DIGESTIBILITY, GROWTH AND ECONOMIC PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FED ON A MIXTURE OF PLANT PROTEIN DIETS IN CAGES

ANNE MUNANIE MAUNDU (M.Sc.)

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DECLARATION

DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other University.

Signature..... Date..10/08/2020.....

Anne Munanie Maundu
I84/29588/2014
Department of Zoological Sciences

SUPERVISORS

We confirm that work reported in this thesis was carried out by the candidate under our supervision.

Signature..... Date..10-08-2020.....

Prof. Rekha Rani Sharma
Department of Zoological Sciences
Kenyatta University

Signature..... Date 10/08/2020

Dr. Joshua Muli Mutiso
Department of Zoological Sciences
Kenyatta University

Signature..... Date..10/08/2020.....

Dr. Jonathan Mbonge Munguti
National Aquaculture Research Development and Training Centre, Sagana
Kenya Marine and Fisheries Research Institute

DEDICATION

This thesis is dedicated to my parents Josephine and Julius Kisalu, who made sure I get quality education and to my husband Caesar Ngule, our little boy, Rens Weka and girl, Rayna Kasiki for their endless love and support throughout my studies.

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ACRONYMS AND ABBREVIATIONS

AD	Apparent digestibility
ADC	Apparent digestibility coefficient
ANF	Anti nutritional factors
ANOVA	Analysis of Variance
AOAC	Association of analytical chemists
CF	Crude fibre
CF	Condition factor
CP	Crude protein
CSM	Cottonseed meal
Cr_2O_3	Chromic or Chromium (III) oxide
DM	Dry matter
DO	Dissolved oxygen
EAA	Essential amino acids
EE	Ether extracts
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FM	Fishmeal
FSM	Fresh water shrimp meal
FO	Fish oil
LnWf	Natural logarithm of the final weight
LnWi	Natural logarithm of the initial weight
NFE	Nitrogen free extracts
pH	Concentration of hydrogen ions
PUFA	Poly unsaturated fatty acids

PPSM	Plant protein supplement mixture
SBM	Soybean meal
SEM	Standard error of the mean
SFM	Sunflower meal
SGR	Specific Growth Rate
TC	Total costs
TVC	Total variable costs
USD	United States dollar

ABSTRACT

Fish feed is one of the critical components in aquaculture production and accounts for over 60% of total operational costs with protein component being the most expensive ingredient. Traditionally, fishmeal (FM) has been the primary dietary animal protein source. However, with dwindling capture fisheries, FM has become increasingly scarce and expensive due to its demand from human consumers and livestock feed manufacturers. This in turn makes the cost of fish feeds expensive leading to low profit margins in farmed fish. Therefore, there is need to identify alternative, low cost, and nutritionally balanced sources of protein for the growth of the industry. Although plant-based protein sources are viable alternative in replacing FM, there have been no studies on mixture of plant proteins to establish their economic utility in fish farming. This study evaluated the effects of replacing freshwater shrimp (*caridina nilotic*a) meal (FSM), with varying levels of soybean (*Glycine max*) meal (SBM), cottonseed (*Gossypium spp*) meal (CSM) and sunflower (*Helianthus annuus*) meal (SFM) on growth performance, digestibility, whole body composition and economic returns in diets of Nile tilapia (*Oreochromis niloticus).* Fingerlings averaging 25g in body weight were stocked in net cages installed in three 800m^2 fertilized earthen ponds. Each pond had 15 cages evaluating five diets with three replicates for a culture period of six months. Three experimental set ups were designed to evaluate the efficiency of a combination of SBM with other plant protein sources in replacing FSM in fish diets. In trial 1, five isonitrogenous (30% CP) and isocaloric (3.5 kcal g^{-1}) diets were formulated, substituting Fresh water shrimp meal with Soybean meal at rates of 0, 25, 50, 75 and 100%.Trial 2 similar diets as above were formulated replacing fresh water shrimp meal with a combination of SBM, CSM and SFM at rates 0, 25, 50, 75 and 100%. In Trial 3, similar diets as in experiment 2 were formulated replacing FM with a combination of CSM and SFM at rates 0, 25, 50, 75 and 100%. All fish were fed twice daily at 10% of their body weight. Data were expressed as means and standard error of the mean. Growth and proximate composition were analyzed using one-way ANOVA at $p< 0.05$, and differences among treatment means identified using Tukeys Multiple Range Test. Results from the study in trial 1, showed that fish fed on D0 had higher final weight ($p<0.05$) than those fed on D1, D2 and D3, while D4 had the lowest weight. In trial 2, fish fed on D1 showed growth performance that did not differ significantly from fish fed D0. However, highest FM replacement (100%), significantly ($p<0.05$) reduced growth performance. In trial 3, D0 and D1 had significantly ($p<0.05$) higher mean weights than the rest of the treatments. In the three trials, similar survival was observed among treatments, but digestibility of protein decreased significantly ($p<0.05$) with increasing inclusion levels of PPSM in the diets. In trial 1, the ash content of carcass decreased significantly with increased levels of SBM. In trial 2, fish accumulated increasing levels of ash and crude fat with increasing levels of PPSM. In trial 3, crude fat increased significantly across all dietary treatments while ash content decreased with increasing levels of PPSM. Diet D3 in trial 1 was more economically viable although it was not significantly different ($p>0.05$) from D1 and D2. In trial 2 and 3, D0 and D1 were not significantly different hence D1 was more viable because it was cheaper than D0. Based on these findings, the present study concludes that the use of either pure fishmeal or fishmeal containing a mixture of 25% of plant proteins diets leads to similar growth performance in *O. niloticus*, the fishmeal containing the mixture of 25% plant proteins remarkably reduces the production costs and achieves higher profits than when the pure fishmeal is used. The present study therefore, recommends that for desirable net returns plant proteins can be used in fish farming.

