Muscles fatty acid composition (%) of tilapia

| | Dietary treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| C10:0 | $0.2^{a}\pm0.01$ | $0.2^{a}\pm0.06$ | $0.3^{a}\pm0.06$ | $0.3^{b}\pm0.01$ | $1.2^{b}\pm0.07$ | $1.2^{b}\pm0.01$ | $0.2^{a}\pm0.05$ |
| C12:0 | $0.5^{a}\pm0.07$ | $0.6^{a}\pm0.5$ | $0.7^{a}\pm0.21$ | $0.9^{ab}\pm0.03$ | $1.6^{c}\pm0.05$ | $1.4^{bc}\pm0.21$ | $0.8^{a}\pm0.17$ |
| C14:0 | $1.5^{a}\pm0.64$ | $1.7^{a}\pm0.73$ | $2.5^{bc}\pm0.21$ | $3.2^{de}\pm0.44$ | $2.8^{cd}\pm0.22$ | $3.6^{e}\pm0.50$ | $2.1^{ab}\pm0.27$ |
| C16:0 | $10.7^{a}\pm0.21$ | $10.7^{a}\pm0.67$ | $11.3^{b}\pm0.42$ | $13.2^{c}\pm0.57$ | $14.7^{d}\pm0.53$ | $15.2^{e}\pm0.53$ | $12.9^{c}\pm0.43$ |
| C18:0 | $5.9^{d}\pm0.4$ | $5.9^{d}\pm0.97$ | $4.7^{c}\pm0.13$ | $4.1^{b}\pm0.07$ | $2.15^{a}\pm0.01$ | $4.2^{bc}\pm0.32$ | $4.7^{c}\pm0.02$ |
| C20:0 | $2.2^{d}\pm0.01$ | $1.7^{bcd}\pm0.8$ | $1.3^{ab}\pm0.6$ | $1.1^{a}\pm0.9$ | $1.4^{abc}\pm0.46$ | $0.9^{a}\pm0.13$ | $1.9^{cd}\pm0.6$ |
| **[a]∑SFAs** | $21.3^{a}$ | $21^{a}$ | $21.1^{a}$ | $23.03^{a}$ | $24.05^{ab}$ | $26.8^{b}$ | $22.7^{a}$ |
| C14:1 | $0.7^{a}\pm0.06$ | $0.7^{a}\pm0.36$ | $0.65^{a}\pm0.14$ | $0.5^{a}\pm0.11$ | $0.6^{a}\pm0.72$ | $0.7^{a}\pm0.48$ | $0.7^{a}\pm0.44$ |
| C16:1 | $0.9^{a}\pm0.13$ | $1.4^{ab}\pm0.25$ | $1.5^{b}\pm0.02$ | $1.7^{b}\pm0.34$ | $1.4^{ab}\pm0.54$ | $1.6^{b}\pm0.9$ | $1.3^{ab}\pm0.25$ |
| C18:1 | $17.3^{a}\pm0.5$ | $18.5^{b}\pm0.42$ | $21.3^{c}\pm0.24$ | $23.1^{d}\pm0.26$ | $27.1^{e}\pm0.13$ | $30.5^{f}\pm0.27$ | $23.4^{d}\pm0.5$ |
| **[b]∑MUFAS** | $19.1^{a}$ | $20.78^{b}$ | $23.5^{c}$ | $25.34^{d}$ | $29.19^{e}$ | $32.8^{f}$ | $25.5^{d}$ |
| C18:2 | $11.6^{e}\pm0.3$ | $11.1^{d}\pm0.16$ | $10.75^{c}\pm0.42$ | $10.5^{b}\pm0.18$ | $15.9^{f}\pm0.23$ | $9.8^{a}\pm0.14$ | $10.6^{cd}\pm0.34$ |
| C20:4 | $2.2^{a}\pm0.3$ | $2.4^{ab}\pm0.21$ | $2.4^{abc}\pm0.17$ | $2.8^{bd}\pm0.18$ | $3.1^{d}\pm0.22$ | $3.1^{d}\pm0.24$ | $2.1^{a}\pm0.13$ |
| **∑n-6** | $13.9^{d}$ | $13.5^{c}$ | $13.2^{bc}$ | $13.4^{c}$ | $19^{e}$ | $12.9^{ab}$ | $12.7^{a}$ |
| C18:3 | $9.1^{f}\pm0.3$ | $8.05^{e}\pm0.18$ | $7.7^{de}\pm0.23$ | $6.5^{c}\pm 0.13$ | $2.9^{b}\pm0.81$ | $2.2^{a}\pm0.52$ | $7.3^{d}\pm1.1$ |
| C20:5 | $8.5^{e}\pm0.2$ | $6.4^{d}\pm0.13$ | $6.2^{d}\pm0.3$ | $5.3^{c}\pm0.24$ | $3.6^{b}\pm0.5$ | $2.9^{a}\pm0.6$ | $5.9^{d}\pm0.4$ |
| C22:6 | $12.2^{d}\pm0.8$ | $11.3^{c}\pm0.32$ | $11.2^{c}\pm0.51$ | $11.3^{c}\pm0.7$ | $5.4^{b}\pm0.21$ | $4.4^{a}\pm0.94$ | $11.5^{c}\pm0.61$ |
| **[d]∑n-3** | $29.7^{e}$ | $25.8^{d}$ | $25.2^{d}$ | $23.4^{c}$ | $12.1^{b}$ | $9.6^{a}$ | $24.8^{d}$ |
| **[c]∑PUFAS** | 43.7f | 39.3e | 37.4d | 36.6c | 31.1b | 22.5a | 37.5d |
| **n-3/n-6** | $2.1^{f}$ | $1.9^{e}$ | $1.8^{d}$ | $1.7^{c}$ | $0.6^{a}$ | $0.7^{b}$ | $1.9^{e}$ |

Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acids, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acids, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acids.[a]∑SFAs: Total saturated fatty acids [b]∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids, d∑n-3: Total omega-3 fatty acids, ∑**n**-6: Total omega 6 fatty acids

In tilapia liver, PUFA were the predominant group of fatty acids (Table 15). This was attributed to both n-3 and n-6 fatty acids which were the predominating group of fatty acids in the tilapia liver. The high dietary level of linseed oil in diet 1 evidently contributed to the significantly high polyunsaturated fatty acids in in diet 1 and that tissue n-3 compositions reduced with decreased dietary concentration of linseed oil. Tilapia liver EPA ranged between 3.1±0.5% and 7.7±0.3% with diet 1 recording significantly high EPA composition.

This pattern was same with n-3/n-6 ratios in which tilapia liver ratio was significantly high in diet 1 (2.09) compared to washout diet (0.60) (Table 15).

However, there was no significant difference in DHA composition in diets 1, 2, 3, 4 and commercial diets with values 10.8±0.91%, 10.9±0.4%, 10.5±0.21%, 10.6±0.6% and 10.6±0.23% respectively (Table 15) even though DHA was the dominant LC-HUFA in Nile tilapia liver. Palmitic, C16:0 and stearic, C18: 0 acids were the predominant saturated fatty acids whereas linoleic acid, C18.2, n-6 was the predominant n-6 fatty acid in the Nile tilapia liver. The tissue composition of palmitic acid relatively increased from diet 1 (10.4±0.56%) to diet 5 (14.9%).

**Table 15: Liver fatty acid composition (%) of tilapia**

| | Dietary treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| C10:0 | $0.7^{a}$±0.2 | $0.3^{a}$±0.1 | $0.4^{a}$±0.1 | $0.4^{a}$±0.1 | $1.4^{b}$±0.1 | $1.8^{b}$±0.6 | $0.2^{a}$±0.2 |
| C12:0 | $0.4^{a}$±0.62 | $0.6^{ab}$±0.6 | $0.9^{b}$±0.21 | $1.1^{b}$±0.5 | $2.0^{c}$±0.04 | $1.6^{c}$±0.1 | $1.0^{b}$±0.1 |
| C14:0 | $1.5^{a}$±0.3 | $1.7^{a}$±0.2 | $1.9^{a}$±0.24 | $3.7^{d}$±0.15 | $3.1^{c}$±0.13 | $4.1^{d}$±0.7 | $2.4^{b}$±0.34 |
| C16:0 | $10.4^{a}$±0.56 | $11.05^{a}$±0.7 | $11.1^{a}$±0.37 | $13.8^{b}$±0.8 | $14.9^{c}$±0.21 | $15.4^{c}$±0.6 | $13.5^{b}$±0.3 |
| C18:0 | $7.1^{f}$±0.31 | $5.4^{e}$±0.2 | $5.1^{de}$±0.2 | $3.5^{b}$±0.4 | $2.2^{a}$±0.73 | $4.6^{cd}$±0.2 | $4.2^{c}$±0.5 |
| C20:0 | 2.1c±0.3 | $1.7^{bc}$±0.2 | $1.5^{bc}$±0.2 | $1.3^{ab}$±0.6 | $1.3^{ab}$±0.41 | $0.8^{a}$±0.6 | $1.6^{bc}$±0.9 |
| **$^{a}$∑SFAs** | $21.76^{a}$ | $20.95^{a}$ | $20.8^{a}$ | $24^{ab}$ | $24.9^{b}$ | $28.49^{c}$ | $23.1^{ab}$ |
| C14:1 | $0.8^{ab}$±0.01 | $0.7^{ab}$±0.2 | $0.7^{ab}$±0.2 | $0.4^{a}$±0.13 | $0.9^{ab}$±0.19 | $0.8^{ab}$±0.6 | $1.1^{b}$±0.30 |
| C16:1 | $0.8^{a}$±0.65 | $1.5^{bc}$±0.1 | $1.6^{bc}$±0.13 | $1.8^{c}$±0.04 | $2.1^{cd}$±0.6 | $2.4^{d}$±0.6 | $1.3^{ab}$±0.12 |
| C18:1 | $16.2^{a}$±1.62 | $21.0^{b}$±0.1 | $22.3^{c}$±0.81 | $23.4^{d}$±0.18 | $27.4^{e}$±0.18 | $31.1^{f}$±0.8 | $23.2^{d}$±0.23 |
| **$^{b}$∑MUFAS** | $17.9^{a}$ | $23.3^{b}$ | $24.6^{bc}$ | $25.7^{c}$ | $30.4^{e}$ | $34.3^{e}$ | $25.6^{c}$ |
| C18:2 | $10.4^{d}$±0.4 | $9.9^{c}$±0.14 | $9.7^{b}$±0.3 | $9.3^{a}$±0.4 | $15.6^{e}$±0.51 | $7.4^{a}$±0.9 | $8.8^{b}$±0.61 |
| C20:4 | $3.0^{b}$ | $3.3^{b}$ | $3.7^{c}$ | $4.0^{d}$ | $4.5^{e}$ | $2.4^{a}$ | $2.6^{a}$ |
| **∑n-6** | $13.5^{c}$ | $13.3^{c}$ | $13.5^{c}$ | $13.4^{c}$ | $20.2^{d}$ | $9.9^{a}$ | $11.5^{b}$ |
| C18:3 | $8.6^{d}$±0.18 | $8.3^{d}$±0.4 | $7.6^{c}$±0.7 | $6.5^{b}$±0.16 | $2.5^{a}$±0.8 | $2.1^{a}$±0.6 | $7.6^{c}$±0.11 |
| C20:5 | $7.7^{e}$±0.3 | $6.5^{d}$±0.12 | $6.4^{d}$±0.7 | $5.8^{c}$±0.19 | $3.7^{b}$±0.17 | $3.1^{a}$±0.5 | $6.4^{d}$±0.13 |
| C22:6 | $10.8^{c}$± 0.91 | $10.9^{c}$±0.4 | $10.5^{c}$±0.21 | $10.6^{c}$±0.6 | $4.9^{b}$±0.31 | $3.4^{a}$±0.2 | $10.6^{c}$±0.23 |
| **$^{d}$∑n-3** | $27.2^{d}$ | $25.8^{de}$ | $24.6^{d}$ | $23.1^{c}$ | $11.2^{b}$ | $8.5^{a}$ | $24.7^{d}$ |
| **$^{c}$∑PUFAS** | $41.7^{e}$ | $40.1^{d}$ | $39.1^{d}$ | $37.4^{c}$ | $32.4^{b}$ | $19.4^{a}$ | $37.2^{d}$ |
| **n-3/n-6** | $2.09^{d}$ | $2.01^{d}$ | $1.9^{c}$ | $1.79^{c}$ | $0.60^{a}$ | $0.9^{b}$ | $2.24^{e}$ |

Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acids, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acids, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acids. $^{a}$∑SFAs: Total saturated fatty acids $^{b}$∑MUFAs: Total monounsaturated fatty acids $^{c}$∑PUFAs: Total Polyunsaturated fatty acids. $^{d}$∑n-3: Total omega-3 fatty acids, ∑**n-**6: Total omega 6 fatty acids

### 4.7.2 Effect of experimental diets on the fatty acid profiles in African catfish

Fatty acid profiles in catfish tissues followed the pattern in tilapia with PUFAs being the predominant group of fatty acids in catfish tissues. Catfish muscles recorded PUFA range of between 20.6% and 39.5 % with significantly high composition recorded in diet 1(Table 16). Omega 3 fatty acids were significantly high in catfish liver of groups fed diet 1(21.6%) with diet 5 and washout diets recording relatively low n-3 values (9.7% and 7.7% respectively) (Table 17).Catfish muscle n-3 composition ranged between 8.72% and 22.63%. DHA was the predominant omega 3 fatty acid in both the catfish tissues with values 10.4± 0.28% and 9.7±0.34% respectively (Table 16 and 17).

In both the catfish tissues, EPA values were relatively lower than DHA composition in all the experimental subjects (Table 16 and 17) with diet 5 and washout diets recording relatively low EPA values in catfish muscles ( 3.5±0.6% and 3.4±0.13% respectively) with 3.5±0.17% and 3.1± 0.51% in catfish muscles. The tissue composition of linoleic acid (C18:2) and α-linolenic acid C18:3) were relatively lower in the fish tissues (Tables 14-17) compared to diet (Table 8). Interestingly, increasing dietary linolenic acid did not translate to increase in tissue linoleic acid but translated to increase in tissue arachidonic acid in diets supplemented with both linseed oil and sunflower oil (diet 1-4), indicating that α-linolenic acid suppresses conversion of linoleic acid to arachidonic acid. lpha linolenic is a metabolic precursor of DHA and EPA and increases in dietary amount this fatty acid translated to increase in DHA and EPA. Nevertheless, arachidonic values were significantly higher in tissues of fish fed diet 5 in both catfish and tilapia.

**Table 16: Muscles fatty acid composition (%) of catfish**

| | Dietary treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| **Diets** | **Diet1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| C10:0 | $0.2^{a}\pm0.16$ | $0.3^{a}\pm0.02$ | $0.4^{a}\pm0.03$ | $1.1^{b}\pm0.17$ | $0.3^{a}\pm0.07$ | $0.3^{a}\pm0.03$ | $0.5^{ab}\pm0.13$ |
| C12:0 | $0.5^{a}\pm0.26$ | $0.8^{ab}\pm0.18$ | $0.9^{ab}\pm0.7$ | $1.1^{d}\pm0.3$ | $1.6^{c}\pm0.08$ | $1.6^{c}\pm0.02$ | $0.5^{a}\pm0.2$ |
| C14:0 | $1.12^{a}\pm0.8$ | $1.3^{ab}\pm0.7$ | $1.6^{bc}\pm0.8$ | $2.1^{cd}\pm0.3$ | $3.5^{e}\pm0.5$ | $3.6^{e}\pm0.25$ | $2.2^{d}\pm0.4$ |
| C16:0 | $11.4^{a}\pm0.49$ | $12.3^{b}\pm0.54$ | $15.3^{c}\pm0.4$ | $16.3^{d}\pm0.8$ | $16.1^{d}\pm0.4$ | $11.6^{a}\pm0.6$ | $12.6^{b}\pm0.31$ |
| C18:0 | $6.5^{f}\pm0.3$ | $6.3^{f}\pm0.43$ | $5.8^{de}\pm0.1$ | $5.5^{d}\pm0.2$ | $3.3^{b}\pm0.2$ | $4.43^{c}\pm0.14$ | $2.5^{a}\pm0.06$ |
| C20:0 | $1.8^{c}\pm0.01$ | $1.4^{bc}\pm0.6$ | $1.3^{abc}\pm0.9$ | $1.3^{ab}\pm0.4$ | $1.1^{ab}\pm0.08$ | $1.3^{abc}\pm0.3$ | $0.8^{a}\pm0.1$ |
| **[a]∑SFAs** | $21.9^{ab}$ | $22.5^{bc}$ | $25.6^{cde}$ | $27.5^{e}$ | $26.02^{de}$ | $23.12^{bcd}$ | $19.35^{a}$ |
| C14:1 | $0.9^{abc}\pm0.03$ | $0.7^{abc}\pm0.3$ | $0.6^{ab}\pm0.2$ | $0.5^{a}\pm0.12$ | $1.2^{c}\pm0.18$ | $1.1^{bc}\pm0.25$ | $0.9^{abc}\pm0.04$ |
| C16:1 | $1.4^{c}\pm0.12$ | $2.4^{d}\pm0.02$ | $2.6^{d}\pm0.6$ | $3.3^{e}\pm0.14$ | $0.2^{a}\pm0.01$ | $1.1^{bc}\pm0.25$ | $0.7^{ab}\pm0.12$ |
| C18:1 | $17.4^{a}\pm0.63$ | $19.7^{b}\pm0.5$ | $26.1^{e}\pm0.46$ | $28.6^{f}\pm0.21$ | $22.9^{c}\pm0.6$ | $32.43^{g}\pm0.9$ | $24.8^{d}\pm0.32$ |
| **[b]∑MUFAS** | $19.81^{a}$ | $22.9^{b}$ | $29.2^{e}$ | $32.5^{f}$ | $24.4^{c}$ | $34.8^{g}$ | $26.5^{d}$ |
| C18:2 | $14.9^{d}\pm1.4$ | $14.8^{d}\pm1.2$ | $14.7^{d}\pm1.57$ | $12.8^{b}\pm0.8$ | $17.9^{e}\pm0.3$ | $9.3^{a}\pm0.12$ | $13.7^{c}\pm0.8$ |
| C20:4 | $1.9^{a}\pm0.32$ | $2.2^{b}\pm0.14$ | 2.4c±0.27 | 2.4c±0.17 | 3.1d±0.3 | 2.5c±0.23 | 2.3b±0.21 |
| **∑n-6** | $16.9^{d}$ | $17.1^{d}$ | $17.2^{d}$ | $15.3^{b}$ | $21.1^{e}$ | $11.9^{a}$ | $16.1^{c}$ |
| C18:3 | $7.9^{e}\pm0.7$ | $6.3^{d}\pm1.13$ | $6.1^{d}\pm0.8$ | $4.4^{c}\pm0.13$ | $3.2^{b}\pm0.12$ | $2.6^{a}\pm0.5$ | $6.4^{d}\pm0.2$ |
| C20:5 | $4.2^{bc}\pm0.46$ | $4.5^{cd}\pm0.83$ | $4.4^{cd}\pm0.4$ | $3.8^{ab}\pm0.4$ | $3.5^{a}\pm0.6$ | $3.4^{a}\pm0.13$ | $4.9^{f}\pm0.6$ |
| C22:6 | $10.4^{f}\pm0.28$ | $9.5^{de}\pm0.58$ | $9.1^{d}\pm0.3$ | $7.8^{c}\pm0.7$ | $3.7^{ab}\pm0.23$ | $2.6^{a}\pm0.35$ | $9.8^{e}\pm0.53$ |
| **[d]∑n-3** | $22.63^{f}$ | $20.41^{de}$ | $19.5^{d}$ | $16.6^{c}$ | $10.51^{b}$ | $8.72^{a}$ | $21.21^{e}$ |
| **[c]∑PUFAS** | $39.5^{e}$ | $37.5^{d}$ | $36.7^{c}$ | $31.9^{b}$ | $31.6^{b}$ | $20.6^{a}$ | $37.3^{cd}$ |
| n-3/n-6 | $1.34^{e}$ | $1.19^{d}$ | $1.13^{c}$ | $1.08^{c}$ | $0.5^{a}$ | $0.73^{b}$ | $1.32^{e}$ |

Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid ,C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid , C18:1 Oleic acid , C18:2 Linoleic acid, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid . [a]∑SFAs: Total saturated fatty acids [b]∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids. [d]∑n-3: Total omega-3 fatty acids, ∑**n**-6: Total omega 6 fatty acids

Catfish liver DHA was relatively lower than muscle DHA with composition ranging between 1.9±0.47 and 9.7±0.34% in the liver. Catfish fed diet 1 had significantly high liver EPA (4.4±0.32%), DHA (9.7±0.34%), n-3 (21.6) and n3/n6 (1.29) values. These values decreased relative to decrease in linseed oil concentration in the diet. Palmitic acid was the predominant saturated fatty acid in catfish liver ranging between 10.6±2.2% and 15.6±0.73%.

In the liver, oleic acid was the predominant monounsaturated fatty acid with highly significant value (33.8±0.13) observed in washout diet (Table 17).

Linoleic acid, C18:2 and arachidonic acids were the observed n-6 fatty acids with diet 5 recording significantly high n-6 fatty acids (24.8%). Liver α-linolenic composition reduced significantly from diet 1 (7.4±0.12) to diet 5 (2.2±0.12). This pattern of fatty acid composition reduction was also observed in n-3/n-6 ratios, DHA, n-3 fatty acids and PUFAS (Table 17).

**Table 17: Liver fatty acid composition (%) of catfish fed on diets containing varied concentrations of linseed and olive oil**

| | Dietary treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| C10:0 | $0.2^{a}$±0.03 | $0.3^{a}$±0.03 | $0.4^{ab}$±0.1 | $0.8^{b}$±0.13 | $0.4^{ab}$±0.04 | $0.2^{a}$±0.01 | $0.4^{ab}$±0.11 |
| C12:0 | $0.5^{ab}$±0.03 | $0.6^{abc}$±0.4 | $0.8^{bcd}$±0.8 | $1.1^{cd}$±0.12 | $1.3^{de}$±0.09 | $1.7^{e}$±0.01 | $0.2^{a}$±0.03 |
| C14:0 | $0.9^{a}$±0.2 | $1.2^{a}$±0.3 | $1.3^{ab}$±0.07 | $1.7^{bc}$±0.02 | $1.7^{bc}$±0.04 | $1.8^{c}$±0.25 | $2.8^{d}$±0.26 |
| C16:0 | $10.6^{a}$±2.2 | $11.8^{b}$±0.8 | $14.8^{d}$±0.28 | 15.4e±0.81 | $15.6^{e}$±0.73 | 10.6a±0.26 | $13.4^{c}$±0.13 |
| C18:0 | $6.8^{d}$±0.3 | $6.4^{cd}$±0.1 | $6.1^{bc}$±0.17 | $5.9^{bc}$±0.02 | $5.5^{b}$±0.63 | $5.5^{b}$±0.3 | $1.6^{a}$±0.12 |
| C20:0 | $2.1^{c}$±0.3 | $1.7^{bc}$±0.03 | $1.3^{b}$±0.8 | $1.7^{bc}$±0.2 | $0.7^{bc}$±0.01 | $1.4^{b}$±0.4 | $0.4^{a}$±0.01 |
| **[a]∑SFAs** | $21.3^{a}$ | $22.2^{ab}$ | $24.9^{bc}$ | $26.8^{c}$ | $25.4^{c}$ | $21.5^{a}$ | $19.12^{a}$ |
| C14:1 | $1.1^{ab}$±0.6 | $0.7^{a}$±0.04 | $0.6^{a}$±0.12 | $0.5^{a}$±0.06 | $0.7^{a}$±0.34 | $0.7^{a}$±0.28 | $1.4^{b}$±0.23 |
| C16:1 | $1.3^{bc}$±0.21 | $2.1^{d}$±0.25 | $2.5^{de}$±0.72 | $2.7^{e}$±0.51 | $0.2^{a}$±0.02 | $0.9^{b}$±0.48 | $1.4^{bc}$±0.21 |
| C18:1 | $16.3^{a}$±0.15 | $19.1^{b}$±0.75 | $25.1^{d}$±0.92 | $27.2^{e}$±0.21 | 20.5c±0.31 | $33.8^{f}$±0.13 | $24.6^{d}$±0.61 |
| **[b]∑MUFAS** | $18.8^{a}$ | $21.8^{b}$ | $28.3^{c}$ | $30.5^{d}$ | $21.6^{b}$ | $35.5^{e}$ | $27.4^{c}$ |
| C18:2 | $15.5^{d}$±0.25 | $14.6^{c}$±0.33 | $14.4^{c}$±0.72 | $13.7^{b}$±0.4 | $21.5^{e}$±0.12 | $11.2^{a}$±0.34 | $15.3^{d}$±0.31 |
| C20:4 | $1.9^{a}$±0.21 | $2.1^{ab}$±0.13 | $2.3^{b}$±0.3 | $2.6^{b}$±0.26 | $3.2^{d}$±0.23 | $2.8^{c}$±0.17 | $1.7^{a}$±0.11 |
| **∑n-6** | $17.5^{c}$ | $16.7^{b}$ | $16.8^{bc}$ | $16.4^{b}$ | $24.8^{d}$ | $14.3^{a}$ | $17.1^{c}$ |
| C18:3 | $7.4^{e}$±0.12 | $6.7^{d}$±0.63 | $5.2^{c}$±0.15 | $4.8^{c}$±0.8 | $3.5^{b}$±0.9 | $2.2^{a}$±0.12 | $7.1^{de}$±0.4 |
| C20:5 | $4.4^{b}$±0.32 | $4.3^{b}$±0.51 | $4.2^{b}$± 0.3 | $4.3^{b}$±0.7 | $3.1^{a}$± 0.51 | $3.5^{a}$±0.17 | $4.3^{b}$±0.21 |
| C22:6 | $9.7^{f}$±0.34 | $8.2^{de}$±0.81 | $7.7^{cd}$±0.21 | $7.5^{c}$±0.37 | $3.1^{b}$±0.91 | $1.9^{a}$±0.47 | $8.3^{e}$±0.31 |
| **[d]∑n-3** | $21.6^{e}$ | $19.3^{d}$ | $17.2^{c}$ | $16.7^{c}$ | $9.7^{b}$ | $7.7^{a}$ | $19.7^{d}$ |
| **[c]∑PUFAS** | $40.1^{e}$ | $37.1^{d}$ | $35.1^{c}$ | $34.1^{b}$ | $35.5^{c}$ | $23.1^{a}$ | $37.8^{d}$ |
| n-3/n-6 | $1.29^{e}$ | $1.22^{d}$ | $1.08^{c}$ | $1.08^{c}$ | $0.43^{a}$ | $0.61^{b}$ | $1.21^{d}$ |

Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). Fatty acids: C10:0 Capric acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acid, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid. [a]∑SFAs: Total saturated fatty acids [b]∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids. [d]∑n-3: Total omega-3 fatty acids, ∑**n**-6: Total omega 6 fatty acids

### 4.8 Feeding period and polyunsaturated fatty acids (PUFA) in fish

It is of interest to know the duration of time required for dietary intervention to obtain desirable fatty acid profile in fish tissue. For this reason, experiments were conducted to determine changes in fatty acid profiles of tilapia and catfish tissues during three months of feeding on the experimental diets.

