

**PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) FED ON
OILSEED MEALS WITH CRUDE PAPAIN ENZYME**

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**A Thesis Submitted to the Graduate School in Partial Fulfilment of the
Requirements for the Award of the Degree of Doctor of Philosophy in Animal
Science of Chuka University**

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DECLARATION AND RECOMMENDATION

Declaration

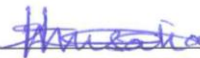
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DEDICATION

This work is dedicated to my loving parents Mr. Joseph Kirimi M'Nkanata and Mrs. Lydia Ntue Kirimi for their prayers, support and encouragement and to my brothers and sisters.

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ABSTRACT

The greatest challenge to increased aquaculture production in Kenya is the high cost of fish feed. The objective of this study was to determine the effect of replacing fish meal with oilseed meals and improving the availability of nutrients using crude papain enzyme in Nile tilapia diets. A control diet (D1) of 30% crude protein and 2900Kcal DE/kg was formulated using fishmeal (FM), soybean meal (SBM), canola meal (CM) and sunflower meal (SFM). The test diets were formulated by replacing 10% CP of FM by SBM (D2), CM (D3) and SFM (D4), respectively. The diets were analysed for proximate, fatty acid and amino acid composition. The level of inclusion of crude papain enzyme was tested at 0.02%, 0.04%, 0.06% and 0.08% by the *in vitro* enzyme assay using pH drop method. One hundred and one days feeding trial was conducted in a 4x2 factorial design on 4 diets (D1, D2, D3 & D4) with (0.06%) and without (0%) the enzyme using seven hundred and twenty Nile tilapia fingerlings (7±3g). The fingerlings were randomly distributed into eight groups of three replicates of 30 fingerlings per net hapa (2x1x1m). Fish were fed twice daily at 5% of their biomass at 10am and 4pm in two equal meals. They were weighed fortnightly and slaughtered at the end of feeding trial for carcass quality analysis and sensory evaluation. Apparent nutrient digestibility was done at the end of feeding trial using chromic oxide as an external marker. Substituting FM with SBM, CM and SFM significantly increased the levels of crude fibre (P<0.05) but reduced ash level. The diet based on SFM recorded highest (16.03%) crude fibre content (P<0.05), while CM based diet recorded highest ether extract content (10.75%). Substituting 10% CP of FM with SBM, CM and SFM, reduced the levels of isoleucine, leucine, arginine, lysine, phenylalanine, histidine and threonine (P<0.05) and increased levels of methionine in diet with SBM. In all the diets, methionine was the 1st limiting amino acid and isoleucine 2nd limiting. Though the diet containing FM exhibited higher (P<0.05) essential amino acid index (EAAI) (0.97), it was not satisfactory because it was limiting in methionine. EAAI reduced (P<0.05) with replacement of fishmeal; SBM (0.78), CM (0.77) and SFM (0.76). Crude papain extract contained (crude protein 66.61%, ash 6.89%, crude fat 7.69%, crude fiber 1.56%, dry matter 93.55% and nitrogen free extract 16.98%). Addition of crude papain at 0.06% had highest (P<0.05) protein digestibility (39.16%). Apparent Digestibility Coefficients (ADC's) increased (P<0.05) for all the nutrients tested upon crude papain enzyme supplementation. Growth performance showed that there was no significant different (P>0.05) on final body weight (47.32g) on 0.06% enzyme and (46.17g) on 0% papain enzyme. However, fish fed FM based diet were larger (56.89g) (P<0.05) than those fed SBM (45.59g) CM (43.89g) and SFM (40.59g). The profit index was highest (2.41) in 0% enzyme and lower in 0.06% enzyme (2.19) (P<0.05). Although 10% CP replacement of FM with SBM, CM and SFM was associated with reduced growth, the economic returns were higher. Final carcass proximate composition increased on enzyme supplementation. Crude papain enzyme supplementation led to increase in saturated and mono saturated fatty acid and decrease in polyunsaturated fatty acid. The overall acceptability of fish decreased (3.87) with 0.06% enzyme. The present results indicates that enzyme supplementation led to increased digestibility and growth of fish but carcass fatty acid, sensory attributes and profit index reduced. Based on this, more research is needed on crude papain enzyme supplementation in Nile tilapia diets.

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ABBREVIATIONS AND ACRONYMS

AA	Amino Acids
ABDP	Aquaculture Business Development Programme
ADC	Apparent Digestibility Coefficient
AND	Apparent Nutrient Digestibility
ADF	Acid Detergent Fibre
ANF	Ant nutritional Factors
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CF	Crude Fibre
CM	Canola Meal
CP	Crude Protein
Cr₂O₃	Chromic Oxide
DE	Digestible Energy
DHA	Decosahexaenoic Acid
DM	Dry Matter
DWG	Daily Weight Gain
EAA	Essential Amino Acid
EAAI	Essential Amino Acid Index
EE	Ether Extracts
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
ERS	Economic Recovery Strategy
ESP	Economic Stimulus Programme
FA	Fatty Acid
FAO	Food and Agricultural Organization of the United Nations
FE	Feed Efficiency
FFEPP	Fish Farming Enterprise Productivity Programme
FM	Fish Meal
GDP	Gross Domestic Product
GE	Gross Energy
GoK	Government of Kenya
HUFA	Highly Unsaturated Fatty Acids

IC	Incidence Cost
IFAD	International Fund for Agricultural Development
LSD	Least Significance Difference
ME	Metabolizable Energy
MMT	Million Metric Tonnes
MUFA	Mono Unsaturated Fatty Acid
NaOH	Sodium Hydroxide
NDF	Neutral Detergent Fibre
NFE	Nitrogen Free Extracts
NI	Nutritional Index
NIR	Near-Infrared Spectroscopy
NRC	National Research Council
NRF	National Research Fund
NSP	Non Starch Polysaccharide
PI	Profit Index
PL	Phospholipids
PRS	Poverty Reduction Strategy
PUFA	Polyunsaturated Fatty Acid
RGR	Relative Growth Rate
RPD	Relative Protein Digestibility
SBM	Soybean Meal
SFA	Saturated Fatty Acid
SFC	Sunflower Cake
SGR	Specific Growth Rate
SR	Survival Rate
TAG	Triacylglycerols
WG	Weight Gain

CHAPTER ONE INTRODUCTION

1.1 Background Information

The aquaculture industry has developed significantly over recent decades and is one of the fastest growing food production sectors in the world compared to other food commodities with an annual increase of approximately 12% (FAO, 2009). Globally, fish provides more than 1.5 billion people with almost 20 percent of their average per capita intake of animal protein, and 3 billion people with 1% of such protein (FAO, 2011) and it is remarkable that one out of every three fish consumed in the world is now farm raised (Gatlin *et al.*, 2007). Farmed food fish contributed a record 42.2% of the total 158 million tonnes of fish produced by capture fisheries (including for non-food uses) and aquaculture in 2012 (FAO, 2014). This compares with just 13.4% in 1990 and 25.7% in 2000 (FAO, 2014). Increasing production capacity of aquacultural resources through intensification seems to be the way forward to meet the ever increasing demand for fish.

Aquaculture development in Africa is insignificant compared to the rest of the world (Changadeya *et al.*, 2003). The Sub-Saharan Africa region continues to be a minor player in aquaculture despite its natural potential. Even aquaculture of tilapia, which is native to the continent, has not developed significantly. Nigeria leads in the region, with reported production of 44 000 tonnes of catfish, tilapia and other freshwater fishes (FAO, 2006). However, there is considerable potential for aquaculture expansion in Africa to improve food security (Kapetsky, 1994; Engle, 1997). Although there is room for enhancing aquaculture production in Africa through improvements in the overall production system, genetics and general farm management principles, the desired growth of aquaculture which is necessary in order to meet the increasing demand for fish is only achievable through cost effective and high quality fish feed (Gabriel *et al.*, 2007).

In Kenya, aquaculture is the fastest growing subsector in the country due to the introduction of Fish Farming Enterprise Productivity Programme (FFEPP) in mid-2009 that targeted the improvement of fish farming in the country (GoK, 2010). Also through the Aquaculture Business Development Programme (ABDP) the GoK under funding from IFAD is supporting smallholder aquaculture fish production to

accelerate and consolidate the expansion of aquaculture production and trade within the country by realizing the productive potential of smallholders. However, despite high adoption of aquaculture in many regions of the country, feed remains the highest challenge due to high cost (Kirimi *et al.*, 2016a). Thus, sustainability of the increased aquaculture production must be supported by a corresponding increase in the production of designed diets for the cultured aquatic animals (Rahman *et al.*, 2013). According to Gabriel *et al.*, (2007), development and management of fish feed, plays a vital role in aquaculture growth and expansion and is a major factor that determines the profitability of aquaculture venture. Nutrition is the most expensive component in aquaculture, particularly under intensive culture, where it accounts for over 50% of operating costs (El-Sayed, 2004).

Considering other livestock, aquaculture is reported to be the single largest user of fishmeal, using in excess of 53 % of global supply (Tacon, 2004; Tacon *et al.*, 2006). Pigs and poultry account for around a quarter of total usage, while other livestock types account for the remainder. According to Jacquet *et al.*, (2010), up to 36% of the world's total fisheries caught each year are ground up into fishmeal and oil to feed farmed fish, chicken and pigs. Tacon (1993) and Glencross *et al.*, (2007) indicated that fish meal is used in aquafeeds for both carnivorous and omnivorous species at levels in excess of 50% particularly in carnivorous species and being too reliant on one ingredient is risky. Fishmeal and fish oil are important feed ingredients in aquaculture, and by 2003 their consumption by the sector had increased to 2.94 million and 0.80 million tonnes, representing 53.2% and 86.8 % of global production, respectively (Tacon *et al.*, 2006). Fish meal is widely sought and considered the most desirable animal protein ingredient in aquaculture feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic fatty acids, energy and minerals (Watanabe *et al.*, 1997; Hardy & Tacon, 2002). Tacon (1993) and Glencross *et al.*, (2007) recommended that as a strategy to reduce the risk of over reliance on fish meal, the identification, development and use of alternatives to fish meal and oil remain a high priority. Ogello *et al.*, (2014) also mentioned that the fast declining status of world fisheries and corresponding increase in aquaculture production exposes great debate on whether it is sustainable to feed fish on fish meal.

According to Olukayode and Emmanuel (2012), the development of sustainable aquaculture depends on the establishment of alternative feedstuffs to fish meal. Various plant feedstuffs are increasingly being considered for use in fish diets to reduce the dependence on fishmeal and other animal protein feedstuffs, and thereby reduce feed costs in aquaculture due to their high availability, low prices and suitable nutritional value. Despite the fact that most plant ingredients are readily available at lower cost than fishmeal, their use within aqua feeds is usually restricted by a relatively low protein content, unbalanced essential amino acid profile, high levels of fibre and starch and presence of one or more ant nutritional factors (NRC, 1993). Medale and Kaushik (2009) noted that inclusion of various protein sources in aquafeeds introduces the notion of limiting essential amino acids. Thus, protein quality of fish meal cannot be obtained from a single plant feed resource. According to Drew *et al.*, (2007), fish meal is the “gold standard” to which plant proteins must be compared in terms of protein quality, fish growth performance, health and cost.

Soybean meal (SBM) is one of the most nutritious of all plant protein due to its high protein content, high digestibility and relatively well balanced amino acid profile, and is widely used as a feed ingredient for many aquaculture species (Lovell, 1988; Storebakken *et al.*, 2000). However, according to FAO (1983) and NRC (1993), amino acids such as methionine and cystine are generally considered to be most limiting in soybean products compared to the quantitative amino acid requirements of most fish species. Soya bean meal is also expensive on the local market. Soybean meal contains proteinase inhibitors which reduce the availability of amino acids (Liu, 1997). Defatted canola (rapeseed) meal has been considered as a potential source of food grade proteins because of its well-balanced amino acid composition, however, like many plant protein sources, canola meal is limiting in lysine but noted for having high levels of methionine and cystine (Bell *et al.*, 1999) that can complement the low levels in SBM. Canola meal is known to contain some anti-nutritive factors like proteinase inhibitors, glucosinolates, phytic acid and tannins (Francis *et al.*, 2001).

Sun flower (*Helianthus annuus*) is a widely cultivated oil seed and its cake/ meal is the residue after oil is extracted from seeds (Dayal *et al.*, 2011). It is a widely used protein source in livestock, poultry and pig rations. Sunflower seed meal is lower in

lysine than soybean meal, but higher in methionine (Senkoylu & Dale, 1999). Silva (1990) reported that sunflower meal can be used in diets in complement with other lysine rich feed sources, but the high level of fibre in sunflower meal contributes to a reduction in the availability of energy of the diets.

The problem of imbalanced amino acid profile in plant based ingredients could be overcome by mixing different ingredients to obtain desired essential amino acid profile in the compounded feed. More so, supplementation of diets with enzymes can help eliminate the effects of ant nutritional factors and improve the availability of dietary energy and amino acids resulting in improved performance of fish (Farhengi & Carter, 2007; Lin *et al.*, 2007; Soltan, 2009; Yildirim & Turan, 2010). Fish being monogastric, cannot efficiently utilize fibre rich ingredients (Maity *et al.*, 2011).

Compounded feeds are nutrient dense, made using blends of various raw materials and additives, formulated according to the nutritional requirements of the species and finally processed (e.g. pelleted, extruded or crumbled). According to Watanabe, (2002), to develop a feed for sustainable fish production, the evaluation of proximate, amino acid composition, digestibility and performance efficiency as well as cost implications and conditions of application is necessary. The aim of this study therefore was to incorporate papain enzyme in a complete and balanced oilseed meals protein based diet in order to improve on the availability of nutrients. This was to improve the performance of Nile tilapia and increase fish production in the country.

1.2 Statement of the Problem

Formulated aquaculture diets are among the most expensive animal feeds on the market in Kenya today. The high cost is due to high level of protein mainly in form of fish meal and fish oil which is scarce and expensive. The scarcity of fish meal is due to increased consumption by humans due to change in eating habits and need for balanced food. There is also more demand for fish meal because of the expanding aquaculture industry, drop in the wild fish catches and in formulating other livestock feed especially in pig and poultry industry. Despite the high cost of FM, its use in the ration has been inevitable because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic

fatty acids, energy and minerals. Since most farmers cannot afford this expensive fish feed, they are opting for non-conventional feed resources which are not meeting the nutritional requirements of fish in terms of essential amino acids and available amino acids. Soybean meal, canola meal and sunflower cake which are readily and locally available can serve as alternative plant protein sources. However, low protein content, unbalanced essential amino acid profile and low availability of nutrients in plant based protein diets is a major limitation. This study therefore aimed to investigate the use of different oilseed meal based protein sources to balance the essential amino acid (EAA) profile and improve nutrients availability by use of papain enzymes in the ration of Nile tilapia.

1.3 Objectives

This study was expected to achieve the following objectives

1.3.1 Overall Objective

To determine the effect of papain enzyme supplementation on oilseed meal based diets as a replacement of FM in the diet of Nile tilapia

1.3.2 Specific Objectives

- i. To determine the effect of replacing fish meal with soybean meal, canola meal and sunflower meal on nutrient composition of diets for Nile tilapia
- ii. To evaluate the protein quality of rations for Nile tilapia containing soybean meal, canola meal and sunflower meal as replacement of fishmeal
- iii. To determine the optimum inclusion level of crude papain in plant protein diets for Nile tilapia
- iv. To determine the nutrient digestibility of oilseed meal diets supplemented with crude papain enzyme fed to Nile tilapia
- v. To determine the effect of papain enzyme supplementation on growth and economic performance of Nile tilapia fed diets containing soybean meal, sunflower cake and canola meal as a replacement of FM.
- vi. To determine the effect of papain enzyme supplementation on the carcass composition and sensory properties of Nile tilapia fed diets containing soybean meal, sunflower cake and canola meal as a replacement of FM.

1.4 Hypotheses

Based on the above specific objectives, this study tested the following hypotheses:

- H₀₁: There is no significant difference of replacing FM with soybean meal, sunflower cake and canola meal, on the nutrient composition of diets for Nile tilapia
- H₀₂: There is no significant difference in protein quality of rations for Nile tilapia containing soybean meal, canola meal and sunflower meal as replacement of fishmeal
- H₀₃: There is no significant difference on the inclusion levels of crude papain on plant protein based diets for Nile tilapia *In vitro*
- H₀₄: There is no significant difference of crude papain enzyme supplementation on growth and economic performance of Nile tilapia fed diets containing soybean meal, sunflower cake and canola meal as a replacement of FM.
- H₀₅: There is no significance difference in the *In vivo* nutrient digestibility of oilseed meal based diets supplemented with crude papain enzyme fed to Nile tilapia
- H₀₆: There is no significant difference of papain enzyme supplementation on the carcass composition and sensory properties of Nile tilapia fed diets containing soybean meal, sunflower cake and canola meal as a replacement of FM.

1.5 Justification of the Study

Africa epitomises recurring famines, acute food shortages, chronically low dietary intakes and food insecurity (The African, 2009) whereas fish and fishery products play a critical role in global food security and nutritional needs of people in developing and developed countries (FAO, 2014). Fish is a major source of high quality dietary protein, essential vitamins, minerals, and other micronutrients required in the human ration. In October 2014, Kenya crossed the threshold to a low middle income country, becoming one of the largest economies in sub Saharan Africa. However, challenges of poverty and income inequalities remain, with food security staying a major concern for the Government of Kenya (IFAD, 2014). According to the global hunger index, notwithstanding slight improvements in the hunger situation, Kenya remains a food insecure country. It is estimated that about 10 million Kenyans suffer from chronic food insecurity and poor nutrition (IFAD, 2014). The agricultural

sector is the backbone of Kenya's economy and the means of livelihood for most of the rural population. In this sector, fisheries industry plays an important role in the economy by providing employment and income to over 2.3 million people (GoK, 2010, Aloo & Margret, 2010). Although the contribution of fisheries sector to the Gross Domestic Product (GDP) is estimated at only 0.5 %, this could be as high as 5% if all the inputs to the fisheries production and value addition are included (Aloo & Margret, 2010) and in the coming years, aquaculture will most likely make a significant contribution to both food security and foreign exchange earnings in Kenya (FAO, 2012). Demand for fish is rising owing to the growing population and the changing feeding habits among Kenyans as they move towards healthy living (GoK, 2010). Since the economic stimulus programme begun in mid-2009 and aquaculture identified as key sub sector to address the problem of food insecurity in Kenya, there has been tremendous growth of this sub sector but one of the most pressing challenge is lack of quality and cost effective fish feeds (Charo-karisa & Gichuri, 2010). However, proper choice of feed ingredients in formulation of fish feed can be a way out of this predicament and plant based protein sources offer the best alternatives to fish meal. Therefore formulation of fish feed with combination of soybean meal, canola meal and sun flower cake as the main protein source to have a balanced ration and supplementation with papain enzyme to increase nutrient availability will highly increase fish yield and contribute significantly to the livelihood of the farmers.

CHAPTER TWO LITERATURE REVIEW

2.1 World Overview of Aquaculture

Aquaculture is the farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production and the individual or corporate ownership of the stock being cultivated (FAO, 2009). Global fish production has grown steadily in the last five decades with food fish supply increasing at an average annual rate of 3.2 percent, outpacing world population growth at 1.6 percent (FAO, 2014). According to FAO (2013), world fisheries and aquaculture production reached 157 million tons in 2012 and is projected to reach about 172 million tons in 2021, with most of the growth coming from aquaculture. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2014). This impressive development has been driven by a combination of population growth, rising incomes and urbanization, and facilitated by the strong expansion of fish production and more efficient distribution channels (FAO, 2014). In this regard, growth in world aquaculture is being driven primarily by population growth, which is predicted to reach about 8 billion by 2030 (Tacon, 2003).

Historically, the oceans were considered limitless and thought to harbour enough fish to feed an ever-increasing human population. The increasing population will place enormous demands on not only the remaining capture fisheries, but also on aquaculture. Therefore in order to breach the gap between demand and supply, aquaculture is seen as the best solution (Agbo, 2008). However, the demands of a growing population, particularly in poorer countries, now far outstrip the sustainable yield of the seas (Tidwell & Allan, 2001). In Sub-Saharan Africa, aquaculture was introduced in the 1950s with main objectives of improved nutrition in rural areas, generation of additional income, diversification of activities to reduce risk of crop failures and the creation of employment in rural areas (Hecht, 2006). About 43% of the African continent is assessed as having the potential for farming tilapia, African catfish and carp. Aquaculture is likely to grow over the next 20 years and the rising demand for FM and fish oil could place heavier fishing pressure on the already threatened stocks of wild fish (Aladetohun & Sogbesan, 2013).

2.2 Fish Farming in Kenyan Economy

Kenya's economy is considered one of Africa's most developed (Rothuis *et al.*, 2011). The agricultural sector is the backbone of Kenya's economy and the means of livelihood for most of our rural population. Sustained agricultural growth is critical to uplifting the living standards of Kenyans as well as generating rapid economic growth. Agriculture sector contributes directly by 26 per cent of the GDP annually and another 25 percent indirectly to the Kenyan economy. The sector accounts for 65 percent of Kenya's total exports and provides more than 70 per cent of informal employment in the rural areas (GoK, 2010). Therefore, the agricultural sector is not only the driver of Kenya's economy but also the means of livelihood for the majority of Kenyan people. The agricultural sector comprises the following subsectors: crops, livestock, fisheries, land, water, cooperatives, environment, regional development and forestry (GoK, 2010).

The fishery resources of Kenya contribute to the national economy through foreign exchange earnings, employment generation, food security support and rural development of Kenya's 2014 estimated population of 44.9 million. The fisheries sector provides employment to 2 million and livelihood for at least 2.3 million people. The sector also brings in valuable foreign exchange to the government, earning some 0.5% of the Gross Domestic Product per annum (FAO, 2015). Aquaculture has great potential in Kenya given its numerous aquatic resources. The country has over 1.14 million hectare potential area suitable for fish farming with capacity to produce over 11 million metric tonnes of fish worth 750 billion Kenya shillings (FAO, 2015).

2.3 Aquaculture Development in Kenya

Fish farming in Kenya began in 1920 initially using tilapia species and later including the common carp and the African catfish. In 1960, the government helped increase the popularity of aquaculture through the "eat more fish" health promotion campaign (Ngugi *et al.*, 2007; Mbugua, 2008). As a result, tilapia fish farming expanded rapidly with the construction of many small ponds. However, the initiative failed in the 1970's due to inadequate fish farming services, lack of quality fingerlings and insufficient training of fish farming workers. Since then, aquaculture in Kenya has taken many different forms, ranging from the small hand-dug 'kitchen ponds', to large

earth ponds of 1000 m² (Ngugi *et al.*, 2007; Mbugua, 2008). Dams and other impoundments used for storing water are often stocked with fish and the most common species farmed are tilapia, catfish, trout and gold fish. Until the mid-1990s, the activity followed a pattern similar to that observed in many African countries, characterized by small ponds, subsistence-level management, and very low levels of production (Ngugi *et al.*, 2007; Mwangi, 2008). By the 1990's there emerged small-scale fish farming (aquaculture) at different levels in Kenya for subsistence (Gitonga & Achoki, 2003).

According to Mbugua, (2008), the government recognized the constraints hindering aquaculture growth and development and realized that the sub sector can play an important role in poverty alleviation of rural populations. It could also play a key role in provision of protein food and reduction of fishing pressure in capture fisheries. Therefore during the preparation of the poverty reduction strategy paper, aquaculture development was identified as a core activity for funding through the medium term expenditure frame work budgeting system. Following this development in addition to the reorganization of the government function, aquaculture has been prioritized and is now one of the core functions of the department of fisheries. Given the fisheries potential, GoK has taken a keen interest and given aquaculture and fisheries in general the priority it deserves (Mbugua, 2008).

The Government of Kenya in its governance role and commitment to the policy of sustainable development as demonstrated in the Poverty Reduction Strategies (PRS), the Economic Recovery Strategy (ERS) for wealth and employment creation, as well as the social pillar of the Kenya Vision 2030 and the Economic Stimulus Programme. In particular, the intersectoral Economic Stimulus Programme (ESP) was launched to address food insecurity and mitigate the effects of the 2007 post-elections violence and the global economic and financial crisis. The ESP was introduced through the 2009/2010 budget, entitled 'Overcoming Today's Challenges for a Better Kenya Tomorrow' (GoK, 2009). Among the numerous intersectoral programmes introduced was the fisheries development programme. The programme consisted of construction of fish ponds in 140 constituencies in Kenya and stocking them with fingerlings. The main aim of introducing fish farming was to contribute towards reducing food

insecurity in the country, improve nutrition, create over 120000 employment and income opportunities (GoK, 2009; Nyonje *et al.*, 2011).

Aquaculture in Kenya can be categorized into three broad divisions; warm fresh water aquaculture dominated by production of various species of tilapia and African cat fish (*Clarius gariepinus*) mainly under semi intensive system using earthen pond; cold fresh water aquaculture involving production of rainbow trout (*Oncorhynchus mykiss*) under intensive system using raceways and tanks; marine water aquaculture (mariculture) which is under developed (Mbugua, 2008)

According to (GoK, 2010), aquaculture is the only sustainable source of fish and has great potential for growth in Kenya due mainly to the presence of a wide variety of water sources such as rivers, springs, dams, lakes and the Indian Ocean. In addition, most of the land that is suitable for other agricultural activities is also suitable for aquaculture as are swampy and marshy areas, which are unsuitable for crop production. Aquaculture can also be integrated with other production activities such as rice farming, poultry and dairy production to increase production efficiency per unit area (GoK, 2010).

2.4 Nile Tilapia in Aquaculture

Tilapia is the third most important cultured fish group in the world after carps and salmonids. Tilapia culture is also one of the fastest growing farming activities with an average annual growth rate of 13.4% during 1970 to 2002. They are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of the total farmed finfish in 2002 (FAO, 2004). The Nile tilapia, *Oreochromis niloticus* (L.), which was introduced in Lake Victoria in 1950s, is the third commercially important fish after introduced Nile Perch, *Lates niloticus* (L.) and endemic pelagic cyprinid *Rastrineobola argentea* and is one of the commercially important species in Kenya and many other tropical and subtropical countries. This is due to its fast growth, resistance to diseases and ability to feed on the lowest trophic level (Pullin, 1988).

However, tilapia is a generic name that is applied to several genera i.e. *Sarotherodon* and *Oreochromis*. Tilapia farming involves the culture of the following species, *Oreochromis niloticus* (Nile tilapia), *O. mossambica*, *O. aureus*, *O. spirulus*, *O. andersonii*, *Tilapia zilli*, *Tilapia rendalli*. The Nile tilapia (*Oreochromis niloticus*) was one of the first fish species cultured. Tilapia have been raised as food for human consumption for a long time; Illustrations from Egyptian tombs suggest that Nile tilapia was cultured more than 3000 years ago (Thomas & Michael, 1999; Agbo 2008). According to Pompa and Masser (1999) tilapia are referred to as “Saint Peter’s fish” in reference to biblical passages about the fish fed to the multitudes. Tilapias are natives of Africa and have been introduced and produced widely around the world. They are primarily fresh water fishes, very tolerant of low water quality and can survive with low dissolved oxygen. They are hardy, grow well under crowded condition, resist diseases, have higher fecundity than most fishes and reproduces freely in ponds. The remarkable success of tilapias as a farmed fish can be attributed mainly to the following factors; they have desirable qualities as a food fish such as white flesh, neutral taste and firm texture, which has made them gain acceptance in a wide variety of human cultures with differing tastes and food preferences (Shiau, 2002).