CHAPTER ONE : INTRODUCTION

1.1 Background information

Fish is an essential source of food in many if not all parts of the world. In the last five decades, fish consumption per capita in the world rose from 9.0 kg in 1961 and 20.2 kg in 2015 respectively (FAO, 2018). This increased growth is attributed to various factors such as increased population growth, effective channels of distribution, health benefits derived from consuming fish and aquaculture development. The increased demand for fisheries products has made both freshwater and marine aquaculture to grow extensively (Rosa *et al.,* 2007). In the last three decades, aquaculture for food security has been growing very fast, therefore, providing income and food for most developing countries (Bell *et al.,* 2009; Filipski and Belton, 2018).

Aquaculture growth in Africa has been slow and feed has been identified among the key limiting factor (Gabriel *et al.,* 2007). Fish feeds are important in fish farming and constitute over 60% of total operational costs (Munguti *et al.,* 2009). To support sustainable aquaculture growth, research geared towards reducing the costs of feeds will play a key role in promoting the aquaculture sector. Animal protein sources have been identified as the most expensive input in fish feeds, specifically fish meal which has been used for decades (Ogello *et al.,* 2014). Although fishmeal and fish oil supplies have been on the decline, their usage in aquaculture is on the rise and this has made the aqua feed industry to face significantly high costs, shortages and demands due to competition with humans for fish meal (Jonni and Janice, 2014). Therefore, there is need to find cheap and readily available alternative protein sources which can substitute fishmeal partially or completely whilst achieving similar or higher growth

performance (Ginindza, 2012). Alternative feed ingredients must be readily available, containing minimal fibre and anti-nutritional factors levels (Gatlin *et al.*, 2007). A country like Nigeria whose aquaculture production has been on the rise, cannot be sustained by its aqua feeds industry. This has resulted in 75% of imported aqua feeds to bridge the growing feeds demand by fish farmers (Udo and Umanah, 2017). In addition, with a decline in capture fisheries, the feed industry faces shortage of feed ingredients because fishmeal has been used for human consumption due to their good nutritional profile (Watanabe, 2002; Ginindza, 2012). In Kenya, the primary protein sources are Omena (*Rastrineobola argentea*) which is directly consumed by humans, and freshwater shrimp (*Caridina nilotica)* a bycatch of Omena fishery (Munguti *et al.*, 2009). Plant- based protein sources usually are readily available and cheaper than animal-based protein sources. Therefore, more studies would be very valuable especially in the production of important fish species for example *Oreochromis niloticus* which is the most farmed and preferred by consumers in Kenya. This study evaluated the effects of a mixture of different plant-based protein sources (soybean meal, cottonseed meal, sunflower meal, and brans of maize, rice and wheat) on digestibility, growth and best economic performance in *O. niloticus*.

1.2 Statement of the problem

Although demand for fish in Kenya has been on the rise due to increased human population, the rate of depletion of natural fish stocks in major lakes such as Lake Victoria through overfishing and natural environmental degradation has led to decreased fish supply. This has necessitated aquaculture to be selected as the best option to close the gap, but fish feeds in aquaculture production are vital components

as they constitute over 60% of the total operational costs. This is because, the commonly used protein source, fishmeal, is expensive hence it affects the cost of fish feeds. Recently, fishmeal supplies have been declining globally, due to the depletion of natural fish stocks and its increased demand has led to increased costs of fish feeds. For example, omena (*Rastrineobola argentea)* a native sardine in east African lakes and freshwater shrimp *(C. nilotica)* meal are the major protein sources used for feed formulation in Kenya. On the contrary, *R. argentea* is directly consumed by humans whereas *C. nilotica* is caught as bycatch of omena fishery in Lake Victoria and has become scanty and costly due to the seasonal closure of the lake (Munguti *et al.,* 2009). At the moment there has been a growing effort to substitute fishmeal with cheaper, protein sources. Plant protein sources with economic potential include oil seeds, legumes and cereal grains and although plants are abundant, affordable and rich in protein, there has been no studies on the possible utilization of plant-based products as alternative fish feed sources.