#### 4.8.1 Effect of feeding period on the content of polyunsaturated fatty acids (PUFA) in tilapia tissues

The tilapia liver and muscles fatty acid composition trends were also related to the dietary fatty acid composition. Muscle EPA and DHA content before and after washout were determined and presented in Figure 9. There was a significant $p<0.05$) reduction in both EPA and DHA muscle composition after washout, however, values in commercial diet were significantly higher in fingerlings fed commercial diets. Polyunsaturated fatty acid compositions of tilapia muscle and liver following 3-months feeding period are presented in tables 18 and 19. In all the experimental diets, there was significant ($P<0.05$) changes in fatty acid composition in tilapia tissues with feeding period.

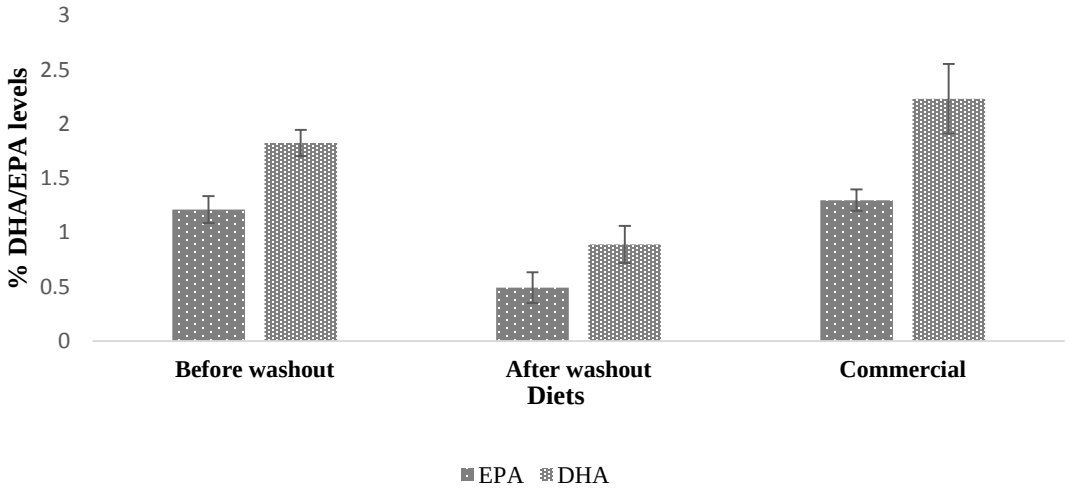



**Figure 9: DHA/ EPA content in Nile tilapia muscles before and after wash out**

Even though there was no particular trend in linoleic acid composition during the feeding period in all experimental diets, significant increase in the tilapia tissues composition of α-linolenic acid C18:3, n-3, arachidonic acid C20:4 n-6, eicosapentaenoic acid (EPA) C20:5, n-3, docosahexaenoic acid (DHA) C22:6, n-3, n-3 and n-3/n-6 ratio was observed (Tables 18 and 19). Tissue composition of α-linolenic acid increased with increased inclusion of linseed oil in the diet with relatively high tissue composition of α-linolenic acid observed in diet 1.The accumulation of α-linolenic acid increased with feeding period in both the tilapia tissues with significantly higher composition observed in month 3 (Tables 18 and 19).

**Table 18: Monthly changes of muscles polyunsaturated fatty acid composition in tilapia**

| | | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Month** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| | 1 | $12.0^{ab}$±0.14 | $13.7^{fgh}$±0.9 | $14.4^{gh}$±0.83 | $15.7^{i}$± 0.62 | $18.8^{j}$±0.63 | $15.7^{i}$±0.47 | $16.5^{i}$±0.82 |
| | 2 | $10.2^{a}$±0.82 | $11.6^{abc}$±0.2 | $14.6^{h}$±0.48 | $18.3^{j}$±0.5 | $19.4^{k}$±0.5 | $18.7^{j}$±0.4 | $16.2^{i}$±0.12 |
| **C18:2** | 3 | $11.9^{bcde}$±0.38 | $12.9^{def}$±0.15 | $13.2^{efg}$±0.45 | $14.3^{gh}$±0.5 | $16.3^{i}$±0.36 | $14.3^{gh}$±0.31 | $12.5^{ede}$±0.14 |
| | 1 | $7.8^{i}$±0.27 | $4.8^{cde}$±0.73 | $4.3^{bcd}$±0.19 | $3.7^{bc}$±0.26 | $3.3^{b}$±0.15 | $1.3^{a}$±0.31 | $5.3^{ef}$±0.41 |
| | 2 | $10.8^{kl}$±0.23 | $9.3^{ij}$±0.45 | $8.9^{i}$±0.61 | $7.4^{gh}$±0.21 | $5.5^{ef}$±0.31 | $4.8^{cde}$±0.13 | $8.5^{hi}$±0.63 |
| **C18:3** | 3 | $11.9^{kl}$±0.1 | $10.3^{l}$±0.63 | $10.2^{jk}$±0.42 | $8.4^{hi}$±0.71 | $6.2^{fg}$±0.37 | $5.3^{ef}$±0.34 | $11.7^{l}$±0.06 |
| | 1 | $1.6^{a}$±0.11 | $1.9^{abc}$±0.37 | $2.3^{bcde}$±0.24 | $2.5^{cdef}$±0.34 | $2.7^{efgh}$±0.31 | $2.8^{efgh}$±0.21 | $1.8^{ab}$±0.13 |
| | 2 | $2.0^{abcd}$±0.23 | $2.5^{cdef}$±0.42 | $2.7^{efgh}$±0.31 | $2.9^{efghi}$±0.27 | $3.2^{ghi}$±0.21 | $3.3^{hi}$±0.43 | $2.4^{bcde}$±0.27 |
| **C20:4** | 3 | $2.6^{defg}$±0.31 | $2.9^{efghi}$±0.33 | $3.1^{fghi}$±0.23 | $3.3^{hi}$±0.14 | $3.5^{i}$±0.41 | $3.5^{i}$±0.22 | $2.8^{efgh}$±0.25 |
| | 1 | $3.5^{hi}$±0.68 | $1.2^{bc}$±0.73 | 0.8b±0.41 | $0.3^{a}$±0.21 | $0.3^{a}$±0.16 | $1.6^{c}$±0.48 | $2.7^{efg}$±0.93 |
| | 2 | $3.6^{hij}$±0.36 | $2.9^{fg}$±0.51 | $2.7^{efg}$±0.21 | $2.2^{d}$±0.23 | $3.2^{gh}$±0.14 | $1.1^{b}$±0.47 | $2.7^{efg}$±0.74 |
| **C20:5** | 3 | $5.9^{kl}$±0.73 | $5.3^{k}$±0.33 | $4.1^{j}$±0.38 | $3.7^{ij}$±0.62 | $2.4^{de}$±0.13 | $1.3^{bc}$±0.41 | $2.4^{def}$±0.41 |
| | 1 | $4.3^{g}$±0.5 | $2.2^{de}$±0.57 | $1.8^{cd}$±0.19 | $1^{abc}$±0.23 | $0.8^{ab}$±0.14 | $0.4^{a}$±0.11 | $3.1^{ef}$±0.51 |
| | 2 | $6.5^{hi}$±0.37 | $4.8^{g}$±0.5 | $3.2^{f}$±0.91 | $2.3^{def}$±0.71 | $2.3^{def}$±0.17 | $1.5^{bcd}$±0.72 | $5.7^{gh}$±0.17 |
| **C22:6** | 3 | $7.9^{ij}$±0.52 | $7.4^{j}$±0.21 | $7.3^{ij}$±0.13 | $6.6^{hi}$±0.5 | $2.5^{def}$±0.13 | $1.8^{cd}$±0.29 | $5.9^{h}$±0.18 |
| **∑n-3** | 1 | $17.2^{g}$±0.34 | $8.2^{bc}$±0.11 | $6.8^{b}$±0.23 | $4.8^{a}$±0.31 | $4.3^{a}$±061 | $3.2^{a}$±0.41 | $10.9^{de}$±0.56 |
| | 2 | $20.6^{hij}$±0.9 | $16.8^{g}$±0.2 | $14.6^{f}$±0.31 | $11.6^{e}$±0.71 | $10.7^{de}$±0.71 | $8.2^{bc}$±0.72 | $16.6^{g}$±0.93 |
| | 3 | $25.3^{k}$±0.37 | $22.6^{j}$±0.17 | $21.2^{ij}$±0.36 | $18.2^{gh}$±0.92 | $10.6^{de}$±0.76 | $9.0^{cd}$±0.43 | $19.5^{hi}$±0.68 |
| | 1 | $28.4^{gh}$±0.31 | $21.7^{cd}$±0.18 | $20.9^{bc}$±0.71 | $20.2^{b}$±0.76 | $22.9^{de}$±0.47 | $18.7^{a}$±0.64 | $27.2^{fg}$±0.88 |
| | 2 | $30.6^{jk}$±0.43 | $28.1^{gh}$±0.37 | $29.0^{hi}$±0.27 | $29.8^{ij}$±0.16 | $32.9^{l}$±0.18 | $26.6^{f}$±0.94 | $32.5^{l}$±0.93 |
| **∑PUFA** | 3 | $37.0^{no}$±0.25 | $35.3^{o}$±1.2 | $34.2^{mn}$±0.39 | $32.3^{m}$±0.92 | $26.7^{kl}$±0.62 | 23.1e±0.23 | $31.8^{kl}$±0.31 |
| | 1 | $12.0^{ab}$±0.41 | $13.7^{fgh}$±0.93 | $14.3^{gh}$±0.93 | $15.7^{i}$±0.26 | $18.8^{j}$±0.63 | $15.7^{i}$±0.74 | $16.5^{i}$±0.82 |
| | 2 | $10.2^{a}$±0.82 | $11.6^{abc}$±045 | $14.6^{h}$±0.48 | $18.3^{j}$±0.9 | $22.4^{k}$±0.5 | $18.7^{j}$±0.54 | $16.2^{i}$±0.23 |
| **∑n-6** | 3 | $11.9^{bcde}$±0.38 | $12.9^{def}$±0.51 | $13.2^{efg}$±0.54 | $14.3^{gh}$±0.23 | $16.3^{i}$±0.36 | $14.3^{gh}$±0.24 | $12.5^{cde}$±0.14 |
| | 1 | $1.5^{def}$±0.64 | $0.8^{bc}$±0.2 | $0.7^{abc}$±0.25 | $0.5^{ab}$±0.14 | $0.4^{a}$±0.12 | $0.4^{a}$±0.12 | $0.9^{c}$±0.15 |
| **n-3/n-6** | 2 | $2.2^{g}$±0.4 | $1.7^{f}$±0.9 | $1.2^{d}$±0.14 | $0.8^{bc}$±0.18 | $0.7^{abc}$±0.1 | $0.6^{abc}$±0.1 | $1.2^{d}$±0.4 |
| | 3 | $2.2^{g}$±0.46 | $1.8^{f}$±0.61 | $1.7^{f}$±0.14 | $1.3^{de}$±0.12 | $0.7^{abc}$±0.14 | $0.7^{abc}$±0.25 | $1.6^{ef}$±0.15 |

Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same column with different superscripts varied significantly ($p<0.05$) for a parcular fatty acid in different diets. C18:2 Linoleic acid, C18.3: α-Linolenic acid, C20:4, Arachidonic acid, C20:5 Eicosapentaenoic acids, EPA, C22: 6, Docosahexaenoic acid, DHA, ∑n-3: Total omega-3 fatty acids, ∑PUFA: Total Polyunsaturated fatty acids, ∑n-6: Total omega 6 fatty acids

The same pattern was also observed with arachidonic acid. Despite lack of correlation between the dietary and tissue linoleic acid and arachidonic, levels of arachidonic acid increased significantly with decrease in dietary linseed oil supplementation and relatively with increase with feeding period (Tables18 and 19). The compositions of DHA, EPA, n-3 and n-3/n-6 also increased with feeding period in both tilapia tissues with significantly high composition observed in diet 1 (Tables 18 and 19). In tilapia muscles, there was a significant increase in EPA with feeding period in diets 1-4, however, in diets 5, washout diet and commercial diet the EPA composition increase were not significant.

Compositions of polyunsaturated fatty acids in tilapia liver followed the same pattern as in the tilapia muscles with significantly high DHA, n-3 and n-3/n-6 values in the third feeding month. In the third month, tilapia liver DHA values ranged between 1.6±0.21 and 7.8±0.95 compared to first feeding month where the DHA values ranged between 0.6±0.23 and 5.4±0.43 (Table 18). Liver n-3 values were also significantly high in the third feeding month with values ranging between 9.3±0.23 and 25.5±0.51 compared to n-3 values in the first feeding month which ranged between 4.1±0.72 and 16.3±0.31. n-3/n-6 ratios also increased with feeding period in both tissues (Tables 18 and 19)

**Table 19: Monthly changes of Liver polyunsaturated fatty acid composition in tilapia**

| | | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Month** | **Diet1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| | 1 | $11.9^{bc}$±0.52 | $13.5^{ef}$±0.4 | $14.1^{fg}$±0.64 | $15.7^{h}$±0.97 | $18.7^{j}$±0.92 | $15.6^{h}$±0.34 | $16.8^{i}$±0.41 |
| | 2 | $10.2^{a}$±0.82 | $11.6^{b}$±0.24 | $14.6^{g}$±0.4 | $18.3^{j}$±0.91 | $22.4^{k}$±0.51 | $18.7^{j}$±0.53 | $16.1^{hi}$±0.45 |
| **C18:2** | 3 | $10.3^{a}$±0.72 | $12.5^{cd}$±0.61 | $13.1^{de}$±0.31 | $13.9^{fg}$±0.82 | $16.2^{hi}$±0.64 | 14.4$^{g}$±013 | $11.7^{b}$±0.24 |
| | 1 | $7.32^{h}$±0.23 | $4.9^{cd}$±0.52 | $4.5^{c}$±0.46 | $3.8^{b}$±0.41 | $3.5^{b}$±0.29 | $1.6^{a}$±0.21 | $6.4^{fg}$±0.23 |
| | 2 | $10.8^{k}$l±0.32 | $9.3^{j}$±0.54 | $8.9^{ij}$±0.69 | $7.4^{h}$±0.53 | $5.5^{de}$±0.51 | $1.8^{a}$±0.13 | $8.5^{i}$±0.7 |
| **C18:3** | 3 | $12.2^{m}$±0.86 | $11.3^{l}$±0.55 | $10.3^{k}$±0.42 | $9.4^{j}$±0.6 | $6.5^{g}$±0.51 | $2.5^{ab}$±0.13 | $10.9^{kl}$±0.94 |
| | 1 | $2.1^{a}$±0.51 | $2.6^{abc}$±41 | $2.9^{bcd}$±0.12 | $3.1^{cdef}$±0.22 | $3.3^{cdefg}$±0.27 | $3.8^{fghij}$±0.13 | $2.3^{ab}$±0.16 |
| | 2 | $3.0^{cde}$±0.81 | $3.3^{cdefg}$±0.24 | $3.5^{defgh}$±0.34 | $3.7^{efghi}$±0.35 | $4.0^{ghij}$±0.43 | $4.2^{hij}$±0.41 | $3.1^{cdef}$±0.24 |
| **C20:4** | 3 | $3.4^{defg}$±0.23 | $3.6^{defghi}$±0.33 | $3.8^{fghij}$±0.16 | $4.0^{ghij}$±0.32 | $4.3^{ij}$±0.31 | $4.5^{j}$±0.23 | $3.5^{defgh}$±0.21 |
| | 1 | $3.8^{i}$±0.12 | $1.8^{e}$±0.68 | $1.0^{c}$±0.24 | $0.2^{a}$±0.01 | $0.5^{b}$±0.17 | $2.0^{ef}$±0.27 | $2.3^{f}$±0.86 |
| | 2 | $3.6^{i}$±0.36 | $2.9^{gh}$±0.51 | $2.7^{g}$±0.21 | $2.2^{f}$±0.31 | $2.2^{ef}$±0.14 | $1.1^{c}$±0.47 | $2.7^{g}$± 0.74 |
| **C20:5** | 3 | $6.3^{k}$±0.52 | $5.2^{j}$±0.59 | $5.1^{j}$±0.22 | $3.7^{i}$±0.24 | $2.1^{ef}$±0.27 | $1.5^{d}$±0.29 | $3.8^{i}$±0.17 |
| | 1 | $5.4^{h}$±0.43 | $3.0^{f}$±0.12 | $1.9^{cd}$±0.31 | $1.4^{b}$±0.12 | $1.2^{b}$±0.19 | $0.6^{a}$±0.23 | $3.1^{f}$±0.61 |
| | 2 | $6.5^{i}$±0.14 | $4.8^{g}$±0.45 | $2.4^{de}$±0.71 | $2.3^{de}$±0.21 | $1.5^{bc}$±073 | $1.2^{b}$±0.24 | $3.3^{f}$±0.91 |
| **C22:6** | 3 | $7.4^{l}$±0.16 | $7.4^{j}$±0.91 | $7.3^{j}$±0.17 | $7.0^{m}$±0.51 | $2.8^{ef}$±0.31 | $1.6^{bc}$±0.21 | $7.8^{kl}$±0.95 |
| | 1 | $16.3^{i}$±0.31 | $9.6^{e}$±0.43 | $7.3^{c}$±0.21 | $5.2^{b}$±17 | $5.0^{b}$±0.62 | $4.1^{a}$±0.72 | 11.6$^{g}$±0.18 |
| | 2 | $20.6^{k}$±0.15 | $16.8^{i}$±0.43 | $14.6^{h}$±0.32 | $11.6^{g}$±071 | $10.7^{f}$±0.24 | $8.2^{d}$±0.92 | $16.6^{i}$±091 |
| **∑n-3** | 3 | $25.5^{n}$±0.51 | $23.8^{m}$±0.51 | $22.4^{l}$±0.34 | $19.8^{j}$±0.62 | $11.1^{f}$±0.24 | $9.3^{e}$±0.23 | $22.3^{l}$±0.61 |
| | 1 | $28.0^{f}$±0.31 | $22.9^{c}$±0.26 | $21.1^{b}$±016 | $20.7^{b}$±0.52 | $23.5^{d}$±0.82 | $19.4^{a}$±0.53 | $28.2^{f}$±0.92 |
| | 2 | $30.6^{i}$±0.41 | $28.1^{f}$±0.71 | $29.0^{g}$±0.36 | $29.8^{h}$±0.23 | $32.9^{j}$±0.41 | $26.6^{e}$±0.91 | $32.5^{j}$±0.9 |
| **∑PUFA** | 3 | $36.6^{n}$±0.83 | $36.1^{m}$±0.71 | $35.3^{l}$±0.51 | $33.5^{k}$±0.94 | $27.1^{e}$±0.17 | $23.4^{d}$±0.62 | $33.8^{k}$±0.34 |
| | 1 | $11.7^{b}$±0.52 | $13.3^{de}$±0.92 | $13.9^{ef}$±0.32 | $15.6^{hi}$±0.43 | $18.6^{k}$±0.41 | $15.4^{h}$±0.85 | $16.6^{j}$±0.94 |
| | 2 | $10.2^{a}$±0.81 | $11.6^{b}$±0.21 | $14.6^{g}$±0.52 | $18.3^{k}$±0.91 | $22.4^{l}$±0.51 | $18.7^{k}$±0.53 | $16.1^{ij}$±0.56 |
| **∑n-6** | 3 | $10.3^{a}$±0.72 | $12.5^{c}$±0.62 | $13.1^{cd}$±0.31 | $13.9^{ef}$±0.81 | $16.2^{ij}$±0.61 | $14.4^{fg}$±0.15 | $11.7^{b}$±0.25 |
| | 1 | 1.4d$^{ef}$±0.96 | $0.8^{abc}$±0.17 | $0.6^{a}$±0.22 | $0.4^{a}$±0.26 | $0.3^{a}$±0.17 | $0.3^{a}$±0.18 | $0.7^{abc}$±0.14 |
| | 2 | 2.3g$^{h}$±0.94 | $1.7^{def}$±0.19 | $1.2^{bcd}$±0.59 | $0.8^{abc}$±0.12 | $0.7^{ab}$±0.12 | $0.6^{a}$±0.17 | $1.3^{cde}$±0.94 |
| **n-3/n-6** | 3 | $2.6^{h}$±0.81 | $2.0^{g}$±0.17 | $1.8^{efg}$±0.22 | $1.5^{def}$±0.29 | $0.7^{abc}$±0.18 | $0.7^{ab}$±0.4 | $2.0^{fg}$±0.27 |

*Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same column with different superscripts varied significantly ($p<0.05$) for a parcular fatty acid in different diets. C18:2 Linoleic acid, C18.3: α-Linolenic acid, C20:4, Arachidonic acid, C20:5 Eicosapentaenoic acids, EPA, C22: 6, Docosahexaenoic acid, DHA, ∑n-3: Total omega-3 fatty acids, ∑PUFA: Total Polyunsaturated fatty acids, ∑n-6: Total omega 6 fatty acids

### 4.8.2 Effect of feeding period on the content of polyunsaturated fatty acids (PUFA) in catfish tissues

Before the feeding experiment began, catfish were fed washout diet for a period of one month. The tissue EPA and DHA content reduced significantly during washout however catfish fed commercial diets still had relatively higher levels of EPA and DHA (Figure 10).

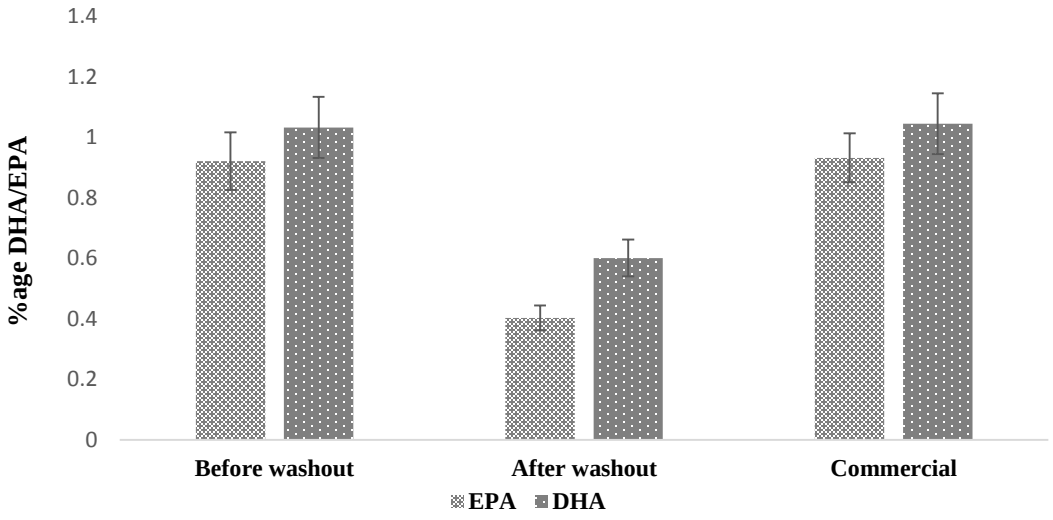



**Figure 10: DHA/EPA contents in African catfish muscles**

The tissue PUFA composition increased significantly with the feeding period. A trend in the increase in tissue fatty acid composition was observed in linolenic, C18:3, arachidonic acid, C20:4, DHA, C22:6, n-3 and n-3/n-6. Alpha-linolenic acid was significantly high in the third feeding month compared to first feeding month in both the catfish tissues ranging between 6.2±0.9 and 11.9±0.3 in the liver while the values ranged between 6.4±0.7 and 11.7±0.5 in the catfish muscles.