2.4.1 Nutritional Requirements of Tilapia

Tilapias are very good aquaculture species partially because they are Omnivorous meaning that they feed on a low trophic level. They are able to produce high quality protein from less refined protein sources thus making them ecologically attractive as sources of animal protein for humans (Jauncey, 1998; Agbo, 2008). The genus *Oreochromis* generally feed on algae, aquatic plants, small invertebrates, detrital material and associated bacterial films. Individual species may have preferences between these materials (Popma & Masser, 1999). *Oreochromis* can utilize any and all of the above feeds when they are available and therefore are considered as opportunistic. This provides an advantage to farmers because the fish can be reared in extensive situations that depend upon the natural productivity of a water body or in intensive systems that can be operated with lower cost feeds (Fitzsimmons, 1997). The best growth performance of tilapia is exhibited when they are fed a balanced diet that provides a proper balance of protein, carbohydrates, lipids, vitamins, minerals

and fibre. Nutritional requirements of fish differ for different species and more importantly vary with life stage. According to Fitzsimmons (1997) fry and fingerlings require diets with higher protein, lipids, vitamins and minerals and lower carbohydrates as they are developing muscle, internal organs and bones with rapid growth. From various studies the protein requirements of juvenile tilapia have been reported to range between 30-56% (Jauncey, 1998; Suresh, 2003). The protein requirements of fish decrease with age and optimum dietary protein requirements for tilapia can be broadly generalised as shown in Table 1.

Table 1: Nile Tilapia (*Oreochromis niloticus*) Dietary Requirements According to Size

Fish size	Fry(<10g)	Fingerling(10-30g)	Grow out (>30g)	>300g	Breeding
Crude protein	40-50	28-35	25-30	20-25	>40
Crude lipid	6-13	6-13	4-12	4-12	>6
Crude fibre	<4	<8	<8	<8	<10
Ash	<16	<16	<16	<16	<16
Carbohydrate	>25	>25	>25	>25	>25
Moisture	<10	<10	<10	<10	<10

Source: (FAO, 2015)

Feeding rate (allowance) in practical feeding of fish involves two options. One is to feed the fish to satiation and the other is to feed a restricted ration (Suresh, 2003). Best growth is normally achieved by feeding to satiation. But satiation levels are not necessarily the most economic feeding levels, because food conversion at satiation levels is often poor. Also, it is difficult to determine satiation levels in fish because food consumption occurs in the water medium. This may lead to overfeeding, which is wasteful and deleterious to water quality. As a result, restricted rations are recommended for feeding fish (Suresh, 2003; Agbo 2008). It is also common practice to feed to satiety before determining the rate of feeding.

2.5 Protein and Amino Acids in Fish Nutrition

Fish do not require protein, but amino acids (Wilson, 2002) and it is known that there is no requirement for protein in the diet, *per se*, but actually a requirement for the amino acids found in protein (NRC, 1994). According to McGill (2009), the importance of amino acids as opposed to crude protein in nutrition and feeding of

animals has been recognized and investigated since the early twentieth century, as well as the effects of reduced levels of specific essential amino acids on performance. Fish and most monogastric animals require the same ten essential amino acids (EAA) (Lovell, 1988). Numerous studies have indicated that a number of factors, including: size and age of fish, protein quality, non-protein energy levels, water temperature, salinity, feed allowance, management practices, and amount of natural food in a culture system affect the dietary protein requirements of fin fishes (NRC, 1993; Lim & Webster, 2006; Robinson & Li, 2007).

Protein quality of dietary protein sources depends on the amino acid composition and their availability and limiting amino acid of a protein or whole feed can be defined as the essential amino acid found in the smallest quantity relative to its requirement (Bender, 2005). Fish appear to have a high requirement for dietary protein, about twice of that of other vertebrates. However, the efficiency with which feed is converted into growth is about three-fold higher in fish than in other vertebrates (Bowen, 1987). This is possible through three strategies: i) as fish are poikilothermic, they do not expend energy towards maintaining elevated body temperatures (Medale & Kaushik, 2009); ii) as fish are neutrally buoyant in water, energy requirements to maintain posture and locomotion are greatly reduced; and lastly iii) fish excrete nitrogen mainly through a minimal-cost process, i.e. passive diffusion of ammonia across the gills driven by a gradient between the blood and the surrounding water. In comparison, mammals and birds excrete urea and uric acid, respectively, after energy-expensive syntheses.

Table 2: Essential Amino Acid Requirement of *Oreochromis niloticus* as a Percentage of Dry Diet and Dietary Protein

Amino acid	Requirement as a % of dry diet	Requirement as a % of dietary protein
Lysine	1.43	5.12
Argenine	1.18	4.2
Histidine	0.48	1.72
Threonine	1.05	3.75
Valine	0.78	2.8
Leucine	0.95	3.39
Isoleucine	0.87	3.11
Phenylalanine + tyrosine	1.05	3.75
Tryptophan	0.28	1.00
Methionine + cystine	0.75	2.68

Source: (NRC, 1993)

Nutrient requirements are often defined as the minimum dietary concentration required for maximum performance, and nutritionists frequently provide a margin of safety above the requirement when formulating to avoid deficiencies and the resultant decreases in performance (Sterling *et al.*, 2005). However, it is important to keep in mind that if the diet does not provide nonessential amino acids or an additional source of nitrogen, the nonessentials must be synthesized from other amino acids including those that are essential. Therefore, in order to prevent deficiencies in either essential or nonessential amino acids, it is common to provide an overall protein requirement as well as requirements for the essential amino acids when formulating rations (NRC, 1994; McGill, 2009).

Deficiency of an essential amino acid leads to poor utilization of the dietary protein and consequently reduces growth and decreases feed efficiency (Lee, 2001; Sun *et al.*, 2007, Kirimi *et al.*, 2016a). A protein deficiency, caused by either one or more limiting amino acids or an overall inadequate consumption of protein, will result in decreases in parameters such as rate of growth, nitrogen retention, feed consumption and feed utilization (Church, 1991), while an over-consumption of protein results in the catabolism of amino acids through deamination and excretion as uric acid which is both energetically and economically inefficient (Sklan & Plavnik, 2002), or, in severe cases, ammonia toxicity (Perry *et al.*, 2004). Studies by Yamamoto *et al.*, (2004) and

Kirimi *et al.*, (2016a) indicate that an imbalance in the amino acids profile of the diet depresses feed intake and growth of fish.

According to Kaushik and Seiliez (2010), formulating cost-effective feeds meeting the essential amino acid (EAA) requirements of fish and shrimp can be a challenge and will depend on relevant data on both EAA requirements of the fish species and the EAA supplied with the feed. Meeting the nutritional requirements for fish constitutes a large percentage of the cost of production. The maintenance requirement of EAA may account for a greater proportion of total requirement (maintenance + growth) because amino acids can be involved in a wide variety of other metabolic reactions beside protein synthesis and are subjected to significant endogenous losses (Rodehutsord *et al.*, 1997). Amino acids are also required as precursors for various metabolites, neurotransmitters, hormones and cofactors (NRC, 2011). Therefore, optimization of amino acid balance in aqua feeds is essential if optimal fish growth and production efficiencies are to be realized, particularly when feeding non-fish meal based diets (Gatlin *et al.*, 2007, Kirimi *et al.*, 2016b).

2.6 Lipids and Fatty Acid Nutrition

Lipids are, along with proteins, the major organic components of fish and in most cases of their feeds, also; carbohydrates are, at least quantitatively, less important. Lipids can be classified into several groups or “lipid classes”, with distinctive properties, functions and roles. The main lipid classes in fish tissues and their feeds are triacylglycerols (TAG), phosphoglycerides or phospholipids (PL), sphingolipids, sterols (mainly cholesterol) and wax esters (Sargent, *et al.*, 2002). TAG are a major class of the neutral lipids and consist of three fatty acids esterified in the *sn*-1, *sn*-2 and *sn*-3 positions of L-glycerol, usually saturated or monounsaturated FA located in the *sn*-1 and *sn*-3 and PUFA in *sn*-2 (Sargent, *et al.*, 2002). The fatty acids (FA) are the major constituents of the lipid classes mentioned above (apart from cholesterol). The FA found in fish lipids contain a single carboxyl group and a straight carbon chain, ranging from 14 to 24 carbon atoms, predominantly with even carbon numbers. Depending on the degree of unsaturation, they are classified as saturated and unsaturated FA, with the latter being further divided into monounsaturated or monoenes (one double bond), and polyunsaturated FA with two or more double bonds

(PUFA). In fish, the double bonds are largely in the *cis* configuration and in most cases interrupted. The lipids and their constituent fatty acids (FA) along with their metabolic derivatives, such as the eicosanoids, play significant roles in various functions of the organism, including growth, health, reproduction etc (Sargent, *et al.*, 2002; Tocher, 2003). Dietary lipids supplies essential fatty acids that cannot be synthesized by the organism (Sargent *et al.*, 1995). Dietary lipids are important sources of energy and fatty acids that are essential for normal growth and survival of fish. Dietary lipids provide energy, facilitate the absorption of fat soluble vitamins, play an important role in membrane structure and function, serve as precursors for steroid hormones and prostaglandins, and serve as metabolizable sources of essential fatty acids. Jauncey (2000) suggested that to maximize protein utilization, dietary fat concentration should be between 8% and 12% for tilapia up to 25g and 6% to 8% for larger fish. As with most fish, Tilapia appears to have a requirement for n-6 (linoleic) fatty acids. Dietary lipids should supply at least 1% of the n-6 fatty acids (Teshima, Kanazawa & Sakimato, 1982). The type and amount of lipid used in diet are based on essential fatty acids requirements, economic constraints of feed manufacture and quality of fish flesh desired. Lipids are of great importance not only to fish nutrition but also to human nutrition. It is well documented that the n-3 highly unsaturated fatty acids (HUFA) have numerous beneficial effects on human health and, undoubtedly, fish constitute the best sources of these nutrients in human diets (De Deckere, *et al.*, 1998; Simopoulos, 2003).

2.7 Apparent Nutrient Digestibility

Nutrition involves the ingestion, digestion, absorption and transport of various nutrients throughout the body where the nutrients in food are converted into body tissues and utilized for various activities (Gul *et al.*, 2007). Before feed ingredients can be catabolized for fuels (energy) or utilised in anabolic processes by fish (i.e. animals), they must be digested and absorbed from the digestive system. The efficiency of digestion of feed varies between species due to basic differences in digestive physiology or even between groups within a species due to more minor variations in digestion capability (Degani, & Yehuda, 1999).

Fish nutrition science started nearly 70 years ago. Many researchers since this time have been trying to evaluate fish feed efficiency using digestibility, metabolizability, energy budget and growth (net nutrient deposition) techniques similar to that of warm blooded animals (poultry, sheep, cows, etc). Some of these techniques (digestibility, metabolizability, and energy budget) could not be done without accepting a level of significant error as fish live in a different environment in addition to being cold blooded animals. These factors made these techniques non-additive (Belal, 2005).

According to Plakas and Katayama (1981), a feedstuff may appear from its chemical composition to be an excellent source of nutrients but be of minute actual value unless it can be digested and absorbed in the target fish species. Digestibility is the fraction of the nutrients or energy in the ingested feed stuff that is not excreted in the feces (NRC, 1993) and is the most important aspect in evaluating the efficiency of feedstuffs (Hassan, 2001). According to Hassan (2001), together with chemical analysis, digestibility determination allows more thorough assessment of nutritive value of a particular protein source in a complete fish diet. The nutritive value of mixed rations depends on the nutrient composition of the individual feed components and the ability of the animal to digest and absorb the nutrients. Feed digestibility has a direct impact on efficiency and productivity in aquaculture. In general, the higher the digestibility of feed, the greater the growth performance of the fish. A key aspect of developing diets for fishes is to determine their capacity to digest common feedstuffs (DeSilva & Anderson, 1995). Knowing availabilities of nutrients to fish species aids in selection of appropriate ingredients and formulation of a cost-effective diet (Hajen *et al.*, 1993).

The determination of relative feed efficiency and dietary nutrient digestibility in fish farming is still a complex phenomenon. Usually, indirect methods using an inert marker have been preferred to determine apparent digestibility of feed or feed ingredients for aquatic species (NRC, 1993). Measurement of dietary nutrient digestibility in fish is mainly accomplished by the indirect method, which allows partial collection of feces but requires the addition of an inert marker in the diet. Several methods for fecal collection have been reported for fish but the most widely used are stripping the final portion of the intestine, C dissecting the intestine, or

collecting the feces from the water, after evacuation (Belal, 2005). All methods have advantages and disadvantages regarding fecal contamination with endogenous components, stress responses of fish, nutrient leaching or the need to sacrifice laboratory trained fish. It is important to select an external indicator that is indigestible, without smell or taste and is consumed by the fish in exactly known quantities. It is assumed that the indicator is completely recovered in the faeces. If not, faecal output has to be corrected by the ratio of the total marker in the faeces to the total weight of marker given, which can only be determined by total collection of faeces.

2.7.1 Importance of Digestibility in Animal Nutrition Research

Fish nutrition has improved dramatically in recent years with the development of balanced commercial diets (Gul *et al.*, 2007) and with the intensification of fish culture operations and constant increases in the cost of many conventional feedstuffs, there is need to develop nutritional, economical and efficient feed on the basis of digestibility of respective fish (Asad *et al.*, 2005). Digestibility studies are one of the best methods of evaluating the capacity of a specific species to use the nutrients of a particular food (Hanley, 1987) and provide indications of the potentially available energy and nutrients for maintenance, growth and reproduction by the animal as well as of the levels of indigestible nutrients contributing to aquaculture wastes (Cho, 1993). Asad *et al.*, (2005) indicated that determining the digestibility of nutrients in feedstuffs is important not only to enable formulation of diets that maximize the growth of cultured fish by providing appropriate amounts of available nutrients, but also to limit the wastes produced by the fish. Digestibility estimates also indicate the level of indigestible nutrients (Asad *et al.*, 2005) voided, accounting for a major portion of aquaculture waste (Cho, 1993). Inclusion of highly digestible feedstuffs will minimize nutrient waste in water body. Knowing availabilities of nutrients to fish species aids selection of appropriate ingredients and formulation of a cost-effective diet (Hajen *et al.*, 1993). The digestible nutrients study will help the nutritionist make a least cost diet possible with the ideal protein concept.

2.7.2 Factors Affecting Digestibility

The method used to determine digestibility can affect the value of the coefficients obtained (Cho *et al.*, 1982). Most of the digestibility determinations have been made using chromic oxide, Cr₂O₃ (Austreng, 1978). Nose (1960) and Inaba *et al.*, (1962) used this method to determine crude protein digestibility in rainbow trout. They observed that the digestibility estimations obtained with fecal collection from the tanks were 10% greater compared with that obtained by stripping, indicating that some nitrogen compounds were lost in the water. Similar results were found by Singh & Nose (1967). Lee (2001) compared apparent nutrient digestibility of a diet by using a chromic oxide indicator according to the various fecal collection methods (dissection, stripping or using fecal collection column attached to fish rearing tank), and suggested that stripping or fecal collection column could be a reliable procedure for measuring nutrient digestibility in rockfish.

Each method has positive and negative features. For example, intestinal dissection of the feces requires that the fish be sacrificed. Also, the stripping of feces often results in under estimation of especially the digestibility coefficients for protein due to contamination of the fecal samples with mucus and intestinal cells and, the settling column technique for fecal collection can lead to over estimation of the digestibility coefficients for nutrients due to some leaching of nutrients from the feces (Hajen *et al.*, 1993). The determined digestibility values are often referred to as apparent digestibility values or coefficients as opposed to true digestibility coefficients. This is because there will inevitably be cells, proteins and secretions originating endogenously from the fish that are contained within the fecal samples (Bureau *et al.*, 2002). These compounds increase the estimated protein and energy contents of the feces, and thus under estimate the true digestibility of protein and energy in the ingredients. However, when feed intake is high, the difference between the estimates for 'true' and apparent digestibility coefficients is negligible (Bureau *et al.*, 2002).

2.8 Enzyme Nutrition

Improving the nutrient digestibility and growth performance has been one of the most important nutritional aspects in animal farming, be it in poultry, piggery or pisciculture (Debnath *et al.*, 2005). The efficacy of the diet not only depends on the nutrient composition and nutrient balance, but also on the effective utilization of the

nutrients by the animal (Manush *et al.*, 2013). In the utilization of dietary nutrients, the digestive enzymes play a vital role in catalyzing the hydrolytic reactions splitting the macromolecules into simple absorbable form of molecules (Manush *et al.*, 2013). Enzymes are one of the many types of protein in biological systems and their essential characteristic is to catalyze the rate of a reaction but is not themselves altered by it. They are involved in all anabolic and catabolic pathways of digestion and metabolism (Khattak *et al.*, 2006). Enzymes play a key role in the digestive process. Although enzymes are produced by the animal itself or by the microbes naturally present in the digestive tract, specific activities necessary to break down some compounds in feed are not found or are at low levels in the digestive tract. According to McDonald *et al.*, (2010), not all compounds in animal feed are broken down by animals' own digestive enzymes, and so some potential nutrients are unavailable to the animal. Therefore, exogenous enzymes are added to the diet to break down these compounds (Tuoying, 2003).

According to Plumstead (2013), poultry industry is the largest user of feed enzymes today and its highly integrated nature has driven a fast uptake of feed enzyme technology over the years. Also there is an increasing trend in the swine, ruminant and aquaculture industries to use feed enzymes. In the aquaculture industry, the search for alternative protein sources to replace fish meal, plus concerns regarding the relatively low nutrient digestibility and the presence of an array of anti-nutritional factors in fish meal alternatives, has led to an increasing interest in feed enzymes and research into optimal applications (Plumstead, 2013). Use of exogenous enzymes in animal diet has attracted considerable interest, the effects of which depended on several factors like type and composition of enzyme supplements, characteristics of feed ingredients used, species, age and physiological status of the animal (Kim *et al.*, 2004; Debnath *et al.*, 2005).

Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, often because the animal cannot produce the necessary enzyme to degrade them. However, nutritionists can help the animal by identifying these indigestible compounds and feeding a suitable enzyme (Khattak *et al.*, 2006). Supplementing the feed with specific enzymes improves the nutritional value of feed

ingredients, increasing the efficiency of digestion. Feed enzymes help break down anti-nutritional factors (e.g. fibre, phytate) that are present in many feed ingredients which interfere with normal digestion, resulting in reduced meat or egg production and lower feed efficiency and can also trigger digestive upsets (Benford & Partridge, 2010). According to Benford and Partridge (2010), the benefits of feed enzymes are to improve efficiency and reducing cost by breaking down of anti-nutrients thus allowing the animal to digest its feed more efficiently. This translates to more products per kilogram of feed; better environment by improving digestion and absorption of nutrients, reducing the volume of manure produced and lowering phosphorus and nitrogen excretion; improving consistency by reducing the nutritional variation in feed ingredients, resulting in more consistent feed for more uniform animal growth and egg production; helps to maintain gut health by improving nutrient digestibility hence fewer nutrients are available in the animal's gut for the potential growth of disease-causing bacteria (Benford & Partridge, 2010). Endogenous enzymes found in the digestive tract of fish help to break down large organic molecules like starch, cellulose and protein into the simpler substances. Addition of exogenous enzymes in fish feeds can improve nutrient utilization, thereby reducing nutrient losses (Mahmoud *et al.*, 2014).

2.8.1 Papain Enzyme

Papaya (*Carica papaya*) is commonly known for its food and nutritional values throughout the world (Battaa *et al.*, 2013). It yields a milky sap, often called latex, which is a complex mixture of chemicals. Chief among them is papain, a well-known proteolytic enzyme (Oliver-Bever, 1986). A papain enzyme is abundantly found in the leaves and skin of the green fruits. Papain is a proteolytic enzyme from the cysteine Proteinase family. Papain enzyme, as a proteolytic enzyme, is of crucial importance in many vital biological processes in all living organisms (Tsuge *et al.*, 1999). The proteolytic enzymes attack peptide bonds of proteins and polypeptides, providing free amino acids, dipeptides and tripeptides. Papain shows extensive proteolytic activity towards proteins, short- chain peptides, amino acid esters and amide links. Protease activity in the digestive tract is a key determinant of the digestibility and assimilation efficiency of ingested proteins (Manush *et al.*, 2013). It is very well reported that

proteolytic enzyme of exogenous origin plays an important role in feed digestibility in fishes (Manush *et al.*, 2013).

2.9 Fish Meal Use in Aquafeed

Fishmeal is a protein-rich light brown flour or meal derived from processing (cooking, pressing, drying, grinding) fresh raw fish (usually small pelagic fish not suitable as food fish or by catch) and residues and by-products from fish processing plants (fish offal or fish trimmings) (FIN, 2006; FAO, 2007).

Fish meal is a highly nutritious feedstuff primarily used as high quality protein source as it has an excellent amino acid profile, it is very palatable, has high a nutrient digestibility, is a rich source of energy, essential fatty acids (EFA), vitamins and minerals and has low levels of ant nutritional factors (Gatlin *et al.*, 2007; NRC, 2011). However, the chemical composition of fish meal may vary significantly depending on the source of fish used for its production (Hertrampf and Piedad-Pascual, 2000). Fish meal and fish oil are important components of the feeds for many farm raised species, from pigs and poultry to farmed fish. As ingredients in aquaculture feeds, fish meal and fish oil supply essential amino acids and fatty acids required for normal growth of cultured species including carp, salmon, tilapia, trout, catfish, shrimp, and others. However, the relatively high cost of fish meal due to growing demand for this product as well as pressure on the wild fisheries that supply these products are adding up to make alternative feeds one of the top issues facing the global aquaculture industry, fuelling research on suitable alternative feed ingredients.

2.10 Plant Protein Feed Resources in Aquafeed

According to De Silva (2001) and Gabriel *et al.*, (2007), research in fish nutrition in recent years seems to focus on the replacement of animal protein sources by plant based proteins, with the aim of reducing the cost of supplemental feeds. As the use of fishmeal in the aquaculture industry decreases for various reasons, alternative, more cost-effective feedstuffs are being increasingly used as protein sources in formulated feeds for farmed fish. Various sources have been attempted from plant, microbial, and other animal sources. The non-conventional feed stuff of animal origin are high quality feed ingredients that could compare to some extent with the conventional

types (Gabriel *et al.*, 2007). They are cheaper by virtue of the fact that there is no competition for human consumption. However, the only problem with these feed stuffs is their unavailability in large commercial quantities for the sustenance of aquaculture industry (Gabriel *et al.*, 2007). The different animal products which have been tested as suitable substitutes for fish meal in Tilapia feeds; among them are blood meal, poultry by product meal, hydrolysed feather meal and meat and bone meal (NRC, 1993). However, at least some of these alternative feed ingredients have been reported to have negative consequences on growth and feed utilization of farmed fish, depending on fish species and inclusion level in their diets.

Feed resources of plant origin have a big potential for a wide application in fish feed industry: they are a cheaper source of high quality proteins compared to proteins from animal sources, global bioavailability and they are relatively easily renewed. According to Rumsey (1993), cost effective practical aquaculture feeds can be produced without the use of fish meal with no apparent loss in fish growth in some species such as tilapia. Plant based aquaculture feeds containing soybean meal protein, canola meal, extruded pea seed meal, wheat and corn meal supplemented with lysine and methionine has been used in the formulation of aquaculture feed for catfish, tilapia and carp without affecting the growth performance of the fish (Tacon *et al.*, 2009). Oilseed cakes and legume seeds are considered suitable as alternative dietary protein sources for fish feed and are available in sub-Saharan Africa on a large scale (Fagbenro *et al.*, 2003). Oilseeds and their byproducts frequently constitute a major source of dietary protein within aquaculture feeds for warm water omnivorous/herbivorous fish species such as those commonly used in African aquaculture, including tilapias (*Oreochromis spp.*) and catfishes (*Clarias spp.*) due to their relatively high protein content and low cost. According to Okoye and Sule (2001), nutrient values estimated from locally available conventional and non-conventional plant sources are high and this justifies continuous investigation and utilization of their nutritional potentials to enhance economic fish production.

However, there has been contradictory information on the usage of plant protein based ingredients as substitute for fish meal. According to NRC (1993), despite the fact that most plant ingredients are readily available at a lower cost than fish meal,

their use within aqua feeds is usually restricted by relatively low protein content, unbalanced essential amino acid profile, high levels of fibre and starch and the presence of one or more ant nutritional factors. Plant protein tends to lower feed intake by reducing diet palatability when replacement levels are high, or by affecting the health of the fish in other ways, such as the condition described as distal enteritis in Atlantic salmon and rainbow trout fed with high soybean meal (Refstie *et al.*, 2000). Stankovic *et al.*, (2011), observed that fish meal and plant protein sources are very different in the amount of proteins, structure of amino acids, energy availability and amount of mineral matter. The main plant protein sources used in compound feeds for aquaculture include oilseeds (soybean meal, rapeseed meal, cottonseed meal, and sunflower seed meal); legumes (peas, lupins, and faba beans); cereals (wheat, rice, maize, barley, and corn); and plant protein concentrates (Tacon, *et al.*, 2011; Oliva-Teles *et al.*, 2015).

Table 3: Oilseed Meals and Their Corresponding Ant nutritional Factors

Ingredient	Ant nutritional factor
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamin, allergens, low in methionine, low palatability, non-starch polysaccharides, oligosaccharides
Canola meal	Protease inhibitors, glucosinolates, erucic acid, phytic acid, tannins, high fibre
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitor, high fibre.

Data extracted from Francis *et al.*, (2001) and Gatlin III *et al.*, (2007).

However, several strategies have been developed to neutralize plant feed stuffs ant nutritional factors and to increase phosphorus and starch availability. These include heat processing, solvent extraction and dehulling, and using exogenous enzymes such as phytases (Glencross *et al.*, 2007; Krogdahl *et al.*, 2010). Some ANF can be inactivated by a variety of methods such as dehulling, germination, soaking and enzyme addition or heat treatment such as autoclaving, roasting and extrusion (Francis *et al.*, 2001).

2.10.1 Canola Meal

Rapeseed (*Brassica napus*) is an important source of edible oil in many countries. Canola, *Brassica napus* Linnaeus, refers to selected varieties of rapeseed that are low

in glucosinolate and erucic acid (Bell, 1993). Canola meal is only second to soybean meal as the most commonly fed protein feedstuff in animal diets around the world (Newkirk & Canada, 2009). Canola seeds have been genetically modified from traditional varieties of rapeseeds by plant breeders to obtain plants with low levels of erucic acid in the oil and low levels of glucosinolates in the non-oil part of the plants (Thomas, 2005; Newkirk & Canada, 2009). Canola is now the third most widely grown genetically modified crop after soybean and maize. It was bred from rapeseed and it differentiates from the latter by its low content in erucic acid; an anti-nutritive fatty acid, which compromised the value of rapeseed oil for years (Tan *et al.*, 2011). Canola meal results from the solvent extraction of canola oil and it is readily available worldwide. The chemical composition of CM has been generally reported based on the residual oil and protein content. The variation in quality depends on origin (with regard to weather and soil condition) and processing (solvent oil extraction or expeller oil extraction). The amino acid profile of canola meal protein is similar to that of herring meal protein and superior to soybean meal protein (Higgs *et al.*, 1995; Mwachireya *et al.*, 1999). It is noted that the balance of amino acids of canola protein is one of the best among commercial vegetable protein sources. Canola meal is considered an important plant protein source for fish meal replacement in diets for both terrestrial animals and aquaculture species. Wide availability, high protein content and a desirable amino acid profile have caused an interest in rapeseed products as fish meal alternative in fish feeds. Many fish species have been shown to have good growth performance when fed with diet containing canola meal. The complementarity of the amino acids profile of canola protein is similar to that of fish meal but better than that of soybean meal as it has higher content of sulphur containing amino acids (methionine and cystine)

Canola meal contains relatively high content of minerals, especially potassium, sulfur, calcium and iron and an especially good source of selenium and phosphorus (Bell *et al.*, 1999). Moreover, it is rich in choline, biotin, folic acid, niacin, riboflavin and thiamin (NRC, 1998). The whole seed contains approximately 21% crude protein while canola meal contains approximately 36% crude protein (Naczek *et al.*, 1998; Uppstrom, 1995). However, unlike peas, canola is rich in sulfur amino acids and lysine (Uppstrom, 1995). The fiber content of canola is primarily found in the hull and

ranges from 12 to 30% in canola meal (Uppstrom, 1995). Protein digestibility of CM has been determined for many fish species and ranges from 23% to 94% despite most values over 80%. The variation can be due to different quality of CM, different fish spp and/ or different methodologies employed in determination of digestibility in different studies. The digestibility of CM protein can be improved by some treatments to reduce anti-nutritional factors. Heat treatment substantially improved protein digestibility due to decrease in the level of glucosinolates.

The nutritional quality of rapeseed products largely depends on their levels of antinutritional factors. Prominent antinutritional factors in rapeseed are glucosinolates, phytic acid, phenolic constituents (e.g. tannins), indigestible carbohydrates fibre and oligosaccharides (Francis *et al.*, 2001). Several processing techniques have been adapted to reduce the level of Antinutrients in rapeseed in order to improve its value for fish nutrition. Dehulling of seeds and utilisation of high temperatures and organic solvents (hexane) during oil extraction as well as sieving of meal decrease content of glucosinolates, phytate, fibre, cellulose, hemicellulose, sinapin and tannins (Mawson *et al.*, 1995; Leming *et al.*, 2004) and increase protein level in meals (Mwachireya *et al.*, 1999). In addition, protein extraction from meals by methanol-ammonia-treatment or ethanol-treatment will further increase protein level and effectively remove glucosinolates, phenolic compounds, soluble sugars, such as sucrose, and some oligosaccharides (Naczka & Shahidi 1990; Chabanon *et al.*, 2007) but will also increase levels of non-digestible fibre (Mwachireya *et al.*, 1999). The phytic acid content of canola meal ranges from 3.1 to 3.7 g/kg (Higgs *et al.*, 1995) and significantly reduces phosphorus digestibility, protein utilization and growth (Spinelli *et al.*, 1983; Forster *et al.*, 1999).

Table 4: The Recommended Practical Inclusion Levels (%) for Canola Meal Usage in Aquaculture Diets

Animal diet type	Inclusion	Reasons for inclusion level
Salmon, trout	20	High performance results reported at 20% inclusion
Catfish	30	No data available beyond 30%
Tilapia	30	No data available beyond 30%
Red sea bream	60	No data available beyond 60%
Shrimp and prawns	15–30	High performance results reported with 15–30% inclusion

Source: (canola council of Canada, 2015)

2.10.2 Soybean Meal

Soybean (*Glycine max*) is one of the world's largest sources of plant protein and oil. Soybean protein has high crude protein and a balanced amino acid profile most of which tend to be deficient in cereal grains which constitute large portions of diets of monogastric animals. When compared to other protein sources, soybean boasts being the standard by which other protein sources are compared. Among the major oilseed meal sources of protein, soybean ranks highest in value based on quality of protein which is reflective of its balance of amino acids and their digestibility. Soybean meal, a byproduct of the oil milling industry also has rich nutritive value when compared to other protein sources. Soybean meal is the by-product after removal of oil from soybeans and it is the major protein source used in aquaculture feeds, not only because of its high protein content but also due to its worldwide availability (Hertrampf & Piedad-Pascual, 2000). Another soybean product commonly referred to as full-fat soybean meal is produced by heat treatment of whole soybeans. This product has a crude protein content of approximately 38% (as-fed basis) and a lipid level of approximately 18% (Lim & Akiyama, 1992). The most used soy products in production of feed for aquaculture are oil-free soy meal and soy protein concentrate. According to Gatlin (2002), other products such as high fat soy meal, mechanically extracted soy cake, soy flour extracted from solvent are used depending on the technology of production. Soy flour lacks in lysine, methionine and cystine which can be overcome by the addition of synthetic amino acids or by a compatible combinations with other feeds. It is also deficient in the content of vitamin B complex (Stankovic *et al.*, 2011). Soybean meal (SBM), because of its availability, consistent quality, and high protein content with good amino acid profile and low cost, is the

most studied plant feedstuff in aquaculture diets (Lim & Dominy, 1989). However, it is considered limiting in methionine and contains some anti-nutrients such as trypsin inhibitor, hemagglutinin and anti-vitamins (Tacon, 1993). Protease inhibitors have been reported to hinder the activity of the proteolytic enzymes trypsin and chymotrypsin in monogastric animals which in turn lowers protein digestibility. The discrepancy among researchers regarding the use of SBM as a protein source for fish may be related to the quality and processing of SBM, variation in diet formulation, and differences in fish species, size and culture systems (Elangovan & Shim, 2000). Soybean meal also contains considerably higher energy and lower fiber content than other oilseed meals. The high concentration of protein and energy, and the low fiber content make soybean meal an ideal feed ingredient in formulating balanced rations that provide optimum growth, production and reproductive performance of monogastric animals. Soybean meal is less expensive than fishmeal and is readily available for constitution of animal feeds. However, the price of soybean meal is higher than that of other plant source protein such as cotton seed, canola and sunflower meals. This may be attributed to the higher percent crude protein, better quality protein and highly digestible amino acids in soybean meal when compared with other plant source proteins.

Soybean composition and processing conditions affect the nutritional quality of soybean meal (Grieshop & Fahey, 2001). On the other hand, Dudley (1999) emphasized the importance of accurate information on soybean meal composition and the availability of key nutrients in formulating balanced animal feeds. These include the quality, balance, and availability of amino acids and the processing conditions that are used in soybean processing to soybean meal or other byproducts. Methods of processing soybean and variations in processing also contribute to the overall quality of the soybean products. These include extrusion and expelling, solvent extraction (Woodworth *et al.*, 2001), roasting and Jet-sploding (Marty *et al.*, 1994; Subuh *et al.*, 2002), and micronization (Marty *et al.*, 1994; Subuh *et al.*, 2002). These methods lead to variations in nutrient composition of the final product (s). In addition to the various methods used in the production of soybean products, there are also variations in the parameters used in the production of soybean meal and soybean protein concentrates, which is reflected in the nutrient composition of the final products. These include the

combinations of heat, timing, moisture and the quality of the soybean. These variations can be minimized through implementation of good quality control mechanisms during processing.

2.10.3 Sunflower Cake

Sunflower (*Helianthus annuus*) is an important oil seed crop of the world and it ranks third in production next to groundnut and soybean (Byrareddy *et al*, 2008). It is the fourth oilseed crop produced globally, 30.7 million metric tonnes. The sunflower (*Helianthus annus* L.) is a plant of the asteraceae family, which originates from North America and has been cultivated in large areas throughout the world (Braga *et al* 2017). Sunflower is an annual which grows from 0.6 to 4.5 m high depending upon the variety and environmental conditions. There are three types of cultivated sunflowers. The first produce large, thick-hulled, chewy seeds that are used in the confectionery trade or in gourmet foods. The second produce medium sized seeds with attractive black and white stripes and are used in bird feeds. The third type of sunflower produces small, thin hulled seeds with high oil content. After oil extraction the resulting cake meal contains about 1.5% residual oil, 30% crude protein, 6.2% ash and 24% crude fibre (with hulls) (Hesley, 1994). This feed ingredient is a good source of protein with amino acid availabilities similar to those of soybean meal (SBM), and much higher than those in cottonseed meals. It is a good source of methionine and arginine. Its lysine content is relatively low but this can be resolved with supplemental lysine. The nutrient content of sunflower seeds depends on the variety and growing conditions, which in turn affect the nutrient content of the sunflower seed meal produced after oil extraction (Senkoylu & Dale, 1999). The nutritional quality of sunflower meal (metabolizable energy, fibre content and protein quality) is affected by the processing method of oil extraction (Mandarino, 1997).

There are two main systems of oil extraction - expeller (hydraulic or screw press) and solvent. In the conventional screw press method dehulled seeds are cooked for one-half hour at 115.6°C (240 °F), conditioned for three minutes at 126.7°C (260 °F), and then passed through a screw press. This extraction procedure cakes with oil content as low as 4% can be obtained. The temperature used in the process will affect the quantity of oil extracted from the sunflower seeds. Lowering processing temperatures

from 115.6°C in the cooker and 126.7°C in the conditioner to 93.3°C in the cooker and 104.4°C in the conditioner resulted in an increase in the oil content of the residual cake. Lower processing temperatures, however, also result in a higher quality of extracted oil and higher quality residual oilcake. Oil can be extracted from decorticated, undecorticated or semi-decorticated sunflower seeds. The use of decorticated seeds decreases the fibre content of the oil meal and thus increases its nutritive value. At the same time inclusion of at least some of the hulls increase the efficiency of oil extraction.

Solvent extraction is a more effective method of oil extraction than mechanical extraction. Processing time and temperature of the sunflower seeds affects lysine available in the final meal. High temperatures during oil extraction can damage the protein. The result is a reduction in the availability of amino acids, especially lysine (Senkoylu & Dale, 1999). Unlike most other oilseed meals, sunflower seed meal has not been found to have anti-nutritional factors (Senkoylu & Dale, 1999) but according to (Rezaei & Hafezian, 2007), SFM does not contain high concentrations of ant nutritive factors. However, the relatively high fibre and lignin contents and low level of lysine limits its use in high performance feeds. Sunflower contains a variety of ant nutritional factors, the most important of which is chlorogenic acid, which is to function as an effective trypsin inhibitor (Kanto, 1988). However, the use of SFM in poultry diets is limited by variations in its chemical composition and the two main components apparently restricting its use are high fiber/low energy and low lysine contents (Senkoylu & Dale, 1999).

2.11 Effects of Diet on Fish Meat Quality

The quality of fish and fishery products has become a major concern in fish industry all over the world (Huss *et al.*, 2003). Fish are a highly nutritious food source since they are rich in protein, healthy fatty acids, minerals such as calcium, iron, selenium, and zinc, and vitamins A, B₃, B₆, B₁₂, D and E (Sidhu, 2003). However, farmed fish have been slowly accepted by consumers since they have been thought to have lower quality and poor flavor compared to their wild counterparts (Haard, 1992; Rasmussen, 2001). According to Noor *et al.*, (2011), diet has influence on the organoleptic quality of cultured fish and improvement of feed and nutrition in aquaculture practices may

provide an opportunity to further enhance the quantity as well as nutritional quality of fish. Nutritional quality and organoleptic acceptability in terms of colour, flavour, texture, appearance and shelf life may be affected by environmental degradation and quality of nutrition and feed provided during culture especially in semi- intensive and intensive systems compared to wild fish (Grigorathis, 2003).

In animal nutrition, one of the main tasks is providing sufficient quantities of food for human population, food of exceptional value as excellent source of proteins, fats, vitamins and minerals which are necessary in human nutrition. Reduction on the feed costs is a major concern in fish farming. However, replacement of fishmeal with lower cost feedstuffs requires the assessment not only the growth performance but also the meat quality resulting. Fish growth and its associated qualities depend on quality and quantity of feed (Khalid *et al.*, 2014). Nutrition is the most important tool that may be utilized to modify properties such as fatty acid profile, fat content, flavor, color and texture to deliver a more nutritious and appealing product to consumers (Haard, 1992; Rasmussen, 2001).

CHAPTER THREE

SUBSTITUTING FISH MEAL WITH OILSEED MEALS ON DIETS NUTRIENT COMPOSITION FOR NILE TILAPIA (*Oreochromis niloticus*)

3.1 Introduction

Fish do not have a true protein requirement but require a balanced combination of essential amino acids (EAA) and nonessential amino acids (NRC, 2011, Wilson, 2002). Supplying the essential amino acids requirements of cultured fish is extremely important because of significant effects of these nutrients on muscle deposition and feed cost (Small & Soares, 1999). However, formulating cost effective feeds that meet the essential amino acid requirement can be a challenge (Kaushik & Seiliez, 2010). This is because the use of high quality proteins to meet these requirements tends to be limited by their price (El-Sayed, 2006; Webster & Chhorn, 2006).

Fishmeal is considered the most desirable animal protein ingredient in aqua feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic fatty acids (Hardy & Tacon, 2002). However, plant protein feedstuffs have been used to replace fishmeal due to their more constant availability, and lower costs, despite presenting lower protein content, amino acid imbalances, antinutritional factors, and, digestibility and palatability than fishmeal (Hardy, 2010). A deficiency in certain essential amino acids is one of the major issues with plant protein sources, as it requires supplementation with other feedstuffs (Ogunji, Rahat, Summen, & Schulz, 2008). Among the plant protein feedstuffs, soybean meal, canola meal and sunflower meal offer the best substitute for fishmeal due to their relatively high crude protein content, availability and low cost.

When considering the value of different protein sources, the quality depends on the quantity of essential amino acids, the balance among the respective amino acids, on which the utilization of the protein depends. As a result, different methods of protein evaluation give different results for the nutritive value (Malomo & Alamu, 2015). Ramarao, Norton, & Johnson (1964) argued that essential amino acid requirements should serve as a standard for evaluating the quality of protein in foods, rather than the total essential amino acids content of the food. There are several reports now to

confirm that amino acid profiles of whole body tissue of a given species of fish resemble those of the dietary requirements (Mambrini & Kaushik, 1995). Protein chemical score (CS) has been used to compare the essential amino acid content in the test protein when the requirement is already established (Hepher, 1988). It is based on the concept that utilisation of a dietary protein depends upon the level of the EAA in greatest deficit which is termed the 'first limiting EAA' (Jauncey, 1998). However, since other essential amino acids may also have effects on protein utilization, this resulted in the development of the essential amino acid index (EAAI) (Bunda, Tumbokon, & Serrano, 2015.).

Although the protein component in fish feed has always been a major concern both for the fish feed manufacturers and fish farmers, fish like all other vertebrates require essential fatty acids (EFA) for normal growth, development and reproduction (Sargent *et al.*, 1999; Tocher, 2010). According to Ariful Alam *et al.*, (2014), recent study and publication about farmed tilapia omega 3 and 6 fatty acid balance in fillet depending on feed provided and concern about human health hazard deserves some attention and justification. Essential fatty acids are unsaturated fatty acids that must be provided preformed in the diet (Bell *et al.*, 1986, NRC, 1993).

The objective of this study therefore was to evaluate the protein quality and dietary fatty acid composition of plant protein feed ingredients (soybean meal, canola meal and sunflower meal) and their effect on protein quality when used as substitutes for fish meal in Nile tilapia rations.

3.2 Materials and Methods

The experiment involved collection of the feed ingredients, processing, analysis and formulation of the diets.

3.2.1 Study Site

The experiment was conducted at Chuka University, Animal Nutrition Laboratory, University of Nairobi and Fletcher Scientific Solutions, Nairobi.

3.2.2 Preparation of Diets

The feed ingredients were sourced from local feed dealers. Four isonitrogenous diets (30% CP) and isocaloric (2900Kcal/kg) were formulated using the ingredients; Fish meal (*Rastrionaebola argentea*), soybean meal, canola meal, sunflower cake, maize grain and wheatbran. All the ingredients were readily available. The ingredients were ground using hammer mill to be uniform. The measured ingredients was mixed thoroughly by hand in desired proportion before adding some water to form a dough and pelleted using a pelletizer machine particle size 4.5 mm diameter. The pellets were dried under shade and packed in polythene bags to prevent attacks by moulds and other pest

The main objective when formulating a fish diet is to provide a nutritionally balanced mixture of ingredients to support maintenance, growth, reproduction and health of the animal at an affordable cost (NRC, 1993, Kirimi *et al.*, 2016). Excel software was used to balance the ingredients.

Table 5: Ingredient Composition and Calculated Crude Protein (%) of the Diets Supplemented to *Oreochromis niloticus* Containing Soybean meal, Canola meal and Sunflower Meal as a Replacement of Fishmeal.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	16.5	9	9	9
Soybean meal	13	24	15	16
Canola meal	16.5	16	31	15
Sunflower cake	18	19	18	43
Maize grain	18	16	13	10
Wheat bran	18	16	14	7
Total	100	100	100	100
Calculated crude protein (%)	30.17	30.06	30.15	30.06
Calculated Digestible Energy(Kcal/kg)	2997.08	2965.08	2949.63	2878.11

3.2.4 Analysis of Samples

Chemical analysis of ingredients and diets was done in order to ascertain their nutritive value more so in the protein quality.

3.2.4.1 Proximate Analysis

The proximate analysis of ingredients and diets were carried out in triplicates following AOAC (1995) procedures: The analyses involved the following nutrients: crude protein (CP), ether extracts (EE), ash, nitrogen free extracts (NFE), crude fibre (CF), Neutral detergent fibre (NDF) and acid detergent fibre (ADF)

Moisture content was determined in triplicate for each sample by air-drying 2g of the samples in an oven at 105°C for 8 hours in clean individually marked dishes. After 8hrs the dishes were transferred to a dessicator and left to cool. They were then weighed again accurately. The loss in weight was the moisture content and what was left is the dry matter (DM) of the sample.

For ash content, approximately 2g of sample was weighed into a pre-weighed crucible and placed in a muffle furnace set at 550°C for 4hrs. After 4hrs the furnace was switched off to cool to about 100°C and then transferred into a dessicator for cooling to room temperature. The dishes were weighed accurately. The increase in the final weight of crucible after incineration represented the ash and was expressed as percentage of the original sample.

The crude protein was determined using the kjeldahl method in triplicates as described in AOAC (1995). Five grams of air dried sample was put in micro-kjeldah tube in triplicates and marked. About 15 mls of concentrated sulphuric acid was added to each tube and then a tablet of selenium catalyst. The tubes were then placed on a digestion rack and heat was switched on. The samples were digested for approximately 1hr until they turned clear or blue-green appearance. The solution was then transferred into conical flask to which 15ml of 0.1NHcl was added followed by 2-3 drops of mixed indicator. It was then connected to a distiller for 5 minutes and removed. Titration was done using 0.1 N sodium hydroxide and the titre value noted.

The method for ether extraction was that of solvent extraction using soxhlet extractor as described in AOAC (1995). For each sample three clean round bottomed flasks were set in oven at 105⁰c for 1 hr then cooled in dessicator and weighed. Two grams of sample was weighed into a thimble and corked with cotton. The thimbles and the contents were put into the Soxhlet extractor unit together with the flask. The soxhlet

extractor was half filled with the extracting solvent i.e. petroleum ether and the whole set up was placed in a heating system. The system was left to run for 16 hrs. Extraction involved boiling, rinsing and evaporation. Extracted lipid in the glass flask was dried in an oven at 105°C before weighing. Crude lipid was quantified as the loss in weight after extraction of the sample with petroleum ether (40-60°C).

Crude fibre, 2 grams of air dried sample was put in a 500ml graduated glass beaker. About 25mls of hot water was put in the beaker before adding 25mls 2N H₂SO₄. The volume of water was increased to 200ml. The content was boiled for 30 minutes, removed and filtered using a filter stick packed with glass wool then washed three times with hot water. Again about 100mls of hot water was added before adding 25ml of 1.78N KOH and volume increased to 200ml with hot water. Again it was boiled for 30 minutes, removed, filtered and washed three times. The residue and the glass wool were transferred into silica dish and washed with 5ml ethyl alcohol. The dish with the content was dried in oven for 2hrs cooled and weighed accurately. The oven-dried sample was then ashed in the muffle furnace at 550°C for 4hrs then cooled in desiccator and weighed. Nitrogen free extracts (NFE) was estimated by subtracting the total of moisture, crude protein, ether extracts, ash and crude fibre from 100. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined by the method of Waldern (1971) and Van Soest *et al.*, (1991)

3.2.4.2 Amino Acid Analysis

Amino acid analysis of the samples was performed by MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range of 780–2500 nm. NIR spectra of foods comprise of broad band's arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C-H, O-H and N-H chemical bonds (Osborne, 2006). Approximately 30-50 g of the sample was put into the sample cup, which was later put on the integrating sphere for measurement. The calibration models were created by INGOT[®] and Bruker Germany. Samples were analyzed for calibration and cross validation of the calibration performed.

Chemical Score (CS)

This is a parameter used to numerically compare essential amino acid (EAA) profiles of ingredients or diets to the requirements of the target species. It is based on the concept that utilisation of a dietary protein depends upon the level of the EAA in greatest deficit. EAA with CS of 100% or more meet or exceed the requirement for EAA and those with less than 100% are in deficit. The amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake (WHO/FAO/UNU, 2002).

$$\text{CS (\%)} = (\% \text{ EAA in ingredient or diet} / \% \text{ EAA requirement for fish}) \times 100$$

(Jauncey, 1998)

Essential Amino Acid Index (EAAI)

EAAI is useful as a rapid tool to evaluate food formulations for protein quality, although it does not account for differences in protein quality due to various reactions (Nielsen, 2002).

$$\text{EAAI} = n / \{(aa_1/AA_1) (aa_2/AA_2) \dots (aa_n/AA_n)\}$$

Where EAAI is the n th root of the essential amino acids in the test diet (aa) to the content of each of those amino acids in the reference tissue (AA) and n is the total number of amino acids evaluated (Tidwell *et al.*, 1993).

3.2.5 Data Analysis

Proximate, fatty acids, amino acids, chemical scores and essential amino acid index data were subjected to analysis of variance (ANOVA) using SPSS statistical package version 17.0 at $P = 0.05$ confidence level to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

3.3 Results

Proximate, fatty acid and amino acid analysis of the experimental ingredients and diets is as reported below

3.3.1 Ingredients Proximate Composition

Results of the proximate nutrient composition of six feed ingredients are as shown in Table 6. Fishmeal had highest crude protein content of (62.60%) followed by soybean meal (47.38%). Sunflower meal recorded highest figures for crude fibre (36.38%), Acid detergent fibre (22.45%) with fishmeal recording the lowest crude fibre content (1.04%). Also Fish meal had highest level of ash content (15.22%) but lowest figures for ether extracts (1.41%). Canola meal had the highest lipid content (23.88%) and wheat bran lowest lipid content (4.30%). Maize meal recorded lowest figures for crude protein (10.65%), Acid detergent fibre (3.52%). However, wheat bran had highest levels of Neutral detergent fibre (46.95%)

Table 6: Proximate Composition of Feed Ingredients (%) Used to Formulate Diets For Nile tilapia

	Fish Meal	Soybean meal	Canola Meal	Sunflower meal	Maize meal	Wheat bran
Proximate composition (%)						
Dry matter	92.33±0.25 ^{cb}	92.37±0.07 ^{bc}	91.07±0.05 ^e	94.42±0.21 ^a	88.42±0.11 ^f	90.10±0.03 ^d
Crude protein	62.60±0.38 ^a	47.38±0.32 ^b	34.39±0.18 ^c	24.81±0.03 ^d	10.65±0.27 ^f	16.04±0.43 ^e
Ether extract	7.49±0.32 ^d	9.27±0.30 ^c	23.88±0.24 ^a	13.31±0.10 ^b	4.73±0.23 ^{ef}	4.30±0.17 ^{fe}
Ash	15.22±0.59 ^a	8.96±0.26 ^b	5.50±0.26 ^{dec}	5.08±0.14 ^{ed}	1.41±0.19 ^f	6.17±0.24 ^{cd}
Crude Fiber	1.04±0.09 ^f	15.88±0.32 ^{bc}	15.58±0.20 ^{cb}	36.38±0.20 ^a	3.79±0.28 ^e	14.41±0.22 ^d
Nitrogen Free Extract	5.92±0.32 ^f	10.88±0.26 ^{ed}	11.72±0.32 ^{de}	14.83±0.47 ^c	67.85±0.44 ^a	49.18±0.75 ^b
Neutral Detergent Fiber	34.24±0.20 ^d	28.16±0.38 ^e	21.07±0.14 ^f	43.03±0.30 ^b	40.79±0.23 ^c	46.95±0.18 ^a
Acid Detergent Fiber	15.22±0.21 ^b	9.89±0.22 ^e	11.99±0.22 ^{dc}	22.45±0.27 ^a	3.52±0.32 ^f	12.28±0.19 ^{cd}

Values are expressed as mean ± SE.^{a,b,c,d,e,f} Values in the same row having different superscript letters are significantly different (P<0.05).

3.3.2 Ingredients Fatty Acid Composition

Results of the diets fatty acid composition is shown in Table 7. Fish meal recorded highest concentration of fatty acids. Palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1n-9) were detected in all the ingredients with fishmeal recording the highest concentration. Eicosatrienoic acid (20:3n-3), Eicosapentaenoic acid (20:5n-3) and Docosahexaenoic acid (22:6n-3) were abundant in fishmeal only. α -linolenic acid (C18:3n-3, Eicosanoic acid (C20:0), Docosanoic acid (C22:0) were only detected in soybean meal

Table 7: Fatty Acid Composition (mg/100g) of Ingredients used to Formulate Diets for *Oreochromis niloticus*

	Fish Meal	Soybean meal	Canola Meal	Sunflower meal	Maize meal	Wheat bran
Fatty Acid						
Myristic acid (14:0)	4.67	ND	ND	1.11	ND	ND
Palmitic acid (16:0)	21.7	3.09	11.23	15.35	13.02	17.63
Stearic acid (18:0)	9.32	2.35	2.16	5.26	1.98	1.04
Palmitoleic acid (16:1n-7)	8.27	ND	ND	1.66	ND	ND
Oleic acid (18:1n-9)	14.97	20.49	33.78	33.73	33.91	38.63
Erucic acid (22:1n-9)	2.81	ND	ND	ND	ND	ND
Nervonic acid (24:1n-9)	0.61	ND	ND	ND	ND	ND
Eicosatrienoic acid (20:3n-3)	0.11	ND	ND	ND	ND	ND
Eicosapentaenoic acid (20:5n-3)	7.09	ND	ND	ND	ND	ND
Docosahexaenoic acid (22:6n-3)	14.64	ND	ND	ND	ND	ND
α -linolenic acid (C18:3n-3)	ND	5.2	ND	ND	ND	ND
Eicosanoic acid (C20:0)	ND	0.59	ND	ND	ND	ND
11-Eicosenoic acid(C20:1)	ND	0.19	ND	ND	ND	ND
Docosanoic acid (C22:0)	ND	0.17	ND	ND	ND	ND
Linolenic acid (C18:3)	ND	ND	0.66	ND	0.88	2.47

ND: means not detected

3.3.3 Amino Acid Composition of Ingredients

Amino acid composition of ingredients is as shown in Table 8. Amino acid results revealed that Fish meal (*Rastrionaebola argentea*), recorded highest level for essential amino acids. Fish meal had highest level of lysine (7.81 mg/100g) followed by canola meal (4.01 mg/100g) with maize meal recording the least (1.42 mg/100g). Methionine content was higher in fishmeal (2.89 mg/100g) compared to the oilseed meals.