In the catfish liver, the DHA composition range between 1.8±0.3 to 7.9±0.3 in the third feeding month compared to the first feeding month when the DHA values ranged between 0.6±0.2 and 3.7±0.1. The tissue compositions for PUFA, n-3 and n-3/n-6 increased significantly with the feeding period (Table 20 and 21)

**Table 20: Monthly changes of Liver polyunsaturated fatty acid composition in catfish**

| | | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Month** | **Diet1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| | 1 | $12.3^{abc}\pm0.3$ | $13.3^{e}\pm0.5$ | $14.6^{fg}\pm0.5$ | $15.3^{gh}\pm0.9$ | $18.4^{kl}\pm0.4$ | $15.8^{hi}\pm0.2$ | $16.7^{j}\pm0.2$ |
| | 2 | $11.6^{a}\pm0.4$ | $12.2^{abc}\pm0.5$ | $17.8^{k}\pm0.2$ | $18.8^{l}\pm0.8$ | $22.3^{m}\pm1.8$ | $14.5^{fg}\pm0.19$ | $16.3^{ij}\pm0.2$ |
| **C18:2** | 3 | $11.9^{ab}\pm0.4$ | $12.9^{cde}\pm0.8$ | $13.2^{de}\pm0.1$ | $14.3^{f}\pm0.9$ | $16.3^{ij}\pm1.3$ | $14.3^{f}\pm0.2$ | $12.5^{bcd}\pm0.4$ |
| | 1 | $7.1^{g}\pm0.1$ | $5.3^{cd}\pm0.4$ | $4.7^{c}\pm0.4$ | $3.3^{b}\pm0.7$ | $3.6^{b}\pm0.4$ | $1.9^{a}\pm0.1$ | $5.3^{c}\pm0.4$ |
| | 2 | $10.1^{j}\pm0.3$ | $9.3^{i}\pm0.2$ | $8.6^{h}\pm0.3$ | $6.3^{ef}\pm0.2$ | $5.9^{de}\pm0.3$ | 6.7fg±0.3 | $7.4^{g}\pm0.3$ |
| **C18:3** | 3 | $11.9^{k}\pm0.3$ | $10.3^{j}\pm0.5$ | $10.2^{j}\pm0.1$ | $8.3^{h}\pm0.8$ | $6.2^{ef}\pm0.9$ | $6.3^{ef}\pm0.4$ | $11.7^{k}\pm0.5$ |
| | 1 | $1.9^{a}\pm0.2$ | $2.4^{abc}\pm0.2$ | $2.7^{bcd}\pm0.2$ | $2.9^{cdefg}\pm0.3$ | $3.1^{defgh}\pm0.1$ | $3.6^{ghijk}\pm0.2$ | $2.2^{ab}\pm0.2$ |
| | 2 | $2.8^{cde}\pm0.2$ | $3.2^{cdefgh}\pm0.1$ | $3.3^{defghi}\pm0.2$ | $3.5^{efghij}\pm0.1$ | $3.8^{hijk}\pm0.15$ | $4.1^{ijk}\pm0.12$ | $2.9^{cdef}\pm0.13$ |
| **C20:4** | 3 | $3.2^{defgh}\pm0.2$ | $3.4^{defghij}\pm0.2$ | $3.6^{fghij}\pm0.1$ | $3.8^{hijk}\pm0.1$ | $4.1^{jk}\pm0.2$ | $4.3^{k}\pm0.2$ | $3.3^{defgh}\pm0.2$ |
| | 1 | $3.9^{g}\pm0.1$ | $2.9^{ef}\pm0.1$ | $1.5^{a}\pm0.14$ | $0.4^{a}\pm0.11$ | $0.5^{a}\pm0.14$ | $0.7^{a}\pm0.14$ | $2.2^{cd}\pm0.5$ |
| | 2 | $2.4^{de}\pm0.1$ | $4.4^{h}\pm0.1$ | $2.2^{cd}\pm0.13$ | $2.8^{ef}\pm0.1$ | $3.2^{f}\pm0.14$ | $1.8^{bc}\pm0.1$ | $2.4^{de}\pm0.4$ |
| **C20:5** | 3 | $5.9^{j}\pm0.3$ | $5.3^{i}\pm0.3$ | $4.1^{gh}\pm0.3$ | $3.7^{g}\pm0.3$ | $2.4^{de}\pm0.3$ | $1.3^{b}\pm0.2$ | $2.5^{de}\pm0.2$ |
| | 1 | $3.7^{hi}\pm0.1$ | $3.3^{gh}\pm0.12$ | $1.7^{cd}\pm0.2$ | $1.3^{bc}\pm0.1$ | $0.6^{a}\pm0.2$ | $1.0^{ab}\pm0.12$ | $3.3^{h}\pm0.12$ |
| | 2 | $5.4^{j}\pm0.2$ | $3.9^{i}\pm0.2$ | $2.8^{fg}\pm0.1$ | $2.3^{ef}\pm0.2$ | $2.5^{f}\pm0.2$ | $1.2^{b}\pm0.11$ | $5.3^{j}\pm.02$ |
| **C22:6** | 3 | $7.9^{n}\pm0.3$ | $7.4^{m}\pm0.3$ | $7.3^{m}\pm0.4$ | $6.6^{l}\pm0.4$ | $2.5^{f}\pm0.15$ | $1.8^{de}\pm0.3$ | $5.9^{k}\pm0.4$ |
| | 1 | $14.5^{h}\pm0.8$ | $11.4^{f}\pm0.4$ | $7.8^{c}\pm0.5$ | $4.8^{b}\pm0.2$ | $4.5^{b}\pm0.4$ | $3.5^{a}\pm0.5$ | $10.8^{ef}\pm0.5$ |
| | 2 | $17.8^{ij}\pm0.4$ | $17.5^{i}\pm0.3$ | $13.4^{g}\pm0.8$ | $11.2^{ef}\pm0.4$ | $11.4^{f}\pm0.8$ | $9.5^{d}\pm0.4$ | $14.6^{h}\pm0.4$ |
| **∑n-3** | 3 | $25.3^{n}\pm0.5$ | $22.6^{m}\pm0.7$ | $21.2^{l}\pm0.6$ | $18.2^{j}\pm1.1$ | $10.6^{e}\pm0.7$ | $9.0^{d}\pm0.5$ | $19.5^{k}\pm0.4$ |
| | 1 | $26.6^{g}\pm0.4$ | $24.4^{f}\pm0.4$ | $22.2^{c}\pm0.4$ | $19.8^{b}\pm0.5$ | $22.7^{d}\pm0.13$ | $18.1^{a}\pm0.5$ | $27.2^{g}\pm 0.5$ |
| | 2 | $29.2^{h}\pm0.3$ | $29.4^{h}\pm0.3$ | $31.1^{i}\pm0.8$ | $29.8^{h}\pm0.9$ | $33.5^{k}\pm1.1$ | $23.8^{e}\pm0.3$ | 30.7i±0.13 |
| **∑PUFA** | 3 | $37.0^{n}\pm0.2$ | $35.3^{m}\pm0.2$ | $34.2^{l}\pm0.2$ | $32.3^{j}\pm0.4$ | $26.7^{g}\pm0.8$ | $23.1^{d}\pm0.6$ | $31.8^{j}\pm1.0$ |
| | 1 | $12.2^{ab}\pm0.1$ | $13.2^{cd}\pm0.1$ | $14.5^{e}\pm0.2$ | $15.1^{fg}\pm0.1$ | $18.3^{jk}\pm0.10$ | $15.6^{gh}\pm0.14$ | $16.5^{i}\pm0.2$ |
| **∑n-6** | 2 | $11.6^{a}\pm0.3$ | $12.2^{ab}\pm0.3$ | $17.8^{j}\pm0.2$ | $18.8^{k}\pm0.2$ | $22.3^{l}\pm0.2$ | $14.5^{ef}\pm0.4$ | $16.3^{i}\pm0.4$ |
| | 3 | $11.9^{ab}\pm0.2$ | $12.9^{cd}\pm0.2$ | $13.2^{d}\pm0.3$ | $14.3^{e}\pm0.3$ | $16.3^{hi}\pm0.17$ | $14.3^{e}\pm0.3$ | $12.5^{bc}\pm0.2$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **n-3/n-6** | 1 | $1.3^{f}\pm0.1$ | $0.9^{e}\pm0.03$ | $0.6^{abcd}\pm0.01$ | $0.4^{abc}\pm0.1$ | $0.3^{ab}\pm0.01$ | $0.3^{a}\pm0.01$ | $0.7^{de}\pm0.2$ |
| | 2 | $1.6^{gh}\pm0.1$ | $1.5^{fgh}\pm0.1$ | $0.8^{de}\pm0.03$ | $0.7^{cde}\pm0.1$ | $0.6^{bcd}\pm0.15$ | $0.7^{de}\pm0.13$ | $0.9^{e}\pm0.17$ |
| | 3 | $2.2^{i}\pm0.3$ | $1.8^{h}\pm0.1$ | $1.7^{h}\pm0.2$ | $1.4^{fg}\pm0.3$ | $0.7^{de}\pm0.1$ | $0.7^{cde}\pm0.2$ | $1.6^{gh}\pm0.4$ |

*Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same columnwith different superscripts varied significantly ($p<0.05$) for a parcular fatty acid in different diets.C18.2: Linoleic acid, C18:3 α-Linolenic acid, C20:4 Arachidonic acids, C20:5 Eicosapentaenoic acid, EPA, C22:6 Docosahexaenoic acid, DHA, ∑ω3 Total omega-3 fatty acids, ∑PUFA Total Polyunsaturated fatty acids, ∑ω6 Total omega 6 fatty acids

**Table 21: Monthly changes of muscle polyunsaturated fatty acid composition in catfish**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Month** | **Diet1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Washout** | **Commercial** |
| **C18:2** | 1 | 12.4$^{bc}$±0.19 | 13.4$^{d}$±0.4 | 15.0$^{e}$±0.4 | 16.1$^{f}$ ±0.4 | 18.6$^{h}$±0.4 | 15.2$^{e}$±0.5 | 17.5$^{g}$ ±0.1 |
| | 2 | 10.3$^{a}$±0.2 | 13.3$^{d}$±0.2 | 18.3$^{h}$±0.3 | 20.6$^{i}$ ±0.6 | 22.7$^{j}$±0.3 | 18.3$^{h}$±0.6 | 17.4$^{g}$ ±0.3 |
| | 3 | 12.1$^{b}$±0.8 | 12.9$^{cd}$±0.8 | 13.3$^{d}$±0.2 | 15.0$^{e}$ ±0.5 | 16.1$^{f}$ ±0.1 | 14.6$^{e}$±0.9 | 11.8$^{b}$ ±0.3 |
| **C18:3** | 1 | 7.5$^{f}$±0.24 | 5.1$^{c}$±0.5 | 3.9$^{b}$±0.9 | 3.4$^{b}$ ±0.87 | 3.7$^{b}$±0.5 | 1.5$^{a}$±0.5 | 5.2$^{c}$ ±0.6 |
| | 2 | 10.1$^{hi}$±0.6 | 9.6$^{h}$±0.6 | 8.0$^{f}$ ±0.9 | 6.2$^{de}$ ±0.50 | 6.3$^{de}$±0.50 | 5.9$^{d}$±0.34 | 6.7$^{e}$ ±0.9 |
| | 3 | 11.7$^{k}$±0.5 | 10.3$^{ij}$±0.4 | 10.0$^{hi}$±0.8 | 8.9$^{g}$ ±0.60 | 6.4$^{de}$ ±0.6 | 6.4$^{de}$±0.7 | 10.8$^{j}$±0.6 |
| **C20:4** | 1 | 1.3$^{a}$±0.32 | 1.6$^{abc}$±0.23 | 2.0$^{bcde}$±0.4 | 2.2$^{cdefg}$ ±0.2 | 2.4$^{efghi}$±0.7 | 2.5$^{efghi}$±0.2 | 1.4$^{ab}$±0.5 |
| | 2 | 1.6$^{abcd}$±0.12 | 2.1$^{cdef}$±0.6 | 2.3$^{efghi}$±0.6 | 2.5$^{efghij}$ ±0.4 | 2.8$^{ghijk}$±0.2 | 2.9$^{hijk}$±0.1 | 2.0$^{bcde}$ ±0.2 |
| | 3 | 2.3$^{defgh}$±0.19 | 2.6$^{efghijk}$±0.2 | 2.8$^{fghijk}$ ±0.2 | 3.0$^{ijk}$ ±0.19 | 3.2$^{jk}$ ±0.14 | 3.2$^{k}$±0.17 | 2.5$^{efghij}$±0.4 |
| **C20:5** | 1 | 3.5$^{f}$ ±0.23 | 2.1$^{bcd}$±0.6 | 0.4$^{a}$±0.4 | 0.4$^{a}$ ±0.3 | 0.5$^{a}$ ±0.01 | 0.2$^{a}$±0.16 | 2.2$^{cd}$ ±0.3 |
| | 2 | 3.5$^{f}$±0.38 | 3.1$^{ef}$±0.8 | 2.6$^{de}$±0.7 | 2.7$^{de}$ ±0.9 | 2.6$^{de}$ ±0.5 | 1.9$^{bc}$±0.60 | 3.2$^{ef}$ ±0.6 |
| | 3 | 5.2$^{h}$±0.6 | 4.6$^{g}$±0.15 | 4.2$^{g}$±0.9 | 4.3$^{g}$ ±0.50 | 3.1$^{ef}$ ±0.12 | 1.5$^{b}$±0.15 | 3.2$^{ef}$±0.8 |
| **C22:6** | 1 | 3.6$^{f}$±0.5 | 2.9$^{e}$±0.13 | 1.5$^{bc}$±0.5 | 1.1$^{b}$ ±0.8 | 0.6$^{a}$ ±0.23 | 0.5$^{a}$±0.8 | 3.5$^{f}$ ±0.4 |
| | 2 | 5.0$^{gh}$±0. 45 | 3.7$^{f}$±0.11 | 2.1$^{d}$±0.8 | 2.2$^{d}$ ± 0.10 | 2.2$^{d}$ ± 0.01 | 1.8$^{cd}$±0.4 | 5.4$^{h}$±0.1 |
| | 3 | 8.3$^{k}$±0.72 | 7.3$^{j}$±0.7 | 7.2$^{j}$±0.6 | 6.1$^{i}$ ±0.50 | 2.3$^{d}$ ±0.14 | 1.5$^{bc}$±0.3 | 4.7$^{g}$±0.8 |
| **∑n-3** | 1 | 14.5$^{h}$±0.70 | 10.0$^{e}$±0.3 | 5.7$^{c}$±0.9 | 4.8$^{b}$ ±0.7 | 4.7$^{b}$ ±0.4 | 2.1$^{a}$±0.6 | 10.8$^{f}$±0.4 |
| | 2 | 18.4$^{j}$±1.10 | 16.2$^{i}$±0.1 | 12.6$^{g}$±0.30 | 10.8$^{f}$ ±0.9 | 10.8$^{f}$ ±0.5 | 9.3$^{d}$±0.9 | 15.1$^{h}$±1.1 |
| | 3 | 24.9$^{m}$±1.1 | 21.8$^{k}$±0.1 | 21.1$^{k}$±0.5 | 18.9$^{j}$ ±0.6 | 11.3$^{f}$±0.9 | 9.0$^{d}$±0.3 | 18.4$^{j}$±1.3 |
| **∑PUFA** | 1 | 26.8$^{d}$±0.19 | 23.3$^{c}$±0.6 | 20.7$^{b}$±1.3 | 20.9$^{b}$ ±1.1 | 23.2$^{c}$±0.9 | 17.3$^{a}$±1.1 | 28.3$^{e}$±0.8 |
| | 2 | 28.4$^{e}$±0.7 | 29.2$^{f}$±0.7 | 30.6$^{gh}$±0.8 | 31.3h ±1.1 | 33.3$^{j}$±0.5 | 27.5$^{d}$±0.40 | 32.3$^{i}$ ±0.2 |
| | 3 | 36.8$^{m}$±0.33 | 34.5$^{l}$±0.6 | 34.2$^{kl}$±1.2 | 33.7$^{jk}$ ±0.6 | 27.2$^{d}$ ±0.9 | 23.4$^{c}$±0.6 | 30.1$^{g}$±0.4 |
| **∑n-6** | 1 | 12.4$^{bc}$±0.9 | 13.4$^{d}$±0.4 | 15.0$^{e}$±0.4 | 16.1$^{f}$ ±0.4 | 18.6$^{h}$±0.2 | 15.2$^{e}$±0.5 | 17.5$^{g}$±0.6 |
| | 2 | 10.3$^{a}$±1.2 | 13.3$^{d}$±0.2 | 18.3$^{h}$±0.3 | 20.6$^{i}$ ±1.2 | 22.7$^{j}$±0.2 | 18.3$^{h}$±0.6 | 17.4$^{g}$±0.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | 12.1$^{b}$±0.8 | 12.9$^{cd}$±0.8 | 13.3$^{d}$±0.2 | 15.0$^{e}$ ±0.5 | 16.1$^{f}$±0.5 | 14.6$^{e}$±0.9 | 11.8$^{b}$±0.3 |
| **n-3/n-6** | 1 | 1.4$^{bcdef}$±0.27 | 1.0$^{abcd}$ ±0.03 | 0.6$^{ab}$±0.03 | 0.5$^{a}$ ±0.02 | 0.5$^{a}$±0.02 | 0.3$^{a}$±0.1 | 0.8$^{abc}$±0.01 |
| | 2 | 2.0$^{fg}$±0.52 | 1.4$^{bcdef}$ ±0.7 | 0.9$^{abc}$±0.03 | 0.7$^{abc}$ ±0.01 | 0.7$^{abc}$ ±0.02 | 0.7$^{abc}$±0.1 | 1.1$^{abcde}$±0.1 |
| | 3 | 2.3$^{g}$±0.16 | 1.9$^{efg}$ ±0.4 | 1.8$^{efg}$±0.4 | 1.5$^{cdefg}$ ±0.1 | 0.9$^{abc}$ ±0.05 | 0.8$^{abc}$±0.01 | 1.8$^{defg}$±0.2 |

*Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same columnwith different superscripts varied significantly ($p<0.05$) for a parcular fatty acid in different diets.C18: 2 Linoleic acid, C18:3 α-Linolenic acid, C20: 4 Arachidonic acids, C20: 5 Eicosapentaenoic acid, EPA, C22: 6 Docosahexaenoic acid, DHA, ∑n-3 Total omega-3 fatty acids, ∑PUFA Total Polyunsaturated fatty acids, ∑n-6 Total omega 6 fatty acids

Arachidonic acid also showed the same pattern with tissue compositions increasing with feeding period. Tissue EPA and DHA compositions also increased with feeding time with significantly high accumulations observed in the third feeding month. DHA values ranged between 1.5±0.3 and 8.3±0.72 in the third feeding month compared to a range of between 0.5±0.8 and 3.6±0.5 in the first feeding month catfish muscle.

### 4.9 Expression of ELOVL and FADS2 genes

#### 4.9.1 Effect of experimental diets on tilapia ELOVL and FADS2 genes

Relative expression of the two most important enzymes in the biosynthetic pathway for long chain polyunsaturated fatty acids, Δ6-desaturase (FAS2) and elongase (ELOVL) was determined for each experimental diet and fish species. Total RNA was extracted from the liver of fish from each experimental group. The integrity of the RNA checked by running an agarose gel (Figure 11).

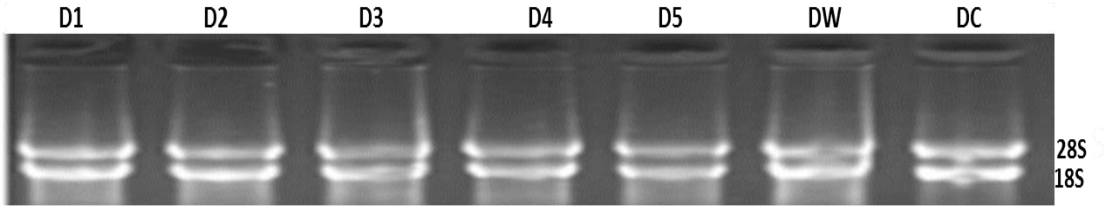



**Figure 11: Total RNA extracted from liver of tilapia fed different diets**

The 260/280 ratio for the RNA extracted from tilapia liver ranged between 1.81 to 1.96 while the 260/230 ratio ranged between 1.99 to 2.1. RNA concentration ranged between 687.1µg/ml to 1184.9µg/ml (Table 22).

**Table 22: Concentration and purity of total RNA isolated from tilapia (TL) fed different diets (D)**

| Sample ID | RNA concentration | A260 | A280 | 260/280 | 260/230 | Factor |
|---|---|---|---|---|---|---|
| TLD1 | 687.1 | 13.74 | 7.24 | 1.90 | 2.01 | 50 |
| TLD2 | 770.0 | 15.40 | 8.50 | 1.81 | 1.99 | 50 |
| TLD3 | 732.2 | 14.64 | 7.47 | 1.96 | 2.03 | 50 |
| TLD4 | 874.0 | 17.50 | 9.63 | 1.82 | 2.01 | 50 |
| TLD5 | 1077.3 | 21.55 | 11.62 | 1.85 | 2.09 | 50 |
| TLDW | 1184.9 | 23.70 | 12.35 | 1.91 | 2.00 | 50 |
| TLDC | 892.0 | 17.84 | 9.34 | 1.91 | 2.04 | 50 |

To assess the effectivity of the primers, conventional PCR was carried out for both tilapia FADS2 genes and ELOVL gene before real time PCR was carried out (Figure 12 and 13). Amplification conditions were as described in chapter three. There was amplification for ELOVL genes in samples from all experimental diets (Figure 12). Low band intensity was observed in sample D2 and D3 may be due to low concentration of the amplicons in the sample.

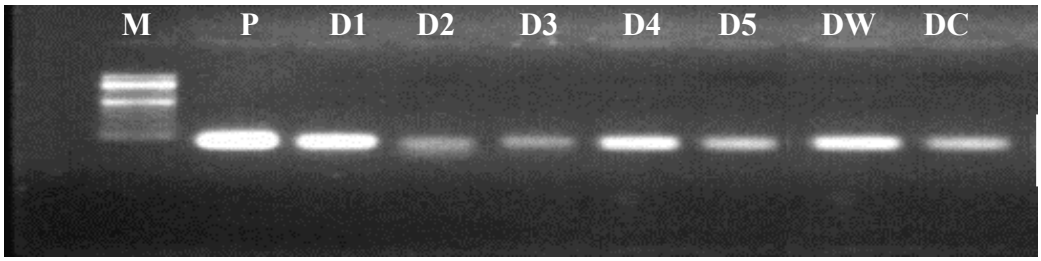



**Figure 12: PCR products for tilapia ELOVL gene**

Similarly, Primers for the FADS genes successfully annealed and amplified samples from all the experimental diets (Figure 13). The intensity of the bands was relatively similar in all the samples amplified.

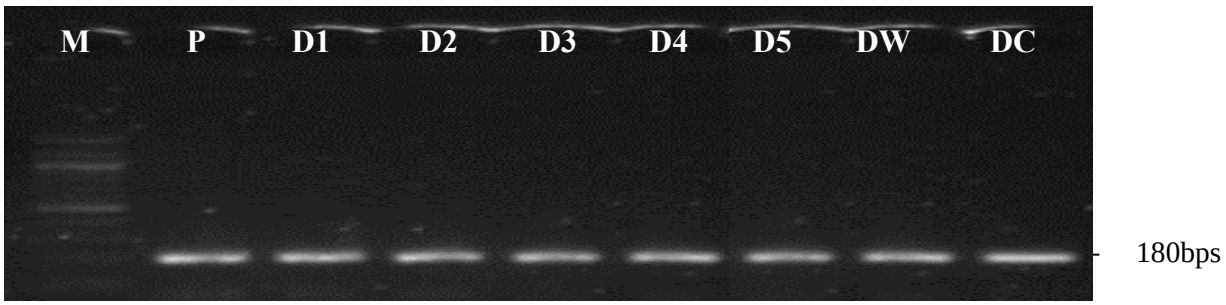



**Figure 13: PCR products for tilapia FADS2 genes**

Amplification curves for the FADS and ELOVL are presented in the Figure 14. The curves showed the levels of expression of FADS and ELOVL genes by threshold cycles (CT) values.Three curves of same colour represent ELOVL gene expression for specific dietary treatment. The lower the cycle numbers the higher the expression while the higher the cycle number the lower the expression.

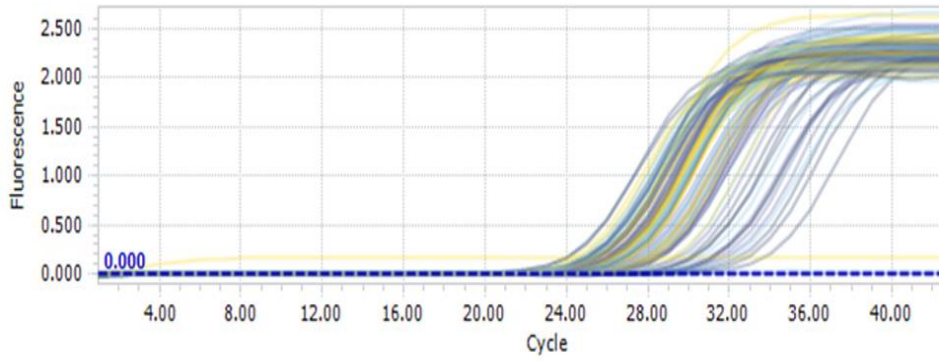



**Figure 14: Amplification curves for ELOVL genes**

To assess the homogeneity of the qPCR products, including the presence of primer–dimers and the specificity of the qPCR reaction, dissociation/melting curves for the ELOVL and β-actin genes were generated (Figure 15). The curves showed that the ELOVL primers were specific for the target gene and generated distinct melting curves. Primers for the β-actin were also specific producing all the melting curves around same temperature.