Table 8: Amino Acid Composition (Mg/100g Protein) of Feed Ingredients used to Formulate Diets for *Oreochromis niloticus*

	Fish Meal	Soybean meal	Canola Meal	Sunflower meal	Maize meal	Wheat bran
Essential Amino Acids						
Lysine	7.81±0.07 ^a	3.01±0.01 ^d	4.01±0.01 ^b	3.14±0.01 ^c	1.42±0.00 ^f	1.75±0.01 ^e
Methionine	2.89±0.01 ^a	0.61±0.01 ^{de}	0.61±0.01 ^{ed}	0.51±0.01 ^f	2.16±0.01 ^b	1.44±0.01 ^c
Cysteine	0.95±0.01 ^e	0.66±0.00 ^f	1.16±0.00 ^c	1.54±0.02 ^b	1.04±0.00 ^d	1.82±0.02 ^a
Histidine	2.43±0.01 ^b	1.26±0.01 ^f	1.57±0.00 ^e	5.44±0.01 ^a	2.12±0.01 ^c	1.81±0.01 ^d
Arginine	5.87±0.04 ^a	3.39±0.01 ^b	3.05±0.02 ^c	2.96±0.00 ^d	2.42±0.01 ^f	2.81±0.01 ^e
Threonine	4.28±0.01 ^a	1.96±0.04 ^f	2.1±0.06 ^e	3.87±0.00 ^b	2.6±0.01 ^d	3.16±0.01 ^c
Valine	5.4±0.01 ^b	2.24±0.01 ^f	2.34±0.01 ^e	6.27±0.02 ^a	4.09±0.01 ^d	4.93±0.01 ^c
Isoleucine	4.55±0.07 ^a	2.36±0.01 ^c	2.55±0.01 ^e	0.97±0.02 ^f	3.26±0.01 ^d	3.83±0.01 ^b
Leucine	7.55±0.04 ^b	3.69±0.01 ^f	3.78±0.01 ^e	10.06±0.01 ^a	7.15±0.01 ^c	6.85±0.02 ^d
Phenylalanine	4.2±0.01 ^c	2.71±0.02 ^f	3.84±0.01 ^d	5.83±0.01 ^a	4.24±0.01 ^b	3.79±0.01 ^e
Tryptophan	1.15	0.68	0.62	ND	ND	ND
Non-Essential AA						
Tyrosine	3.32	ND	ND	ND	3.25	2.32
Aspartic acid	9.29	ND	ND	ND	3.46	2.83
Serine	3.83	ND	ND	ND	6.47	6.61
Glutamic acid	12.88	ND	ND	ND	13.21	17.11
Proline	4.28	ND	ND	ND	8.06	12.61
Glycine	5.74	ND	ND	ND	4.17	6.01
Alanine	6.24	ND	ND	ND	10.14	4.32

Values are expressed as mean ± SE. ^{a,b,c,d,e,f}. Values in the same row having different superscript letters are significantly different (P<0.05).

ND- Not detected

3.3.4 Diets Proximate Composition

Proximate composition of the four experimental diets is shown in Table 9. Crude protein values for diet 1, 2, 3 and 4 were near isoproteinous (30.57%, 30.76%, 30.34 and 31.35% respectively ($P>0.05$). Diet 3 recorded highest level of Ether extracts 10.75% ($P<0.05$) and low for diet 1 (7.55%). Diet 1 had highest ash content (6.16) but low in crude fibre content (11.06%) with diet 4 recording highest crude fibre content (16.03%). However all the diets recorded almost the same amount of neutral detergent fibre although diet 4 recorded slightly lower figure (23.08%). Acid detergent fibre were within the same range but diet 4 recorded highest (11.86%)

Table 9: Proximate Composition of the Diets (%), for Nile tilapia Containing either Soybean meal, Canola Meal or Sunflower meal as a Replacement of 10% (On CP basis) of Fishmeal

	Diet 1	Diet 2	Diet 3	Diet 4
Proximate composition				
Dry matter	90.9±0.07 ^{dbc}	91.31±0.16 ^{dbca}	91.00±0.09 ^{dbca}	91.56±0.19 ^{abc}
Crude protein	30.57±0.43 ^a	30.76±0.53 ^a	30.34±0.31 ^a	31.35±0.33 ^a
Ether Extracts	7.55±0.27 ^{cd}	7.67±0.18 ^{cd}	10.75±0.28 ^a	9.63±0.18 ^b
Ash	6.16±0.03 ^{abc}	5.60±0.24 ^{abc}	5.40±0.21 ^{dbc}	5.81±0.17 ^{abc}
Crude fibre	11.06±0.08 ^d	12.18±0.12 ^c	13.37±0.17 ^b	16.03±1.00 ^a
Nitrogen free Extracts	42.45±0.21 ^{ab}	42.79±0.65 ^{ab}	37.44±0.56 ^{cd}	36.09±0.51 ^{cd}
Neutral detergent fibre	24.07±0.22 ^{ca}	24.41±0.31 ^{abc}	24.41±0.23 ^{bac}	23.08±0.34 ^d
Acid detergent fibre	8.37±0.25 ^{cd}	8.23±0.30 ^{dc}	11.83±0.20 ^{ba}	11.86±0.47 ^{ab}

Values are expressed as mean ± SE. ^{a, b, c, d} Values in the same row having different superscript letters are significantly different ($P<0.05$).

3.3.5 Diets Fatty Acid Composition

The fatty acid composition of the diets is presented in Table 10. Soybean meal based diet had a high level of palmitic acid (16:0) compared to the other diets. However canola meal based diet recorded the highest level of saturated fatty acid. Diet 1 (FM based) recorded highest amount of polyunsaturated fatty acid (32.06 mg/100g) followed by sunflower meal based diet (30.27 mg/100g). Total monounsaturated acid also reduced in fishmeal based diet.

Table 10: Fatty Acid Composition (mg/100g) of the Formulated Diets for Nile tilapia Containing either Soybean meal, Canola Meal or Sunflower meal as a Replacement of 10% (On CP basis) of Fishmeal.

Fatty Acids	Diet A	Diet B	Diet C	Diet D
Saturated				
Myristic acid (14:0)	4.14±0.01 ^d	4.47±0.01 ^b	4.36±0.01 ^c	5.11±0.01 ^a
Palmitic acid (16:0)	15.05±0.01 ^c	15.35±0.01 ^a	14.77±0.01 ^d	15.16±0.00 ^b
Stearic acid (18:0)	2.98±0.01 ^c	2.12±0.01 ^d	3.22±0.01 ^b	3.26±0.01 ^a
Total saturated	22.21	21.95	22.36	23.55
Mono-unsaturated				
Palmitoleic acid (16:1n-7)	5.36±0.01 ^{ba}	5.17±0.01 ^c	4.81±0.01 ^d	5.37±0.01 ^{ab}
Oleic acid (18:1n-9)	12.75±0.01 ^d	13.50±0.01 ^b	13.13±0.01 ^c	13.73±0.01 ^a
Eicosenoic acid (20:1n-9)	2.50±0.01 ^d	2.71±0.01 ^b	2.66±0.01 ^c	2.82±0.01 ^a
Erucic acid (22:1n-9)	4.54±0.01 ^c	6.12±0.01 ^b	6.90±0.01 ^a	5.36±0.01 ^d
Nervonic acid (24:1n-9)	0.81±0.01 ^b	0.86±0.01 ^a	0.32±0.01 ^d	0.49±0.01 ^c
Total mono-unsaturated fatty acid	25.98	28.32	27.81	27.76
Polyunsaturated				
Linoleic acid (18:2n-6)	15.64±0.01 ^a	14.71±0.01 ^c	15.23±0.01 ^b	12.24±0.01 ^d
Gamma-linolenic acid (18:3n-6)	0.17±0.00 ^{ab}	0.11±0.00 ^d	0.13±0.00 ^c	0.15±0.00 ^{ba}
Arachidonic acid (20:4n-6)	0.62±0.01 ^{ab}	0.53±0.01 ^{cd}	0.52±0.01 ^{dc}	0.61±0.01 ^{ba}
Eicosatrienoic acid (20:3n-3)	0.10±0.01 ^a	0.10±0.01 ^a	0.11±0.01 ^a	0.10±0.01 ^a
Eicosapentaenoic acid (20:5n-3)	6.89±0.01 ^b	6.33±0.01 ^c	7.14±0.01 ^a	5.93±0.01 ^d
Docosahexaenoic acid (22:6n-3)	8.64±0.01 ^d	7.64±0.01 ^a	7.02±0.01 ^c	6.87±0.01 ^d
Total polyunsaturated fatty acids	32.06	29.87	30.15	30.27

Values are expressed as mean ± SE.^{a, b, c, d} Values in the same row having different superscript letters are significantly different (P<0.05).

3.3.6 Diets Amino Acid Composition

Amino acid composition of the diets is as shown in Table 11. Diet 4 had lowest content of essential amino acid methionine (0.86mg/100g), lysine (6.83 mg/100g), phenylalanine (2.54 mg/100g), histidine (1.50 mg/100g) and valine (2.40 mg/100g) (P<0.05). Diet 1 recorded highest values (P<0.05) for essential amino acid lysine (8.12 mg/100g), phenylalanine (3.42 mg/100g), histidine (2.32 mg/100g), valine (2.79 mg/100g) and threonine (2.65 mg/100g). Methionine content of diet 2 was highest (1.05mg/100g) followed by diet 1 (0.93mg/100g), diet3 (0.92 mg/100g) with diet 4(0.86 mg/100g) recording least. Statistically, methionine content of diet 1 (0.93 mg/100g) and diet 3 (0.92 mg/100g) was similar (P>0.05).

Table 11: Amino Acid Composition (mg/100g Protein) of the Diets (%), Formulated for Nile tilapia Containing either Soybean meal, Canola Meal or Sunflower meal as a Replacement of 10% (On CP basis) of Fishmeal.

	Diet1	Diet 2	Diet 3	Diet 4
Essential Amino Acids				
Isoleucine	2.3±0.01 ^b	1.34±0.01 ^c	1.23±0.01 ^d	1.42±0.00 ^a
Leucine	4.43±0.01 ^a	3.12±0.00 ^d	3.89±0.01 ^b	3.57±0.01 ^c
Arginine	5.52±0.01 ^a	4.55±0.01 ^d	4.61±0.01 ^c	4.73±0.01 ^b
Valine	2.79±0.01 ^a	2.46±0.01 ^b	2.42±0.00 ^{cd}	2.40±0.01 ^{dc}
Methionine	0.93±0.01 ^{bc}	1.05±0.01 ^a	0.92±0.01 ^{cb}	0.86±0.01 ^d
Lysine	8.12±0.01 ^a	7.35±0.01 ^b	7.19±0.01 ^c	6.83±0.01 ^d
Phenylalanine	3.42±0.01 ^a	2.57±0.01 ^{bc}	2.56±0.00 ^{cbd}	2.54±0.01 ^{dc}
Histidine	2.32±0.01 ^a	1.65±0.00 ^c	1.70±0.01 ^b	1.50±0.01 ^d
Threonine	2.65±0.01 ^a	2.30±0.01 ^b	2.15±0.01 ^d	2.25±0.01 ^c
Non-essential amino acids				
Aspartic acid	5.13±0.01 ^a	4.68±0.01 ^b	3.58±0.01 ^c	3.04±0.01 ^d
Glutamic acid	8.76±0.01 ^a	7.53±0.01 ^b	7.08±0.01 ^d	7.35±0.01 ^c
Proline	0.18±0.00 ^a	0.11±0.00 ^{cd}	0.11±0.00 ^{dc}	0.13±0.00 ^b
Serine	3.31±0.01 ^a	2.64±0.01 ^c	2.52±0.00 ^d	2.67±0.01 ^b
Glycine	4.25±0.01 ^a	3.22±0.00 ^c	3.11±0.01 ^d	3.31±0.01 ^b
Tyrosine	1.00±0.00 ^a	0.78±0.01 ^b	0.66±0.01 ^d	0.73±0.01 ^c
Alanine	0.19±0.00 ^b	0.11±0.00 ^d	0.15±0.00 ^c	0.21±0.00 ^a

Values are expressed as mean ± SE.^{a,b,c,d}. Values in the same row having different superscript letters are significantly different (P<0.05).

3.3.7 Chemical Scores and Essential Amino Acid Index of Feed Ingredients

Ingredients chemical scores are as shown in Table 12. Fishmeal recorded highest chemical scores for all essential amino acids which were above 100. In all the ingredients, chemical scores for lysine were below 100 except for fishmeal (152.47). The chemical score for oilseed meals were below 100 apart from SFM histidine (316.47), threonine (103.11), valine (224.04), leucine (296.75) and phenylalanine+tyrosine (155.46), SBM (leucine (108.75) and CM (leucine (111.50) and phenylalanine+tyrosine (102.47)). Maize meal and wheat had scores above 100 except lysine (27.79 and 34.25) and Arginine (57.54 and 66.98) respectively.

Table 12: Chemical Scores (%) and Essential Amino Acid Index (EAAI) of Ingredients (%) used to Formulate Diets for *Oreochromis niloticus*

	Fish Meal	Soybean meal	Canola Meal	Sunflower meal	Maize meal	Wheat bran
Amino Acid						
Lysine	152.47±1.30 ^a	58.79±.22 ^d	78.32±.22 ^b	61.32±.19 ^c	27.79±.06 ^f	34.25±.28 ^e
Methionine +Cystine	143.28±.21 ^a	47.63±.33 ^f	66.29±.33 ^e	76.49±.98 ^d	119.52±.33 ^c	121.39±.89 ^b
Histidine	140.39±.46 ^a	73.06±.77 ^e	91.08±.19 ^f	316.47±.70 ^a	123.25±.67 ^c	105.43±.70 ^d
Arginine	139.76±.96 ^a	80.64±.28 ^b	72.7000±.51 ^c	70.39±.15 ^d	57.54±.21 ^f	66.98±.20 ^e
Threonine	114.22±.38 ^a	52.18±1.1 ^f	56.00±1.46 ^e	103.11±.09 ^b	69.24±.23 ^d	84.17±.17 ^c
Valine	192.97±.24 ^b	79.88±.31 ^f	83.45±.31 ^e	224.04±.66 ^a	145.95±.31 ^d	176.07±.41 ^c
Isoleucine	146.30±2.09 ^a	75.77±.39 ^e	81.99±.19 ^d	31.19±.67 ^f	104.82±.32 ^c	123.15±.32 ^b
Leucine	222.81±1.04 ^b	108.75±.43 ^f	111.50±.45 ^e	296.75±.17 ^a	211.01±.43 ^c	202.16±.49 ^c
Phenylalanin e+Tyrosine	200.53±1.46 ^a	72.26±.55 ^f	102.47±.40 ^e	155.46±.15 ^d	199.91±.23 ^b	162.84±.44 ^c
EAAI	1.48±0.01 ^a	0.70±0.01 ^f	0.80±0.01 ^e	1.14±0.00 ^b	0.93±0.00 ^d	1.02±0.06 ^c

Values are expressed as mean ± SE^{a,b,c,d,e,f}. Values in the same row having different superscript letters are significantly different (P<0.05).

3.3.8 Chemical Scores and Essential Amino Acid Index of Diets

Chemical scores and essential amino acid indices for the diets are as shown in Table 13. Diet containing FM recorded chemical scores above 100 for all essential amino acids except Isoleucine (73.95), methionine + cysteine (34.58) and threonine (70.58). Replacement of FM with oil seed meals recorded scores below 100 except Arginine, Lysine and leucine (CM and SFC). The values for methionine were all below 100. Fish meal diet recorded highest essential amino acid index (0.97) while its replacement by SFM had the lowest (0.76).

Table 13: Chemical Scores (%) and Essential Amino Acid Index (EAAI) of Diets (%), Formulated for Nile tilapia Containing either Soybean meal, Canola Meal or Sunflower meal as a Replacement of 10% (On CP basis) of Fishmeal.

	Diet 1	Diet 2	Diet 3	Diet 4
Chemical Scores				
Isoleucine	73.95±0.18 ^b	42.98±0.28 ^c	39.44±0.28 ^d	45.55±0.11 ^a
Leucine	130.58±0.97 ^a	91.94±0.10 ^d	114.85±0.43 ^b	105.31±0.17 ^c
Arginine	131.51±0.08 ^a	108.41±0.16 ^d	109.84±0.16 ^c	112.54±0.08 ^b
Valine	99.76±0.31 ^a	87.74±0.31 ^b	86.55±0.12 ^{cd}	85.83±0.24 ^{dc}
Methionine +Cystine	34.58±0.25 ^{bc}	39.06±0.25 ^a	34.33±0.21 ^{cb}	32.21±0.25 ^d
Lysine	158.59±0.11 ^a	143.49±0.17 ^b	140.37±0.17 ^c	133.46±0.17 ^d
Phenylalanine+Tyrosine	118.04±0.23 ^a	89.24±0.44 ^b	86.05±0.32 ^d	87.38±0.09 ^c
Histidine	134.88±0.34 ^a	96.12±0.19 ^c	99.03±0.51 ^b	87.21±0.58 ^d
Threonine	70.58±0.18 ^a	61.24±0.23 ^b	57.24±0.23 ^d	60.09±0.18 ^c
Essential Amino Acid Index(EAAI)	0.97±0.00 ^a	0.78±0.00 ^{bc}	0.77±0.08 ^{cb}	0.76±0.00 ^d

Values are expressed as mean ± SE. ^{a,b,c,d} Values in the same row having different superscript letters are significantly different (P<0.05).

3.4 Discussion

3.4.1. Proximate Nutrient Composition of the Ingredients

Generally, the proximate composition of the ingredients in this study was within the values obtained by Oduho *et al.*, (2005); Kirimi *et al.*, (2016); Maina *et al.*, (2007). However, the differences in nutrient composition of feeds and feed stuffs could be attributed to the place of origin, production, processing methods and even adulteration by unscrupulous traders (Anjum *et al.*, 2012, Kirimi *et al.*, 2016a). In the present study, the ingredients fishmeal, soybean meal, canola meal and sun flower meal had protein contents of (62.60%, 47.38%, 34.39% and 24.81%) respectively; these values characterize them as protein feedstuffs (Tacon & Barg, 1999; Lovell, 1998)

The crude protein value of fishmeal (*Rastrionaebola argentea*) recorded in this study was within the range of values obtained by Kirimi *et al.*, (2016a) but higher than that obtained by Maina *et al.*, (2007) who reported a CP of 55%. Fish meal processed from whole fish is of high protein quality above 60% (Prado *et al.*, 2016). This could have attributed to the high crude protein content of fish meal in this study. According to Drewet *et al.*, (2007), Anjum, *et al.*, (2012), the crude protein content of fish meal range from 50% to 70% depending on fish species, the source and processing method. Ash content was (15.22%); a figure close to that obtained by Kirimi *et al.*, (2016) but above that obtained by Oduho *et al.*, (2005). Contamination with sand and organic

matter from the drying surfaces may cause the differences in ash and crude fiber contents. Fish meal obtained from fish leftovers has high ash content (Millamena, 2002; Boscolo *et al.*, 2004) which is a limitation when included at high levels in feed as it raises the phosphorus. Ether extract for fish meal was 7.55%, a figure above that recorded by kirimi *et al.*, 2016a (5.07%) but below that of Oduho *et al.*, (2005). The difference in ether extract is possibly due to age or seasonal variation. According to Okot (1995) pelagic fishes like *Rastrionaebola argentea* have two oil cycles, the first beginning December-January (low oil content) which rises to a peak in May-June.

In the present study, the crude protein (47.38%) of soybean meal was within the range reported by NRC (1993), but lower than those by Zambom, *et al.*, (2001) who observed the value of 51.41% crude protein. This variation in protein content in soybean meal is mostly due to method of oil extraction. According to Li *et al.*,(2000a), expeller process of oil extraction from soybeans results in a meal containing approximately (42% crude protein), solvent-extraction (44% crude protein) if hulls are included or 48% crude protein without the hulls (NRC, 1993). However, full-fat soybean meal produced by heat treating whole soybean contain approximately 38% crude protein (Lim & Akiyama, 1992). Despite high CP in soybean meal recorded in this study, research by kirimi *et al.*, (2016a) recorded 11% CP which was attributed to adulteration by unscrupulous dealers.

Canola meal had a protein content of (34.39%), (Table 2), which was below the figures reported by (Adewole *et al.*, 2016). The low CP content of canola meal in this study may be attributed by inadequate oil extraction which was recorded to be high (23.88%). Dry season conditions create lower oil content and higher level of protein content (Spragg & mailer 2007). According to Bell and Keith (1991), the proximate composition of canola meal may vary due to cultivars, environment conditions during growing and harvesting periods and crushing conditions.

Sunflower meal had a protein content of (24.81%) which is lower than 28% CP reported by Maina *et al.*, (2007). This may be attributed to the high fibre content (36.38%) which according to Maina *et al.*, (2007), protein concentration in sunflower cake is inversely proportional to the fibre content. The chemical composition of

sunflower seed depends on the weather, soil, variety and method of cultivation of the crop (Karunajeewa *et al.*, 1989; Senkoğlu & Dale, 1999). The fat content was (13.31%). This difference in values for the crude fat may be due to type of processing method used before ether extraction (Akande, 2011). The crude fibre reported in sunflower was (36.38%) and varies between 14% and 39% (Villamide & San, 1998).

The crude protein content of maize meal was (10.65%). The nutritive value of maize protein varies according to cultivar, type of grain (dent, flint, dent/flint), growing conditions (Korniewicz *et al.*, 2000), grain drying temperature (Kaczmarek *et al.*, 2007), starch structure (Svihus *et al.*, 2005), and presence of ant nutrients, primarily, phytate, enzyme inhibitors, and resistant starch (Cowieson, 2005).

3.4.2. Proximate Nutrient Composition of the Diets

The crude protein content of the four diets in the present study was near isoproteinous (Table 9). This was attributed to initial analysis of ingredients prior to formulation of diets in order to balance for the CP. Dietary protein plays a significant role in supplying amino acids for the biosynthesis of the body proteins which are essential for the growth of fish (Alam *et al.*, 2016). Though the protein content in the four diets was isoproteinous, the concentration of protein ingredients (fish meal, soybean meal, canola meal and sunflower meal) varied because of differences in CP. This was in attempt to compensate for the varying nutrients among the ingredients. It's worth noting that this variation of major protein ingredient (Fish meal, soybean meal, canola meal and sunflower meal) in each diet led to considerable changes in other nutrients (Table 5). However, the diets in the present study meet the protein requirement for Nile tilapia fingerlings weighing 10-30g and 30g to market size which according to Jauncey, (1998); Suresh, (2003) is 25% to 30% CP. Luquet (1991) reviewed several studies and recommended a protein content of 30 -35% as the optimum for tilapia.

The crude fat content of diets observed in this study was a reflection of the crude fat content in the major protein ingredients used (FM, SBM, CM and SFM). Diet 3 recorded highest amount of crude lipid (10.75%). This was attributed to the high levels of canola meal in diet 3 where it formed the bulk of the protein with a lipid content of (23.88%). Conversely, diet 1 recorded the lowest crude fat content (7.55%)

followed closely by diet 2(7.67%). In the two diets (diet 1 and 2), fishmeal and soybean meal formed the bulk of protein with crude fat content of (7.49%) and (9.27%) respectively. According to NRC, (1993), dietary lipids are important sources of energy and of essential fatty acids (EFA) needed for normal growth and development and assist in the absorption of fat-soluble vitamins. In the present study, the dietary lipid content was within the levels recommended (5% to 12%) for tilapia (Suresh, 2003). However, it is important to profile the fatty acid in the diets of Nile tilapia in order to ascertain the presence of essential fatty acids. This is because the fatty acid composition of the dietary lipid has a significant influence on the tissue fatty acid composition of the fish (Watanabe, 1982; Henderson & Tocher, 1987; Sargent *et al.*, 1989).

The crude fibre content in the present study significantly varied among the diets ($P < 0.05$). This variation in crude fibre was due to varying of ingredients in order to balance for the crude protein. The high fibre content in diet 4 (16.03%) could have been attributed by the high fibre content in sunflower meal (36.38%) where it formed the bulk of the protein. This was followed by diet 3 where canola meal (15.58% crude fibre) formed the bulk of protein. In diet 1, the low fibre content recorded (11.06%) was due to high fish meal content which had low fibre content (1.04%). Crude fibre in the feed gives it the physical bulkiness, improves binding and moderates the passage of feed through the alimentary canal (Ayuba & Iorkohol, 2012, Obeng *et al.*, 2015). However, monogastric animals including fish are generally unable to digest fibre because they do not secrete cellulase (Bureau *et al.*, 1999). According to De Silva & Anderson (1995), the recommended fibre content in fish feed is 8-12%. Based on this, the fibre content for diet 1 and 2 were within the range for Nile tilapia diets. However, diet 3 and 4 was far above the recommended (13.37% and 16.03%) respectively. High levels of fibre content reduces the total dry matter, lowers the digestibility of nutrients, slows growth, adds to the faecal waste which affects the water quality and hence fish performance (De Silva & Anderson, 1995, Lovell, 1998).

Cell wall carbohydrates can be quantified by determination of neutral detergent fibre (NDF), which includes cellulose, hemicellulose and lignin as the major components (Van Soest *et al.*, 1991). In the present study NDF was closely related among the diets

though diet 4 had a significant difference ($P < 0.05$) with others. NDF represents the structural fibre, which is only partially digestible, and lignin is the fraction of NDF that is totally indigestible. On the other hand, diet 3 and 4 recorded higher figures for acid detergent fibre (ADF) (11.83% and 11.86%) respectively. Based on the high NDF compared to the low ADF figures reported in this study, it implies that a higher portion of the crude fibre in the diets would be digested. However, according to Adewole (2016), the detergent methods were originally developed by Van Soest' for forage analysis. When the same neutral detergent fibre method is used for fibre analysis of cereal grains or protein supplements of monogastric animal diets, a significant underestimation of NSP (Non starch polysaccharide) and thus total dietary fibre content occurs.

The ash content in this study slightly varied among the diet with diet 1 recording slightly higher figure (6.16%). This could be attributed to the high fish meal inclusion with (15.22%) ash content. However, Fish, unlike most terrestrial animals, can absorb some minerals (inorganic elements) not only from their diets but also from their external aquatic environment (NRC, 1993).

3.4.3 Fatty Acid Composition of Ingredients and Diets

According to Watanabe (1982); Sargent *et al.*, (1989), it is important to profile the fatty acid in the diets of Nile tilapia in order to ascertain the presence of essential fatty acids. This is because the fatty acid composition of the dietary lipid has a significant influence on the tissue fatty acid composition of the fish. In the present study, fishmeal recorded highest concentration of fatty acids and this is in agreement with Pike, (1999) that fish meal provides fat rich in long chain omega-3 fatty acids. In the oilseed meals (soybean meal, canola meal and sunflower meal), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) were not detected. This agrees with Turchini *et al.*, (2011) that none of the vegetable oils contain omega 3 (n-3) long chain polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, but they are rich in other fatty acids, mainly stearic acid (18:0), linoleic acid (18:2 n-6) and gamma-linolenic acid (18:3 n-6). Diet 1 recorded lower levels of linoleic acid (12.2 mg/100g), attributed to the low amount detected in fishmeal compared to other ingredients (Table 7). Linoleic acid (18:2n-6) and linolenic (18:3n-3) acids, once obtained from the feed, can be further elongated and desaturated to

produce highly unsaturated fatty acids (HUFA), such as arachidonic acid (ARA)(20:4n-6), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sprecher *et al.*, 1995; Sprecher, 2000). However, the extent to which n-3 HUFA are produced by the elongation and desaturation of 18:3n-3 depends on fish species, life stage and fish size, water temperature and feed, including the dietary lipid source of the fish (Tocher *et al.* 2000; Tocher *et al.*, 2003). Polyunsaturated fatty acid (PUFA) must be provided in the diets. Based on this, the three very long chain polyunsaturated fatty acids i.e docosahexaenoic acid (22:6n-3), eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6) were detected in the diets. Fishmeal based diet recorded higher values for total polyunsaturated fatty acid (32.1mg/100g) attributed to the higher quantity of FM in diet 1. It's worth noting that although most of the fatty acids were not detected in the oilseed meals, they appeared in the diets. This could be due to their presence in minute quantities which could not be detected but upon mixing of the different ingredients, they rose to detectable levels. According to Ariful Alam *et al.*, (2014), research on the fatty acid requirements of Nile tilapia has produced contradictory results. This could be attributed to various factors such as length of experiment, nutritional history of the experimental fish, size of fish, source of dietary lipids and water temperatures. However, considering that fish are not able to synthesize both the omega-3 and omega-6 series of fatty acids and require them in their diets and based on fatty acid requirements determined for other fish species, it is recommended that a provision of both omega-3 and omega-6 polyunsaturated fatty acid should be included in the Nile tilapia feeds (Ariful Alam *et al.*, 2014).

3.4.4. Ingredients Amino Acid Composition

Fish meal (*Rastrionaebola argentea*) displayed better and higher amino acid profile than other ingredients and according to Drew *et al.*, (2007); fish meal is the “gold standard” to which plant proteins must be compared in terms of protein quality. The amino acids results in this study are comparable to those reported by Maina *et al.*, (2007) and Oduho *et al.*, (2005). Amino acid composition of fishmeal obtained in this study revealed that among the EAA, lysine had the highest concentration with values of (7.81 mg/100g), closely followed by arginine with a value of (7.55 mg/100g). High levels of essential amino acids in fish meal as in the present study makes it more

desirable in fish feed. However, high costs and scarcity is the major limitation to its use (Kirimi *et al.*, 2016a).

Soybean meal in the present work recorded low levels for methionine and cysteine. This is in agreement with the report by NRC (1993) that methionine and cysteine are the two most limiting amino acids in soybean meal. However, Banaszkievicz (2000) reported low levels of tryptophan. NRC, (2012) reported slightly higher levels for methionine (0.66) and cystine (0.70) but lower levels for lysine (2.96) and phenylalanine (2.40). The present study agrees with Storebakken *et al.*, (2000) that soybean is widely used plant protein ingredient in many aquaculture species due to relatively well balanced amino acid.

The essential amino acid composition of canola meal figures recorded in this study was below those obtained by Adem *et al.*, (2014). However, levels of methionine and cystine (0.61 and 1.16 mg/100g) recorded in this study contradicts with the findings of Chen *et al.*, (2015) who reported (0.91) and (1.21) for methionine and cystine respectively but lower (0.71) than that recorded by NRC (2012). More so, levels for lysine were higher than those recorded by NRC, (2012) for lysine (4.01), leucine (3.78) and phenylalanine (4.01). According to Bell and Keith, (1991); Newkirk, (2009), the concentration of amino acids in canola meal varies depending on varieties, environmental factors, canola seed composition, and amount of residual oil and carbohydrates in the meal. Level of heat duration contributes to degradation of heat sensitive amino acids such as lysine (Newkirk, 2009).

Amino acid profile for maize meal was slightly lower than those reported by Lasek *et al.*, (2012) except for methionine which was higher. Cystine (1.04 mg/100g) was the first limiting amino acid in maize meal closely followed by Lysine (1.42) and tryptophan was not detected. This agrees with the observation of Olson and Frey (1987) that maize meal is deficient in the essential amino acids tryptophan and lysine and this limits the amount of protein available for monogastric animals. In contrast, Paes and Bicudo (1995) argued that specialty maize with increased levels of the essential amino acids lysine and tryptophan has been produced in developing countries as a strategy to improve protein quality of maize. However, the level of

aromatic amino acid (phenylalanine, 4.24 and tyrosine, 3.25 mg/100g) was high which corroborate with Makanjuola and Olakunle (2017) that maize meal is high in aromatic amino acids.

In the present study sunflower meal amino acid analysis showed that, methionine recorded the lowest figure (0.51 mg/100g) hence the most limiting amino acid in all the ingredients. However, this observation contradicts the findings of Vieira *et al.*, (1992) who reported that lysine was the first limiting amino acid in broiler sunflower meal based diets. The levels of leucine and valine was high, a similar report made by Akande (2011). This variation in chemical composition of sunflower meal may be due to method of processing and oil extraction (Karunajeewa *et al.*, 1989; Viera *et al.*, 1992). According to Akande (2011), the concentration of sulfur amino acids drastically decreases with heat processing.

3.4.5 Diets Amino Acid Composition

In the diets, all the essential amino acids except tryptophan were detected though there was slight variation among them (Table 11). This variation in amino acid contents of the diets could have been attributed to the varying proportion of the ingredients in order to balance for the crude protein and more so amino acids. Diet I displayed a better amino acid profiles with higher levels of leucine, arginine, valine, lysine, histidine, threonine and phenylalanine ($P < 0.05$). The high concentration of essential amino acids in diet 1 could be attributed to the high levels of fish meal used i.e double the amount used in diet 2, 3 and 4. According to De Silva and Anderson (1995), the EAA profiles of plant proteins used in feed formulation are usually poor, implying they are deficient of one or more EAAs, compared to the requirements of the animal. This could have contributed the low amino acid levels observed in diet 2, 3 and 4 in the present study where soybean meal, canola meal and sunflower meal formed the bulk of protein. However, mixing of different ingredients as in the case of present study, might have contributed to rising of some EAAs in the four diets i.e methionine, lysine, valine and argenine. According to Jubie *et al.*, (2015), animal nutritionist recommend varied sources of protein for reasons that each source could be complimentary with another source which ultimately could balance the essential amino acids.

In the present study, the concentration of sulfur containing amino acids (methionine and cystine) in the diets decreased with substitution of fishmeal though diet 2 recorded slightly higher levels (1.08) ($P < 0.05$). However, methionine levels in the four diets were low (Table 11) against the recommended (2.68) for Nile tilapia (NRC, 1993). This could have been attributed by low levels of methionine recorded in the ingredients (Table 8). This agrees with Lovell, (1989); Furuya *et al.*, (2004) that methionine is an essential amino acid for fish and it is usually the first limiting amino acid in many fish diets. Methionine acts as methyl donor, precursor of several substrates, including nucleic acids, proteins, phospholipids, biogenic amines, carnitine, cystine, choline, polyamines and other metabolic intermediates (Mato *et al.*, 1997; Zhou *et al.*, 2006). Despite low levels of cysteine detected in the ingredients, it was not translated into the diets. This could have been caused by presence of negligible quantities after formulating the diet which was not sufficient to be detected. However, there exists a relationship among amino acids such that cystine can be formed metabolically from dietary methionine at a rate sufficient to meet the requirements of fish but the reverse sequence of reactions does not occur (NRC, 1993). In the present study, cystine requirement could not be met by methionine because it was deficient.

The results of this study indicate that Lysine levels in all the diets were above the recommended by Santiago and Lovell (1988) of 5.1 g/100 g crude protein for Nile tilapia. This could have been attributed by the high levels of lysine in all the ingredients except in maize meal and wheatbran (Table 8). Thus, lysine requirements were met. However, excess lysine reported in this study could lead to amino acid toxicity. It has been reported that excessive levels of amino acids may become toxic and have an adverse effect on growth because the disproportionate amount of one amino acid affects the absorption and utilization of other amino acids (Murthy & Varghese 1996).

Phenylalanine+tyrosine were present in the four diets (Table 11). However, only diet 1 met the requirement of (3.75) for Nile tilapia recommended by NRC (1993). Phenylalanine, an aromatic indispensable amino acid is required for normal growth and metabolic processes. It is the sole precursor of tyrosine. Phenylalanine can be

converted to tyrosine by tetrahydrobiopterin-dependent phenylalanine hydroxylase in liver and kidneys but phenylalanine cannot be synthesized back from tyrosine (NRC, 1993). Inclusion of adequate levels of tyrosine reduces the phenylalanine requirement. Hence, to satisfy the total aromatic amino acid requirement of fish, feeds should be formulated with required amount of phenylalanine and adequate tyrosine.

Histidine is an essential amino acid for hemoglobin synthesis in aquatic and terrestrial animals and is important for growth, tissue formation and repair as well as for the maintenance of osmoregulation and myelin sheaths that act as protectors for nerve cells (Ahmed, 2013). In the present study, only diet 1 met the requirements for histidine (2.32) against the recommended (1.75). This is attributed to the low levels of histidine in the ingredients.

Valine levels in the present study were slightly lower than the recommended levels (2.8). Chung and Bakerv (1992) described that valine deficiency reduces the use of other limiting amino acids for protein deposition, and is thus essential. Isoleucine was significantly low in all the diets. According to Khan and Abidi (2007), the determination of essential amino acid requirements, besides methionine, lysine, and threonine is mainly important in low protein diets and those developed from plant foods, and Isoleucine is highlighted because of its important role in protein synthesis and in energy metabolism of skeletal muscle. Antagonism between branched-chain amino acids arises in animals from an excess of leucine over isoleucine and valine; the first two steps of the catabolic breakdown of all three branched-chain amino acids are catalyzed by the same enzymes (NRC, 1993). Based on this, in the present study, there could be antagonism of the branched chain amino acids because leucine content in all the diets was in excess of isoleucine and valine. Diets with branched amino acid deficiencies result in decreased response from lymphocytes and stimulating agents, increased susceptibility to infections, and growth reduction (Calder, 2006).

3.4.6. Chemical Score and Essential Amino Acid Index

The essential amino acid producing the lowest chemical score is termed the 'first limiting EAA' the next lowest chemical score 'second limiting EAA (Jauncey, 1998). In the present study (Table 12), Fishmeal as an ingredient recorded highest chemical

scores for most essential amino acids and no essential amino acid was limiting. This is in agreement with De Silva and Anderson, (1995) that there is no single foodstuff that can serve as an alternative to fish meal. Generally, the oil seed meals were poor sources of EAA for fish apart from leucine, Phenylalanine + Tyrosine (CM and SFM), valine (SFM), Threonine (SFM and histidine (SFM) (Table 12). Among the oilseed meals, sunflower meal was a better source of EAA apart from isoleucine which was the 1st limiting amino acid. In wheatbran and maize meal, lysine was the first limiting amino acid. Sunflower had highest EAAI among the oilseed meals (1.14) and this was mainly due to high levels of leucine (10.06%), valine (6.27%) and histidine (5.44%).

In all the diets, methionine had the lowest chemical scores hence the first limiting amino acid and isoleucine second limiting amino acid. The Essential Amino Acid Index (EAAI) recorded in the present study varied significantly among the four diets ($P < 0.05$) with diet 1 recording highest (0.97). This variation in EAAI is attributed to the corresponding fluctuations in respective amino acids in the diets. It's worth noting that in SFM based diet (4) EAAI was lowest (0.76) despite high EAAI in sunflower meal (1.14). This scenario could be attributed to the low CP in the SFM (24.81%) hence large quantity was required to balance for the CP in SFM based diet. Also the low levels of methionine (0.51) and isoleucine (0.97) in sunflower could have contributed to this. The findings in the current research closely agree with the study done by Kirimi *et al.*, (2016a) where diet with more fishmeal recorded highest EAAI. This is because the more fishmeal used translated to more amino acids in the diet. Based on Oser (1959) criterion for classifying protein quality, diet 1(0.97) could be considered good quality protein while diet 2 (0.78), diet 3(0.77) and diet 4(0.76) useful protein sources.

3.5 Conclusion

Proximate nutrients results show that all the diets meet CP requirement for Nile tilapia. There was an increase in crude fibre content by substituting FM with CM and SFM that was due to their high CF and low CP content, thereby requiring high levels to give the required CP content (30%) of the ration. Fatty acid profile of the diets improved on FM inclusion. However, considering the results of amino acid profile of the diets, chemical scores and the Essential Amino Acid Index, it can be concluded

that all the diets were not satisfactory in the content of essential amino acids despite higher EAAI for diet 1. This is because they were all limiting in methionine, with chemical score lower than the standard. However, substituting fishmeal with soybean meal, canola meal and sunflower meal gave useful protein sources.

CHAPTER FOUR

ENZYME ASSAY TO DETERMINE OPTIMUM CRUDE PAPAIN LEVEL ON PLANT PROTEIN DIETS FOR NILE TILAPIA (*Oreochromis niloticus*)

4.1 Introduction

Improving the nutrient digestibility and growth performance has been one of the most important nutritional aspects in animal production, be it in poultry, piggery or pisciculture (Debnath *et al.*, 2005). In the utilization of dietary nutrients, the digestive enzymes play a vital role in catalyzing the hydrolytic reactions splitting the macromolecules into simple absorbable form of molecules (Manush *et al.*, 2013). According to McDonald *et al.*, (2010), Asmare (2014), not all compounds in animal feed are broken down by animals' own digestive enzymes, and so some potential nutrients are unavailable to the animal. However, although enzymes are produced by the animal itself or by the microbes naturally present in the digestive tract, specific activities necessary to break down some compounds in feed are not found or are at low levels in the digestive tract. Based on this, animal nutritionists help the animal by identifying these indigestible compounds and feeding a suitable enzyme (Khattak *et al.*, 2006). Enzymes are protein in biological systems which catalyze the rate of a reaction but they remain unchanged. They are involved in all anabolic and catabolic pathways of digestion and metabolism (Khattak *et al.*, 2006). Feed enzymes help break down anti-nutritional factors (e.g. fibre, phytate) that are present in many feed ingredients which interfere with normal digestion, resulting in reduced production and lower feed efficiency and can also trigger digestive upsets (Benford & Partridge, 2010). Poultry industry is the largest user of feed enzymes today and its highly integrated nature has driven a fast uptake of feed enzyme technology over the years. Also there is an increasing trend in the swine, ruminant and aquaculture industries to use feed enzymes (Plumstead, 2013).

In the aquaculture industry, the search for alternative protein sources to replace fish meal, plus concerns regarding the relatively low nutrient digestibility and the presence of an array of anti-nutritional factors in fish meal alternatives, has led to an increasing interest in feed enzymes and research into optimal applications (Plumstead, 2013). However, few studies have evaluated enzyme supplementation in feed for aquatic

organisms, and many dietary recommendations for aquatic organisms are based on results obtained for non-ruminants animals (Castillo & Gatlin, 2014).

Papain crude extract is a proteolytic enzyme that can break down peptide bonds of a protein molecule (Aravind *et al.*, 2013). The extract is typically derived from the sap of unripe papaya fruits or directly from the fruit which are still hanging on the tree (Li *et al.*, 2010; Jeana *et al.*, 2013; Macalood *et al.*, (2013); Nitsawang *et al.*, 2006). This enzyme has an important role in protein hydrolysis into amino acids which can be absorbed by the Intestine (Muchtadi *et al.*, 1992). Papain is characterized by its ability to hydrolyse large proteins into smaller peptides and amino acids which can be readily absorbed by the body (Ming *et al.*, 2002). Therefore, addition of exogenous enzymes like crude papain in fish feeds can improve nutrient utilization, thereby reducing nutrient losses (Mahmoud *et al.*, 2014). However, until now, no enzyme assay has been carried out to get a precise level of crude papain extract for use in Nile tilapia diets. In this regard, the method of *In vitro* protein digestibility can be employed to determine the best suited concentration of crude papain extract in plant protein based diets for Nile tilapia. According to Lazo *et al.*, (1998), *In vitro* enzyme assays are less expensive, less time consuming and easier method for determining protein digestibility by enzyme. It allows for close observations of the dynamics of the breakdown of protein by using only small amount of raw materials (Dimes & Haard, 1994). The current study therefore was conducted to determine chemical composition, enzymatic activity and optimal crude papain enzyme concentration for use in plant protein based diets for Nile tilapia.

4.2 Materials and Methods

This experiment involved extracting crude papain latex, processing, protease activity determination and evaluation of level of inclusion using *in vitro* protein digestibility assay.

4.2.1 Study Site

The experiment was conducted at Chuka University Animal nutrition and Biochemistry laboratory and Fletcher Scientific Solutions.

4.2.2 Preparation of Diets

The method of preparation of the experimental diets was as described in 3.2.2 in chapter 3 (Table 5).

4.2.3 Collection of Crude Papain Latex

Latex of *Carica papaya* was collected from locally grown plants in Imenti South District, Meru County. Initially, 4 to 6 longitudinal incisions 3 mm deep were made on the unripe mature fruit surface from fruit stalk end to the tip of the fruit by using a stainless steel knife. The exuded latex was allowed to run down the fruit and drip into aluminum tray. The latex was then sun dried (40°C for 14 h) (Adu, *et al.*, 2009). Using laboratory mortar and pestle, the dried latex was then ground to form a greenish or grey powder known as papain (Adu *et al.*, 2009; Panuphong & Pawinee, 2012; Battaa, *et al.*, 2013).

4.2.4 Research Design

A 4 X 4 factorial design was adopted considering 4 diets with papain enzyme at 4 levels (0.02%, 0.04%, 0.06% and 0.08%). The experimental units were replicated in triplicate.

4.2.5 Amino Acid and Proximate Analysis

The proximate analysis of ingredients and diets were carried out in triplicates as described in AOAC (1995). Amino acid analysis of the samples was performed by MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range of 780–2500 nm.

4.2.6 Enzymatic Activity Testing of Crude Papain Extract

Protease activity was determined using the Hammersten casein as substrate. The samples were passed through 60 mesh sieve to obtain uniform sample size. Approximately 0.12 g sample was weighed and 10 ml of each buffer was added. The mixture was stirred for 30 min and then centrifuged for 5 min at 12,000 rpm to obtain a clear supernatant and then diluted with the same buffer. The diluted enzyme solution was allowed to react with the substrate of desired pH for 10 min at 55°C. The reaction

was stopped by addition of trichloroacetic acid and the amount of tyrosine released was determined spectrophotometrically using a standard curve at 280 nm. Analysis was based on one unit of protease activity which releases 1.0 micromole of tyrosine.

4.2.7 Determination of *In vitro* Relative Protein Digestibility using Crude Papain Extract

In vitro methods for the protein digestibility assay were conducted using the pH drop method. At first the diets (except casein) were finely ground with a mortar and pestle to pass through a 180- μ m mesh screen. The diets were soaked with water for overnight at 4⁰ C. An equivalent amount of each diet that provided 312.5 mg of crude protein, determined by the respective material's proximate analysis was mixed with 50ml of distilled water and 0.02%, 0.04%, 0.06% and 0.08% of crude papain enzyme to produce suspension of 8mg crude protein per ml. The mixture was kept at pH 8 with the addition of dilute sodium hydroxide (NaOH) or hydrochloric acid (HCl) and temperature of 37.5⁰ C. The pH was recorded at every minute interval for 10 minutes by pH meter (HI 211 pH/ORP Meter, HANNA instruments). Casein was chosen as the reference protein because of its high protein digestibility (about 99%) (Akiyama *et al.*, 1989). The protein digestibility (PD) was calculated as the percentage of magnitude of pH drop ($-\Delta$ pH) ratio of the ingredient and casein (Lazo, 1994).

The RPD of the diets substrate under different enzyme treatment was calculated following the equation as follows:

$$[(-\Delta \text{pH}_{\text{Ingredients}} \div -\Delta \text{pH}_{\text{Casein}})] \times 100 \text{ (Lazo } et al., 1998)$$

Where

$-\Delta$ pH is the magnitude of pH decline in each assay

4.2.8 Data Analysis

Proximate, enzymatic activity and relative protein digestibility data were subjected to a two way analysis of variance (ANOVA) using SPSS statistical package version 17.0 at P= 0.05 confidence level to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

4.3 Results

Chemical analysis, enzymatic activity and *in vitro* digestibility data is reported below.

4.3.1 Proximate Analysis of Dried Crude Papain Extract

Proximate analysis of crude papain is as shown in Table 14 .Crude protein content was highest (66.61%) and crude fibre recording least (1.57%). The ash content was (6.89%) with ether extract recording (7.69%).

Table 14: Proximate Analysis of Dried Crude Papain Extract

Crude papain	Proximate composition					
	Dry Matter	Crude Protein	Ether Extract	Crude Fibre	Ash	Nitrogen Free Extract
	93.55±.08	66.61±.38	7.69±.36	1.57±.33	6.89±.22	16.98±.62

4.3.2 Amino Acid Profile of Crude Papain Extract

The concentration of amino acid in the crude papain is shown in Table 15. Glycine (23mg/100g) recorded highest concentration with cysteine (1 mg/100g) being least. Methionine was not detected. Isoleucine and lysine recorded the same concentration (9 mg/100g).

Table 15: Amino Acid Profile of Crude Papain Extract

Amino acid	Concentration (mg/100g)
Essential amino acid	
Arginine	10
Histidine	2
Leucine	8
Isoleucine	9
Lysine	9
Cysteine	1
Phenylalanine	4
Threonine	7
Tryptophan	3
Valine	14
Non-essential amino acid	
Glycine	23
Tyrosine	15
Glutamine	8
Asparagine	10
Serine	10
Aspartic acid	7
Glutamic acid	7
Proline	8

4.3.3 Protease Activity of Crude and Pure Papain

The protease activity of crude papain and pure papain (standard) is shown in Table 16. Crude papain recorded highest activity of (1.9 u/mg) and pure papain (1.5 u/mg).

Table 16: Protease Activity of Crude and Pure Papain

Enzyme	pH	Protease Activity [†] (units/mg)
Crude Papain	7.5	1.9±0.13
Pure Papain	7.5	1.5±0.11

[†]One unit of protease activity is the amount of enzyme that releases 1.0 micromole of tyrosine per minute

4.3.4 Relative *In vitro* Protein Digestibility

Results for the diets and enzyme *In vitro* protein digestibility are as shown in Table 17. Diet 1 recorded highest *In vitro* protein digestibility (39.96%) with diet 4 recording least (33.27%). Diet 2 recorded (35.84%) closely followed by diet 3 (34.82%). In the enzyme concentration, 0.06% recorded highest *In vitro* protein digestibility (39.16%) with 0.02% concentration recording least (30.23%). Enzyme

concentration of 0.08% was the second in *In vitro* protein digestibility (38.65%) and 0.04% recording (35.86%).

Table 17: Relative *In vitro* Protein Digestibility of the Four Diets and Crude Papain Enzyme at Different Concentrations

		Relative Protein Digestibility
Diet(D)		
	1	39.96±1.55 ^a
	2	35.84±1.12 ^{bc}
	3	34.82±1.17 ^{cbd}
	4	33.27±0.94 ^{dc}
Enzyme Concentration (EC)		
	0.02	30.23±0.64 ^d
	0.04	35.86±0.89 ^c
	0.06	39.16±1.16 ^{ab}
	0.08	38.65±1.00 ^{ba}
Level of significance		
Diet		P<0.05
EC		P<0.05
DXEC		P>0.05

Values are expressed as mean ± SE. ^{a, b, c, d}. Values in the same column between diets or enzyme having different superscript letters are significantly different (P<0.05).

4.4 Discussion

4.4.1 Chemical Composition and Enzymatic Activity of Crude Papain

Proximate analysis of dried crude papain showed that crude protein content was high (66.61%) than that obtained by Macalood *et al.*, (2013). This variation in proximate composition of crude latex is probably due to species difference, soils and stage of latex extraction as reported by Abu-Goukh *et al.*, (2010); Bari *et al.*, (2006) that there is increase in protein as fruit matures. Amino acid profile reported in this study was above figures obtained by Laurentius *et al.*, (2016) except for cysteine which was below (2.83). However, in both studies, glycine was the predominant amino acid and no methionine was detected in the extract. The variation in amino acid profile of crude papain extract could be as a result of the source of latex, origin and variety of the paw paw tree.

The crude papain in the present experiment recorded higher protease activity (1.9u/mg) than the refined papain (1.5u/mg). This could be attributed to duration and storage conditions of the refined papain prior to use. Papain losses activity with longer

storage period. However, in the case of crude papain, it was harvested and activity done after a shorter duration of storage hence could not have lost activity. The protease activity of crude papain (1.9u/mg) at pH 7.5 in this experiment was lower than that recorded by Macalood *et al.*, (2015) who reported activity of 2.66u/mg at pH 5.5 but (0.285u/mg) at pH 9. However, the activity recorded in this research was higher than (0.95u/mg) recorded by Rostika *et al.*, (2018). This variation in protease activity can be attributed to the differences in pH. According to Edwin and Jagannadham (2000), Ghosh, (2005), the optimum pH for activity of papain is in the range of 3.0 - 9.0 which varies with different substrate. The conditions of acidity for the optimum action of papain are found to be pH 5. In the present experiment protease activity was tested at near neutral pH (7.5) and according to Macalood *et al.*, (2015), papain is more active in slightly acidic medium than in a basic hence probable reason for the slightly low activity reported in this study. Several studies supported the idea that papain exhibits its greatest activity at an acidity equal to the concentration of the hydrogen ion of 10^{-5} N (Macalood *et al.*, 2013).

In the present work, crude papain latex was sun dried for 14 hrs following the procedure by Adu, *et al.*, (2009). This could not have affected the protease activity because papain as a cysteine hydrolase is active under a wide range of conditions and very stable even at elevated temperatures (Cohen *et al.*, 1986). However, at temperatures over 55°C, papain activity declines due to changes that occur at the active site (Sumner *et al.*, 1993). Studies done on different methods to dry out raw papain found differences in enzymatic activity which was attributed to the loss of activity due to changes in the enzyme native structure during the drying process (Devakate *et al.*, 2009; Sloth *et al.*, 2008; Puig *et al.*, 2008). In this study, the latex was collected from greener, mature and unripe fruits. This could have contributed to the higher activity recorded at pH 7.5. The hydrolytic activity of the latex depends upon the state of development of the fruit, the greener the fruit; the more active is the papain (Amri & Mamboya, 2012). A fully grown yellow fruits contains little latex and almost no enzymes (Becker, 1995). The high protein content (66.61%) of crude papain recorded in this study could also have led to increased protease activity. According to Abu-Goukh *et al.*, (2010); Bari *et al.*, (2006), increase in proteolytic

activity in mature fruits is associated with an increase in protein content of the fruit as it ripens.