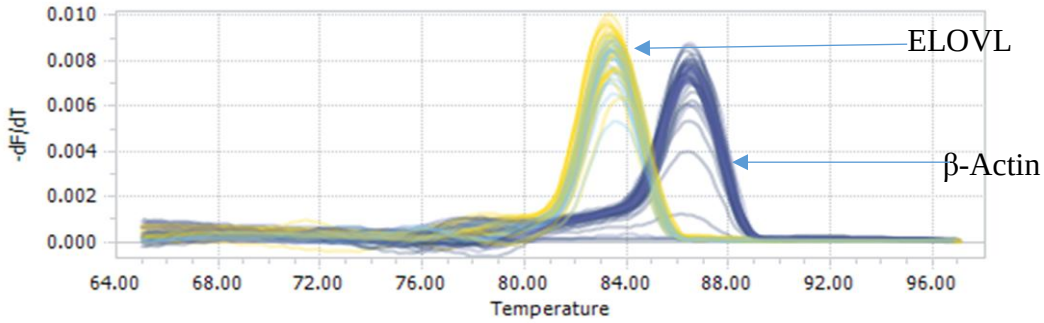



**Figure 15: Melting curves for ELOVL genes**

Relative expression of FADS2 genes in liver was not significantly different in the tilapia fed diet 1-3 however; the expression of FADS2 was significantly low in the diet 4, diet 5 and commercial diet. The relative expression of FADS in washout diet was relatively lower compared to the expression of β-actin (Figure 16)

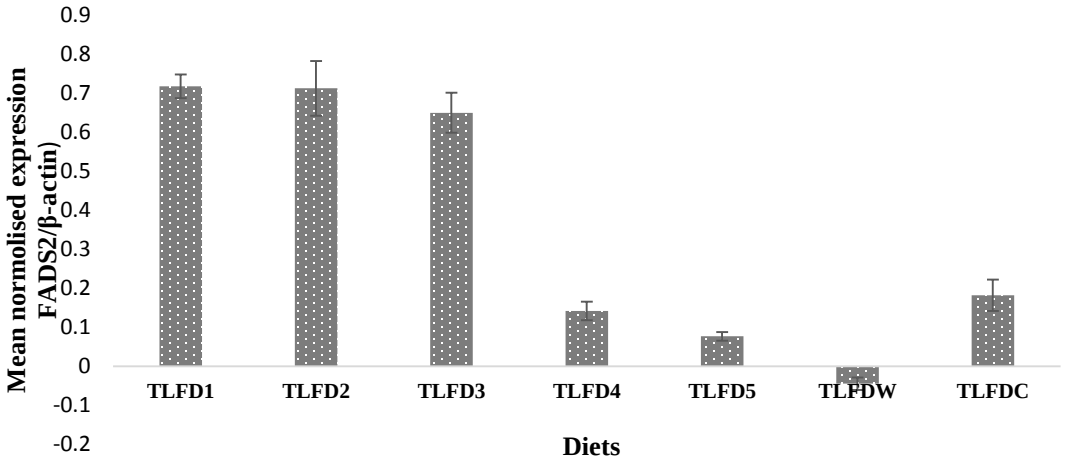



**Figure 16: Tilapia Fatty acid desaturase gene expression**

The tilapia ELOVL genes were significantly highly expressed relative to housekeeping gene (β-actin) in all the experimental diets. However, the expression varied significantly among the experimental diets. Expression of ELOVL genes in diet 5 and washout diet were significantly low. The ELOVL expression in commercial diet, diet 1, diet 2 and diet 3 were not significantly different however, the expression of ELOVL gene in diet 1 was relatively high and that there was a relative reduction in expression levels from diet 1 to washout diet (Figure 17).

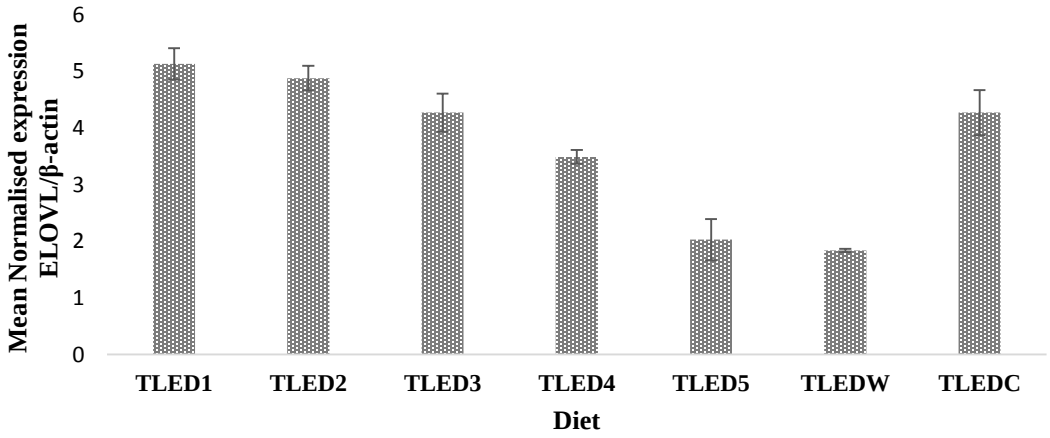



**Figure 17: Figure: Tilapia elongase (ELOVL) gene expression.**

### 4.9.2 Effect of experimental diets on catfish ELOVL and FADS2 genes

As earlier mentioned, the expression of elongase and Δ6-deasurase enzymes were estimated by the abundance of their mRNA in the fish liver. Data on the purity and concentration of the extracted RNA is shown in Table 23. Extracted RNA had two distinct bands representing 18S and 28S (Figure 18). The 260/280 rations for the catfish RNA ranged between 1.85 and 1.99 while the 230/260 ratios ranged between 1.98 and 2.14 which are near the recommended purity levels for extracted RNA. RNA concentrations ranged between 5501µg/ml and 1029.91µg/ml.

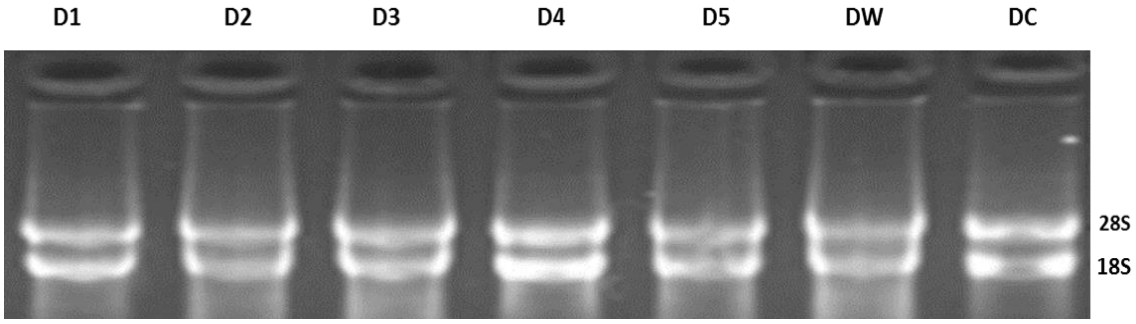



**Figure 18: Total RNA from liver of catfish fed different diets**

**Table 23: Concentration and purity of total RNA isolated from Catfish**

| Sample ID | RNA concentration | A260 | A280 | 260/280 | 260/230 | Factor |
|---|---|---|---|---|---|---|
| CFD1 | 775.9 | 15.52 | 8.09 | 1.92 | 2.08 | 50 |
| CFD2 | 1029.9 | 23.6 | 11.91 | 1.98 | 2.04 | 50 |
| CFD3 | 955.1 | 19.1 | 9.76 | 1.96 | 1.99 | 50 |
| CFD4 | 889 | 17.8 | 9.5 | 1.87 | 2.13 | 50 |
| CFD5 | 839.9 | 16.8 | 8.55 | 1.97 | 2.03 | 50 |
| CFDW | 807.9 | 16.06 | 8.05 | 1.99 | 2.05 | 50 |
| CFDC | 550.1 | 11 | 5.94 | 1.85 | 2.14 | 50 |

Conventional PCR was carried out to assess the effectivity of the primers with catfish ELOVL and FADS2 genes. Primers for both ELOVL and FADS genes successfully amplified the target gene and the amplification products are presented as shown in Figures19 and 20.

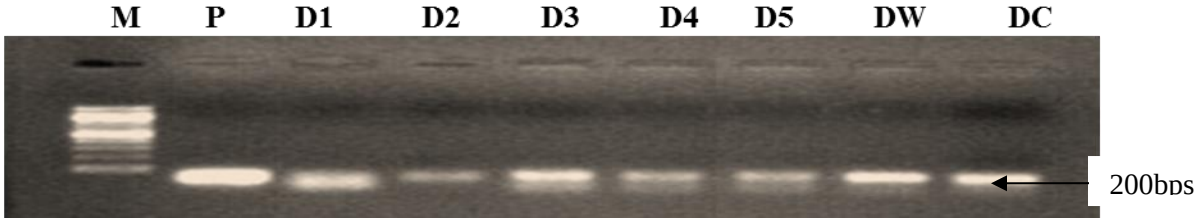



**Figure 19: PCR products for liver ELOVL gene obtained from catfish**

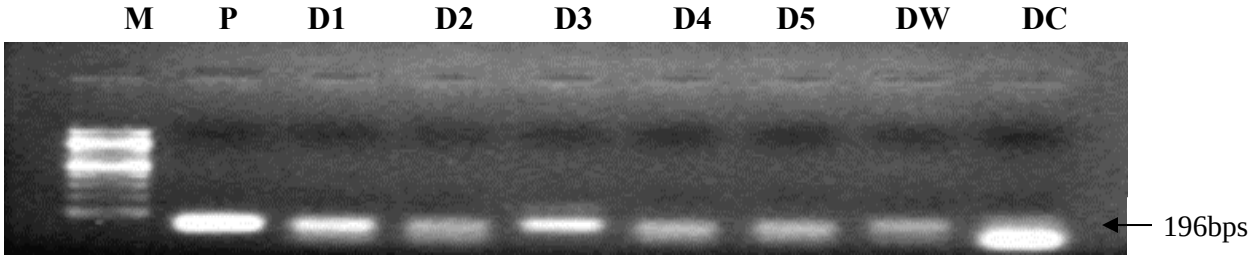



**Figure 20: PCR products for liver FADS2 gene obtained from**

Amplification and dissociation curves were also obtained to estimate the threshold cycles and ascertain homogeneity of qPCR products respectively (Figures 21 and 22).

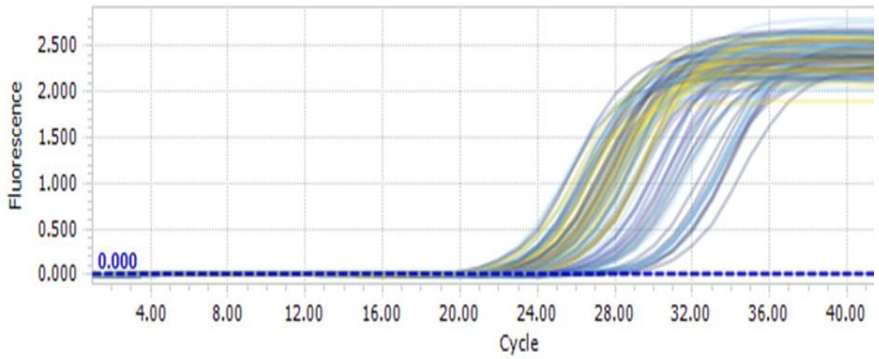



**Figure 21: Amplification curves for FADS genes**

The experiment was set up in triplicates thus three amplification curves with similar colour represent particular dietary experiments. The level of expression was estimated rom the threshold cycle number (Ct values)

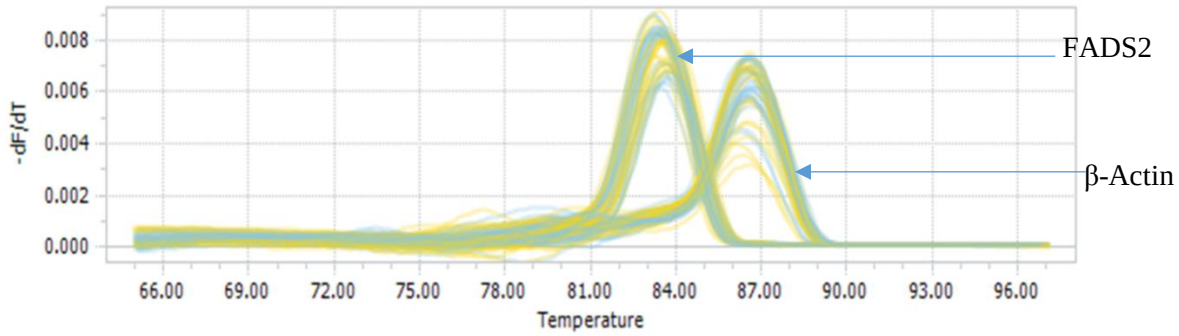



**Figure 22: Melting curves for FADS2 and β-Actin gene**

The expression of the catfish ELOVL genes varied significantly with experimental diets.ELOVL was significantly expressed in diet 1 relative to the expression of β-actin. The expression levels reduced significantly from diet 1 to washout diet (Figure 23). Expression of ELOVL gene in commercial diet and diet 3 were not significantly different.

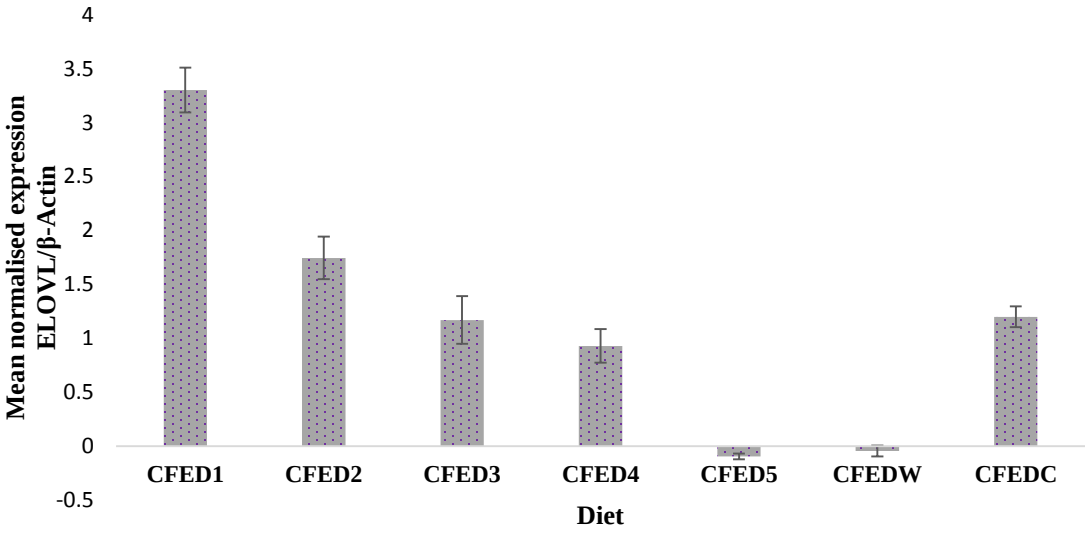



**Figure 23: Catfish elongase (ELOVL) gene expression for different diets**

The expression of FADS2 gene in catfish was significantly high in diet 1 however the expression reduced significantly from diet 1 to washout diet.

The expression of FADS2 gene in diet 5 and washout diet was lower relative to the expression of β-actin (Figure 24).

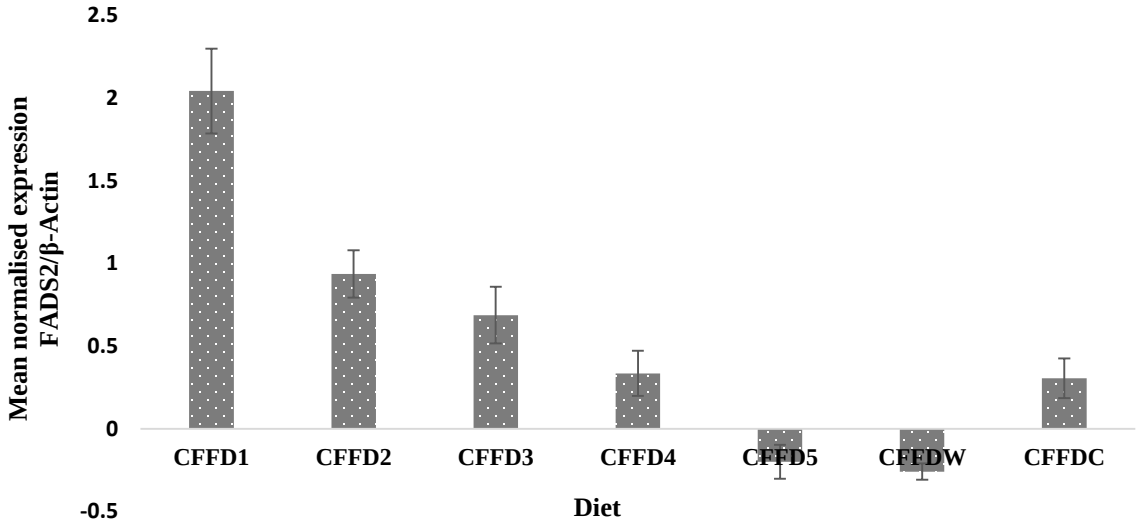



**Figure 24: Catfish fatty acid desaturase (FADS2) gene expression**

# CHAPTER FIVE

# DISCUSSION

## 5.1 Fatty acid profiles of wild fish and fish obtained from selected farms.

There is a nutritional deficit of n-3 PUFA in the human diet; therefore, a high consumption of food containing these fatty acids is recommended (Hossain, 2011). Due to stagnating wild fisheries and growing human population, aquaculture is expected to fill the gap in supplies of fish as food for humans, as demand continues to increase (FAO, 2008). However, there is concern whether there are nutritional differences, especially in n-3 fatty acids, between wild-caught and farm raised fish. Significant variations were observed in fatty acid profiles in fish sampled from different fish farmers in the current study. Palmitic acid was the most abundant saturated fatty acid in both farmed and wild fish. This is in agreement with the literature where C16:0 was reported as the most abundant SFA, in *L. niloticus, C. gariepinus* and *O. niloticus* from Lake Kainji, Nigeria, in four fish species from Maga Lake in Far North Region of Cameroon, and three freshwater fish species in Malaysia (Effiong and Fakunle, 2013; Tenyang*et al*, 2016; Zuraini *et al.*, 2006). Likewise, Aggelousis and Lazos (1991) showed that in freshwater fish from Greece, the most abundant FAs were palmitic acids. Zenebe et al., (1998a, 1998b) also reported palmitic acid as the dominant saturated fatty acid in *O. niloticus* collected from five Ethiopian lakes. Meanwhile, Osman *et al.*, (Osman *et al*., 2001) reported that C16:0 was not the most abundant FA present in some fish from Malaysian waters.

The other major SFA found in both fish species was stearic acid (18:0) ranging between 8% -12.5% in wild Nile tilapia and 9.1% -11.1% in wild African catfish. In farmed fish, stearic acid was the second most abundant fatty acid with highest composition (15%) observed in African catfish sampled from SMTUF (Muranga).

The same farm (SMTFU) had highest (16.6%) stearic acid in its tilapia sample. Other authors have also found stearic acid as the second major SFA (Osibona, 2011; Mohamed and Al -sabahi, 2011; Tenyang *et al*, 2016).

Total MUFAS ranged between 25.9% and 34.8% in wild tilapia compared to 29% - 31.3% in wild catfish. These value were higher than values reported by Zenebe *et al* (1998a; 1998b), whose values ranged between 2.02%-24.6% in wild Nile tilapia and 2.34%-9.43% in catfish. In the sampled farmed fish, tilapia MUFAS ranged between 25.9% and 34.8% whereas catfish MUFAS ranged between 26.7% and 32.05%. The most abundant MUFA was oleic acid (C18:1 n-9), a findings supported by several other studies (Tenyang *et al*, 2016; Osibona, 2011; Effiong and Fakunle, 2013). Oleic acid values from current study ranged between 20.6% -22.1% in wild tilapia and 21.1% -26.2% in catfish higher than values reported by Zenebe *at al* (1998a, 198b). Palmitoleic acid (C16:1, n-7) was the other major MUFA and ranged between 6%-7.2% in wild Nile tilapia and 5.1%-6.9% in African catfish. According to Andrade *et al.* (1995) the most dominant MUFAs in freshwater fish from south Brazil were oleic and palmitoleic acids.The findings in the current study are comparable to results from other studies where palmitoleic acid was also found to be the second major MUFA (Effiong and Fakunle, 2013; Tenyang *et al*, 2016; Osibona, 2011). Palmitoleic acid and oleic acid were also the predominant MUFAS in samples obtained from different fish farms. In wild tilapia, PUFA ranged between 11.7% to 16.4% whereas in wild African catfish, the values ranged between 14. 5% to 16.6%. In farmed African catfish and Nile tilapia, PUFAS varied between 12.8%-18.4% and 13.1%-19.4% respectively. Omega-6 PUFAS dominated n-3 PUFAS in both wild and farmed fish in the current study. However, n-3 PUFAS dominated in experimental diets 1-4, showing that inclusion of linseed oil at even 25% of the total oil has a greater effectin altering the tissue n-3: n-6 ratio

The principal n-6 PUFA found in both the fish species were linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, C20:4 n-6). Current finding support other

studies where LA and AA were found as the principal n-6 PUFA in fresh water fish collected from different lakes (Effiong and Fakunle, 2013; Tenyang *et al*, 2016; Osibona, 2011).

In contrast Mohamed and Al-Sabahi (2011) did not find any AA in *O. niloticus* collected from river Nile, Sudan. Linoleic acid (LA) is the precursor of AA, a substrate for eicosanoid production which is also involved in the regulation of gene expression (Ou *et al.*, 2001) LA is also found as a structural component of cell membranes and is important in cell signaling. In current study, arachidonic acid ranged between 0.5% to 0.85% in wild Nile tilapia and 0.53% to 1.11% in African catfish. In farmed fish, AA ranged between 0.5% -0.9% in tilapia and catfish. These values are within the range reported by Zenebe *et al* (1998a, 1998b). Arachidonic acid is a precursor for prostaglandin and thromboxane biosynthesis (Rahnan, *et al*., 1995). Arachidonic acid can facilitate the blood clotting process and attach to endothelial cells during wound healing. The inclusion of the fish in human diets might help in the wound healing process of the consumer (Erkihun *et al*., 2017).

The n-3 PUFA are considered to be of major importance in terms of human health. DHA, EPA and α-linoleic acids (ALA) were the n-3 PUFAs analysed in the current study. In wild tilapia their values ranged as follows: DHA, 2%-3.1%; EPA, 0.8%-1.4% and ALA, 0.43%-0.9%. In wild African catfish, the n-3 fatty acid values ranged as follows: DHA, 1.52%-2.3%, EPA, 0.93%-1.8% and ALA, 0.72%-1.2%. These values were comparable to those obtained in a study by Zenebe *et al* (1998a, 1998b). In their study, Zenebe and others reported a DHA range of 2.69%-11.55%; EPA range of 0.48%-1.9% and ALA range of 0.2%-1.8% in wild *O.niloticus.* In wild catfish, they reported the following ranges, DHA, 3.4%-5.8%, EPA 0.53%-2.6% and ALA 0.48%-1.52%. the current findings are similar to findings by Efiong and Faculae (2013), Osibona (2011) and Kwetegyeka *et al*. (2008), who reported that the most abundant LC n-3 PUFAs in freshwater species is DHA. In the current study, DHA was found to be higher in Nile tilapia compared to African catfish.

Efiong and Faculae (2013) similarly found that DHA was higher in *O. niloticus* than *L. niloticus* and *C. gariepinus* collected from Lake Kainji, Nigeria. In a similar study Tenyang et al. (2016) found higher content of DHA in *O. niloticus* compared to the other four fish species collected from Maga Lake, Cameroon.

The higher content of DHA and total n-3 PUFA in *O. niloticus* might be attributed to the feeding habits and diverse food items consumed by the fish. *O. niloticus* feeds lower in the food chain, mainly on microalgae, which are excellent sources of EPA, DPA and DHA. For example, studies by Mfilinge *et al*. (2005) and Meziane *et al*. (2007) reported that diatoms and dinoflagellates contain higher concentrations of EPA and DHA, respectively, and have been used as markers of diatoms and dinoflagellates in the aquatic food web. *O. niloticus* feed by consuming diverse species of phytoplankton, zooplankton and macrophyte. Based on this feeding chain, it is more likely that the EPA, DPA, and DHA contained in the herbivorous fish species were transferred to the carnivorous ones via the food chain. Thus, expansion of diet and diversity of microalgae species might contribute to *O. niloticus* having higher content of DHA. In addition, more DHA in *O. niloticus* could be as a result of desaturation and elongation of FAs. The ability to elongate and desaturate FAs is not the same in all species of fish. *O. niloticus* have the ability to bio convert stearic acid, oleic acid, and other FAs, which belong to group C: 18 fatty acids, to highly unsaturated FAs (Kwetegyeka*et al.,* 2008). Conversion of ALA to EPA and DHA is limited and varies according to the intakes of other FAs (Burdge *et al*., 2003). Thus, a typical intake of ALA may be less able to satisfy the physiological requirements for LC n-3 PUFAs than LC n-3 FAs (Erkihun*et al*., 2017). There was variation in fatty acids profiles in the wild fish sampled in the current study. The weight of sample wild fish varied thus FA variation might have been attributed to by weight difference.