4.4.2 *In vitro* Protein Digestibility

In testing of the *In vitro* protein digestibility, there was a general decrease in pH when different concentrations of crude papain were added to the substrate (diets) at pH 8.0. During proteolysis, protons are released from the cleaved peptide bonds at alkaline pH, resulting in a decrease in pH (Moyano *et al.*, 2014). There was no significance in the interactions between diets and concentrations of enzyme used (Table 17) and so the interpretation of results was based on the main effects (diet and enzyme concentration). In the present study, ranking of diets by relative protein digestibility, from highest to lowest indicated that diet 1 provided highest ($P < 0.05$) relative protein digestibility estimates (39.96%). This can be attributed to higher amount of fishmeal (16.5%) in diet 1 compared to (9%) in diet 2, 3 and 4 (Table 5). According to Alarcon *et al.*, (2002), the degree of protein hydrolysis decrease with increasing levels of plant proteins. Fish meal being animal protein ingredient is highly digestible and has low fibre content hence availing sufficient dietary protein as substrate for protease enzyme (Kemigabo *et al.*, 2017).

There was a general decrease *in vitro* protein digestibility with corresponding increase in crude fibre content of the diets ($P < 0.01$). According to Boisen and Eggum (1991), enzyme activity is influenced by fibre which reduces *in vitro* enzyme activities. The mechanism of inhibition by most fibres might be due to absorption of enzymes into the fibre matrix (Schneeman, 1978), or unspecific bindings to the fibres (Ikeda & Kusano, 1983). Thus, in the present study, the activity of papain enzyme could have been inhibited by dietary fibre in the diets and other indigestible residues from food (Moron *et al.*, 1989). Therefore the decreasing relative protein digestibility for each assay despite enzyme concentration was related to increase in fibre content of the diets.

In the present study, different concentrations of crude papain (0.02%, 0.04%, 0.06% and 0.08%), were used in order to optimize the enzyme concentration. According to Anderson *et al.*, (1993), Lazo *et al.*, (1998), enzyme concentrations influence the

sensitivity of *In vitro* assays. Less than optimum concentrations and combinations of enzymes may result in over estimates or under estimates of protein availability in feed ingredients (Lazo *et al.*, 1998). Successive increase in the concentration of enzyme from 0.02% to 0.06% ($P < 0.05$) led to increased relative *In vitro* protein digestibility. However, further increase in enzyme concentration from 0.06% to 0.08% ($P > 0.05$) led to smaller increase in relative protein digestibility. Such decrease in reaction rate may be due to end product inhibition as a result of increased Enzyme-Substrate (E: S) ratio (Adler-Nissen, 1986). According Robbins (1978); Gauthier *et al.*, (1986), proteolysis often leads to an accumulation of digestion products and their subsequent interactions, which ultimately result in inhibition of the enzymatic reactions. Therefore, there is need for optimization of the Enzyme-substrate ratio which yields an accurate account of digestibility as in the present study. According to Savoie (1994), the limitations of employing closed *In vitro* assays as in the present experiment, is related to the potential for inhibition of the enzyme activity by reaction products and indigestible food residues. This is in line with the theory of substrate enzyme reaction which states that “at relatively low concentrations the rate of enzyme catalyzed reaction increases linearly with substrate concentration but is asymptotic at relatively higher substrate concentrations” (Sousa *et al.*, 2015, Kemigabo *et al.*, 2017).

It's worth noting that, despite different concentration of enzyme, the relative *In vitro* protein digestibility figures for the diets were generally low across the diets compared to the standard ingredient (casein) (Akiyama *et al.*, 1989). This could have been attributed to the cross-binding of proteins from different ingredients which probably yield fewer degradable reaction products as a result of diet formulation process (Tonheim *et al.*, 2007). Also the buffering capacity of different protein sources as in the case of present study interferes with the *In vitro* protein digestibility. According to Pedersen and Eggum (1981), Moughan *et al.*, (1989), Urbano *et al.*, (2005), the components of some food materials interfere with the pH drop due to their buffering capacity. This may have contributed to variation in observed proteolytic activity despite dietary protein in the diet being fixed (30% CP). However, the slightly higher ash content (6.16) in diet 1 was unlikely to affect the *in vitro* protein digestibility. Hsu *et al.*, (1977) reported no effect of ingredient buffering capacity on *In vitro* protein

digestibility, among materials that had high ash content (6-10 times) with greater carbonate buffering capacity than other ingredients tested.

4.5 Conclusion

Based on the enzymatic activity and *In vitro* protein digestibility, it can be concluded that crude papain extract from unripe paw paw fruits can be incorporated in plant protein based diets for Nile tilapia at a concentration of 0.06%. However, the crude fibre content of the diets should be minimal for the enzyme to be more effective. More research need to be carried out on the crude papain extract inclusion in plant protein based diets for Nile tilapia and incorporation of cellulase in order to act on the fibre in the diets.

CHAPTER FIVE

NUTRIENTS DIGESTIBILITY AND GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) FED ON OILSEED MEALS SUPPLEMENTED WITH PAPAIN ENZYME

5.1 Introduction

According to Machena and Moehl (2001); Agbo (2008), the need to increase aquaculture production requires corresponding increases in more efficient dry diets. Dietary protein is the major and most expensive component of formulated aqua feeds (Wilson, 2002). Fishmeal is the desirable animal protein ingredient in fish feeds due to high protein content, balanced amino acid profile, high digestibility and palatability, and essential n-3 polyenoic fatty acids (Hardy & Tacon, 2002, Kirimi *et al.*, 2016b). However, substitution of fishmeal by plant alternatives is a necessity which is being driven by both economic and sustainability (Shahabuddin *et al.*, 2015). The use of plant protein sources in fish feeds has expanded considerably in recent years to meet the demand for feeds and sustain the development of worldwide aquaculture production (Tacon & Metian, 2015). However, the essential amino acid compositions of alternative protein sources for fish are generally not comparable with that of fish meal. A deficiency in certain essential amino acids (EAA) is one of the major issues with plant protein sources as it requires supplementation with other feedstuffs (Ogunji *et al.*, 2008). Therefore, combining different alternative protein sources which possess different limiting amino acids which could complement each other has been strongly recommended (Jackson *et al.*, 1982; Tacon & Jackson, 1985).

Much research has been done to evaluate new protein sources to partially or wholly replace fishmeal in diets for fish. Among the plant protein sources, Soybean meal is one of the most suitable alternative ingredients for replacing fishmeal in fish diets. It has high protein content, highly digestible, relatively well-balanced amino acid profile and reasonable price (Kikuchi, 1999; NRC, 1993; Storebakken *et al.*, 2000). Rapeseed (*Brassica napus* L.) is an important source of edible oil in many countries. Canola meal is only second to soybean meal as the most commonly fed protein feedstuff in animal diets around the world (Newkirk, 2009). The whole seed contains approximately 21% crude protein while canola meal contains approximately 36% crude protein (Naczek *et al.*, 1998; Uppstrom, 1995). Sunflower is cultivated extensively due to its adaptability to a wide range of climatic and soil conditions

(Ravindran & Blair, 1992). Its seeds are inexpensive to process, and the cake remaining after oil extraction is used as a protein supplement in animal diets (Daghir *et al.*, 1980, Maina *et al.*, 2007). The crude protein content of the cake ranges from 25 to 45% (air-dry basis) depending on the extent of dehulling and the efficiency of the oil extraction process (Maina *et al.*, 2007).

However, concerns regarding the relatively low nutrient digestibility and the presence of an array of anti-nutritional factors in fish meal alternatives, has led to an increasing interest in feed enzymes (Plumstead, 2013). Carica papaya plants are locally available and unripe fruits contain crude papain, a proteolytic enzyme that can break down peptide bonds of a protein molecule (Aravind *et al.*, 2013). The enzyme in the feed hydrolysis protein into amino acids, increase the absorption of protein and digestion rate in the digestive tract of fish (Rostika *et al.*, 2018; Muchtadi *et al.*, 1992). This will increase feed utilisation efficiency hence more growth as more amino acids are availed for absorption. The current study therefore was conducted to evaluate the effect of crude papain inclusion at 0.06% on oilseed meals based diets on nutrient digestibility, growth and economic performance of Nile tilapia.

5.2 Materials and Methods

This experiment involved a feeding trial where Nile tilapia fingerlings were fed diets formulated in chapter 3.

5.2.1 Study Site

The experiment was conducted at Iriene Primary School fish pond in Imenti South, Meru County. The fish were raised in net cages, of dimension 2m ×1m ×1m suspended in liner pond.

5.2.2 Preparation of Diets

The method of preparation of the experimental diets was as described in 3.2.2 in chapter 3 (Table 5). However, the crude papain extract was supplemented at the rate of 0.06%. The enzyme in powder form was dissolved in 50ml water and then reconstituted to 500mls of water. The solution was mixed thoroughly with the formulated marsh feed to form a paste and pelleted.

5.2.3 Experimental Design

In a 4x2 factorial design the study was based on 4 diets (FM, SBM, CM, and SFM based) with papain enzyme levels of 0% and 0.06%. The experiment was conducted in 24 net hapas dimension 2m x 1m x 1m. Seven hundred and twenty sex-reversed Nile tilapia fingerlings from Sagana fish farm of average weight 7 ± 3 g were selected and acclimatized for two weeks during which time the fish were not fed on prepared rations. Feeding with the experimental rations began after the initial weight of the fish was taken. After acclimatization the fingerlings were randomly picked and transferred into the twenty four hapa nets at a rate of 30 fingerlings per unit net hapa in a completely randomized design. They were further divided randomly into eight groups with three replicates.

5.2.4 Feeding and Data Collection

Feed was given to the fish at a rate of 5% of body weight throughout the experimental period twice daily i.e. morning (10 am) and evening (4 pm) in two equal meals. The amount of supplementary feed provided was adjusted accordingly after weighing the fish at each sampling done fortnightly. Water parameters were monitored weekly (dissolved oxygen, pH and temperature) using multiparameter water quality meter, Hanna D.O model H19147. Fortnightly, fish from each net hapa were weighed (g) and total length (cm) recorded for the entire experimental period. Also the amount of feed consumed daily was recorded.

5.2.5 Evaluation of the Fish Performance

A number of parameters were used to determine the growth response of the fish.

Survival rate (SR) was calculated as the number of fish that survived during the experimental period expressed as a percentage of the stocked fish. It was calculated by subtracting the number of dead fish during the culture period from the fish stocked and then expressing it as percentage (Charo *et al.*, 2006).

Survival rate (%) = (Initial number of fish stocked - mortality) / initial number of fish stocked x 100

The daily weight gain (DWG) was taken as the difference between the final body weight and the initial body weight of fish over a period of time.

$$\text{Daily weight gain (DWG \%)} = \frac{(\text{final body weight} - \text{initial body weight})}{\text{Initial body weight} \times \text{days of experiment}} \times 100$$

Relative growth rate (RGR) was the change in weight of fish expressed as a percentage of the final average weight (Otubusin *et al.*, 2009)

$$\text{RGR (\%)} = (\text{Wf} - \text{Wi}) / \text{Wf} \times 100$$

Where Wf= final average weight at the end of experiment

Wi = Initial average weight at the beginning of experiment.

Specific growth rate (SGR), the instantaneous change in weight of fish expressed as the percentage increase in body weight per day over any given time interval, was calculated by taking natural logarithms of body weight change expressed as percentage per day (Khalafalla *et al.*, 2010)

$$\text{SGR (\% per day)} = [(\text{Ln final body weight} - \text{Ln initial body weight}) / 100] / \text{Experimental period}$$

Where Ln= natural logarithm

Efficiency of feed utilisation (FE) was calculated as the live weight gain (g) per feed consumed (g). It often serves as a measure of efficiency of the diet. Feed efficiency describes the influence of feed administration on the fish consuming the feed and the utilization of feed administered so that it can improve the fish growth (Gusrina 2008). The higher the FE the better the feed quality and the efficiency at which this feed is transformed into meat, enabling lower production cost required to produce the fish meat (Effendi, 2004).

Feed utilisation efficiency (Guroy *et al.*, 2005)

$$\text{Feed efficiency (FE) \%} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$$

5.2.6 Apparent Nutrient Digestibility

The apparent nutrient digestibility was done by indirect method using chromic oxide as a marker.

5.2.6.1 Experimental Diets

Chromic oxide was added to the diet at 1% level as an external marker for nutrient digestibility study.

5.2.6.2 Feeding and Faecal Collection

Apparent nutrient digestibility was performed at the end of feeding trial using fish weighing 40 ± 2 g. The experiment took 30 days of faecal collection. The procedure was carried out in glass aquaria of dimension (0.9m \times 0.6m \times 0.5m). Each dietary treatment was done in triplicate.

Fish were acclimated to the experimental system for 7 days before the start of the experiment. Fish were fed to satiation twice a day on each of the experimental diets. Faeces collection started 4 days after feeding to allow evacuation of all previously ingested materials. The aquaria were cleaned to remove any uneaten feed and water completely exchanged in each glass aquarium every day. Faecal collection was done by siphoning. Care was taken to avoid breaking of the thin fecal strings in order to minimize nutrient leaching. After collection of faecal material, samples from the two glass aquaria were pooled together and oven dried at 50°C for 5 hrs and kept in a dry container for composition analysis by acid digestion and spectrophotometric methods. Water temperatures were maintained at 25-30⁰c and was warmed using heater. Adequate oxygen supply was ensured by artificial aeration using air pump through capillary system. All the fish that died were replaced by others of similar weight from a reserve tank to ensure that there were fish to provide the required amount of faecal samples.

5.2.6.3 Digestibility Determination

Faecal samples were analyzed in triplicates for nutrients (dry matter, crude protein, ether extract and nitrogen free extracts) following the procedure by AOAC (1995) in order to determine the apparent nutrient digestibility of the different treatments.

Chromic Oxide in the diets and faeces was determined according to the method of Furukawa and Tsukahara (1966). The procedure involved digestion of the sample by concentrated nitric acid and oxidising chromic oxide with 70% Perchloric acid. The orange colour formed by the oxidation of chromium III to chromium IV is read on a spectrophotometer (Uvikon 860 Kontron instruments) at 350nm against distilled water. 100 mg of sample was weighed into a Kjeldahl flask. 5 ml of concentrated nitric acid were added to the flask and the mixture was boiled (using an electric 60mantle) gently for about 20 minutes (taking care not to boil dry). After cooling the sample, 3ml of 70% perchloric acid was added to the flask. The mixture was then gently heated again until the solution turned from a green to an orange colour after which it was left to boil for a further 10 minutes to ensure oxidation was complete. The solution was transferred to a 100 ml volumetric flask and diluted to volume. The absorbance of the solution was determined by spectrophotometer (Uvikon 860 Kontron instruments) at 350nm against distilled water. Chromic III oxide was calculated according to the formular:

$$\text{Chromic Oxide (\%)} = \left[\frac{\text{absorbance} - 0.0032}{0.2089} \right] / \text{sample weight} \times 100$$

Apparent digestibility coefficients of the test diets were calculated by the indicator method of difference. Apparent digestibility coefficients (ADCs) for dry matter, crude protein, crude lipid, and nitrogen free extracts in the diets were determined using the formular (De Silva 1989; Bureau *et al.*, 2002):

$$\text{Apparent Nutrient Digestibility (AND) (\%)} = 100 - \left\{ 100 - (\% \text{Chromium oxide in feed} / \% \text{chromium in faeces}) \times (\% \text{ of nutrient in faeces} / \% \text{ of nutrient in diet}) \right\}$$

5.2.7 Economic Analysis

Economic analysis was conducted to assess the cost effectiveness of using oilseed meals as substitute for fishmeal and supplementing with crude papain extract. Only the cost of feed was used in the calculation with the assumption that all other operating costs remained constant. Cost of the feed was calculated using market prices of ingredients. Ingredients prices varied as shown in Table 18.

Table 18: Cost of Feed Ingredients (kshs) used in Formulating Diets Containing Oilseed Meals as a Replacement of Fishmeal in Concentrate Supplement

Ingredient	Price(Kshs) per kg
Fishmeal	250
Soybean meal	100
Canola meal	70
Sunflower meal	50
Maize meal	30
Wheatbran	30
Crude papain enzyme	15/g

Incidence cost is the cost of feed used to produce a kg of fish (relative cost per unit weight gain) and the lower the value, the more profitable using that particular feed (Nwanna, 2003; Abu *et al.*, 2010).

IC= Cost of feed/ weight of fish produced

Profit index (PI) was calculated as:

PI= Weight or value of fish produced/cost of feed (Abarike, Attipoe & Alhassan, 2012)

5.3 Data Analysis

Data on growth performance parameters, economic analysis and apparent nutrient digestibilities were subjected to a two way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.0 at P = 0.05 confidence level, to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

The basic linear model for factorial design was:

$$Y_{ijkl} = \mu + A_i + B_j + (AB)_{ij} + e_{ijkl}$$

Where Y_{ijkl} is the observation on the i^{th} EU

μ = is the overall population mean,

A_i = is the effect due to diet, where $i= 1-4$

B_j = is the effect due to papain enzyme $j=1-2$

$(AB)_{ij}$ = is the effect of interaction of the diet and papain enzymes

e_{ijkl} = is the random error term.

5. 4 Results

Growth performance, water quality parameters, economic analysis and apparent digestibility coefficients data is as shown below.

5.4.1 Physico-Chemical Parameters

Data on physico-chemical parameters are as shown in Table 19. Water temperatures in the hapas ranged from 22.06°C to 29°C with a mean of 25.53°C. The pH ranged from 7.42 to 10.01 mean of 8.72. Dissolved oxygen ranged between 2.5mg/l to 5.3mg/l with a mean of 3.9mg/l.

Table 19: Range and Average of Physico-Chemical Parameters of Water during 101 Days Experimental Period

Parameter	Range	Mean
Temperature (°C)	22.06-29.0°C	25.53°C
Dissolved oxygen (mg/l)	2.5-5.3mg/l	3.9mg/l
pH	7.42-10.01	8.72

5. 4. 2 Apparent Digestibility Coefficients of the Diets

The results of apparent digestibility coefficients (ADC's) for dry matter, crude protein, crude lipid and nitrogen free extracts are shown in Table 20. Apparent digestibility coefficient for dry matter was statistically similar ($P>0.05$) though FM based diet recorded highest (76.98%). ADC for protein was highest in FM based diet (94.45%), SBM (93.63%) closely followed by CM (93.47%) and SFM (93.29%) recording least. Enzyme treated diets recorded significantly higher ($P<0.05$) ADC's for all the nutrients.

Table 20: Apparent Digestibility Coefficients (%) of the Diets

	<u>Diet (D)</u>				<u>Enzyme (%) (E)</u>		<u>Significance</u>		
	FM	SBM	CM	SFM	0	0.06	D	E	DXE
DM	76.98±1.82 ^b	75.63±2.01 ^d	77.50±1.44 ^a	76.28±1.6 ^c	72.97±0.45	80.23±0.47	P>0.05	P<0.05	P>0.05
CP	94.45±0.94 ^a	93.63±1.07 ^{bcd}	93.47±1.11 ^{cbd}	93.29±0.85 ^{db}	91.53±0.22	95.88±0.19	P<0.05	P<0.05	P>0.05
EE	92.13±0.42 ^d	94.34±0.48 ^{ba}	94.80±0.79 ^{ab}	93.47±0.31 ^c	92.71±0.31	94.66±0.42	P<0.05	P<0.05	P<0.05
NFE	77.35±0.44 ^{cb}	78.17±0.95 ^{ab}	76.44±1.74 ^d	77.79±0.21 ^{bca}	75.71±0.57	79.17±0.38	P<0.05	P<0.05	P<0.05

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

5. 4.3 Growth Performance

Data on fish growth performance are presented in Table 21. The highest final average body weight (56.89g) was recorded in FM based diet with SFM recording lowest (40.59g). However, SBM (45.59g) and CM (43.89) were statistically similar ($P>0.05$). Survival rates were high and statistically the same ($P>0.05$) for all the diets. Fish fed FM based diet had highest daily weight gain (0.49g/d) followed by SBM (0.37g/d), CM (0.36g/d) and SFM (0.33g/d) ($P<0.05$). The highest feed conversion efficiency was recorded in FM diet (0.44) and SFM recorded lowest (0.36). Crude papain enzyme treated diets had highest final body weight (47.32g) with none treated diets recording (46.17g). Also daily weight gain, specific growth rate and relative growth rate were higher in enzyme treated diets. However, Feed conversion efficiency was similar ($P>0.05$)

Table 21: Growth Performance Parameters of *Oreochromis niloticus* Fed on Oilseed Meals as Replacement of Fishmeal

	<u>Diet (D)</u>				<u>Enzyme (%) (E)</u>		<u>Significance</u>		
	FM	SBM	CM	SFM	0	0.06	D	E	DXE
IBW	7.32±0.85 ^a	7.42±0.06 ^a	7.36±0.08 ^a	7.54±0.06 ^a	7.43±0.05	7.39±0.06	P>0.05	P>0.05	P>0.05
FBW	56.89±1.37 ^a	45.59±0.91 ^{bc}	43.89±2.12 ^{cb}	40.59±1.60 ^{cd}	46.17±2.14	47.32±2.10	P<0.05	P>0.05	P>0.05
DWG	0.49±0.01 ^a	0.37±0.01 ^b	0.36±0.02 ^c	0.33±0.01 ^d	0.38±0.02	0.39±0.02	P<0.05	P>0.05	P>0.05
SGR	0.88±0.01 ^a	0.78±0.01 ^{bc}	0.77±0.02 ^{cbd}	0.72±0.02 ^{dc}	0.78±0.02	0.79±0.02	P<0.05	P>0.05	P>0.05
RGR	86.90±0.47 ^a	83.68±0.35 ^{bc}	83.03±0.88 ^{cbd}	81.26±0.88 ^{dc}	83.42±0.79	84.02±0.74	P<0.05	P>0.05	P>0.05
SR	97.78±1.4 ^a	98.67±0.84 ^a	99.33±0.67 ^a	97.22±2.77 ^a	99.11±0.62	97.39±1.44	P>0.05	P>0.05	P>0.05
FCE	0.44±0.01 ^{ac}	0.38±0.01 ^{bcd}	0.39±0.02 ^{cabd}	0.36±0.02 ^{dbc}	0.39±0.01	0.39±0.01	P<0.05	P>0.05	P>0.05

Note, Values are expressed as mean ± SE. ^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

5. 4.4 Economic Analysis

Data on economic performance is shown in Table 22. The total cost of feed was highest ($P < 0.05$) for FM based diet (Ksh 308.29) which reduced on substitution with oilseed meals. The incidence cost was lowest on SFM based diet (Ksh 169.15) closely followed by CM with FM based diet recording highest but statistically similar ($P > 0.05$). The total biomass harvested per hapa was highest for FM based diet (1.70kg) with the estimated value of Ksh 681.33, SBM (1.37kg) with a value of kshs 548.06, CM (1.35kg) value of Ksh 540.80 and lowest for SFM diet (1.30kg) with the lowest estimated value of (kshs 518.40). The profit index was highest for SFM diet (2.38), CM (2.36), SBM (2.25) and FM had the lowest profit index (2.21). However, upon crude papain enzyme supplementation, total cost of the feed increased incidence cost (182.84) and subsequent decrease in profit index (2.19) but total harvested biomass increased (1.46kg).

Table 22: Economic Analysis of *Oreochromis niloticus* Fed on Oilseed Meals Supplemented with Crude Papain Enzyme

	Diet (D)				Enzyme (%) (E)		Significance		
	FM	SBM	CM	SFM	0	0.06	D	E	DXE
Feed input(kg)	3.42±0.05 ^a	2.99±0.02 ^b	2.84±0.04 ^{cd}	2.73±0.03 ^{dc}	2.95±0.08	3.04±0.08	P<0.05	P<0.05	P>0.05
feed (costs(Kshs)	308.29±9.94 ^a	243.13±6.78 ^b	229.97±8.97 ^c	219.39±7.40 ^d	232.84±10.33	267.55±10.93	P<0.05	P<0.05	P>0.05
Harvested biomass (kg)	1.70±0.04 ^a	1.37±0.02 ^{bcd}	1.35±0.04 ^{cbd}	1.30±0.01 ^{dc}	1.39±0.05	1.46±0.05	P<0.05	P>0.05	P>0.05
Biomass value (Kshs)	681.33±17.87 ^a	548.06±10.76 ^b	540.80±17.42 ^c	518.40±5.96 ^d	559.20±20.26	585.10±21.88	P<0.05	P>0.05	P>0.05
Incidence cost	181.13±5.12 ^a	177.51±4.18 ^a	170.54±6.72 ^a	169.15±4.42 ^a	166.33±3.10	182.84±2.66	P>0.05	P<0.05	P>0.05
Profit index	2.21±0.06 ^{abc}	2.25±0.05 ^{bac}	2.36±0.09 ^{cba}	2.38±0.06 ^{dbc}	2.41±0.04	2.19±0.03	P>0.05	P<0.05	P>0.05

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

5. 5 Discussion

5.5.1 Apparent Digestibility Coefficients for the Diets

The results of the present study (Table 20) indicate that the dry matter, protein, lipid, and nitrogen free extracts of the experimental diets were well digested by Nile tilapia. The Apparent protein digestibility values for all the diets were above 90% which is in line with NRC (1993) that the digestion coefficients for protein rich feed stuffs are usually in the range of 75 to 95%. Although SFM based diet had relatively high crude fibre (16.03%), the apparent digestibility coefficient was high. This is because tilapia as in other species, protein digestion is relatively high, even in feed containing high fibre (Anderson *et al.*, 1991). However, high dietary crude fibre content as in the present work (Table 9) may accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of protein (Hossain *et al.*, 2000). Based on this, SFM based diet recorded slightly low apparent protein digestibility coefficient which might have been due high concentration of crude fiber. Despite similar crude protein levels in the diets, there was slight variation in the apparent nutrient coefficients. This could be attributed to varying proportion of different ingredients which have different digestibility figures. According to Jauncey (1982), higher ADC values are obtained in diets with higher protein contents due to decrease in the proportion of metabolic faecal nitrogen with the rise in protein content in the diet. The highest protein digestibility was found in FM based diet because fishmeal formed the bulk of protein. Fishmeal protein is highly digestible hence preferred animal protein ingredient in fish feed.

Upon crude papain enzyme supplementation in the present study, ADC increased ($P < 0.05$) (Table 20). Papain enzyme is a protease enzyme capable of hydrolyzing protein complex compound into simple elements (amino acids). According to Rostika *et al.*, (2018), the addition of papain as an exogenous enzyme into the feed improves the feed's protein hydrolysis. This result in the increased feed digestibility hence improved amino acid absorption into the body for growth. El Moussaoui *et al.*, (2001) and Azarkan *et al.*, (2003) argued that Papaya latex may cause the better absorption of amino acids because of cysteine proteinases (digestive enzymes) constitute as much as 80% of the enzyme fraction in papaya latex. Thus, it is clear from the ADC values recorded in the present study that 0.06% crude papain enzyme supplemented in the

diets played a considerable role in the digestion process in Nile tilapia. The results of the present study agree with work done by Singh *et al.*, (2011) and Muchlisin *et al.*, (2016) where fingerlings of *Cyprinus carpio* and Keureling fish (*Tor tambra*) respectively were supplemented with papain showed higher protein digestibility values as compared to control feed where no enzyme supplementation was used. Rachmawati *et al.*, (2018) also reported increased protein digestibility when papain enzyme was supplemented to post larvae of freshwater lobster (*Cherax quadricarinatus*) at 0.2, 0.3 and 0.4 percent pure papain. The ADC for crude lipid in all the diets was relatively high and this agrees with Aksnes and Opstvedt (1998), that when lipid is administered either alone or in a mixed diet, routinely gives digestibility values ranging from 85% to 95% for fish.