This observation is consistent with previous studies indicating that fatty acid profile in fish is age specific (Parlov *et al,* 2009, Nemova *et al* 2015a; Nemova *et al* 2015b). Svetlana *et al* (2016) observed that the levels of n-3 fatty acids, DHA and n-3/n-6 ratios in *Atlantic salmon* increase in two year feeding period confirming the increased accumulation of essential fatty acids in fish with age. In the present study, fatty acid profiles varied between farms.

Regarding the fatty acid differences, in general, most of the studies have reported higher content of total PUFAs and n-3 PUFAs in wild than those of the farmed ones (Hossain, 2011). However, these results are in contrast to what has been found by other authors (Mnari *et al*., 2007; Hossain *et al*., 2012), who found higher proportion of total PUFAs and n-3 PUFAs in farmed fish than in wild specimen of different fish species. Fatty acid profile variations in farmed fish from different fish farms were attributed to the differences in dietary lipid compositions of aqua feeds given to the fish by different fish farmers. JSF fed fish on imported commercial feeds whereas UNIF fed their fish with locally formulated feeds which included freshwater shrimps, sunflower cake, wheat bran and cassava as binders. SMTUF alternated locally prepared feeds with imported commercial aqua feeds.

Biochemical composition of fish is strongly affected by the composition of their feed (Orban *et al*., 2007) supporting the hypothesis that differences in feed composition influenced differences in tissue fatty acids in fish sampled from fish farms. In addition, the lipid content and fatty acid composition of fish are affected by various factors including genetic, seasonal and environmental factors, and the nutritional quality of dietary components (Alemu *et al*., 2013). Farmed fish may have superior fatty acid content than wild fish because fish farming can overcome the problem of variation in the fatty acid composition of fish due to dietary variance (Savage, 2009).Aqua feeds can be supplemented with proteins and lipids to make the body composition more favorable for human health and nutrition.

Justi *et al*., (2003) reported that *Oreochromis niloticus*, which received a diet with addition of 5% flaxseed oil (high in n-3 fatty acids) showed highest index for n-3 PUFA (DHA 56.2%)thus the best n-3/n-6 ratio. Thus, it shows that it is possible to favorably change the body composition of fish through changes in diets (Steffens, 1997). Sargent *et al*. (1989) also agreed that it is technically possible to produce a fish with a ratio of n-3/n-6 PUFA that is optimal for human nutrition. Proper choice of dietary lipid sources would allow the fatty acid composition of cultured fish to be tailored to address the beneficial health aspects and consumer's demand (Alasalvar *et al*., 2002).

### 5.2 Formulation, proximate and fatty acid composition of experimental diets

Sustainability of aquaculture relies on the development and establishment of alternative feedstuffs to fish meal (Olukayode and Emmanuel, 2012). This is because, aqua feeds take up between 40-60% of the fish farm's production costs and is a major constraint to fish farming in resource poor regions (Ali *et al.,* 2009). Fagbenro, (1999) concur that the success of fish farming heavily depends on the provision of suitable and economical fish feeds formulated from locally available feedstuff especially agricultural by-products to reduce the price of complete feeds. Aside from fishmeal, other ingredients used in feed formulations include soya bean meal, oilseed meals, animal by-products and grains and by-products thereof. Soya bean meal has been widely used to replace fishmeal due to its steady supply (Hlophe *et al*., 2011) and balanced amino acid profile among plant-based sources. However, this ingredient is limited by its high fat content and trypsin inhibitor (Abowei & Ekubo 2011), as well as its unsustainable and variable availability through competition with human consumption and climate change (Kumar *et al.,* 2010).

In the current study locally available ingredients; rice bran, freshwater shrimp, wheat flour, linseed oil, sunflower oil and olive oil, were used for fish feed formulation (Table 6). The experimental diets were formulated based on the protein and energy requirements for Nile tilapia and African catfish as suggested by Abdel-Tawwab*et*

*al*., (2010). Studies have identified several alternative protein sources for fish meal in aqua feeds and determined the essential nutrients in fish meal and ways of incorporating them into the alternatives (Tacon *et al*., 1983).

The use of locally available materials such as wheat bran, maize bran, rice bran, *Caridina niloticus*, cotton cake, sunflower cake among others, as components of aqua feeds, have been recommended and their suitability determined (Liti *et al.*, 2001; Munguti *et al.*, 2014). However, oilseed meals, such as sunflower and cottonseed cakes, are deficient in lysine and methionine compared with soya bean meal, while groundnut cake (peanut) with high protein content at 45% becomes poisonous when mouldy due to mycotoxin (aflatoxin) contamination (Abowei & Ekubo 2011). Studies have revealed that warm water fishes require both n- 6 and n- 3 fatty acids while cold- water fishe require only n- 3 fatty acids for optimum growth and development (Takeuchi, 2008). Both n-3 and n-6 were provided in the formulated diets through dietary inclusion of sunflower and linseed oils (Table 6). Whereas optimal use of the fish resources (trash fish) and exploiting under utilized ocean resources such as Antarctic krill have been proposed, the most significant option could be the use of terrestrial animal meals and plant protein-rich derivatives (Tacon and Metian, 2008). Animal by-products, which are produced from animal waste tissues, offer cheap alternatives as they do not compete with human consumption and usually have high protein content (El Sayed & Tacon 1997). However, several drawbacks as to their use have been documented. For years, meat and bone meal (MBM) has been included in commercial fish diets for up to 20% inclusion, but has been prohibited due to the outbreak of bovine spongiform encephalopathy in the 1990s. Meanwhile, feather meal has been reported to have poor digestibility and variations in quality (Ayadi *et al.*, 2012). Grains and by-products thereof are primary carbohydrate sources which include rice bran and wheat bran. Rice bran is a good nutrient source and available at a reasonable cost, yet significant fibre and fat content limit its binding capacity during pelleting. Wheat

bran is an excellent binder and is more commonly used in salmonids due to its higher protein content than whole grains.

However, its use is limited as it competes with human consumption as well (Ruforum, 2011). In the current study, fresh water shrimp (*Caridina niloticus*) was used as the main protein source. However, experts have proposed other protein source such as single cell proteins, earthworm, insects, snails, maggots, and frogs as potential fish meal replacers (Tacon *et al.,* 1983). The use of *C. niloticus* as a protein source in tilapia aquafeeds has been evaluated (Liti *et al*., 2006). In other experiment using the *C. niloticus* protein source, it has been demonstrated that the growth performance of juvenile *C. gariepinus* fed diets formulated with *C. nilotica* was comparable with fish fed Artemia salina (Chepkirui-Boit *et al*. 2011).

The aquafeed formulation in the current study promoted growth performance comparable to findings by Abdel-Tawwab *et al*., (2010) and Hern_andez *et al.*, (2010). Dietary fatty acid composition varied among the diets with α-linolenic composition in diet 1 being significantly high. Alpha linolenic composition reduced with reduction of dietary linseed oil inclusion from diet 1 to diet 4.

Dietary fatty acid composition depended on the type and concentration (v/w) of vegetable oils included in the diet. Both n-3 and n-6 fatty acids were included in the diet with n-3 fatty acids ranging between 5.9% and 19.7% whereas n-6 fatty acid ranging between 20% to 28% (Table 8). Linoleic acid was the major n-6 fatty acids originating from the dietary oil (Table 6). Linseed oil was the principle source of dietary α-linolenic acid whereas *Caridina niloticus* were the dietary source of DHA and EPA.In the current study, the n-6 fatty acid requirement were met in the formulated diet with linoleic acid values ranging between 20-28% (Table 8). These values were above the recommendations by Takeuch *et al* (1983) but lower than dietary n-6 fatty acid used by Molner *et al* (2012), which ranged from 20.4% to 33.9%. Likewise, n-3 series of fatty acids such as C18:3n-3, C20:5 n-3, and C22:6n-3 have also been suggested to be a dietary essential for Nile tilapia, blue tilapia, *O.*

*aureus*, and *O. niloticus* × *O. aureus* hybrids (El-Sayed *et al*., 2005; Yildirim-Aksoy *et al*., 2007).

### 5.3 Effect of experimental diets on growth parameters and survival of tilapia and catfish

Good quality of fish feed is a function of how well the feed meets the nutrient requirement of a fish to promote its growth and survival. Fish growth is significantly influenced by a number of factors including the level of protein in the feed (Goda *et al*., 2007, Keremah and Beregha 2014, Corn'elio *et al.,* 2014), fiber level in the feed (Adewolu et al. 2010, Agbabiaka et al. 2013), carbohydrate and lipid ratio (Ali and Jauncey (2004a), lipid level (Agokei *et al.,* 2011, Ali *et al.,* 2012) among many other factors. Protein is considered as the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth.

For instance, *Clarius gariepinus* require at least 40% dietary protein for maximum growth of (Henken *et al.,* 1986, Van Weerd 1995) whereas in *Oriochromis niloticus*, dietary protein requirement has been stated to be between 32% to 50% for juvenile tilapia and for larger tilapia 25 to 30% (El-Saidy and Gaber, 2005; Ali *et al.,* 2008; Nguyen *et al.,* 2009; Abdel-Tawab *et al.,* 2010). This protein requirement of tilapia differs with each life stage of the fish (Jauncey and Ross 1982). The protein content in the experimental diets ranged between 44.2% and 45.9% (Table 6) which were within the recommended range for optimal growth as reported by Nguyen *et al.,* (2009); Abdel-Tawab *et al.,* (2010) and Ali *et al.,* (2012).

High level of fiber content in feed has been observed to slow the growth of *C. gariepinus* fingerlings (Adewolu *et al.* 2010, Agbabiaka *et al.* 2013). In the results of Agokei *et al.* (2011), significant higher growth performance of *C. gariepinus* juveniles was found in the diet that contained <2% fiber content. The fiber content in experimental diets for the current study was > 2% which might have contributed to lower growth performance of fish with experimental diets compared to

commercial diet whose fiber content was approximately 2% (Table 6). Impact of dietary ash level on fish growth has also been investigated. Ash content in the current study ranged between 3.7%-13.9%.

Ali and Jauncey (2004b) noted a better growth performance of *C. gariepinus* on diet containing 9.3% ash content, while Alam *et al.,* (2012) suggested that ash content in the feed of *C. gariepinus* should not be less than 8%. High ash content of >12% in feed has been reported to produce better growth performance in *Clarias* species (Kiriratnikom and Kiriratnikom 2012, Corn´elio *et al.,* 2014). Dietary oils are also important dietary component of aqua feeds because they are the source of essential fatty acids (EFA). Long-chain highly unsaturated fatty acids (HUFAs) such as docosahexaenoic acid (DHA, 22:6 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and arachidonic acid (ARA, 20:4 n-6), are required by fish for their normal growth and reproduction (Sargent *et al.*, 1999).

Results from the current study showed that high dietary linseed oil (>50% total replacement) as in diets 1-3, lowered growth performance and survival rate in both the fish species, however, survival rate was relatively higher in catfish (Tables 12 and 13). The survival rate increased from 94% in diet 1 to 97.67% in diet 4 in tilapia while it increased from 95% in diet 1 to 98% in diet 5 in catfish (Table 12 and 13). Findings in the current study are consistent with earlier reports that high dietary linseed oil lowered the growth performance of tilapia (Li *et al* 2016; Francis *et al*., 2006) and that high dietary n-3 polyunsaturated fatty acids (PUFAs) depressed growth in hybrid tilapia (Huang *et a*l, 1998) and tilapia zillii (Kanazwa *et al*; 1980). Ng *et al*., (2003) reported that in catfish, cod liver oil rich in HUFA resulted in significantly lower growth than some vegetable oils, for example, sunflower oil; refined, bleached, deodorized olein; crude palm oil; and crude palm kernel oil.

Other studies have also reported poor growth in African catfish (Hoffman and Prinsloo, 1995) and hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* (Huang et al. 1998; Ng et al. 2001), fed cod liver oil. The growth of both catfish and

tilapia were significantly affected by the dietary treatment in the current study. Earlier studies have established that both n-3 and n-6 polyunsaturated fatty acids are essential for optimal growth of Nile tilapia (Chen *et al*. 2013) and hybrid tilapia, *O. niloticus x O. aureus* (FAO, 2014) and that freshwater fish have a dietary requirement for n-3 and n-6 fatty acids, predominantly in the form of α-linolenic and linoleic acids (Izquierdo *et al*., 2003; Tocher, 2003). In addition, the incorporation of vegetable oils in fish feeds, replacing fish oil should not alter growth performance since the needs of essential fatty acids (EFAs) are covered (Corraze & Kaushik, 2009). Dietary linoleic (C18.2) and α-linolenic (C18.3) acids were provided in this study through sunflower and linseed oil respectively.

However, high levels of linseed oil (>50%) in the diets 1-3 (Table 5) might have resulted into n-3/n-6 imbalance exceeding the physiological tolerance of both catfish and tilapia for n-3 fatty acids leading to an oxidative stress induced by cellular incorporation of fatty acids and possible damage of immune response (Li *et al*, 2016). Fish nutritional status is known to influence the immune system and its resistance to disease (Xu*et al*., 2010). The possible immunosuppression caused by high α-linolenic acid inclusion or the unbalanced n-3/n-6 ratio in the diet 1-3 has been reported in various fish species (Sargent *et al*., 2002).

As a rule, diet fatty acids may affect the immune response by conditioning, plasma membrane fatty acid composition and its subsequent effects on the membrane's physical qualities (Tocher, 1995). Dietary lipids also influence cytokines and eicosanoid production, synthesized from the precursor's eicosapentaenoic (20:5 n-3, EPA) and arachidonic (20:4 n-6, ARA) acids, which are the key cell messengers in the inflammation process (Rowley*et al*., 1995). In addition, dietary lipids influence transcription regulation of genes responsible for immune responses (Montero*et al*., 2008). The stimulatory or inhibitory of the immune system by dietary fatty acids in freshwater fish has been reported to depend directly on the quantity and quality of

the dietary fatty acids and on their ratio, such as EPA: ARA ratio (Lin and Shiau, 2003).

Different dietary fatty acid ratios were used in this study and might have influenced the relative differences in growth and survival rates among the feeding groups. In addition, it has been established that growth-promoting effects of n-6 fatty acids are superior to those of the n-3 series in red belly tilapia (Lim *et al*., 2009) confirming the relatively high growth rate in both tilapia and catfish at high dietary sunflower oil content in this study. For instance, sunflower alone (100%) promoted better growth in both fish species than linseed (100%). However, in tilapia, 25:75 ratios of linseed and sunflower mixture promoted better growth followed by a ratio of 50:50 (linseed: sunflower). Diet 3 (50:50, linseed and sunflower) promoted better growth in catfish however differences in the specific growth rate was not significant among the experimental diets (Tables 11-13). Thus different sunflower: linseed oil ratios differently affected growth of tilapia and catfish.

Commercial diet promoted better growth in both catfish and tilapia. This is consistent with previous findings that imported commercial feeds produce better growth performance in various species of fish when compared with locally formulated feeds. These include the work of Shapawi *et al.* (2011) who compared growth performance of humpback grouper *Cromileptis altivelis* fed on farm-made feeds and coppens and Ahmed *et al.* (2012) who showed that commercial feeds enhanced better growth performance of the fingerlings of *Labeo rohita*. Commercial feeds are nutritionally complete with possible additives such as growth promoters and antibiotics. Feed additives are edible substances that are added to animal feeds in small quantity to enhance the feed quality so that it enhances growth performance and reduces mortality in fish (Dada, 2015). Combination of these factors might have promoted significantly higher specific growth rate and survival in fish fed commercial diets.

Sargent *et al*., (2002) reported that the immune system's development, maintenance and efficiency depend on a complete and balanced nutrition and several nutrients, such as vitamins and fatty acids or additives such as probiotics, have shown an immune-modulatory activity in fish. *Caridina niloticus* was the principal protein source in the formulated diet and might have been lacking some essential amino acids necessary for fish growth. Amino acids regulate key metabolic pathways that are crucial to maintenance, growth, reproduction, and immune responses in fish (Peng *et al.,* 2008). There was no growth promoting factor or antibiotics added in the experimental diets thus the possible cause of low growth rate and reduced survival rate in this study compared to commercial feeds.

The weight gains were between 750% and 1689% in tilapia and 440% and 958% in catfish. This demonstrate that diets formulated using *C. niloticus* does not compromise the overall growth of *O. niloticus* and *C.gariepinus*. The overall growth performance in this study was relatively higher than findings in previous studies with *C. niloticus* as the protein source (Mugo-Bundi *et al.,* 2013). The growth performance of Nile tilapia and African catfish in terms of final mean weight at harvest, weight gain and SGR under the experimental diets in this study were comparable with other studies (Abdel-Tawwab *et al*., 2010; Hern_andez *et al.,* 2010).

In the latter studies, average per cent weight gain ranged from 297% to 2634% and SGR ranged from 1.3% to 1.5% day_1. However, the specific growth rates in this study were relatively lower than that observed by Abdel-Tawwab *et al* (2010) and Hernandez *et al.,* (2010). The observed growth response of the Nile tilapia and catfish in this study indicated ability to utilize locally available feed ingredients presumably reflecting the high digestive capacity of these species for a wide range of food items (Degani, Viola & Yehuda 1997). However, the reduced growth performance with experimental diets could also be as a result of anti-nutritive factors in the locally formulated feed ingredients which might not have been removed, bad

formulation and inadequate processing of the local feed might all have synergistic effects on the growth performance of the fish.

The growth and survival rates were relatively high in catfish compared to tilapia. This could have been because catfish is known to be hardy and thrives in diverse environments. It can adapt its feeding habits depending on food availability and able to withstand adverse environmental conditions, and is highly fecund and easily spawned under captive conditions (Nyina-Wamwiza *et al*, 2010). These features might have the contributing factors for the relatively high survival rate in catfish. In conclusion, high linseed oil lowers the survival rate in both catfish and tilapia

### 5.4 Effect of experimental diets on fatty acid profiles in catfish and tilapia

From this study, it was evident that dietary lipid composition (Tables 8) influenced tissue lipids composition (Tables 14-17), an observation made in earlier studies (Ng *et al.,*2003; Vientiane *et al.*, 2005; Ji *et al.,* 2011). Tissue α-linolenic acid (C18:3) and oleic acid (C18:1) composition reduced with reduction of linseed oil and olive oil composition in the diet respectively (Tables 8, 14,15,16,17). Oleic acid (C18:1) was the dominant fatty acid in all tissues in both catfish and tilapia fed experimental diets. Palmitic acid was the dominant saturated fatty acid in both diets and fish tissues. This study confirms earlier studies that both C18:1 and C16:0 are preferentially retained in tissues and their retention may be related to energy storage as both of these two fatty acids are preferred substrates for β-oxidation in fish (Torstensen *et al.,* 2000, Kowalska *et al.,* 2010a). Palmitic acid (16:0, PA) dominance is also attributed to the fact that it can be provided in the diet or synthesized endogenously from other fatty acids, carbohydrates and amino acids (Gianfranca *et al*., 2017). In addition, the plasma and tissue concentration of palmitic acid is not significantly influenced by palmitic acid dietary intake (Innis and Dyer, 1997; Song *et al.,* 2017), suggesting that the homeostatic control of palmitic acid concentration is regulated by its production. However, oleic acid is formed from elongation and desaturation of palmitic acid (Heath *et al.,* 2001).

Interestingly, in the wild fish and fish sampled from selected fish farms, palmitic acid rather than oleic acid was the predominant fatty acid. Oleic acid has many health benefits (Wu *et al.,* 2012; Carrillo *et al.,* 2012**)** over palmitic acid (Saadatian-Elahi *et al.,* 2004; Bassett et al., 2016) therefore the experimental diets used in the current study might promote health in terms of dominating oleic acid over palmitic acids in fish tissues. The concentrations of saturated fatty acids (SFA) were lower than that of polyunsaturated fatty acid (Tables 14-17) in all experimental tissues partially confirming report by Menoyo *et al.,* (2005) that linseed and sunflower oils offers low SFA concentration. The composition of omega-3 fatty acids, especially docosahexaenoic acid DHA), C22:6, increased with the increased composition of linseed in the diet in all the tissues (Tables 14-17) indicating a possible selective deposition and retention of DHA in muscles of both tilapia and catfish at concentration higher than that in the diets (Tables 14-17). The retention trend in this study partially confirm reports in Atlantic salmon (Bell *et al.,* 2003), rainbow trout (Caballero *et al.,* 2002), African catfish (Ng *et al.,* 2003) and turbot (Regost *et al.,* 2003).

Bell (2001) suggested that the underlying mechanism for this selective retention of DHA include high specificity of fatty acyl transferases for DHA and relative resistance of DHA to beta-oxidation due to its complex metabolic pathway. Findings in this study are also consisted with report by Li *et al.,* (2016) that increased inclusion of linseed in tilapia feed significantly increased DHA levels and that feeding diets containing linseed oil can increase EPA/DHA content in tilapia (Justi*et al.,* 2003; Tonial *et al.,* 2009).

Agata *et al.,* (2012) reported DHA composition of 17.1% with 6.7% dietary inclusion of linseed oil in pikeperch fed for 56 days. Molnar *et al.,* (2012) observed 11.39% DHA composition in tilapia fillet fed 5% linseed diet inclusion for 42 days compared to 14.1% DHA composition in tilapia fed fish oil inclusion for the same period of time. In the present study, DHA composition of 4.4-12.2% was recorded in

tilapia muscle (Table 14), 3.4-10.8% in tilapia liver (Table15), 2.6-10.4% in catfish muscles (Table16) and 1.94-9.7% in catfish liver (Table 17) at linseed oil inclusion of 14% for a feeding period of 150 days. In addition, the DHA values from this study partially compare with reports by Menoyo *et al.,* (2005) who observed DHA value of 13.09% on replacement of linseed oil with fish oil at 100% inclusion in Atlantic salmon as well as study by Aguiar *et al.,* (2011) who reported a DHA value of 12.5% in tilapia head with linseed oil diet inclusion at 5% for a feeding period of 150 days.

On the contrary, the aforementioned findings are inconsistent with report by Turchini *et al.,* (2011) that there is a decreased concentration of n-3 PUFA when diets of greater linseed oil are fed to fish. The total n-3 fatty acids were higher in tilapia muscles (9.6%-29.7%) than in catfish muscles (8.7%-22.6%) (Tables 14 and 17) which indicate a probable more efficiency in conversion of $C_{18}$ PUFAS to HUFAS in tilapia. This finding is partially consistent with the study by Kwategyeka *et al*., (2008), that wild tilapia had more n-3 PUFA (31.2%-32.0%) than catfish (24.0%-24.6%). In the current study, wild tilapia had relatively higher n-3 fatty acids compared to catfish (Tables 9 and 10) confirming findings by Kwategyeka *et al*., (2008).

The difference in n-3 composition between catfish and tilapia in this study is probably related to the difference in lipid storage capacity in their tissues and also preferences in selective retention and mobilization of specific fatty acids (Mourente and Bell, 2006). Studies indicate that some fish species can bio-convert C18:3 and C18:2 into highly polyunsaturated fatty acids and that physiological accumulation of fatty acids in fish tissues occur when dietary supply of the same is high (Stubhaug et al, 2005). In addition, the extents at which fish can desaturase/elongate $C_{18}$ polyunsaturated fatty acid, PUFA to highly polyunsaturated fatty acids, HUFA vary with species (Sargent*et al*., 2002). Notably, most marine species are carnivorous and considered to have weak capacity to bio-convert $C_{18}$ PUFAS to HUFAS and thus require preformed HUFAs in their diet (NRC, 2011).

This is inconsistent with findings in this study where catfish, a carnivorous fish species, recorded substantial DHA composition (Tables 16-17). There was no significant difference ($p<0.05$) in the n3/n6 ratios between commercial diet and diet 1 in both catfish tissues (Tables 16-17) partially confirming a report by Molner *et al.*, (2012) that n3/n6 ratios in fish fed linseed oil are not significantly different from fish fed diets containing fish oil. The n3/n6 ratios between commercial diet and diet 1 varied significantly in the tilapia tissues (Tables 14-15).

However, tilapia tissue n3/n6 ratios reported in the current study were lower than values reported in earlier studies on European sea bass, 3.26 (Mourente and Bell, 2006), Atlantic salmon, 4.59 neutral lipids and 6.72 polar lipids (Menoyo, 2005), and pike perch, 3 (Kowalska *et al*., 2013). EPA composition was the lowest among the n-3 fatty acids in all the experimental subjects and diets. This finding is consistent with earlier finding that EPA may selectively be used as a substrate for β-oxidation (Karapanogiotidits *et al*., 2007) or converted into DHA(Sprecher,2000) resulting into its apparent low composition in the tissues.Alpha-linoleic acid (C18:2) and linolenic acid (C18:3) values in the fish tissues (Tables 14-17) were lower than values in the experimental diets (Table 8) suggesting their selective utilization when present at high concentrations (Ng *et al.*, 2003).

In addition, α-linoleic acid is metabolic precursors of EPA and DHA resulting into possible reduction in the fish tissue. The concentrations of DHA, a metabolic product of C18:3, significantly increased in the fish tissue suggesting a possible conversion of C18:3 to DHA. On the other hand, the availability of arachidonic acid in the tissue suggests that linoleic acid may have undergone selective utilization for beta-oxidation or both arachidonic acid and linoleic acid were selectively used for eicosanoid production. The current study was not able to accurately account for low tissue concentration of arachidonic acid (C20:4), a desaturation product of C18:2 (Tables 14-17). However, these low values of C20:4 relative to the dietary C18:2 was recently reported (Li *et al.*, 2016) and attributed to a number of roles played by

C20:4 including its role in eicosanoids formation and resistance to stressors which are prevalent under intensive culture system (Bell and Sargent, 2003; Li *et al.*, 2016). In addition,previous studies indicate that excessive dietary supply of C18 polyunsaturated fatty acids may create selective competition disrupting bioconversion thus low levels of arachidonic acid (Ruyter *et al*., 2006).

Linoleic acid, C18:2, n-6 and α-linolenic acid, C18:3 n-3 are metabolized by same sequential desaturation and elongation enzymes (Vinsentainer, 2007) thus excessive dietary supply may create selective competition disrupting their bioconversion and resulting into low or physiological tissue levels of C20:4 (Ruyter *et al.*, 2006, Li *et al*., 2016). This phenomenon was observed in the current study where even though 100% linseed oil gave high amount of DHA, the percentage conversion of α-linolenic acid to DHA seemed higher with lower linseed oil content (Tables 14-17) thus positive economic implication to the farmer. In conclusion, linseed oil is a possible candidate for fish oil replacement in tilapia and catfish feeds because increased linseed oil in diet improved fish tissue DHA and n3/n6 ratio pointing its relevance in fish feeds and effect on the overall fatty acid profile in fish tissue. Worth noting, is the dynamics of results obtained from different studies for same and/or different fish species. This suggest that factors influencing tissue fatty acids are not limited to dietary lipids and feeding period and that particular studies are unique in their own ways. Alemu *et al* (2013) noted that the lipid content and fatty acid composition of fish are affected by various factors including genetic, seasonal and environmental factors, and the nutritional quality of dietary components.

**5.5 Effect of feeding period on the content of polyunsaturated fatty acids (PUFA) in fish tissues**

This study established an increased accumulation of polyunsaturated fatty acids with feeding period of 3 months in catfish and tilapia (Tables 17-18). In the current study, tilapia tissue DHA, n-3 fatty acids and n-3/n-6 ratios increased as follows DHA (4.3%-7.1%, diet 1 muscles; 5.4%-7.4% diet 1 liver), n-3 fatty acids (17%-25% in diet 1 muscle; 5.4%-7.4% in diet 1 liver) and n-3/n-6 ratios (1.5-2.2 in diet 1

muscles; 1.4-2.6 in diet 1 liver) (Table 18 and 19). Same pattern of increase in the composition of polyunsaturated fatty acid with feeding period was observed in other experimental diets.

Studies have shown that fatty acid composition of fish tissues is affected by species, environmental factors, size, age and diets (Saito *et al*, 1999; Kiessling *et al*; 2001). In the current study, tilapia was reared under same exogenous conditions therefore culturing period and diet were the possible factors that could affect fatty acid profile of fish under study. From findings in the current study, DHA, total omega 3 and n3/n6 ratio increased significantly ($P<0.05$) from month 1 to month 3 in both tissues (Tables 18-19). This observation is consistent with previous study indicating that fatty acid profile in fish is age specific (Parlov *et al*, 2009, Nemova *et al* 2015a; Nemova *et al* 2015b). A study on Atlantic salmon indicated that in two year feeding period, the levels of n-3 fatty acids increased from 24%-29.5%, DHA increased from 5.4%-8.6% and n-3/n-6 ratio increased from 2.5-3 (Svetlana *et al.,* 2016). The length of feeding time also determines the incorporation of n-3 PUFA into fish fillet (Justi *et al.*, 2003). Tonial *et al.,* (2009) established that the shortest time period required for inclusion of linseed oil into adult fish muscle to raise n-3 PUFA is 45 days. The feeding period in the current study was 90 days which was beyond the average period established by Tonial *et al.,* (2009) for incorporation of dietary lipid into fish tissues.

**5.6 Effect of experimental diets on the expression of ELOVL and FADS2 genes**

There was significant variation in the mRNA abundance for ELOVL and FADS2 genes among the experimental diets. This study demonstrated higher relative mRNA abundances of ELOVL and FADS2 genes to β-actin in both catfish and tilapia liver, especially in groups fed diets with elevated (>25%) levels of linseed oil resulting into increased n-3 PUFAs in these groups. From the data obtained, relative expression of hepatic FADS2 mRNA was significantly ($p<0.05$) higher in tilapia groups fed diets 1, 2 and 3 compared to diet 4, 5 and washout diets. The expression

pattern was similar with tilapia ELOVL genes in which expression reduced significantly ($p<0.05$) from diet 1 to washout diet.

Coincidentally, n-3 PUFA particularly, DHA (C22:6 n-3) was significantly higher in all the groups fed diet 1 and that the tissue content of this fatty acid reduced with the reduction in dietary linseed oil composition. Same pattern was observed in catfish where relative expression of ELOVL and FADS2 genes reduced significantly from diet 1 to washout diet. This observation suggests that there was an active conversion of dietary ALA to tissue DHA as indicated by pattern of tissue DHA content behavior in relation to dietary lipid. Tissue arachidonic acid (C20:4 n6) also increased relative to dietary LA further supporting the possibility of the experimental fish species to actively utilize dietary lipids. The conversion of dietary ALA and ALA into the tissue LC-HUFA requires the involvement of fatty acyl elongases and desaturases possibly resulting into the increased expression of ELOVL and FADS2 genes in this study. Further, mRNA expression levels for FADS2 and ELOVL genes also followed the same pattern of tissue DHA levels and dietary linseed oil composition pointing a possible direct relationship between dietary lipid, tissue lipid content and gene expression. This dietary regulation of ELOVL and FADS2 expression and the activity of the LC-PUFA biosynthesis pathway has been demonstrated in freshwater and salmonid fish species (Monroig, 2011). However, dietary ALA influence on ELOVL and FADS2 genes is species specific (Wei-Chung, 2011). Tocher and Dick (1999, 2000) demonstrated that LC-PUFA biosynthesis activity was modulated by different $C_{18}$ PUFA in carp cells. In other studies, LC-PUFA biosynthesis was increased in salmonids, (Tocher *et al.* 1997, 2001), and expression of LC-PUFA biosynthesis genes, especially Δ6 Fad, was induced in salmon liver and intestine in fish fed diets containing vegetable (Zheng *et al.*, 2004b, 2005a, b;). Increasing dietary ALA, C18:3 upregulated hepatocyte ELOVL and FADS2 mRNA abundance's in barramundi (Wei-Chung, 2011). In contrast, liver and intestinal Δ6 Fad expression and activity was generally not significantly affected in Atlantic cod fed diets containing either vegetable or fish oil

(Tocher *et al.*, 2006), possibly reflecting differences in the Fad gene promoters in cod and salmon (Zheng *et al.*, 2009b).

Regulation of FADS2 and ELOVL genes has also been investigated in marine fish larvae, a developmental stage particularly sensitive to suboptimal LC-PUFA supply in the diet (Izquierdo, 2000). FADS2 appeared to be up-regulated in larval gilthead sea bream fed on microdiets formulated on rapeseed and soybean oil with low EPA and DHA compared to larvae fed on diet formulated with fish oil (Izquierdo *et al.*, 2008). A recent study on nibe croaker, a marine sciaenid teleost, reported increased expression of a putative FADS2 in response to *Artemia* diets containing low $C_{20\text{-}22}$ LC-PUFA (Yamamoto *et al.*, 2010). The expression of an Elovl-like elongase, however, did not show nutritional regulation in this species. In conclusion, there are evidences that dietary lipid regulate expression of ELOVL and FADS2 genes. The extent of regulation depends on the composition of individual dietary fatty acids and fish species.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

The following inferences were drawn from the current study;

1. Fatty acid profiles of wild fish varied with their bodyweight. Fish with greater body weight recorded higher accumulation of n-3 fatty acids. Relatively higher n-3 fatty acids were recorded in tilapia than in catfish. Fatty acid profiles of farmed fish varied with dietary composition given by individual farmers.
2. The level of dietary inclusion of linseed significantly affects growth performance and survival of fish. High dietary linseed oil (>50%) lowered growth and survival of both tilapia and catfish.
3. It was evident that there is a direct effect of dietary lipid composition on tissue fatty acid composition of fish. There was an apparent relationship between dietary linseed oil levels and tissue n-3 PUFA. The tissue n-3 PUFAs increased with increase in dietary ALA which was principally provided by linseed oil. Fresh water fish like Nile tilapia and African catfish easily utilizes dietary LA and ALA for biochemical synthesis of highly polyunsaturated fatty acids, such as DHA and EPA,
4. The accumulation of polyunsaturated fatty acid depends on the feeding period. As observed in this study, the longer the feeding period the high the tissue content of n-3 polyunsaturated fatty acids.
5. Dietary lipids significantly influence expression of ELOVL and FADS2 genes which are responsible for the synthesis of long chain fatty acids. Data from this study showed that levels of expression of these genes depended on the levels of dietary precursors such as LA an ALA. Therefore, careful choice of dietary composition and longer feeding period is key to fish with superior quality for god human health.

## 6.2 Recommendation

The use of vegetable oil in place of fish oil in aqua feed formulation is now established. This can go along in the improvement of the quality of farmed fish in terms of nutritive value at a lower cost. The use of locally available materials for fish feed formulation can definitely reduce the aquaculture production cost thus increased income among fish farmers. In addition, the quality of fish produced in terms of the omega 3 fatty acid composition can be improved through aqua feeds supplementation with dietary source of linolenic acid. This study focused on a very limited aspect on aquaculture and fatty acid study. Desirable fatty acid profiles were obtained with >50% dietary linseed oil with reduced growth and survival rates. More research is needed to achieve both good growth rates and fatty acid profiles. In addition, the research focused on the mRNA levels of ELOVL and FADS2 genes. More research is needed to assay for desaturases and elongases to establish their concentrations in the crude extract of fish liver for every experimental diet.

## REFERENCES


Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E. and Shalaby, A.M.E. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.). Aquaculture. 298:267-274

Aberoumand Ali (2009). Identification of Fatty Acids in Edible Wild Plants by Gas chromatography Food Analytical Methods Volume 2, Issue 3, pp 208–211

Adewolu, M.A., Ikenweiwe, N.B. and Mulero, S.M. (2010). Evaluation of an Animal Protein Mixture as a Replacement for Fishmeal in Practical Diets for Fingerlings of *Clarias gariepinus* (Burchell, 1822). Israel Journal of Aquaculture-Bamidgeh 62(4): 237-244.

Agaba, M., Tocher, D.R., Dickson, C., Dick, J.R. and Teale, A.J. (2004). Zebrafish cDNA encoding multifunctional fatty acid elongase involved in production of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids. *Mar. Biotechnol*. 6, 251-261.

Agaba, M.K., Tocher, D.R., Dickson, C.A., Zheng, X., Dick, J.R. and Teale, A.J. (2005). Cloning and functional characterisation of polyunsaturated fatty acid elongases from marine and freshwater teleost fish. *Comp. Biochem. Physiol*. 142B, 342–352.

Agata Kowalska, Zdzisław Zakęś,Andrzej Krzysztof Siwicki, Barbara Jankowska,Sylwia Jarmołowicz and Krystyna Demska-Zakęś(2012). Impact of diets with different proportions of linseed and sunflower oils on the growth, liver histology, immunological and chemical blood parameters, and proximate composition of pikeperch *Sander lucioperca* (L.).*Fish Physiol Biochem*. 38(2): 375–388.

Agbabiaka, L.A., Okorie, K.C. and Ezeafulukwe, C.F. (2013). Plantain peels as dietary supplement in practical diets for African catfish (*Clarias gariepinus* Burchell 1822) fingerlings. Agriculture and Biology Journal of North America 4(2): 155-159. DOI: 10.5251/abjna.2013.4.2.155.159

Aggelousis, G. and Lazos, E.S. (1991). Fatty acid composition of the lipids from eight freshwater fish species from Greece. J Food Comp Anal.; 4:68–76. Doi: 10.1016/0889-1575(91)90049-C.

Aggelousis, G. and Lazos, E.S. (1991). Fatty acid composition of the lipids from eight freshwater fish species from Greece. J Food Comp Anal. 4:68-76.

Agokei, E.O., Oparah, C.A., Aranyo, A. and Apapa, U. (2011). Growth of *Clarias gariepinus* juveniles fed five commercial feed. Continental Journal of Fish and Aquatic Science 5(3): 1-5.

Aguiar, A.C., Cottica, S. M., Boroski, M., Oliveira, C. C., Bonafe, E. G., Franca, P. B. and Visentainer, J. V. (2011). Quantification of Essential Fatty Acids in the Heads of Nile Tilapia (Oreochromis niloticus) Fed with Linseed Oil. *Journal of the Brazilian Chemical Society*, 22: 643-647,

Akio Kihara (2012). Very long-chain fatty acids: elongation, physiology and related disorders. *J Biochem.* 152(5):387-95

Alam, M. K., Habib, M.A.B. and Tahmid, M.S. (2012). A survey on commercial fish feed used at Fulpur area in Mymensingh District. *Journal of Bangladesh Agricultural University* 10(1): 175-178. DOI:10.3329/jbau. v10i1.12111

Alemu, L. A., Melese, A. Y. and Gule lat, D. H. (2013). Effect of endogenous factors on proximate composition of nile tilapia (Oreochromis Niloticus L.) fillet from Lake Zeway. *American Journal of Research Communication*, 1(11): 405-410

Ali, A., Al-Ogaily, S.M., Al-Asgah, N.A., Goddard, J.S. and Ahmed, S.I. (2008). Effect of feeding different protein to energy (P/E) ratios on the growth performance and body composition of Oreochromis niloticus fingerlings. *Journal Appl. Ichthy*. 24:31-37.

Ali, H., Azad, M. K., Anisuzzaman, M., Chowdhury, M M. R, Hoque, M. and Sharful, M. I. (2009). "Livelihood status of the fish farmers in some selected areas of Tarakanda Upazila of Mymensingh district", J. *Agrofor. Environ*. Vol. 3, No. 2, pp. 85-89

Ali, M. Z. and Jauncey, K. (2004a). Effect of feeding regime and dietary protein on growth and body composition of *Clarias gariepinus* (Burchell, 1822). *Indian Journal of Fisheries* 51 (4): 407-416

Ali, M. Z. and Jauncey, K. (2004b). Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell, 1822). *Aquaculture International* 12(2): 169-180. DOI: 10.1023/B: AQUI.0000032065.28059.5b

Ali, M. Z., Zaher, M., Alam, M.J. and Hussain, M.G. (2012). Effect of dietary carbohydrate to lipid ratios on growth, feed conversion, protein utilisation and body composition in climbing perch, *Anabas testudineus*. International Journal of Fisheries and Aquaculture 4(1): 1-6.

Alimuddin Yoshizaki, Kiron, V., Satoh, S., Takeuchi, T. and Yoshizaki, G. (2008). Cloning and over-expression of a masu salmon (Oncorhynchus masou) fatty acid elongase-like gene in zebrafish. *Aquaculture* 282, 13–18

Alimuddin Yoshizaki, Kiron, V., Satoh, S. and Takeuchi, T. (2005). Enhancement of EPA and DHA biosynthesis by over-expression of masu salmon Δ6-desaturase-like gene in zebrafish. Transgenic Res. 14, 159–165.

Alimuddin Yoshizaki, Kiron, V., Satoh, S. and Takeuchi, T. (2007). Expression of masu salmon Δ5desaturase-like gene elevated EPA and DHA biosynthesis in zebrafish. Mar. Biotechnol. 9, 92– 100

Al-Ruqaie Ibrahim M. (2007). Extruded Leftover Food as Animal Feed: I. Effect of Extruded Feed on Growth and Feed Utilization of Tilapia (*Oreochromis niloticus*) in Saudi Arabia. *Pakistan Journal of Biological Sciences, 10: 3248-3253.*

Alvarez, M.J., Diez, A., Lopez-Bote, C., Gallego, M. and Bautista, J. M. (2000). Short-term modulation of lipogenesis by macronutrients in rainbow trout (Oncorhynchus mykiss) hepatocytes. *Br J Nutr* 84, 619–628

Anderson, D. B., R. G. Kauffman and L. L. Kastenschmidt (1972). Lipogenic enzyme activities and cellularity of porcine adipose tissue from various anatomical locations. J. Lipid Res. 13:593.

Andrade, A.D., Rubira, A.F., Matsushita, M. and Souza, N.E. (1995). Fatty acids in freshwater fish from south brazil. *J. Am. Oil Chem. Soc*. 72:1207-1210.

AOAC. (1995). Official methods of analysis, 16th edition. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Bahurmiz, O.M. and Ng, W.K. (2007). Effect of dietary palm oil source on growth, tissue fatty acid composition and nutrient digestibility of red hybrid tilapia, (*Oreochromis sp*.), raised from stocking to marketable size. *Aquaculture* 262:382-392.

Barlow, S. (2000). Fishmeal and oil: sustainable feed ingredients for aquafeeds. *Global Aquacult. Advocate* 4:85–88

Barlow, S.M. and Pike, I.H. (2001). Aquaculture feed ingredients in year 2010: fish meal and fish oil. In: Aquavision _98. 2nd Nutreco Aquaculture Business Conference. (Nash, C.E. & Julien, V. eds), pp. 71–74, Stavanger, Norway, 13–15 May 1998 Nutreco Aquaculture, Stavanger, Norway.

Bassett, J. K., Hodge, A. M., English, D. R., MacInnis, R. J., and Giles, G. G. (2016). Plasma phospholipids fatty acids, dietary fatty acids, and breast cancer risk. Cancer Causes Control 27, 759–773. doi: 10.1007/s10552-016-0753-2

Beisenherz, G., T. Biicher and Barbade, K. H. (1955). Glycerophosphate dehydrogenase from rabbit muscle. *In* S. P. Colowick and N. O. Kaplan (Ed.) Methods in Enzymology. Vol. 1. Academic Press, New York. p. 391.

Bell, J. G., D. R. Tocher, B. M. Farndale, A. H. McVicar, and J. R. Sargent (1999). Effects of essential fattyacid-deficient diets on growth, mortality, tissuehistopathology and fatty acid compositions in juvenileturbot (*Scophthalmus maximus*). *Fish Physiology andBiochemistry* 20:263–277.

Bell, J. G., Tocher, D. R., Henderson, R. J., Dick, J. R., and Crampton, V. O. (2003b) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J. Nutr*. 133:2793–2801.

Bell, J. G., McGhee, F., Campbell, P. J. and Sargent, J.R. (2003a). Rapeseed oil as an alternative to marine fish oil in diets of postsmolt Atlantic salmon (*Salmo salar*): Changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out." *Aquaculture* 218:515–528.

Benoit Stephen, William L. Holland and Deborah J. Clegg (2009). Palmitic acid mediates hypothalamic insulin resistance by altering PKC-q q subcellular localization in rodents. *J Clin Invest.*119 (9):2577-2589.

Bharadwaj, K.G., Hiyama, Y., Hu, Y., Huggins, L.A., Ramakrishnan, R., Abumrad, N.A., Shulman, G.I., Blaner, W.S. and Goldberg, I.J. (2010). Chylomicron- and VLDL-derived lipids enter the heart through different pathways: in vivo evidence for receptor- and non-receptor-mediated fatty acid uptake. *J. Biol. Chem*., 285, pp. 37976-37986

Bimbo, A. P. (2007). Current and future sources of raw materials for the long-chainomega–3 fatty acids market. *Lipid Technology*,19(8), 176−179

Bligh, E.C. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 913–917.

Bonaa, K. H., Bjerve, K. S., Straume, B., Gram, I. and Thelle, D. (1990). *N. Engl. J. Med.*322: 1037-1043.

Boris Nemets, Ziva Stahl and Belmaker R.H. (2002). Addition of Omega-3 Fatty Acid to maintenance medicationtreatment for recurrent unipolar depressive disorder. *American Journal of Psychiatry;* 159:477–479

Bouraoui, L. J., Sánchez- Gurmaches, L., Cruz- Garcia, J. Gutiérrez, L., Benedito-Palos, J. and Pérez- Sánchez, I. Navarro (2011). Effect of dietary fish meal and fish oil replacement on lipogenic and lipoprotein lipase activities and plasma insulin in gilthead sea bream (Sparus aurata). *Aquaculture nutrition* 17; 54–63

Bray, G. A. (1960). A simple efficient liquid scintillatorfor counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* 1:279.

Brouwer IA, Zock PL, Camm AJ, et al. SOFA Study Group. Effect of fish oil on ventricular tachycardia and death in patients with implantable cardioverter defibrillators. JAMA 2006; 295:2613– 2619. [PubMed: 16772624

Brummett, R.E., Lazard, J. and Moehl, J. (2008). African aquaculture: Realizing the potential. Food Policy. 33(5):371–385

Bublitz, C. and Wieland O. (1962). Glycerokinase. *In S*.P. Colowick and N. O. Kaplan (Ed.) Methods inEnzymology. Vol. 5. p. 354. Academic Press, NewYork.

Burdge, G.C., Finnegan, Y.E., Minihane, A.M., Williams, C.M. and Wootton, S.A. (2003). Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13 C] α-linolenic acid to longer-chain fatty acids and partitioning towards β-oxidation in older men. Br J Nutr 90:311-321.

Burge, G. C., and Calder, P. C. (2005). Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Repro. Nutr. Dev*. 45:581-597.

Caballero, M. J., Obach, G. Rosenlund, D. Montero, M. and Gisvold Izquierdo (2002). Impact of different dietary sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214:253–271.

Cahu, C. P. and Salen, M.de Lorgeril (2004). Farmed and wild fish in the prevention of cardiovascular diseases: Assessing possible differences in lipid nutritional values. Nutrition, Metabolism and Cardiovascular Diseases14 Pages 34-41

Calder, P.C. (2006). n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition*, 83: 1505-1519.

Calder PC, Yaqoob P. Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors 35, 266-272

Carrillo, C., Cavia Mdel M and Alonso-Torre S (2012). Role of oleic acid in immune system; mechanism of action; a review. Nutricion hospitalaria.;27(4):978-90

Chesnut, C. H., M. Azria, S., Silverman, M. Engelhardt, M.Olson, and L. Mindeholm (2008). Salmon calcitonin: A review ofcurrent and future therapeutic indications.*Osteoporos. Int*.,19: 479–491

Clement, L., Poirier, H., Niot, I., Bocher, V., Guerre-Millo, M., Krief, S., Staels, B. and Besnard, P. (2002): Dietary *trans*-10,*cis*-12 conjugated linoleic acid induces hyperinsulinemiaand fatty liver in the mouse. *J Lipid Res* 43:1400 –1409,

Cocchi, M. Tonello, L. Martínez, Á.J., Lercker, G. and Caramia, G.M. (2009) Extra virgin olive oil and oleic acid. *Nutr. clín. diet. hosp*.; 29(3):12-24

Cocchi Massimo, Luca Sardi, Lucio Tonello and Giovanna Martelli (2009) I Disordini Dell'Umore Giocano Un Ruolo Sul Benessere Del Suino? Italian Journal of Animal Science, 8:4, 691-704

Codabaccus, M.B., Bridle, A.R., Nichols, P.D. and Carter, C.G. (2012). Restoration of fillet n-3 long-chain polyunsaturated fatty acid is improved by a modified fish oil finishing diet strategy for atlantic salmon (Salmo salar L.) smolts fed palm fatty acid distillate. *J Agric Food Chem*.; 60(1):458-66

Connor William E. (2000). Importance of n−3 fatty acids in health and disease *The American Journal of Clinical Nutrition*, Volume 71, Issue 1, Pages 171S–175S,

Corn'elio, F.H.G., Cunha, D.A., Silveira, J., Alexandre, D., Silva, C. and Fracalossi, D.M. (2014). Dietary protein requirement of juvenile Cachara Catfish, *Pseudoplatystoma reticulatum*. Journal of the World Aquaculture Society 45(1): 45-54. DOI: 10.1111/jwas.12090

Crawford, M., Galli, C., Visioli, F., Renaud, S., Simopoulos, A.P. and Spector, A. A. (2000). Role of plant-derived omega–3 fatty acids in human nutrition. *Ann. Nutr. Metab*. 44:263-265.

Cunnane S.C., Ryan M.A. and Nadeau C.R., (2003). Why is carbon from some polyunsaturates extensively recycled into lipid synthesis? *Lipids* 38 477-484.

Dada, A.A. (2015): Improvement of tilapia (Oreochromis niloticus Linnaeus, 1758) growth performance fed three commercial feed additives in diets. *Journal of Aquaculture Research and Development* 6, 325-327.

Dakshinamurti, K. and Desjardins, P. R. (1969). Acetyl-CoA carboxylase from rat adipose tissue. *Biochem. Biophys. Acta* 179:221.