The difference in protein digestibility may be due to differences in chemical composition, origin and processing of various feed ingredients, method of faeces collection and fish species (Koproco *et al.*, 2004). In relation to the technique of faecal collection employed, Cho *et al.*, (1982) argued that the method used to determine digestibility can affect the value of the coefficients obtained. In his study Singh and Nose (1967) established that digestibility estimations obtained with fecal collection from the tanks were 10% greater compared with that obtained by stripping, indicating that some nitrogen compounds were lost in the water. This could have contributed to the relatively high coefficients figures obtained in the present work since the breakup of faeces by fish movement may have led to leaching of nutrients and therefore overestimation of digestibility of nutrients. However, to minimize on this, upon removal of uneaten feed, faeces were continuously siphoned out from the glass aquaria. According to Bureau and Hua, (2006); Forster (1999), variability in apparent digestibilities may also be due to differences in chemical composition, origin and processing methods of various feed ingredients. Based on this, the ingredients used to formulate the four rations were the same hence effects of origin and processing was minimized.

5. 5.2 Growth Performance of Nile Tilapia in Enzyme Free Diets

In this study, water quality parameters i.e temperature, pH and dissolved oxygen were within recommended range for optimum biochemical reactions in digestion and

metabolic utilization of feeds to enhance growth rate. Hence any variation observed in growth performance of Nile tilapia fingerlings in the present work was as a result of the dietary treatments. Generally, there was progressive mean weight gain observed in all the dietary treatments. The high nutritional composition in the diets in the present study could be attributed to the increase in body weight. Thus high nutritive value of feed promotes better growth and higher yield in fish (Madu *et al.*, 2003). However, fishmeal based diet had highest growth performance (56.48g) ($P < 0.05$) compared to the oilseed meals (SBM, CM and SFM) based diets, results consistent with those of Kirimi *et al.*, (2016b). This can be attributed to the high and balanced levels of essential amino acids in diet 1 where fishmeal formed the bulk of the 30% CP despite limiting in methionine (Table 11). Desilva and Anderson (1995) stated that the quality of feed is a function of how well it meets nutrients requirement of fish. The results of the present study are in agreement with Hardy and Tacon (2002), Kirimi *et al.*, (2016a), that fish meal is the most desirable animal protein ingredient in aqua feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic fatty acids. However, high costs and scarcity is the major limitation to its use (Kirimi *et al.*, 2016a).

There was a decline in growth performance of Nile tilapia on substitution of fishmeal (*Rastrionaebola argentea*) with oilseed meals (SBM, CM and SFM). However, among the oilseed meal based diets; SBM performed better in terms of growth rate (45.59g), although there was no significant difference ($P > 0.05$). This agrees with the review done by Lim and Dominy (1989); Ogello *et al.*, (2014) that SBM is the best plant protein source in terms of protein content and EAA profile considered as a partial or total FM alternative for tilapia, though with varying results. The low growth performance observed in SBM based diet compared to FM diet could be attributed to amino acid imbalances specifically methionine and isoleucine which were the most limiting. This study agrees with research by Jackson *et al.* (1982) who reported growth reduction when 50% or more fishmeal was replaced with SBM, attributed to the methionine deficiency and the presence of trypsin inhibitors. This trend is similar to that reported by Shiau *et al.*, (1987) who recorded significant growth reduction in the hybrid tilapia when soybean meal substituted 24% of fishmeal at 30% and 32%

dietary protein level. However, Shiau *et al.*, (1987); Liti *et al.*, (2006) reported that soybean meal could fully substitute fishmeal without a significant reduction in tilapia growth if the diets contained sub-optimal (24%) levels of protein. However, based on the present study, soybean meal cannot totally replace fishmeal in Nile tilapia diets because 10% CP replacement of FM with SBM depressed growth. The contradiction among researchers regarding the use of SBM as a protein source for fish may be related to the quality and processing of SBM, fish species, size and culture systems (Ogello *et al.*, 2014). In general, based on the present work, it is evident that substituting fishmeal with oilseed meals reduced growth performance of Nile tilapia. This agrees with Liti *et al.*, (2006) that substituting animal protein with plant protein at higher levels than the optimal dietary protein reduces the growth of tilapia, while growth is not affected below the optimal levels.

Canola meal based diet in the present study performed better (43.89g) than sunflower but with no significant different ($P>0.05$). The decrease in growth of fish with an increased canola meal level in the diet can be attributed to the sub-optimal levels of essential amino acid (methionine and isoleucine). Also the mustard smell of CM based diet was still noticeable which might have adversely affected the acceptance of the diets. This is in agreement with Adem *et al.*, (2014) that despite lower glucosinolate content in canola meal, the typical mustard smell of glucosinolate is still noticeable in the feed affecting acceptance by the fish. In the present study, a decrease in growth performance has been reported at inclusion level of 31% i.e (10% of the 30% dietary protein). This is in contrast with Enami (2001) who indicated that protein from canola can replace up to 10% of protein from fishmeal in the diets for tilapia. Jackson *et al.*, (1982) reported a significant reduction in weight gain with rapeseed inclusion levels of 63% and higher. Based on the present results therefore, apart from the reduced amino acids, the unfavourable taste resulted in reduced feed intake and growth performance (Luo *et al.*, 2012; Chang *et al.*, 2010; Zhou & Yue, 2010).

Sunflower based diet performed poorly of all oilseed meals in substituting fishmeal in the present study with final body weight of (40.59g). Decline in performance of Nile tilapia recorded in SFM based diet is due to poor amino acid profile with methionine and isoleucine being the 1st and 2nd limiting respectively. Low growth performance is

also attributed to higher crude fiber content than the other diets. Shiau and Kwok (1989) indicated that increased dietary fiber can reduce growth in tilapia. According to De Silva and Anderson (1995), Lovell (1998) the recommended fibre content in fish feed is 8-12% hence far above (16.03%) in the present study. A high level of fiber content reduces the total dry matter, lowers the digestibility of nutrients and slows growth. Jackson *et al.*, (1982) reported good growth in tilapia (*Sarotherodon mossambicus*) fed rations containing 35.2% sunflower meal replacing 50% of the fish meal protein. It's worth noting that vitamin and mineral premix was deliberately left out in the present experiment during diet formulation. This was to avoid introduction of exogenous amino acids present in most premixes in order to minimise sources of variation. However, the fertilized liner pond offered fish extra nutrition from natural food. This source of nutrients may have provided an extra supply of limiting essential amino acids, though it could not compensate for the deficient nutrients in the oilseed meal based diet (Kirimi *et al.*, 2016b, Munguti *et al.*, 2014). The weight of fish at the start of experiment was not significantly different ($P>0.05$) but mean final body weight within the same group was high. This large fluctuation in final weight gain in the same group despite similar dietary treatment might have been genetical due to inbreeding of brooder stock in the hatchery hence stunted growth observed in some fish. Feed conversion efficiency varied among the diets with FM based diet recording highest. This was likely to be as a result of the improved nutrient and energy utilization (Rostika *et al.*, 2015). However, the values obtained ranged between 0.36 (36%) to 0.44 (44%). According to Rostika *et al.*, (2015), feed might be considered in good category when the feed efficiency value is more than 50 % or even close to 100 %. Thus in the present study, the feed efficiency obtained could not be considered in a good category.

5. 5.3 Growth Performance of Nile Tilapia on Crude Papain Enzyme Treated Diets

There was slight increase in growth performance when 0.06% crude papain enzyme was incorporated in the diets though no significant difference statistically ($P>0.05$) (Table 21). This can be attributed to the addition of crude papain extract in the diets which increased nutrient availability (Table 20). Papain is a protease enzyme that hydrolyzes proteins to short peptides in diet, which is the key factor to increase

protein digestibility and fast absorption, and helps to increase growth factors through fast metabolism (Wong *et al.*, 1996). According to Howard (2010) and Rostika *et al.*, (2015) protease enzyme from papain is effective in reducing the energy for activating metabolism process hence increase in growth rate.

Results of this study were also in line with those conducted by previous researchers. Nile tilapia yielded better results in terms of percent weight gain and specific growth rate in diets supplemented with crude papain in the form of papaya leaf (Munguti *et al.*, 2014). Better growth performance of common carp was also reported when fed with 3% papain in feed and 10% papaya leaf mixed with feed which was attributed to increased protein digestion due to papain (Tagare, 1992). Diet supplementation with 0.1% of papain resulted in better growth of post larvae of *Macrobrachium rosenbergii* (Patil, 2014). However, based on literature from these studies, they lacked the protease activity data of the papain enzyme to justify and standardize concentration to be used in Nile tilapia diets hence there could be underestimate or over estimate. In contrast, the protease activity in the present study at the inclusion level of 0.06% was determined (1.9units/mg). Despite low weight gain in addition of crude papain enzyme in the present study, there was no preference of the diet with or without the enzyme. Thus inclusion level of 0.06% had no negative effect on palatability of the feed. In the present study, survival rate was high and within the most range (97.39% to 99.33%) and statistically similar ($P>0.05$) due to the fish positive reactions to the enzyme added feed. However, the low mortality recorded in the present study was not linked to diet or crude papain enzyme inclusion but presence of a predator in specific hapas that fed on fish. Based on this, the crude papain extract inclusion at 0.06% had no negative effect on survival rate of fish and this corroborates with studies done by Singh *et al.*, (2011) where similar trend was observed. The high survival rate in the present study is attributed to good management procedures and optimum physico-chemical parameters within the culture system (Kirimi *et al.*, 2016b).

The markedly low growth performance in the diets despite crude papain enzyme supplementation in the present work could be attributed to crude fibre content in the diets. According to Moron *et al.*, (1989); Boisen and Eggum (1991), fibre reduces enzyme activities. Thus, the activity of papain enzyme could have been inhibited by

dietary fibre in the diets hence low increase in growth observed. Addition of protease enzyme at the right dose to the feed is helpful in accelerating the fish growth (Rostika *et al.*, 2018). However, considering the growth performance results of this study, it is not possible to affirm if the papain enzyme dosage was insufficient or if the enzyme added in the diets was masked by the fibre content in the diet. In the current study, different ingredients were used to formulate the diets in order to balance and compensate for the deficient amino acids. However, this could have negatively impacted the activity of the enzyme hence low increase in growth observed. According to Tonheim *et al.*, (2007), cross-binding of proteins from different ingredients yield fewer degradable reaction products as a result of diet formulation process. It's worth noting that crude papain extract had appreciable amount of amino acids, however, comparing the inclusion level in the diet (0.06%), it could not have brought a difference in the ration amino acid profile. Also the inclusion levels were similar across the diets.

5.5.4 Economic Analysis

The success of aquaculture invariably depends on the provision of suitable and economical fish feed. Therefore, development of a more sustainable aquaculture feed production will depend on identifying and establishing alternative feedstuffs to fishmeal (Olukayode & Emmanuel, 2012). In the present study, oilseed meals have been used as substitute for fishmeal and resulted in varying economic performance in terms of incidence costs and profit index. The costs of producing a kg of feed in the present work was highest on FM based diet which translated into higher incidence cost. This is attributed to the high costs of fishmeal (kshs 250) (Table 18). On the other hand, sunflower based diet was the least in production costs attributed to the low market price of sunflower (Kshs 50) as it formed the bulk of protein in SFM based diet. The economic analysis in the present study indicates positive net returns for all the diets. The difference in economic performance for the rations in the present study was due to varying major protein sources with different prices. The economics of feed production indicated that the costs of the diets were minimized by replacing FM with the oilseed meal mixtures. Sunflower and canola meal based diet recorded highest profit index though they had lower final weight gain. This was attributed to the relatively low market prices of canola meal and sunflower meal (Table 18). To

increase fish farm profits, the cost of feed must be reduced and considerable effort be focused on finding alternatives to fishmeal from both plant and animal protein sources (Hossain *et al.*, 2002). However, production of fish using the diets will take longer for the fish to reach market size. This was in agreement with Ogunji (2004), Kirimi *et al.*, (2016) that in relation to culture period, when alternative protein sources are used in tilapia feeds, the rate of fish growth is reduced leading to increased rearing time. The low cost of the protein sources would reduce the entire cost of raising the fish, compensating for the delayed growth and time lost, consequently, increasing profitability. However, this might not be entirely good for intensive commercial farmers who are looking for high profits at the shortest possible time but immensely benefit small to medium scale semi-intensive farmers.

Supplementation with enzymes has the potential to improve the use of amino acids in the diet and reduce rearing costs (Wang *et al.*, 2006). In view of this, *Papaya carica* is widespread in Kenya and can be harvested with least costs to incorporate in Nile tilapia diets in order to improve on the availability of amino acids. In the present study, though there was slight weight gain on crude papain enzyme supplementation, the increased incidence cost increased and led to subsequent decrease in profit margin (Table 22). The decrease in profit margin was attributed to the slight weight gain of fish despite addition of crude papain extract hence increased incidence costs.

5.6 Conclusion

Plant proteins were inferior compared to FM but showed higher profit margins. Upon supplementation of crude papain extract in plant protein based diet, apparent digestibility coefficients and growth performance of fish increased. However, the profit margins decreased with enzyme supplementation. More research is needed on the use of crude papain enzyme in Nile tilapia diets with a focus on reducing the fibre content by incorporating fibre degrading enzyme.

CHAPTER SIX

CARCASS COMPOSITION AND SENSORY EVALUATION OF NILE TILAPIA FED ON OILSEED MEALS SUPPLEMENTED WITH CRUDE PAPAIN ENZYME.

6.1 Introduction

In Aquaculture, reduction of the feed costs has been a major concern and attempts have been made to partially or wholly replace the fish meal component in fish feed with alternative feed resources both animal and plant in origin. However, the value and the feasibility of an alternative feedstuff to fishmeal cannot simply be evaluated by its ability to maintain growth, as fish market value for human consumption depends, in large part, on the perceived quality (Amberg & Hall, 2008; Ng & Bahurmiz 2009; Sealey *et al.*, 2011). In view of this, the main goal of fish nutrition as a scientific discipline is to produce feeds that support good growth rates while maintaining fish health and quality, resulting in a safe and healthy product for the consumer (Singh *et al.*, 2008). Fish make up more than 50% of the total animal protein intake in under developed countries providing an important source of essential fatty acids (EFA) and other important micronutrients (FAO, 2016). Fish are rich in vitamins, minerals, fatty acids and proteins (including all essential amino acids). Its high nutritional value makes it an important food source for humans (Murray & Burt, 2001). However, farmed fish have been slowly accepted by consumers since they have been thought to have lower quality and poor flavor compared to their wild counterparts (Haard, 1992; Rasmussen, 2001). It has been shown that the composition of the fish carcass is not only genetically controlled, but the type of diet also affects significantly both the composition and sensory quality of the meat (Oliveira, 2002). Exogenous factors that affect fish body composition include the diet of the fish (composition, frequency) and the environment in which it is found (salinity, temperature). However, the main exogenous factor affecting proximate composition is the diet. Various studies have examined the effects of temperature, light, salinity, pH and oxygen concentration on the proximate composition of fish but these factors would seem to have very limited effects (Albeti, 2017). Based on this, improvement of feed and nutrition in aquaculture practices may provide an opportunity to further enhance the quantity as well as nutritional quality of fish (Noor *et al.*, 2011).

Many scientists are studying the safe utilization of nutrients and their interactions when alternative feed ingredients from plants are used to substitute for traditional (and expensive), fish meal and oil, as well as evaluating alternative marine ingredients (Sing *et al.*, 2008). Among the plant protein sources, vegetable oils represent most suitable alternative ingredients for replacing fishmeal in fish diets. However, according to Abou *et al.*, (2013), increasing interests are focused on the fatty acid composition of fish, especially when evaluating the suitability of vegetable sources (soybean meal, canola meal and sunflower meal) to replace fishmeal in fish diets. The fatty acid composition of fish tissue changes in response to the fatty acid composition of the diet, as shown in previous studies in which dietary fish meal were replaced with plant ingredients (Leaver *et al.*, 2008, Benedito-Palos 2008). However, due to the relatively low nutrient digestibility, addition of crude papain as an exogenous enzyme into the feed can improve the feed's protein hydrolysis. This will result in increased feed efficiency and improved growth. Hence efficient feed improves body deposition. This study therefore was carried out to determine the carcass proximate, fatty acid composition and sensory attributes of Nile tilapia fed on oilseed meal diets supplemented with crude papain enzyme.

6.2 Materials and Methods

This experiment involved preparation of carcass and fillets for both proximate, fatty acid analysis and sensory evaluation.

6.2.1 Study Site

The experiment was conducted at Fisheries department Nkubu and laboratory analysis done at University of Nairobi and Fletcher scientific solutions

6.2.2 Preparation of Diets

The diets were prepared as described in 3.2.2 in chapter 3

6.2.4 Sample Collection for Carcass Analysis

Both at the beginning and end of feeding trial (final day of sampling), 10 fish samples were selected randomly from each group for carcass proximate and fatty acid analysis. Fresh fish were immediately descaled, eviscerated (gutted). Fillets were removed from the muscles for fatty acid analysis. The samples were packed in a cool box, preserved with clean ice blocks and transported to the deep freezer at -4°C . Both whole carcass and fillets samples were minced using meat mincer and homogenized in preparation for carcass proximate and fatty acid analysis.

6.2.5 Analysis of Samples

Chemical analysis of whole carcass and fillets was done in order to ascertain the meat quality.

6.2.5.1 Carcass Proximate Analysis

The proximate analysis of the carcass were carried out in triplicates following the procedure AOAC (1995) as described in chapter 3.3.1

6.2.5.2 Fillet Fatty Acid Analysis

Fatty acid analysis of fillets was performed by MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range of 780–2500 nm. NIR spectra of foods comprise of broad band's arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C-H, O-H and N-H chemical bonds (Osborne, 2006). Approximately 30-50 g of the sample was put into the sample cup, which was later put on the integrating sphere for measurement. The calibration models were created by INGOT[®] and Bruker Germany. Samples were analyzed for calibration and cross validation of the calibration performed.

6.2.6 Sample Collection and Preparation for Sensory Analysis

After 101 days of the experimental trial, fish for the sensory test were randomly selected from the net cages for each group and transferred into fresh clean water

where they were starved for one day. During the day of sensory test, three fish from each group were selected, gutted, descaled and washed in tap water

6.2.7 Organoleptic/Sensory Evaluation

Organoleptic test was done following the procedure by Kirimi *et al.*, (2015). Fish were blind coded with three digit numbers and then double wrapped with aluminium foil (5mx45.5cm heavy duty). Two litres of water was put in a sufuria and the gas heat used to boil the water to 100°C. The wrapped fish in the aluminium foil were put in the boiling water and the sufuria covered with a lid. The temperature was measured by inserting a thermometer in the boiling water. They were left for 20 minutes to cook under steam and then the heat put off. The steamed samples were removed from the sufuria and cooled to a room temperature. Eight member panel (gender: 4 women, 4 men; age group 30-56 years) were selected from fisheries and agriculture department Nkubu. All volunteers were selected on the basis of their interest and availability. Prior to evaluation, a session was held to familiarize the panelists with the product. The panel was then asked to read through the questionnaires and understand the meaning of each attribute (texture, taste, aroma, appearance and juiciness) to avoid any misinterpretation (Kilcast & Subramaniam, 2000). The panelists were not allowed to discuss their findings with one another during the evaluation session.

Steamed and fresh fish were presented to the 8 semi-trained panelists in plates coded with three digit random numbers, along with distilled water to wash their mouth between the samples. Panelists evaluated whole fish first from each group coded with three digit numbers that did not indicate treatment. The panelists individually evaluated changes in skin, gills, texture and odour.

The assessment was based on 5 point hedonic scale (where 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = like very much). The descriptors for various sensory attributes were defined and the panelists were asked to rate their acceptance for general appearance, texture and colour while the attributes of steamed fish samples were, aroma, taste and juiciness. The samples were evaluated for aroma by sniffing when the aluminium foil was first opened partially. Panelists were advised to have at least 2 minutes break before tasting the next sample. The descriptions of sensory attributes for both fresh and steamed fish are as shown in Table 23.

Table 23: Description of Sensory Attributes used for the Fresh and Steamed Fish Evaluation

Attribute	Description of the attribute
Appearance	Bright, iridescent pigmentation, colour, size, shape, clarity, surface,
Texture	Rigid body, firm and elastic
Aroma (odor)	Aroma is the odor of a food product
Taste (flavour)	Intensity of perceived taste of typical steamed fish flesh
Juiciness	Intensity of juiciness of steamed fish flesh while chewing
Overall acceptability	Overall impression of the fried fish flesh based on above attributes

Partially adopted from Khan *et al.*, (2011)

6.2.8 Data Analysis

Carcass proximate composition, fatty acid and sensory evaluation data were subjected to analysis of variance (ANOVA) using SPSS statistical package version 17.0 at P= 0.05 confidence level to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

6.3 Results

Carcass proximate, fatty acid and sensory evaluation data is as shown below.

6.3.1 Carcass Proximate Composition

Results of the whole fish body proximate composition at the start and end of the study is presented in Table 24. There was a change in whole body proximate composition at the end of experiment compared with that at the start of the experiment. Initial crude protein content was 60.19% while lipid was 17.23%. However, at the end of experiment, there was increase in crude protein content both in enzyme treated diets and non-treated diets. SBM recorded higher figures for CP (64.49%) closely followed by FM based diet (63.84%). FM based diets recorded lowest lipid content (16.83%) than oilseed meals. SFM recorded highest concentration of the lipid content (20.62%). The ash content ranged from 13.60% SBM to 14.50% SFM. However, on crude papain treated diets, there was a slight increase in protein content (63.97%) and lipid content (19.58%)

Table 24: Carcass Proximate Composition (%) of Nile Tilapia Fed on Oilseed Meals Supplemented with Crude Papain Enzyme

	<u>Diet (D)</u>					<u>Enzyme (%) (E)</u>		<u>Significance</u>		
	Initial	FM	SBM	CM	SFM	0	0.06	D	E	DXE
DM	91.06±0.01	91.92±0.11 ^{cb}	92.08±0.12 ^{bc}	93.04±0.19 ^a	90.97±1.18 ^d	92.58±0.21	91.42±0.57	P<0.05	P<0.05	P<0.05
CP	60.19±0.19	63.84±0.63 ^{bc}	64.49±0.21 ^a	63.19±0.25 ^{dc}	63.48±0.20 ^{cbd}	63.53±0.22	63.97±0.33	P<0.05	P<0.05	P<0.05
EE	17.23±0.54	16.83±0.17 ^d	19.55±0.11 ^c	20.00±0.32 ^b	20.62±0.47 ^a	18.91±0.33	19.58±0.58	P<0.05	P<0.05	P<0.05
ASH	14.16±0.16	13.89±0.39 ^{cdb}	13.66±0.58 ^{dc}	13.96±0.37 ^{bc}	14.50±0.09 ^a	14.77±0.06	13.24±0.23	P<0.05	P<0.05	P<0.05
CF	0.88±0.13	1.00±0.14 ^{cb}	1.27±0.12 ^{ab}	1.17±0.17 ^{bca}	0.73±0.04 ^d	1.16±0.10	0.93±0.10	P<0.05	P<0.05	P<0.05
NFE	4.06±0.40	4.44±0.37 ^a	1.04±0.41 ^{cd}	2.27±0.71 ^b	0.73±0.25 ^{dc}	1.64±0.52	2.60±0.52	P<0.05	P<0.05	P<0.05

Values are expressed as mean ± SE. ^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

6.3.2 Fillets Fatty Acid Composition

Fatty acid composition of the fillets is shown in Table 25. Fillets fatty acid composition closely resembled dietary fatty acid composition though no definite pattern for all the fatty acids. Palmitic acid (16:0) constituted the largest proportion of the saturated fatty acid in both enzyme treated and non-treated fish fillets. However, enzyme treated diets recorded highest figure (16.21). Linoleic acid (18:2n-6) was in highest concentration of the polyunsaturated fatty acid in all the treatments. However, monosaturated fatty acid; Oleic acid (18:1n-9) was highest in both treatments. The effect of enzyme treatment led to increase in saturated and monosaturated fatty acid and decrease in total polyunsaturated fatty acid in all the diets. CM based treatments recorded highest proportion of the total polyunsaturated fatty acid.