Daviglus, M.; Sheeshka, J. and Murkin, E. (2002). Health benefits from eating fish. *Comments Toxicology*,8: 345 – 374.

de Lorgeril, M., Salen, P., Martin, J. L., Mamelle, N., Monjaud, I., Touboui, P. and Delaye, J.(1996). Effect of a Mediterranean type ofdiet on the rate of cardiovascular complications in patients withcoronary artery disease. J. *Am. Coll. Cardiol*. 5:103–105

de Lorgeril, M., S. Renaud, Mamelle, N., P. Salen, Martin, J. L., Monjaud, I., Guidollet, J., Touboul, P. and Delaye, J.. (1994). Mediterraneanalpha-linolenic acid-rich diet in secondary preventionof coronary heart disease. Lancet 343:1454–1459.

Dort, J., Sirois, A., Leblanc, N., Côté, C.H. and Jacques, H. (2012). Beneficial effects of cod protein on skeletal muscle repair following injury. *Physiol Nutr Metab*. 7(3):489-98

Drotningsvik, A., Mjos, S. A., Hogoy, I., Remman, T. and Gudbrandsen, O. A. (2015). A low dietary intake of cod protein is sufficient to increase growth, improve serum and tissue fattyacid compositions, and lower serum postprandial glucoseand fasting non-esterified fatty acid concentrations in obeseZucker fa/fa rats.*Eur. J. Nutr*., 54: 1151–1160

Effiong, B.N. and Fakunle, J.O. (2013). Proximate composition and fatty acid profile in some commercially important fish species from lake Kainji, Nigeria. Int J Biol Pharm Allied Sci. 2:849-56.

Eilander, A., Hundscheid, D. C., Osendarp, S. J., Transler, C. and Zock, P. L. (2007) Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies, Prostaglandins Leukotrienes and Essential Fatty Acids, 76(4), 189–203

Ekubo, A. A. and Abowei, J. F. N. (2011). Review of some Water Quality Management Principles in Culture Fisheries. *Research Journal of Applied Science, Engineering and Technology*, 3(12), 1342-1357.

El-Saidy, D.M.S.D. and Gaber, M.M.A. (2005). Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, Oreochromis niloticus (L.) cultured in concrete tanks. *Aquaculture Research*, 36:163-171

Erdemoglu, N., Sener, B. and Choudhary, M.I. (2004). Bioactivity of Lignans from Taxus baccata. Z Naturforsch C 59:494–498

Erkihun Massresha, Hintsa Mateos, Paul Lewandowski and Ashagrie Zewdue (2017). Proximate Composition and Fatty Acid Content of Commercially Important Fish Species from Ethiopian Lakes: A Review. *World Journal of Food Science and Technology*; 1(3): 105-114

Fagbenro, O. A. (1999). Comparative evaluation of heatprocessed winged bean (Psophocarpus tetragonolobus) meals as partial replacement for fishmeal in diets for African catfish (Clarias gariepinus). *Aquaculture,* 170, 297-305.

Fan, F., W.Q. Xue, B.H. Wu, M.G. He, H.L.Xie, W.F.Ouyang, S.L.Tu, andY.M.Chen (2013). Higherfish intake isassociated with a lower risk of hip fractures in Chinesemen and women: A matched case-control study. PLoSOne,8: e56849 doi: 10.1371/journal.pone.0056849

FAO (2012b). World aquaculture production by species groups. Rome. Available: ftp://ftp.fao.org/FI/STAT/summary/default.htm. [Accessed: 10 February 2015].FAO Agriculture and Consumer Protection department (2002). Human vitamin and mineral requirements. Training materials for agricultural planning.

FAO, IFAD and WFP, (2014). The State of Food Insecurity in the World 2014. Strengthening the enabling environment for food security and nutrition. Rome: FAO: FAO

Fernandez M. L. and West K. L. (2005) Mechanisms by which dietary fatty acids modulate plasma lipids. *J Nutr* 135, 2075–2078

Flachs, P., Rossmeisl, M., Bryhn, M. and Kopecky, J. (2009). Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clin Sci* 116: 1-16.

Folch, J., Lee, M. and Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*,226: 497 - 509.

Fonseca-Madrigal, F. V. Karazalos, P.J. Campbell, Bell, J.G and Tocher, D.R. (2005). Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout *Oncorhynchus mykiss,Aquaculture Nutrition* 11 241-250.

Fox., T. E., E.G.H.M. Van den Heuvel, C. A. Atherton1, J.R. Dainty1, D. J. Lewis, N.J. Langford, H.M. Crews, J.B. Luten, M. Lorentzen, F.W. Sieling, P. van Aken-Schneyder, M. Hoek, M.J.J. Kotterman, P. van Dael and S.J. Fairweather-Tait1(2004). Bioavailability of selenium from fish, yeast and selenate: a comparative study in humans using stable isotopes. *European Journal of Clinical Nutrition*, 58, 343–349

Friesen, E. N., Balfry, S. K., Skura, B. J., Ikonomou, M. G., and Higgs, D. A. (2013a). Evaluation of cold-pressedflaxseed oil as an alternative dietary lipid source forjuvenile sablefish (*Anoplopoma fimbria*). *Aquaculture Research* 44:182–199.

Ganga, R., D. Montero, J. G. Bell, E. Atalah, E. Ganuza,O. Vega-Orellana, L. Tort, L. Acerete, J. M.Afonso, T. Benitez-Sanatana, A. F. Vaquero, and Izquierdo, M. (2011). Stress response in sea bream (*Sparusaurata*) held under crowded conditions and fed dietscontaining linseed and/or soybean oil. *Aquaculture.* 311:215–223.

Gianfranca Carta, Elisabetta Murru, Sebastiano Banni, and Claudia Manca (2017). Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications

Ginsberg, H. N., Kris-Etherton, P., Dennis, B., Elmer, P. J., Ershow, A., Lefevre, M., Perason, T., Roheim, P., Ramakrishan, R., Reed, R., Stewart, K., Stewart, P., Phillips, K. and Anderson, N. (1998). Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects. *Arteriosclerosis, Thrombosis and Vascular Biology*18: 441-449.

Glock, G. E. and P. McLean (1953). Further studies on the properties and assay of ghicose-6-phosphate dehydrogenase and 6-phosphoglueonate dehydrogenase of rat liver. *Biochem. J.* 55:400.

Goda A. M., El-Haroun, E. R. and Chowdhury, M. A. (2007). Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. Aquaculture Research 38(3): 279-287. DOI: 10.1111/j.1365-2109.2007.01663.

González-Rovira, A., Mourente, G., Zheng, X., Tocher, D.R. and Pendón, C. (2009). Molecular and functional characterization and expression analysis of a Δ6 fatty acyl desaturase cDNA of European sea bass (Dicentrarchus labrax L.). *Aquaculture* 298, 90–100.

Guerin T. , Chekri R., Vastel C., Sirot V., Volatier J. L., Leblanc J. C. and Noel L. (2011). Determination of 20 trace elements in fish and other sea food from the French market. *Food Chem*. 127:934-942.

Guil-Guerrero, J.L., Maroto, F.F.G. and Gimenez, A.G., (2001). Fatty acid profiles from forty-nine plant species that are potential new sources of g-linolenic acid. *J. Am. Oil Chem. Soc*. 78, 677-684.

Guillou, H., Zadravec, D., Martin, P.G.P. and Jacobsson, A. (2010). The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog. Lipid Res*. 49, 186-199.

Harris, W. S. and Jacobson, T. A. (2009). Omega-3 fatty acids. In: Ballantyne CM, editor. Clinical Lipidology: A Companion to Braunwald's Heart Disease. Philadelphia, PA: Saunders, an imprint of Elsevier Inc., 326 –38.

Harris W. S. (2008) The omega-3 index as a risk factor for coronary heart disease. *Am J Clin Nutr*. 87:1997S–2002S.

Hastings, N., Agaba, M., Tocher, D.R., Leaver, M.J., Dick, J.R., Sargent, J. R. and Teale A.J. (2001). A vertebrate fatty acid desaturase with Δ5 and Δ6 activities. *Proc. Natl. Acad. Sci*. USA 98, 14304-14309.

Hastings, N., Agaba, M.K., Tocher, D.R., Zheng, X., Dickson, C.A., Dick, J.R. and Teale, A.J. (2005). Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from α-linolenic acid in Atlantic salmon (Salmo salar). *Mar. Biotechnol*. 6, 463-474.

Heath, R. J., S.W. White and C.O. Rock (2001). Lipid biosynthesis as a target for antibacterial agents Prog. Lipid Res., 40 (2001), pp. 467-497

Henken, A. M. Machiels, M. M, Deeker, W. and Hogendoorn, H. (1986). The effects of dietary protein and energy content on growth rate and feed utilization of the African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture 58(1-2): 55-74. DOI: 10.1016/0044- 8486(86)90156-0

Holben, D. H., and A. M. Smith (1999). The diverse role of seleniumwithin sialoproteins: A review. Am. Diet. Assoc., 99:836–843

Holick, M. F. (2008a). Diabetes and the Vitamin D connection. *Curr. Diab*. Rep.,8: 393–398

Holick, M. F. (2008b). The vitamin D deficiency pandemic and consequences for no skeletal health: Mechanisms of action. Mol.*Aspects Med*., 29: 361–368

Horton J.D., Goldstein, J.L. and Brown, M. S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver.*J.Clini. Invest*.109, 1125-1131.

Hossain, M.A. (2011). Fish as Source of n-3 Polyunsaturated Fatty Acids (PUFAs), Which One Is Better-Farmed or Wild? Advance Journal of Food Science and Technology, 3, 455-466.

Hunter, J.E., Zhang, J. and Kris-Etherton, P.M (2010). Cardiovascular disease risk of dietary stearic acid compared with Trans, other saturated and unsaturated fatty acids: A systematic review. *Am. J.Clin. Nutr*. 91, 46–63.

Innis, S. M. and Dyer, R. (1997). Dietary triacylglycerols with palmitic acid (16:0) in the 2-position increase 16:0 in the 2-position of plasma and chylomicron triacylglycerols, but reduce phospholipid arachidonic and docosahexaenoic acids, and alter cholesteryl ester metabolism in formula-Fed piglets. *J. Nutr*. 127, 1311–1319.

Izquierdo, M.S., Fernández-Palacios, H. and Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197, 25-42.

Izquierdo, M. S., Robaina, L., Juárez-Carrillo, E., Oliva, V., Hernández-Cruz, C.M. and Afonso, J.M. (2008). Regulation of growth, fatty acid composition and delta 6 desaturase expression by dietary lipids in gilthead seabream larvae (Sparus aurata). *Fish Physiol. Biochem*. 34, 117–127.

Izquierdo, M.S., Socorro, J., Arantzamendi, L. and Hernández-Cruz, C.M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiol. Biochem*. 22, 97-107.

Jakobsson, A., Westerberg, R. and Jacobsson, A. (2006). Fatty acid elongases in mammals: Their regulation and roles in metabolism. *Prog. Lipid Res*. 45, 237-249.

Jauncy K. and Ross B. A. (1982). guide to tilapia feed and feeding University of Stirling, Scotland, UK. 111.

Jaya-Ram, A., Ishak, S.D., Enyu, Y-L., Kuah, M-K., Wong, K-L. and Shu-Chien, A.C. (2011). Molecular cloning and ontogenic mRNA expression of fatty acid desaturase in the carnivorous striped snakehead fish (Channa striata). *Comp. Biochem. Physiol*. 158A, 415–422

Ji, H., Li, J. and Liu, P. (2011). Regulation of growth performance and lipid metabolism by dietary n-3 highly unsaturated fatty acids in juvenile grass carp, Ctenopharyngodon idellus. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.*, 159, 49 –56.

Jordal, A.-E.O., Ø. Lie and B.E. Torstensen (2007). Complete replacement of dietary fish oil with a vegetable oil blend aff ect liver lipid and plasma lipoprotein levels inAtlantic salmon (Salmo salar L.). *Aquaculture Nutrition*, 13; 114–130

Justi, K. C., Hayashi, C., Visentainer, J. V., de Souza, N. E. and Matsushita, M. (2003). The influence of feed supply time on the fatty acid profile of Nile tilapia (Oreochromis niloticus) fed on a diet enriched with n-3 fatty acids. *Food Chemistry*, 80(4), 489-493.

Karapanagiotidis, I., Bell, M., Little, D. and Yakupitiyage, A. (2007). Replacement of Dietary Fish Oils by Alpha-Linolenic Acid-Rich Oils Lowers Omega 3 Content in Tilapia Flesh. *Lipids*, 42 (6): 547-559.

Kennedy, A., Martinez, K., Chuang, C.C., LaPoint, K. and McIntosh, M. (2009).Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. *J. Nutr*. 139, 1–4.

Keremah, R. I. and Beregha, O. (2014). Effect of varying dietary protein levels on growth and nutrient utilization of African catfish *Clarias gariepinus* fingerlings. *Journal of Experimental Biology and Agricultural Sciences* 2(1): 13-18.

Kiriratnikom, S. and Kiriratnikom, A. (2012). Growth, feed utilization, survival and body composition of fingerlings of Slender walking catfish, *Clarias nieuhofii*, fed diets containing different protein levels. *Songklanakarin Journal of Science and Technology* 34(1): 37-43.

Kowalska A., Zakes, Z., Jankowaska, B. and Siwicki, A. (2010a). Impact of diets with vegetable oils on growth, histological structure of internal organs, biochemical blood parameters and proximate composition of pikeperch, *Sander lucioperca*(L). *Aquaculture* 301. 69-77

Kris-Etherton, P. M., Harris, W. S. and Appel, L. J. (2003). Fish consumption, fish oil, Omega-3 fatty acids and cardiovascular disease. Arteriosclerosis Thrombosis Vascular Biology, 23, 20–31.

Kudo, Y., Tanaka, Y., Tateishi. K., Yamamoto, K., Yamamoto, S. and Mohri, D. (2011). Altered composition of fatty acids exacerbates hepatotumorigenesis during activation of the phosphatidylinositol 3-kinase pathway. *Journal of hepatology*.;55(6):1400-8.

Kwetegyeka, J., Mpango, G. and Grahl-Nielson, O. (2008). Varietion in fatty acid composition in muscles and heart tissues among species and populations of tropical fish in Lakes Victoria and Kyoga-*Lipids* 43.1017-1029

Leaver, M.J., Bautista, J.M., Björnsson, T., Jönsson, E., Krey, G., Tocher, D.R. and Torstensen, B.E. (2008a). Towards fish lipid nutrigenomics: current state and prospects for finfish aquaculture. Rev. *Fisheries Sci.* 16, 71-92.

Leaver, M.J., Villeneuve, L.A., Obach, A., Jensen, L., Bron, J.E., Tocher, D.R. and Taggart, J.B. (2008b). Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (Salmo salar). *BMC Genomics* 9, 299.

Li F. J., Lin, X., Lin, S.M., Chen, Y.W. and Guan, T.(2016). Effects of dietary fish oil substitution with linseed oil on growth, muscle fatty acid and metabolism of tilapia (*Oriochromis niloticus*). *Aquaculture nutrition*22, 499-508

Lilian Wambui Kariuki, Peter Masinde, Stephen Githiri, and Arnold N. Onyango (2016). Effect of water stress on growth of three linseed (*Linum usitatissimum* L.) varieties. *Springerplus*. 5(1): 759

Lin Yu-Hung and SHI-YEN Shiau (2007). Eff ects of dietary blend of fish oil with corn oil on growth and non-specific immune responses of grouper, Epinephelus malabaricus. *Aquaculture Nutrition* 13; 137–144

Liti, D.M., R.M. Mugo, J.M. Munguti and H. Waidbacher (2006) Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds . *Aquaculture nutrition,* Volume12, Issue3. Pages 239-245

Livak, K. J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8.

Lowry, O.H., Rosebrough, N.J., Farr. A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol Chem* 193:265–275

Madani, Z., K. Louchami, A. Sener, W. J. Malaisse, and D. A.Yahia (2012). Dietary sardine protein lowers insulin resistance,leptin and TNF-alpha and beneficially affects adipose tissueoxidative stress in rats with fructose-induced metabolic syndrome.*Int. J. Mol. Med*.,29: 311–318

Malde, M. K., S. Bugel, M. Kristensen, K. Malde, I. E. Graff and J. I. Pedersen (2010). Calcium from salmon and cod bone iswell absorbed in young healthy men: A doubleblindedrandomised crossover design.*Nutr. Metabol*., 7: 61. http://www.nutritionandmetabolism.com/content/7/1/61

Marquardt, A., Stöhr, H., White, K. and Weber, B.H.F. (2000). CDNA cloning, genomic structure, and chromosomal localization of three members of the human fatty acid desaturase family. *Genomics 66*, 175-183

Martınez-Valverde, I., M. Jesus Periago, M. Santaella, and G.Ros (2000). The content and nutritional significance of mineralsonfishflesh in the presence and absence of bone. *Food Chem*., 71:503–509

Martins, D. A., L. M. P. Valente, and S. P. Lall. (2009). Apparent digestibility of lipid and fatty acids in fishoil, poultry fat and vegetable oil diets by Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture*

Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Yoshikawa, T., Hasty, A.H., Tamura Y., Osuga J-I, Okazaki H, Iizuka, Y., Takahashi, A., Sone H., Gotoda, T., Ishibashi, S., Yamada, N. (2002) : Dual regulation of mouse 5- and 6-desaturase gene expression by SREBP-1 and PPAR_. *J Lipid Res* 43:107–114.

Menoyo David, Clemente J._Lopez-Bote, Amalia_Diez, Alex_Obach and Jose M. Bautista (2007). Impact of $n-3$ fatty acid chain length and $n-3/n-6$ ratio in Atlantic salmon (*Salmo salar*) diets. Volume 267, Issues 1–4, , Pages 248-259

Menoyo, D., M. S. Izquierdo, L. Robaina, R. Gine´s, C. J. Lo´pez-Bote, and Bautista, J. M. (2004). Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *Br. J. Nutr*. 92:41–52.

Méziane, T., Lee, S.Y., Mfilinge, P.L., Shin, P. K., Lam, M.H. and Tsuchiya, M. (2007). Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. *Mar Biol*. 150:1103-1113.

Mfilinge P. L., Meziane, T., Bachok, Z. and Tsuchiya, M. (2005).Litter dynamics and particulate organic matter outwelling from a subtropical mangrove in Okinawa Island, South Japan. Estuar Coast. Shelf Sci. 63:301-313.

Miller, M.R., Bridle, A.R., Nichols, P.D. and Carter, C.G. 2008b. Increased elongase and desaturase gene expression with stearidonic acid enriched diet does not enhance long-chain (n-3) content of seawater Atlantic salmon (Salmo salar L.). *J Nutr.* 138, 2179-2185.

Miller, M. R., Nichols, P. D. and Carter, C.G. (2008a). n-3 oil sources for use in aquaculture alternatives to the unsustainable harvest of wild fish. Nutr. Res. Rev. 21, 85-96.

Moazzami, A. and Kamal-Eldin, A. (2006). Sesame seed is a rich source of dietary lignans. *Journal of the American Oil Chemists Society*, 83: 719-723

Mohamed, E.H.A. and Al-Sabahi G.N. (2011). Fatty acids content and profile of common commercial Nile fishes in Sudan. *Int. J. Fish. Aquac*. 3: 99-104.

Mohd-Yusof, N.Y., Monroig, Ó., Mohd-Adnan, A., Wan, K.-L. and Tocher, D.R. (2010). Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, Lates calcarifer. *Fish Physiol. Biochem*. 36, 827-843.

Molnar, T. B., Janka Biro, Hancz C., Romvari, R., Varga Daniel, Horn, P. and Szabo Andras (2012). Fatty acid profile of fillet, liver and mesenteric fat in tilapia (*Oriochromis niloticus*) fed vegetable oils supplementation in the finishing period of fattening. *Arch Tierrz* 55: 2.194-205.

Monroig, Ó., Webb, K., Ibarra-Castro, L., Holt, G.J. and Tocher, D.R. (2011a). Biosynthesis of long-chain polyunsaturated fatty acids in marine fish: Characterization of an Elovl4-like elongase from cobia Rachycentron canadum and activation of the pathway during early life stages. *Aquaculture* 312, 145– 153.

Monroig, Ó., Li, Y. and Tocher, D.R. (2011b). Delta-8 desaturation activity varies among fatty acyl desaturases of teleost fish: high activity in delta-6 desaturases of marine species. *Comp.Biochem. Physiol.* 159B, 206-213.

Monroig, Ó., Rotllant, J., Sánchez, E., Cerdá-Reverter, J. M. and Tocher, D.R. (2009). Expression of long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis genes during zebrafish Danio rerio early embryogenesis. *Biochim. Biophys. Acta* 1791, 1093–1101.

Moon, Y. A. and Horton, J. D. (2003). Identification of two mammalian reductases involved in the two-carbon fatty acyl elongation cascade, *J. Biol. Chem*., vol. 278pg. 7335−7343.

Morais, S., Castanheira, F., Martinez-Rubio L., Conceição, LEC and Tocher, D.R. (2012) Long chain polyunsaturated fatty acid synthesis in a marine vertebrate: ontogenetic and nutritional regulation of a fatty acyl desaturase with Δ4 activity. BBA - Mol Cell Biol Lipids 1821:660–671

Mourente, G., Good, J. E. and Bell, J. G. (2005). Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E2 and F2α, immune function and effectiveness of a fish oil finishing diet. *Aquaculture Nutrition*, 11 (1): 25-40,

Munguti J. and Charo-Karisa H. (2011). Fish Feeds and Aquaculture Development in Kenya. In: Samaki News: Aquaculture development in Kenya towards food security, poverty alleviation and wealth creation. Vol. 7. No. 1. pp. 27-29

Munguti, J., Charo-Karisa, H., Opiyo, M. A., Ogello, E. O., Marijani, E. and Nzayisenga L (2012). Nutritive value and availability of commonly used feed ingredients for farmed Nile Tilapia (*Oreochromis niloticus*) land African catfish (*Clarias gariepinus*, burchell) in Kenya, Rwanda and Tanzania. *African Journal of Food Agriculture, Nutrition and Development*;12(3):1-22

Munguti Jonathan Mbonge, Safina Musa, Paul S. Orina, Domitila N Kyule, Mary A. Opiyo,Harrison Charo-Karisa and Erick Ochieng Ogello (2014).An overview of current status of Kenyan fish feed industry and feed management practices,challenges and opportunities. *International Journal of Fisheries and Aquatic Studies* 1(6): 128-137

National Economic Survey, Kenya (2016). Kenya Bureau of Statistics ISBN: 9966-767-54-1

National Research Council (NRC) (2011). Nutrient Requirement of Fish and Shrimp. National Academy Press, Washington, DC.

Navarro, J.C., McEvoy, L.A., Bell, M.V., Amat, F., Hontoria, F., Sargent, J.R. (1997). Effect of different dietary levels of docosahexaenoic acid (DHA, 22:6-3) on the DHA composition of lipid classes in sea bass larvae eyes. *Aquacult. Internat*. 5, 509-516.

Nemetz, P. N. *et al*. (2008). Recent trends in the prevalence of coronary disease: a population-based autopsy study of nonnatural deaths. *Arch Intern Med* 168, 264–270,

Ng, W.K., P.K. Lim and, P.L. Boey (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle *α*-tocopherol concentration of African catfish, *Clarias gariepinus*, *Aquaculture* 215 229-243

Ngugi C. C and Manyala, J. O. (2004). Aquaculture extension services in Kenya. In: Aquaculture Extension Services in Sub-Saharan Africa. Fisheries Department Circular No. 1002. Food and Agriculture Organization of the United Nations, ed. FAO Fisheries Department, Rome, IT, pp. 35-42.

Ngugi, C.C. Bowman, J. R. and Omolo, B. O. (2007). A New Guide to Fish Farming in Kenya. Aquaculture Collaborative Research Support Program, Narobi, KE

Nguyen, T. N, Davis, D. A. and Saoud, I. P. (2009). Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, Oreochromis spp. *Journal World Aquaculture Soc*.; 40:113-121.

Nugteren, D.H. (1965). The enzymic chain elongation of fatty acids by rat-liver microsomes. Biochim Biophys Acta 106:280–290

Nyonje, B.M, Charo-Karisa, H., Macharia, S.K. and Mbugua, M. (2011), Aquaculture Development in Kenya: Status, Potential and Challenges. In Samaki news: Aquaculture development in Kenya towards food security, poverty alleviation and wealth creation

Ochoa, S. (1955). Malic enzyme. *In* S. P. Colowick and N. O. Kaplan (Ed.) Methods in Enzymology. Vol.1. Academic Press, New York. p. 739.

Olsen, R.L. and M.R. Hasan (2012). A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends Food Sci. Technol*., 27 (2). pp. 120-128

OlsenRagnar, L. and Mohammad R. Hasan (2012). A limited supply offishmeal: Impact on future increases in global aquacultureproduction.*Trends in Food Science & Technology* 27: 120-128

Olukayode, A. M. and Emmanuel, B. S. (2012). The potential of two vegetablecarried blood meals as protein sources in African catfish (Clarias gariepinus Burchell) juvenile diet. *Open J. Anim. Sci.* 2(1):15-18

Osibona, A. O. (2011). Comparative study of proximate composition, amino and fatty acids of some economically important fish species in Lagos, Nigeria. *Afr. J. Food Sci*. 5:581-8.

Osman, H., Suriah, A.R. and Law, E.C. (2001). Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *Food chem.* 73:55-60.