Table 25: Fillets Fatty Acid Composition (g/100g) of Nile Tilapia Fed on Oilseed Meals Supplemented with Crude Papain Enzyme

	<u>Diet (D)</u>				<u>Enzyme (%) (E)</u>		<u>Significance</u>		
	FM	SBM	CM	SFM	0	0.06	D	E	DXE
Saturated									
Myristic acid (14:0)	2.83±0.27 ^b	2.62±0.26 ^d	2.79±0.38 ^c	2.91±0.37 ^a	3.50±0.06	2.07±0.03	P<0.05	P<0.05	P<0.05
Palmitic acid (16:0)	15.02±0.80 ^c	14.60±0.21 ^d	15.69±0.42 ^b	15.72±0.28 ^a	14.30±0.21	16.21±0.21	P<0.05	P<0.05	P<0.05
Stearic acid (18:0)	3.61±0.24 ^{dc}	3.62±0.22 ^{cd}	3.65±0.34 ^{bcd}	3.84±0.45 ^a	2.98±0.37	4.37±0.88	P<0.05	P<0.05	P<0.05
Total saturated	21.46	21.84	22.13	22.47	20.78	22.65			
Mono- unsaturated									
Palmitoleic acid (16:1n-7)	6.25±0.23 ^d	6.87±0.28 ^{cab}	7.10±0.18 ^{acb}	7.04±0.22 ^{bca}	6.35±0.12	7.29±0.12	P<0.05	P<0.05	P<0.05
Oleic acid (18:1n-9)	22.49±0.74 ^c	22.07±0.83 ^d	22.99±0.63 ^b	23.22±0.54 ^a	21.16±0.21	24.23±0.61	P<0.05	P<0.05	P<0.05
Eicosenoic acid (20:1n-9)	3.02±0.30 ^a	2.19±0.43 ^b	2.14±0.47 ^c	2.09±0.43 ^d	1.45±0.16	3.27±0.07	P<0.05	P<0.05	P<0.05
Erucic acid (22:1n-9)	1.92±0.29 ^a	1.39±0.47 ^{cb}	1.40±0.49 ^{bc}	1.32±0.46 ^d	0.55±0.12	2.46±0.03	P<0.05	P<0.05	P<0.05
Nervonic acid (24:1n-9)	0.41±0.04 ^a	0.38±0.05 ^b	0.35±0.07 ^c	0.32±0.04 ^d	0.48±0.01	0.25±0.01	P<0.05	P<0.05	P<0.05
Total mono- unsaturated	34.09	32.9	33.98	33.99	29.99	37.5			
Polyunsaturated									
Linoleic acid (18:2n-6)	9.34±0.55 ^b	9.83±0.20 ^a	7.63±0.62 ^d	8.18±0.26 ^c	8.45±0.50	9.04±0.24	P<0.05	P<0.05	P<0.05
Alpha- linolenic acid	1.29±0.14 ^{cd}	1.54±0.08 ^b	4.45±1.48 ^a	1.26±0.06 ^{dc}	1.36±0.06	2.92±0.85	P<0.05	P<0.05	P<0.05
Gamma-linolenic	0.05±0.02 ^d	0.06±0.02 ^d	0.10±0.04 ^{ab}	0.10±0.04 ^{ba}	0.15±0.01	0.00±0.00	P<0.05	P<0.05	P<0.05

acid (<i>18:3n-6</i>)									
Arachidonic acid (<i>20:4n-6</i>)	0.59±0.01 ^a	0.46±0.06 ^d	0.53±0.09 ^b	0.53±0.10 ^c	0.67±0.02	0.38±0.03	P<0.05	P<0.05	P<0.05
Eicosatrienoic acid (<i>20:3n-3</i>)	0.10±0.05 ^b	0.09±0.04 ^c	0.05±0.02 ^d	0.11±0.05 ^a	0.8±0.01	0.00±0.00	P<0.05	P<0.05	P<0.05
Eicosapentaenoic acid (<i>20:5n-3</i>)	4.09±0.11 ^b	3.72±0.18 ^d	4.27±0.32 ^a	3.78±0.27 ^c	4.35±0.13	3.59±0.13	P<0.05	P<0.05	P<0.05
Docosahexaenoic acid (<i>22:6n-3</i>)	7.10±0.48 ^d	7.37±0.60 ^c	7.76±0.28 ^b	8.17±0.50 ^a	8.63±0.14	6.57±0.16	P<0.05	P<0.05	P<0.05
Total polyunsaturated	22.56	23.07	24.79	22.13	24.41	22.5			

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

6.3.3 Sensory Analysis

Results of sensory analysis are as shown in Table 26. There was no significant difference on texture and aroma of fish due to different protein sources ($P>0.05$). However, FM based diet scored higher numerically closely followed by canola meal based diet. The taste of the meat appeared to be best for FM based diet (4.63) with CM and SFM recording similar figures (3.75). Juiciness and overall acceptability was high in FM based diet. Enzyme treated diets scored low for all sensory attributes. However, there was no significant difference for taste and aroma ($P>0.05$) in fish on enzyme treated diets.

Table 26: Sensory Evaluation of Fresh and Steamed Nile tilapia Fed on Oilseed Meals as Replacement of Fishmeal Supplemented with Crude Papain Enzyme

Attribute	<u>Diet (D)</u>				<u>Enzyme (%) (E)</u>		<u>Significance</u>		
	FM	SBM	CM	SFM	0	0.06	D	E	DXE
Appearance	4.13±0.20 ^{bcd}	3.69±0.24 ^{db}	4.05±0.19 ^{abc}	3.94±0.17 ^{cba}	4.22±0.13	3.78±0.15	P>0.05	P<0.05	P>0.05
Texture	4.13±0.18 ^a	3.69±0.19 ^a	4.00±0.16 ^a	4.00±0.18 ^a	4.13±0.13	3.78±0.12	P>0.05	P>0.05	P>0.05
Aroma	3.94±0.25 ^a	3.50±0.24 ^a	3.63±0.20 ^a	3.56±0.27 ^a	4.03±0.17	3.28±0.14	P>0.05	P<0.05	P<0.05
Taste	4.63±0.16 ^a	3.94±0.21 ^{bcd}	3.75±0.19 ^{cbd}	3.75±0.28 ^{dc}	4.12±0.15	3.91±0.17	P>0.05	P>0.05	P<0.05
Juiciness	4.50±0.16 ^a	3.81±0.23 ^{bcd}	3.81±0.21 ^{cbd}	3.69±0.22 ^{dbc}	4.06±0.15	3.84±0.16	P<0.05	P>0.05	P>0.05
Overall acceptability	4.25±0.11 ^a	3.69±0.12 ^{dbc}	3.87±0.13 ^{bd}	3.81±0.19 ^{cd}	3.94±0.10	3.87±0.11	P<0.05	P>0.05	P<0.05

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (P<0.05)

6.4 Discussion

6.4.1 Carcass Proximate Composition

Chemical analysis at the end of the experiment is frequently used to determine the influence of feed on fish composition. According to Amadou and Tidiane (2016), both environment and diet are exogenous factors that affect the proximate composition of cultured fish. Carcass proximate composition was little affected by dietary and enzyme treatments at the termination of the feeding trial. This is in agreement with previous studies (Hemre, *et al.*, 2004) where the dietary changes had small effects on the chemical composition of either the whole fish, or specific tissues, such as the muscle or the liver. The differences between these tissues regarding the extent of compositional changes, to which they may be affected by dietary changes, have also been reported (Lie, *et al.*, 1988; Hemre, *et al.*, 2004). However, there was increase in protein and fat in final carcass composition on dietary treatments. According to Jobling and Johansen (2003), size probably plays a more significant role in influencing the whole body composition, given the differences between the start and end protein, lipid and ash that were shown in the present study

The carcass crude protein content in all the treatments was markedly high and ranged between 60.19% and 64.49%. This is in-line with the report of Steffens (2006), that protein forms the largest quantity of dry matter in fish. These values are within the range reported by Koumi *et al.*, (2009) and Iluyemi *et al.*, (2010). However, the findings in the present study are lower than 70.4% CP reported by Jabir *et al.*, (2011) for Nile tilapia. The high protein content in Nile tilapia as in the present study makes it important living resource of dietary protein as other sea and fresh water fish (Vlieg & Murray, 1988; Zuraini *et al.*, 2006). The increase in protein content above the initial can be attributed to the optimum protein and energy in the feed and proper feeding of fish. However, in case of underfeeding or restricted food supply, fat reserves are first mobilized and this reaches a critical low value before proteins begin to be utilized, ultimately causing a reduction in protein contents (Love 1980; Hassan & Javed 1999). In the present study, fish were fed twice in a day at 5% body weight to optimise feed utilisation. According to Huss, (1988) during periods of heavy feeding, the protein content of muscle tissue increases slightly at first then the fat content may show a marked and rapid increase.

The dry matter content was high in all the treatments but in case of restricted feeding, as protein is being utilized, water moves-in to take its place. Such shift results in increased water content of the fish body. According to Daudpota *et al.*, (2014), fat and water to a certain degree substitute each other such that with increasing fat content, the protein content (% of dry matter) is reduced with a simultaneous increase in dry matter. This scenario is also observed in farmed fish. Less effort is needed to get food, this lead in enhanced adipose deposition resulting in decreased water contents in the fish body (Rasmussen, 2001).

Lipids are generally regarded as the most important constituents, which determine the quality of fish meat (Love, 1988). According to Kasheif and Ibrahim (2011), dietary lipids affect body composition of tilapia. In their study, the biochemical analysis of whole tilapia fish bodies indicated that moisture, ash and protein contents are unaffected by the lipid levels in the diet, however, whole body lipid content increased with the dietary lipid level. In the present work, trends in the whole body lipid concentration slightly varied among the treatments. The marked difference in lipid content of fish may be due to effect of crude papain enzyme supplementation in the feed which might have enhanced fat deposition due to increased feed efficiency. Tilapias have a tendency to store most of the dietary fat. However, they have been described as lean fish (Viola, 1988). According to Hanley (1991), 40% of the body fat in tilapia is distributed around the viscera while muscle contains only 8% of the total fat. Based on this, the fat content recorded in this study could be only 8% because viscera fat was discarded. Shearer (1994) stated that the whole body lipid stores are influenced more by energy intake than by dietary lipid levels. Excess energy in the diets may result in excessive deposition of carcass lipids (Bromley, 1980; Metailler *et al.*, 1981). However, in the present study energy content in the diets (Table 5) was balanced across the treatments.

The ash content ranged from 13.24% to 14.50% in all the treatments. The results reveal significant difference ($P < 0.05$) in body ash content between treatments. The ash content in this study indicates the presence of different minerals and according to Murray and Burt (2001), constitute 1-2% of the edible portion in fish. This implies that Nile tilapia is a good source of minerals. Similar values of ash content in Nile

tilapia have also been reported by Iluyemi *et al.*, (2010) (14.0%) and Jabir *et al.*, (2011) (14.1%).

The narrow range of final carcass proximate composition in the present study could have been attributed by narrow range of dietary nutrients treatments. However, in case of increasing protein levels in the diets, there is increase in whole body protein and decrease in lipid content due to the high carbohydrate and low protein content in the diet having low protein concentration (Daudpota *et al.*, 2014).

6.4.2 Carcass Fatty Acid Composition

In the present study the whole body fatty acid composition closely resembled fatty acid composition in the diets though some slight variation in specific fatty acid. This is in agreement with Al-Souti *et al.*, (2012); Mulligan and Trushenski, (2013) and Visentainer *et al.*, (2005) that fatty acid composition of cultured fish greatly depends on the dietary fatty acids. Fillets from fishmeal based diet recorded low values for total saturated fatty acids attributed to low levels of these fatty acids in the diets (Table 25). Palmitic acid (16:0) and Oleic acid (18:1n-9) were the most abundant saturated fatty acid in fish fillets and this was a reflection of the fatty acid in the dietary treatments. According to study conducted by Satue and Lopez (1996); Suloma *et al.*, (2008), palmitic acid is the predominant saturated fatty acid in Nile tilapia and this agrees with the present study. However, myristic acid was in lowest concentration of all the saturated fatty acid in the fillets. According to Moloney *et al.*, (2001), myristic acid (14:0) and palmitic acid (16:0) are considered to be hypercholesterolemic fatty acids and thereby increase the synthesis of cholesterol, promoting the accumulation of low density lipoprotein, which is a risk factor for cardiovascular diseases. However, when consumed by humans, stearic acid is transformed into oleic acid (monounsaturated), a fatty acid that does not carry any cardiovascular risks (Barendse, 2014; Lima *et al.*, 2017). Oleic acid was the most abundant of the mono-unsaturated fatty acids in both the diets and fillets.

However, linoleic acids in the fillets were much lower than levels in the diets. This is because some dietary linoleic acid might have been converted to long chain polyunsaturated fatty acid by desaturase and elongase enzymes (Torstensen & Tocher,

2010). In this study, the levels of arachidonic acid (20:4n-6) in the fillets resembled those in the dietary treatments. However, study done by Visentainer *et al.*, (2005) where addition of flax seed oil rich in alpha linolenic acid (18:3n-3), was found to moderately increase concentration of arachidonic acid in tilapia fillets. The approach did little to increase LC-PUFAs. This is likely due to fact that tilapia has a limited hepatic capacity to elongate and desaturate 20:5n-3 and 22:6n-3 from dietary ALA precursor (Tocher *et al.*, 2002; Karapanagiotidis *et al.*, 2007)

Soybean meal, canola meal and sunflower meal based fillets had slightly similar levels of polyunsaturated fatty acid. However, they recorded higher levels for saturated fatty acids. Karapanagiotidis *et al.*, (2006) reported on the elevated level of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in the fillet of intensively farmed tilapia due to the increased fat deposition.

In the present study, fish had low levels of decosahexanoic and eicosapentaenoic acid (20:5n-3). Although this might have been contributed by low levels in the diets, fish were frozen at -4°C for a period of about 3 weeks before analysis was done which might have led to some levels of oxidation of PUFA hence low values observed. According to Maina *et al.*, (2003), the highly unsaturated fatty acids are more susceptible to oxidation and may have undergone some degree of oxidation during storage. PUFA predisposes the meat to rancidity with linolenic acid (C18:3) being twice as susceptible to oxidation as compared to linoleic acid (C18:2) (Lima *et al.*, 2017; Nurnberg *et al.*, 2005).

Although diet was the major cause of variation in fillet fatty acid composition in the present study; other factors could also have played a role. According to FAO (2009), Zenebe, (2010), the fatty acids composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location. The present study was conducted in warm season i.e between the month of January and April. According to Cahu *et al.*, (2004), Ng *et al.*, (2007), lipid composition of farmed fish is more constant and less affected by seasonal variations than that of wild fish as its flesh fatty acid profile directly reflects the fatty acid composition of the diet. However, the cold the water temperature, the more efficient fish are at converting

saturated fatty acids into mono saturated and polyunsaturated fatty acids. This is possibly due to need to keep cell membranes fluid at lower temperatures and polyunsaturated fatty acids provide greater membrane fluidity (Desilva *et al.*, 1997).

In relation to the duration of feeding, Justi *et al.*, (2003) found that in Nile tilapia, the length of the feeding time (in a period of 30 days) is directly related to the incorporation of n-3 PUFA into fillet, mainly for α -linolenic acid. Tonial *et al.*, (2009) demonstrated that 45 days is the shortest time period required for the inclusion of linseed oil in tilapia feeds to raise the nutritional value (n-6 to n-3 of muscle tissue) of Nile tilapia. Based on these studies, the length of feeding time (101 days) in present work was sufficient to raise the nutritional value of n-6 to n-3.

6.4.3 Sensory Evaluation

In the present experiment, there were variations observed in the organoleptic quality of Nile tilapia fed diets with and without enzymes. However, to the best of knowledge in this study, no similar studies on the effect of crude papain enzyme on sensory traits of fish are available. In terms of general appearance, there was significant differences among the treatments ($P < 0.05$). Fishmeal based diet scored higher numerically with sunflower based diet scoring lowest. Fish on FM based diet were brighter in colour than those on oilseed meals. This can be attributed to the protein quality of FM based diet as appearance of coat colour reflects the quality of feed. With reference to the textural impression, all the treatments followed the same trend ($P > 0.05$), with sunflower based diet recording firmer and more rigid fresh than others. This could be attributed to the slight variation in crude fibre and fat. According to Venugopal and Shahidi, (1996); Grigorakis *et al.*, (2003), sensory textural properties of the fish, as differences in texture of fish muscle tissue are related to the lipid, protein, fibre and water contents.

Fresh fish has a mild delicious taste and smell that is attributed by various volatile and non-volatile organic compounds (Grosch, 1996). There was no significant different ($P > 0.05$) in aroma (odor) of enzyme treated fish whereas variation was observed as a result of different protein sources ($P < 0.05$) i.e soybean meal, canola meal and sunflower meal. This agrees with study done by Regost *et al.*, (2003) who found

significant difference in the odour of fish fed diets containing soybean oil and fish fed 100% fish oil. However, study done by Bjerkgeng *et al.*, (1997) on organoleptic properties of juvenile Nile tilapia showed that use of soybean meal did not affect flesh quality in terms of general appearance and organoleptic properties. In the present study, although all fish were fresh, FM based diet scored higher for taste than the other treatments. According to Givens (2002), feeding fish with vegetable based diet as in the present work produces a 'flat taste' to the cooked meat and over softens the texture that lead to oozing out. This could have contributed to the FM based diet being preferred more in terms of taste. Lipids noticeably influence the sensation of cooked fish in the mouth of the consumer. Fat-rich tissues usually taste very smooth and succulent (juicy), while on the contrary, when fat levels are low, the sensation of dryness or fibrousness (rough or coarse) describes the tissue better. Consumer preference is biased towards meat that is tenderer. During the chewing process, fat is released, which stimulates salivation and increases the perception of both juiciness and tenderness. Hence, as a result of the lubricating effect of fat, meat with an increased content of fat is perceived as juicier and tenderer (Gustone, 2006). This could have contributed to FM based diet meat being juicier in the present study.

In terms of the overall impression of the fish (acceptability) based on the above attributes, FM based diet seemed to be more acceptable, closely followed by canola meal based diet. SBM based diet was least preferred. This preference was expressed mainly in relation to the taste, juiciness and aroma.

6.5 Conclusion

Based on the carcass proximate and fatty acid, it can be concluded that plant protein sources led to slight increase carcass nutrient composition especially the protein component. However, in terms of fatty acid profile, crude papain supplementation resulted to increase in both saturated and monosaturated fatty acid and decrease in total polyunsaturated fatty acid. Increase in saturated fatty acid is desirable in human due to health concerns. Hence more research needs to be carried out on the effect of crude papain enzyme on the carcass fatty acid profile. In terms of sensory characteristics, crude papain enzyme supplemented fish were less preferred.

CHAPTER SEVEN

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

7.1 Summary

The burgeoning aquaculture subsector in Kenya presents a major opportunity to reduce persistent rural poverty in the country by increasing incomes and tackling diet-related issues. However, the greatest challenge to increased aquaculture production is the high cost of fish feed. Fishmeal is the desirable animal protein ingredient in fish feeds due to high protein content, balanced amino acid profile, high digestibility and palatability, and essential n-3 polyenoic fatty acids but its costs is unbearable. Attempts have been made to find alternatives to fishmeal in fish feed in order to reduce the cost. Plant protein raw materials have been identified as appropriate substitutes for fish meal, due to their high availability, low prices and suitable nutritional value. However, concerns regarding the relatively low nutrient digestibility, imbalanced amino acid profile and the presence of anti-nutritional factors limit their use in aqua feeds. However, plant protein ingredients of different origin vary in their amino acid composition which necessitates combination of more than one ingredient in order to balance for the amino acids.

In this study oilseed meals (soybean meal, canola meal and sunflower meal) were used as major plant proteins to partially replace fishmeal component of fish feed such that each contributed 10% of the 30% crude protein (CP) in diet 2, 3 and 4 respectively. The purpose of combining the different ingredients was to balance for the amino acids because plant protein ingredients of different origin vary in their amino acid composition. However, due to low availability of nutrients in plant protein ingredients, crude papain extract was used as exogenous enzyme. Papain enzyme is a proteolytic enzyme that breaks down peptide bonds of a protein molecule. In the present research all the basic procedures in developing a sustainable feed for fish production were carried out i.e, the evaluation of proximate, amino acid composition, digestibility, performance efficiency and final product quality (meat) as well as cost implications. These formed the basis for this study and each was evaluated in details. The initial stage involved proximate analysis of the feed ingredients to ensure that the raw materials met the basic nutritional requirements of macro nutrients. The crude protein content of fishmeal, soybean meal, canola meal and sunflower meal were

(62.60, 47.38, 34.39 and 24.81) percent respectively. Based on the protein analysis, it is evident that oilseed meals are good sources of protein in aqua feed apart from SFM which had a CP content level below the recommended in a ration. Despite the high protein content of the oilseed meals, they presented high crude fibre content which is a major limitation to their use. However, crude lipid content of these oilseed meals was comparable to fishmeal with canola meal recording the highest content (23.88%). In terms of the ingredients fatty acid profile, it was evident that oilseed meals cannot compare with fishmeal because of more polyunsaturated fatty acid present. In oilseed meals more saturated fatty acids were detected which is undesirable from nutrition aspect. Amino acid profile of the ingredients revealed that fishmeal is superior in all the essential amino acids unlike in oilseed meals which were low in most essential amino acids. This scenario of deficiency in one or more essential amino acid in plant protein based ingredients makes them inferior to fishmeal despite their availability and low price. As stated earlier, the main aim of formulating feed using different ingredients is to complement deficient nutrients in the other ingredient. Based on this, the above ingredients were then used to formulate isonitrogenous fish feed (30% crude protein). However, despite similar crude protein levels of the diets, crude fibre content was markedly higher than the recommended for Nile tilapia especially in sunflower. It is evident that the high fibre content in oilseed meals was translated into the diets. High fibre content in fish feed accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of nutrients.

However, since fish is a monogatric animal, protein quality is of importance when considering this class of animal. It is known that protein quality entails presence of the essential amino acids and in their right balance in the feed. Therefore, amino acid profile of the ingredients and the diets were determined. In this case the diets protein quality was evaluated using two parameters i.e Chemical score (CS) and Essential Amino Acid Index (EAAI). In the feed ingredients protein quality, fish meal recorded highest chemical scores and EAAI with no limiting amino acid. This was closely followed by sunflower meal. However, SBM, CM and SFM diets were limiting in amino acids especially lysine and methionine. Therefore substituting FM with SBM, CM and SFM in the diets reduced the protein quality of the feed. This was portrayed by the EAAI i.e 0.97, 0.78, 0.77 and 0.76 for FM, SBM, CM and SFM based diets

respectively. Although the protein component in fish feed has always been a major concern fish like all other vertebrates require essential fatty acids (EFA) for normal growth, development and reproduction. It is also well established that the dietary fatty acid is reflected on the carcass fatty acid profile. Based on this, it is possible to manipulate the carcass fatty acid depending on the diet. This is of great importance as it concerns to human health. In this regard, analysis of the diets fatty acid profile was done. Dietary saturated fatty acid increased with oilseed meal substitution but polyunsaturated fatty acids reduced.

Despite improving the protein quality of the oilseed meals, the availability of amino acids is of great concern. Therefore, crude papain enzyme was used to enhance availability of amino acids in the diets. The extract was collected from unripe paw paw fruits and processed for inclusion in the formulated diets. However, prior to inclusion in the diets, protease activity was determined which revealed that the crude papain extract had high activity 1.9 u/mg hence can successfully be incorporated in the ration. This led to *In vitro* protein assay to determine the inclusion level in the plant protein based diets. The formulated diets were used as substrate and four levels of enzyme concentration was used i.e 0.02%, 0.04%, 0.06% and 0.08%. The inclusion level of 0.06% was arrived as the best concentration for use. However, since there was no interaction between diets and concentrations of enzyme, the interpretation of the results was based on the main effects (Diet and enzyme concentration).

Nutrient digestibility experiment was performed in order to determine the effect of crude papain extract on the availability of nutrients. Papain as a proteolytic enzyme cleaves the peptide bonds and avails more amino acids for absorption in the body. It is evident based on this work that the enzyme has a capacity to avail more nutrients due to the increased nutrient digestibility recorded. This was also portrayed in the performance of the fish

In the feeding trial four diets were formulated to contain 30% CP and fingerlings of initial weight 7 ± 3 g were used. There was improvement in growth performance on crude papain extract supplementation. This was attributed to increased nutrient digestibility. Therefore, crude papain enzyme has a capacity to improve efficiency of

feed utilisation by availing more amino acids in fish. However, FM based diet performed better while SFM poorer. In terms of carcass composition, there was increase in the crude protein content compared with the initial. Fatty acid composition of the fillets revealed that polyunsaturated fatty acid composition decreased with enzyme supplementation and increased saturated fatty acids.

7.2 Conclusions

Based on the investigations carried out in this research, the major conclusions are as follows: The proximate nutrient composition of the oilseed meals and specifically the crude protein contents was satisfactory though sunflower meal recorded slightly lower CP (24.81%). Therefore they can be classified as good protein sources. Fishmeal remained superior in proximate nutrient composition. However, the major challenge with the oilseed meals was high fibre content especially in sunflower meal which recorded crude fibre content of (36.38%). Replacement of 10% (On CP basis) of Fishmeal with SBM, CM and SFM did not vary the proximate composition of the diets because all the diets were formulated to contain the same crude protein (30%). However, crude fibre content increased with FM replacement and sunflower based diet recorded highest crude fibre content (16.03%) which is undesirable and far above recommended. Protein quality aspect in terms of amino acids revealed that fishmeal as an ingredient maintained the superiority. This was also reflected in the FM based diet as chemical scores and EAAI decreased considerably on FM replacement with SBM, CM and SFM but gave useful protein sources. Considering amino acid profile of the diets, chemical scores and the Essential Amino Acid Index, all the diets were not satisfactory in the content of essential amino acids. This is because they were all limiting in methionine, with chemical score lower than the standard.

Also replacement of FM with oil seed meals increased saturated and monosaturated fatty acids in the diets which is undesirable because this is translated into the carcass. The chemical composition results reveal that to maintain the quality of fish feed, fishmeal component should be partially replaced with oilseed meals. However, mixing the different ingredients improved the quality of the rations though not to the standard of fishmeal based diet.

In vitro enzyme assay has shown that crude papain has considerable protease activity and based on *In vitro* protein digestibility, 0.06% concentration was more effective for incorporating in Nile tilapia diets. However, enzyme activity was largely masked by the crude fibre content.

The apparent nutrient digestibility revealed an increase in digestibility on addition of enzyme at 0.06%. The results show that not only the protein digestibility was improved but also other nutrients hence crude papain enzyme extract can effectively be incorporated in Nile tilapia diets.

The growth performance of Nile tilapia fed on FM based diets performed better than those on SBM, CM and SFM based diet. The low growth performance observed in SBM, CM and SFM was due to the imbalance of essential amino acids. However, on supplementation with crude papain extract, there was slight weight gain. This implies that crude papain increased efficiency of feed utilisation through improved nutrient digestibility hence overall growth of fish.

Based on the carcass proximate and fatty acid, it can be concluded that there was slight increase carcass nutrient composition especially the protein component. However, in terms of fatty acid profile, crude papain supplementation resulted to increase in both saturated and monosaturated fatty acid and decrease in total polyunsaturated fatty acid. Increase in saturated fatty acid is desirable in human due to health concerns. In sensory attributes, crude papain enzyme supplemented fish were less acceptable than unsupplemented. However, FM based diets were more preferred.

7.3 Recommendations

- i. Based on the diets protein quality in the present study, SBM, CM and SFM were limiting in essential amino acids and future research should focus on exogenous amino acid supplementation.
- ii. *In vitro* enzyme assay in the present study focused only on protein digestibility. However, more research should also gear towards effect on other nutrients.

- iii. Crude papain enzyme concentration used in the current research i.e 0.06% might have been insufficient hence low enzyme activity and subsequent slight weight gain observed. Therefore more research needs to be done by increasing the level of crude papain enzyme in the diet *In vivo*
- iv. The fibre content in the diets in the present study was high and this might have inhibited the activity of the enzyme. Future research should incorporate cellulase in order to degrade fibre in plant protein based diets
- v. The saturated fatty acids which are undesirable in human nutrition increased with enzyme supplementation hence more research is needed on its effect on meat composition and histopathology.
- vi. From the economic view, though it was more expensive to rear fish on FM based diet, it appeared that it could take longer time for fish fed on SBM, CM and SFM diets to reach market size because growth performance was slower. However, the addition feeding days could lead to increased cost of production. Therefore a more holistic economic analysis should be employed in future take into account labour, power etc.

7.4 References.

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**APPENDIX I
NACOSTI PERMIT**

CONDITIONS


1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice.

REPUBLIC OF KENYA
NACOSTI
National Commission for Science, Technology and Innovation
RESEARCH CLEARANCE PERMIT
Serial No. **43621**
CONDITIONS: see back page

THIS IS TO CERTIFY THAT:

MR. JAMES GITONGA KIRIMI
OF CHUKA UNIVERSITY, 0-60202
NKUBU, has been permitted to conduct
research in Meru County
on the topic: PERFORMANCE OF NILE
TILAPIA(OREOCHROMIS NILOTICUS)FED
ON PLANT PROTEIN DIET
SUPPLEMENTED WITH PAPAIN ENZYME
for the period ending:
4th April,2018

Permit No : NACOSTI/P/17/40298/16344
Date Of Issue : 4th April,2017
Fee Received :Ksh 2000


Director General
National Commission for Science, Technology & Innovation

Applicant's Signature