Ou, J., Tu, H., Shan, B., Luk, A., DeBose-Boyd, R.A., Bashmakov, Y., Goldstein, J.L. and Brown, M. S. (2001). Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc. *Natl. Acad. Sci*. USA. 98:6027-6032.

Ouellet, V., J. Marois, S. J. Weisnagel, and H. Jacques (2007). Dietarycod protein improves insulin sensitivity in insulin-resistantmen and women.Diab. Care. 30: 2816–2821

Panserat, S., G. Corraze, S. Kaushik and P. Bergot (2003). Cloning and nutritional regulation of a Δ6-desaturase-like enzyme in the marine teleost gilthead seabream *Sparus aurata*, Comparative *Biochemistry and Physiology*. 135 449-460.

Panserat, S., Kolditz, C., Richard N., Plagnes-Juan, E., Piumi F.,Esquerre D., Medale F., Corraze, G. and Kaushik, S. (2008b). Hepatic gene expression pro¢les in juvenile rainbow trout(Oncorhynchus mykiss) fed fishmeal or fish oil-free diets. *BritishJournal of Nutrition*. 100: 953-967.

Patterson, E., R. Wall, G. F. Fitzgerald, R. P. Ross and C. Stanton (2012). Health Implications of High Dietary Omega-6 Polyunsaturated Fatty Acids. *Journal of Nutrition and Metabolism.* Volume 2012, Article ID 539426, 16 pages.

Peng Li, Kangsen Mai, Jesse Trushenski and Guoyao Wu (2008). New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids* DOI 10.1007/s00726-008-0171-1

Peters, J.M., Park, Y., Gonzalez, F.J. and Pariza, M. W. (2001): Influence of conjugatedlinoleic acid on body composition and target gene expression in peroxisomeproliferator-activated receptor _-null mice. *Biochim Biophys Acta*1533:233–242.

Pike, I. (2005). Eco-efficiency in aquaculture: global catch of wild fish used in aquaculture. Int Aquafeed 8:38–40

Pilon, G., J. Ruzzin, L. E. Rioux, C. Lavigne, P. J. White, L. Froyland, H. Jacques, P. Bryl, L. Beaulieu, and A. Marette (2011). Differential effects of variousfish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity inhigh-fat-fed rats. *Metab.Clin. Exper*., 60: 1122–1130

Popa, V.M., Gruia, A. Raba, D.N., Dumbrava, D. Moldovan, Bordean, D. and Mateescu, C. (2012). Fatty acid composition and oil characteristics of linseed from Romania J.*Agroalignment.process. Technology* 18, 136-140

Popma, T. and M. Masser (1999). Tilapia: Life history and biology. Southern Regional Aquaculture Center Pub. SRAC-283 pp. 4

Pruszyriski, T. (2003). Effects of feeding on ammonium excretion and growth of the African catfish (Clarias gariepinus) fry. *Czech J. Anim. Sci*., 48 (3): 106-11

Raclot, T. Groscolas, R. Langin, D. and Ferre P (1997). Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in ratwhite adipose tissues. J Lipid Res 38, 1963–1972.

Rad, F., Kurt, G. I. and Bozaoůlu, A.S. (2003). Effects of spatially localized and dispersed patterns of feed distribution on the growth, size dispersion and feed conversion ratio of the African Catfish (Clarias gariepinus). *Turk J Vet Anim Sci* 28, 851-856

Rahnan, S. A., Huah, T.S., Nassan, O., Daud, N.M. (1995). Fatty acid composition of some Malaysian freshwater fish. Food chem. 54:45-9.

Ralston, N. V. C. (2008). Selenium health benefit values as seafood safety criteriaEcohealth,5: 442–455

Ralston, N. V. C. and L. J. Raymond (2010). Dietary selenium's protective effects against methylmercury toxicity. T*oxicology*, 278: 112–123

Ralston, N. V. C., C. R. Ralston, and L. J. Raymond (2016). Seleniumhealth benefit values: Updated criteria for mercury riskassessments.Biol. Trace Elem. Res.,171: 262–269

Rana, K. (2011). Critical Review and Analysis of the current aquaculture regulatory framework in the Republic of South Africa in relation to international benchmarks. Cape Town.*The Journal of Biochemistry*, Volume 152, Issue 5, (1) Pages 387–395.

Regost, C., J. Arzel, J. Robin, G. Rosenlund and Kaushik S. J. (2003). Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*). Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture*217:465–482.

Rincón-Cervera, Miguel Á. and José, L. Guil-Guerrero (2010). Preparation of stearidonic acid-enriched triacylglycerols from Echium plantagineum seed oil. *Eur. J. Lipid Sci. Technol*. 112, 227–232

Ringø, E., J. B. Lødemel, R. Myklebust, L. Jensen, V.Lund, T. M. Mayhew, and R. E. Olsen (2002). Theeffects of soybean, linseed andmarine oil on aerobic gutmicrobiota of Arctic charr *Salvelinus alpinus* L. before and after challenge with *Aeromonas salmonicida* ssp.*salmonicida*. *Aquaculture Research* 33:591–606.

Rudkowska, I., B. Marcotte, G. Pilon, C. Lavigne, A. Marette, and M. C. Vohl (2010). Fish nutrients decrease expression levels oftumor necrosis factor-alpha in cultured human macrophages.*Physiol. Genomics*,40: 189–194

Ruxton, C. H., Reed, S.C., Simpson, M.J. and Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet 17:449–459

Ruyter, B., Moya-Falcon, C., Rosenlund, G. and Vegusdal, A. (2006). Fat content and morphology of liver and intestine of Atlantic salmon (Salmo salar): Effects of temperature and dietary soybean oil. *Aquaculture* 252, 441-452.

Saadatian-Elahi, M., Norat, T., Goudable, J., and Riboli, E. (2004). Biomarkers of dietary fatty acid intake and the risk of breast cancer: a meta-analysis. *Int. J. Cancer* 111, 584–591. doi: 10.1002/ijc.20284

Sahena, F. Zaidul, I. S. M., Jinap, S. and Saari, N. (2009). PUFAS in fish: Extraction, fractionation, importance in health. *Comp. Rev. Food Sci*. Saf. 8:59-74.

Salati, L. M. and Amir-Ahmady, B. (2001). Dietary regulation of expression of glucose-6-phosphate dehydrogenase. *Annu Rev Nutr* 21, 121–140.

Salem, Jr. N.; Kim, H. Y. and Yergey, J. A. (1986). Docosahexaenoic acid: Membrane function and metabolism. In health effects of Polyunsaturated Fatty Acids in Seafood. *Academic Press, Orlando,* pp. 263-317.

Sampath, H. and Ntambi, J.M. (2005). The fate and intermediary metabolism of stearic acid. Lipids 2005, 40, 1187–1191.

Santigosa, E., Geay, F., Tonon, T., Le Delliou, H., Kuhl, H., Reinhardt, R., Corcos, L., Cahu, C., ZamboninoInfante, J.L. and Mazurais, D. (2011). Cloning, tissue expression analysis, and functional characterization of two Δ6-desaturase variants of sea bass (Dicentrarchus labrax L.). *Mar. Biothechnol.* 13, 22-31.

Sargent, J. R. and Tacon, A. (1999). Development of farmed fish: a nutritionally necessary alternative to meat. Proc Nutr Soc 58:377–383

Sargent, J., cEvoy, A., Est vez, A., Bell, G., Bell, M., Henderson, J., Tocher, D.R. (1999). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179, 217–229.

Sargent, J.R., McEvoy, L.A. and Bell, J.G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* 155, 117–127.

Sargent, J. R., Tocher, D. R. and Bell, J.G. (2002). The Lipids, in: Halver, J.E., Hardy, R.W., (Eds.), Fish Nutrition, third ed., Academic Press, San Diego, pp. 181-257.

Sarker, M.A-A., Yamamoto, Y., Haga, Y., Sarker, M.S.A., Miwa, M., Yoshizaki, G., Satoh, S. (2011). Influences of low salinity and dietary fatty acids on fatty acid composition and fatty acid desaturase and elongase expression in red sea bream Pagrus major. *Fish. Sci*. 77, 385–396.

Sarvenaz Khalili Tilami and Sabine Sampels (2017). Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals. *Reviews in Fisheries Science & Aquaculture* Volume 26, Pages 243-253

Seiliez, I., Panserat, S., Corraze, G., Kaushik, S. and Bergot, P. (2003). Cloning and nutritional regulation of a Δ6desaturase-like enzyme in the marine teleost gilthead sea bream (Sparus aurata). *Comp. Biochem. Physiol.* 135B, 449–460.

Seiliez, I., Panserat, S., Kaushik, S. and Bergot, P. (2001). Cloning, tissue distribution and nutritional regulation of a Δ6-desaturase-like enzyme in rainbow trout. *Comp. Biochem. Physiol*. 130B, 83–93.

Sheridan, M. A. (1988). Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comp Biochem PhysiolB Biochem Mol Biol* 90, 679–690.

Simopoulus, A. P. (2008). The importance of the omega 6/ omega-3fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine*. 233: 674-688.

Song, X., Huang, Y., Neuhouser, M. L., Tinker, L. F., Vitolins, M. Z. and Prentice, R. L. (2017). Dietary long-chain fatty acids and carbohydrate biomarker

evaluation in a controlled feeding study in participants from the Women's Health Initiative cohort. *Am. J. Clin. Nutr*. 105, 1272–1282. doi: 10.3945/ajcn.117.153072

Sprecher H., Luthria, D.L., Mohammed, B.S. and Baykousheva, S.P. (1995): Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J Lipid Res 36:2471–2477.

Sprecher, H (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochemi.Biophysics.Act*a1486, 219-231

Stansby, M. E. (1973), Polyunsaturates and fats in fish flesh. *Journal of the American Dietetic Association, Chicago*, 63, 625-630, 1973.

Stubhaug Ingunn, Douglas R., Tocher, J., Gordon Bell, James R.Dick and Bente E.Torstensen (2007). Fatty acid metabolism in Atlantic salmon (Salmo salar L.) hepatocytes and influence of dietary vegetable oil. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. Volume 1734, Issue 3, Pages 277-288

Suzuki, H., Morikawa, Y. and Takahashi, H. (2001). Effect of DHA oil supplementation on intelligence and visual acuity in the elderly. *Fatty acids and lipids – New findings World Rev. Nutr Diet. Basel, Karger,*88: 68-71.

Suzuki, H., Park, S. J., Tamura, M. and Ando, S. (1998a). Effect of the long term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synapic membrane fluidity in adult mice: A comparison of sardine oil diet with palm oil diet: Mechanism of Ageing and Development. 101: 119-128

Suzuki, H. Takahashi, H. and Kataoka, H. (1998b). Effect of bread supplemented with DHA on the visual acuity in near-sighted patient (myopia). *3rd ISSFAL Congress. Abstract Book* p. 254.

Tacon A. G. J (2004). Use of fish meal and fish oil in aquaculture: a global perspective. *Aquat Resour Cult Dev* 1:3–14

Tacon, A. G. J., and M. Metian (2013): Fish matters: importance ofaquatic foods in human nutrition and global food supply. *Rev. Fisher. Sci*., 21:22–38

Taggart, J.B., Bron, J.E., Martin, S.A.M., Seear, P.J., Høyheim, B., Talbot, R., Carmichael, S.N., Villeneuve, L.A.N., Sweeney, G.E., Houlihan, D.F., Secombes, C.J., Tocher, D.R. and Teale, A.J. (2008). A description of the origins, designs and performance of the TRAITS-SGP Atlantic salmon Salmo salar L. cDNA microarray. *J. Fish Biol*. 72, 2071-2094.

Tanghe, S. and De Smet S. (2013). Does sow reproduction and piglet performance benefit from the addition of n-3 polyunsaturated fatty acids to the maternal diet? Vet J. 197:560–9

Tenyang, N., Ponka, R., Tiencheu, B., Djikeng, F. T. and Womeni H. M. (2016). Proximate Composition, Fatty Acid and Mineral Contents of Four Freshwater Fish from Maga Lake (Far North Region of Cameroon). *Am J Food Sci and Tech*. 4:64-69

Tocher D.R., J. Fonseca-Madrigal, J.G. Bell, J.R. Dick, R.J Henderson and Sargent, J. R. (2002). Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon *Salmo salar*, *Fish Physiology and Biochemistry* 26 157-170.

Tocher, D. R and Dick, J. R. (1999). Polyunsaturated fatty acid metabolism in a cell culture model of essential fatty acid deficiency in a freshwater fish, carp (Cyprinus carpio), *Fish Physiology and Biochemistry*, 21 (3), pp. 257-267

Tocher, D. R. and Dick J. R. (2000). Essential fatty acid deficiency in freshwater fish: the effects of linoleic, alpha-linolenic, gamma-linolenic and stearidonic acids on the metabolism of [1-14C]18:3n-3 in a carp cell culture model, *Fish Physiology and Biochemistry*, 22 (1), pp. 67-75.

Tocher, D. R. (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Fish Sci* 11, 107–184

Tocher, D. R., J. G. Bell, J. R. Dick, R. J. Henderson, F. McGee, D. Michell, and P. C. Morris. (2000). Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parrsmolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiol. Biochem*. 23:59–73.

Tocher, D.R., J.R. Sargent and G.N. Frerichs. (1988). The fatty acid compositions of established fish cell lines after long-term culture in mammalian sera. Fish Physiol. Biochem. 5: 219-227

Torcher, D. R., Agaba, M., Hastings, N., Bell, J. G., Dick, J.R. and Teale, A. J. (2002).Nutritional regulation of hepatocyte fatty acid desaturation andpolyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). *Fish Physiol Biochem* 24 (4):309-320.

Torrejon, C., Jung, U.J. and Deckelbaum, R. J. (2007) N-3 Fatty Acids and Cardiovascular Disease: Actions and Molecular Mechanisms. *Prostaglandins, leukotrienes, and essential fatty acids*.;77(5-6):319-326. doi: 10.1016/j.plefa.2007.10.014.

Torris, C., M. Molin, and M. S. Cvancarova (2016). Lean fish consumption is associated with lower risk of metabolic syndrome: A Norwegian cross sectional study, BMC PublicHealth,16: 347. doi:10.1186/s12889-016-3014-0

Torstensen, B. E., J.G. Bell, G. Rosenlund, R.J. Henderson, I.E. Graff and D.R. Tocher (2005). Tailoring of Atlanticsalmon *Salmo salar* L. flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend, *Journal of Agricultural Food Chemistry* 53 10166-10178.

Torstensen, B. E., Froyland, L. and Lie, O. (2004). Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (Salmo salar L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquacult Nutr 10, 175–192.

Toufique, K.A. and Belton, B. (2014) Is Aquaculture Pro-Poor? Empirical Evidence of Impacts on Fish Consumption in Bangladesh. World Development. 64:609–620. DOI: 10.1016/j.worlddev.2014.06.035. [Accessed: 26 October 2014].

Trattner, S. (2009). Quality of lipids in fish fed vegetable oils. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala

Turchini G.M., W.K. Ng and D.R. Tocher (2010). Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds, CRC Press, Boca Raton, FL, USA

Turchini, G.M., Torstensen, B.E. and Ng, W.K., (2009). Fish oil replacement in finfish nutrition. *Rev. Aquacult*. 1, 10–57.

Turner, E. C., Snaddon, J. L., Fayle, T.M. and Foster, W. A. (2008). Oil Palm Research in Context: Identifying the Need for Biodiversity Assessment. PLoS ONE 3(2): e1572. https://doi.org/10.1371/journal.pone.0001572

Van Weerd, J. H. V. (1995). Nutrition and growth in *Clarias species* - a review. *Aquatic Living Resources* 8: 395-401.

Vance, J. E. and Vance, D. E. (Eds) (2008). Biochemistry of Lipids, Lipoproteins and membranes, Fifth Ed. Elsevier

Visentainer, J. V., N. E. de Souza, M. Makoto, C. Hayashi, and M. R. B. Franco (2004). Influence of diets enriched with flaxseed oil on the α-linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (Oreochromis niloticus). *Food Chemistry*, 90: 557-560. doi: http://dx.doi.org/10.1016/j.foodchem..05.016., 2005.

Wang Chenchen, William S., Harris Mei, Chung Alice H., Lichtenstein Ethan M., Balk Bruce Kupelnick, Harmon S. Jordan and Joseph Lau (2006). N−3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *The American Journal of Clinical Nutrition*, Volume 84, Issue 1, Pages 5–17,

Watanabe, T. (1982). Lipid nutrition in fish. Comp Biochem Physiol B Biochem Mol Biol 273, 3–15.

Watanabe, T., Kiron V. and Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture* 151: 185-207.

Weaver, K. L., Ivester, P., Chilton, J. A., Wilson, M. D., Pandey, P., and Chilton, F. H. (2008). The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. *J. Am. Diet. Assoc*. 108: 1178-1185.

Whelan, J. and Rust, C. (2006). Innovative dietary sources of N-3 fatty acids. *Annual Review of Nutrition,* 26, 75e103.

White, P. (2008). Fatty acids in oilseeds (vegetable oils). in: Chow, C.K.(Ed.), Fatty acids in foods and their health implications. CRC Press, pp. 227–262.

World Health Organization (2003). Diet, Nutrition and the Prevention of Chronic Diseases, WHO Technical Report Series 916, Report of a Joint WHO/FAO Expert Consultation, p. 88

World Health Organization (2005). Avoiding heart attacks and strokes. World Health Organization, Geneva, Switzerland.

Wu, H.T., Chen, W. Cheng, K.C., Ku, P. M., Yeh, C. H. and Cheng, J. T. (2012). Oleic acid activates peroxisome proliferatoractivated receptor delta to compensate insulin resistance in steatotic cells. *The Journal of nutritional biochemistry*. 23(10):1264-70.

Yalcin, S., Solak, K. and Akyurt, I. (2002). Growth of the catfish Clarias gariepinus (Clariidae) in the River Asi (Orontes), Turkey. Cybium, 26(3), 163172.

Yamamoto, Y., Kabeya, N., Takeuchi, Y., Alimuddin, Haga, Y., Satoh, S., Takeuchi, T. and Yoshizaki, G. (2010). Cloning and nutritional regulation of polyunsaturated fatty acid desaturase and elongase of a marine teleost, the nibe croaker Nibea mitsukurii. *Fish. Sci*. 76, 463–472.

Zenebe, T., Ahlgren, G. and Boberg, M. (1998b). Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian Rift Valley. J Fish Biol. 53:987-1005.

Zenebe, T., Ahlgren, G., Gustafsson, I. B. and Boberg, M. (1998a) Fatty acid and lipid content of Oreochromis niloticus L. in Ethiopian lakes—dietary effects of phytoplankton. *Ecol Freshw Fish*. 7:146-58.

Zheng, X., Tocher, D. R., Dickson, C. A., Bell, J.G. and Teale, A. J. (2004),Effects of dietscontaining vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (*Salmo salar*). *Aquaculture* 236(1–4):467-483.

Zheng, X., Ding, Z., Xu, Y., Monroig, O., Morais, S. and Tocher, D. R. (2009a). Physiological roles of fatty acyl desaturase and elongase in marine fish: Characterisation of cDNAs of fatty acyl Δ6 desaturase and Elovl5 elongase of cobia (Rachycentron canadum). *Aquaculture* 290, 122-131.

Zheng, X., Leaver, M. J. and Tocher, D.R. (2009b). Regulation of fatty acyl desaturase (FAD) gene transcription: isolation and characterisation of Δ6 FAD gene promoters of Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.). *Comp. Biochem. Physiol*. 154B, 255-263.

Zheng, X., Seiliez, I., Hastings, N., Tocher, D.R., Panserat, S., Dickson, C.A., Bergot, P., Teale A. J. (2004a). Characterization and comparison of fatty acyl Δ6 desaturase cDNAs from freshwater and marine teleost fish species. *Comp. Biochem. Physiol*. 139B, 269-279.

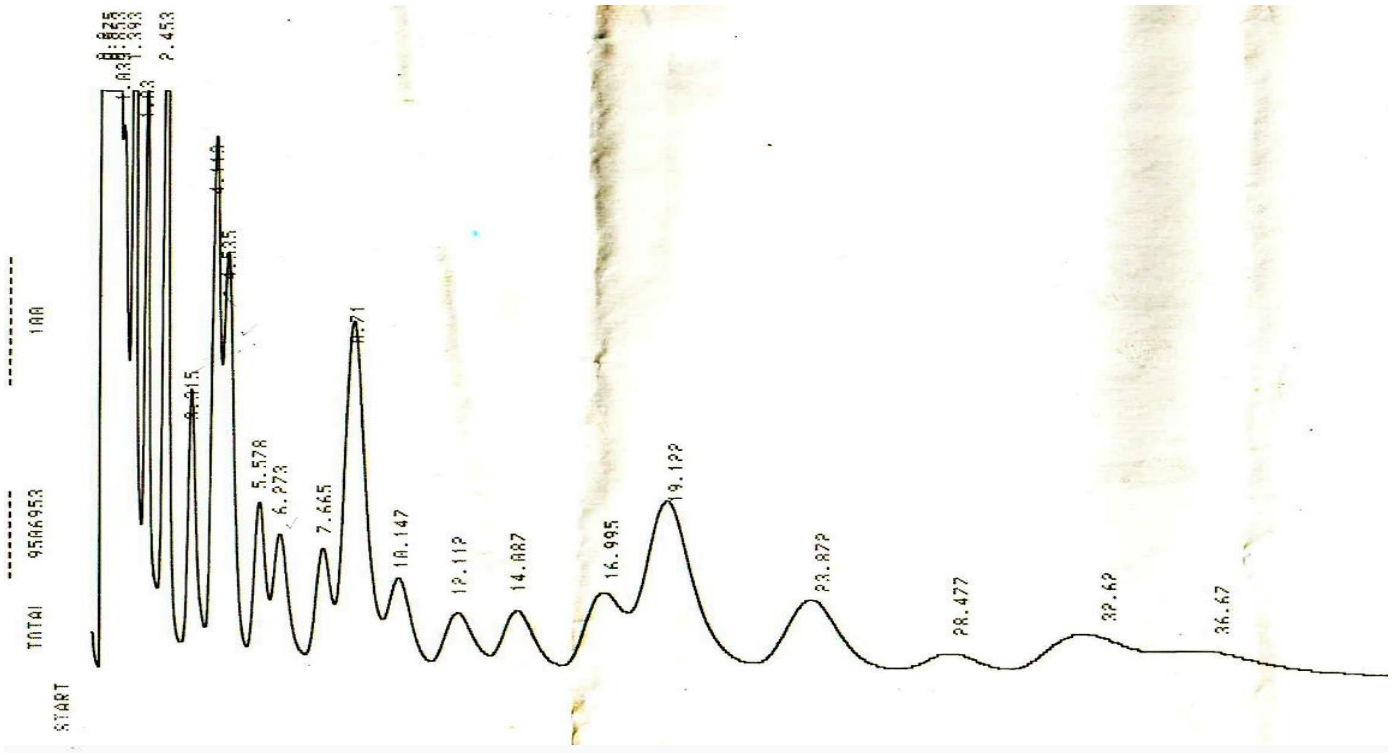
Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J. (2004b). Effects of diets containing vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (Salmo salar). *Aquaculture* 236, 467-483.

Zheng, X., Tocher, D.R., Dickson, C.A., Dick, J.R., Bell, J.G., Teale, A. J. (2005a). Highly unsaturated fatty acid synthesis in vertebrates: new insights with the cloning and characterisation of a Δ6 desaturase of Atlantic salmon. *Lipids* 40 13-24.

Zheng, X., Torstensen, B. E., Tocher, D.R., Dick, J.R., Henderson, R.J., Bell, J.G. (2005b). Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturases and elongase genes in liver of Atlantic salmon (Salmo salar). *Biochim. Biophys*. Acta 1734, 13–24.

Zuraini, A, Somchit, M. N., Solihah, M. H., Goh .M., Arifah, A. K., Zakaria, M. S., Somchit, N., Rajion, M. A., Zakaria, Z. A., Jais, A. M. (2006). Fatty acid and amino acid composition of three local Malaysian Channa spp. fish. *Food Chem.* 97:674-8.

APPENDIX 1: SAMPLE CHROMATOGRAPH




CHROMATOPAC C-8AA FILE 5
SAMPLE NO A METHOD 41
REPORT NO 10099

| PKNO | TIME | AREA | MK | TYNO | CONC | NAME |
|---|---|---|---|---|---|---|
| 1 | 0.3 | 11972888 | S | F | 92.3961 | |
| 2 | 0.575 | 17346 | T | | 0.1339 | |
| 3 | 0.853 | 43784 | T | | 0.3379 | |
| 4 | 1.033 | 4358 | T | | 0.0336 | |
| 5 | 1.393 | 56851 | T | | 0.4387 | |
| 6 | 1.83 | 33814 | T | | 0.2609 | |
| 7 | 2.453 | 63572 | T | | 0.4906 | |
| 8 | 3.315 | 32431 | T | | 0.2503 | |
| 9 | 4.113 | 69264 | TV | | 0.5345 | |
| 10 | 4.535 | 63967 | TV | | 0.4936 | |
| 11 | 5.578 | 26259 | TV | | 0.2026 | |
| 12 | 6.273 | 27004 | TV | | 0.2084 | |
| 13 | 7.665 | 25500 | T | | 0.1968 | |
| 14 | 8.71 | 113760 | TV | | 0.8779 | |
| 15 | 10.147 | 28040 | TV | | 0.2164 | |
| 16 | 12.112 | 20787 | T | | 0.1604 | |
| 17 | 14.087 | 25589 | TV | | 0.1975 | |