1.3 Justification of the study

Aquaculture contributes largely in eliminating hunger and malnutrition through the provision of food fish which are rich in proteins, essential fatty acids, minerals and vitamins. Sub- Saharan Africa obtains more than 30% of protein from fish every year (Olagbemide, 2015). The continued fish demand has resulted in rapid growth of aquaculture (Tidwell and Allan, 2005). However, for the aquaculture industry to be more profitable and viable, cost-effective and high-quality fish feeds are needed (Munguti *et al.,* 2014). For decades, fishmeal has been utilized in fish feeds even though it is costly, due to its balanced essential amino acid profile (EAA), high

palatability and high protein levels (Ogello *et al.,* 2014). However, the cost of fishmeal has doubled in the recent years with up to 36% of worlds total fisheries being used in feed production for chicken and pigs and is also directly consumed by humans (El Sayed, 2006; Ogello *et al.,* 2014). Therefore, with aquaculture on the rise and the demand for reduced production costs, it is important to explore and develop alternative protein sources to replace fishmeal which will reduce the cost of fish feeds making fish farming an attractive venture for potential fish farmers.

Plant proteins appear promising in developing nutritionally balanced and low-cost aquafeeds (Ogello *et al.,* 2014). Examples of some of the dietary protein sources are soybean, cotton and sunflower seed cake, maize germ, cassava, arrow root and papaya leaves (Munguti *et al.,* 2012). The applicability of plant protein sources as efficient fish meal would translate to reliable information to guide in future fish meal formulation if such evaluation studies are conducted in normal commercial fish rearing and production set up as this would provide real time data on fish feed consumption, body mass changes, fish size, and market sale returns. Guided by this requirement, the present study was carried out at the National Aquaculture Research Development and Training Centre, Sagana, due to availability of experimental resources, favourable climatic conditions for culture of Nile tilapia as well as having well trained feed experts.

1.4 Research questions

i). What is the effect of using a mixture of plant protein sources in *Oreochromis niloticus* development performance parameters; overall growth, specific growth rate, feed intake and feed conversion rate.

ii). What is the apparent protein digestibility of the formulated feeds from soybean and cottonseed meal, maize germ and wheat bran on *Oreochromis niloticus?*

iii). What is the effect of using a mixture of plant protein feeds on carcass nutrient composition in *Oreochromis niloticus?*

iv). What is the profitability of using a mixture of plant protein feeds in *Oreochromis niloticus?*

1.5 Hypotheses

i). Fishmeal replacement by a mixture of plant protein sources has no effect on *Oreochromis niloticus* development performance parameters; overall growth, specific growth rate, feed intake and feed conversion rate.

ii). Mixture of plant protein sources has no effect on apparent protein digestibility in *Oreochromis niloticus.*

iii). Usage of a mixture of plant protein sources has no effect on carcass nutrient composition in *Oreochromis niloticus.*

iv). Usage of a mixture of plant protein sources does not affect the profitability of *Oreochromis niloticus.*

1.6 Objectives of the study

1.6.1 General Objective

To determine the apparent digestibility, growth and economic performance of Nile tilapia (*Oreochromis niloticus*) fed on diets of a mixture of plant protein sources in cages installed in fertilized earthen ponds.

1.6.2 Specific objectives

i). To determine the effect of replacing fishmeal with a mixture of plant protein sources on growth performance parameters by *Oreochromis niloticus.*

ii). To determine apparent protein digestibility of plant-protein formulated diets in *Oreochromis niloticus.*

iii). To determine the effect of a mixture of plant protein diets on *Oreochromis niloticus* carcass composition.

iv). To determine the profitability of a mixture of plant protein sources in *Oreochromis niloticus* production

1.7 Significance of the study

Results obtained from this study are important to fish farmers and provides a guide in making decisions relating to fish farming as a business enterprise. In addition, the study also provides guideline to future investigations on the economics of aquaculture farming and better utilization of available production inputs. The discovery of the importance of plant products as fish meal ingredients would, by a function of supply and demand dynamics, lead to increased demand for these plant materials, thereby giving other opportunities for business ventures including cultivation of these plants

as commercial produce. The need for plant material and the cultivation of such plants and vegetables would lead to increased ground cover reducing the effects of soil erosion and mitigating on the negative effects of climate change. These issues would lead to policy formulation at the National level for better economic growth and development.

CHAPTER TWO : LITERATURE REVIEW

2.1 Fish Aquaculture

Aquaculture is a crucial production sector, accounting for approximately 45% of food fish, molluscs, crustaceans and aquatic plants (seaweeds) for human consumption (FAO, 2010). Fish is considered as a good protein source in Asia, particularly in China, freshwater carp culture has been spawned and reared approximately 2500 years ago. In Europe, carp culture was first introduced in the fifteenth century. According to Landau (1992), the first mariculture practice was seen among the Romans over 2200 years ago when they cultured fish and shellfish.

Recent statistics obtained globally from FAO (2018) indicate that global fish production was 171 million tonnes (Figure 2.1) with aquaculture accounting for 47% with an estimated USD 232 billion being from aquaculture production. Generally, Asia has been leading in farmed fish with China being the biggest farmed fish producer globally since 1991 with an estimated output of 61.5 million tonnes in 2018, followed by India, Indonesia, Vietnam, and Bangladesh (FAO, 2018). This growth was attributed to various factors which included population and economic growth, and pre-existing aquaculture practices (Bostock *et al.*, 2010).

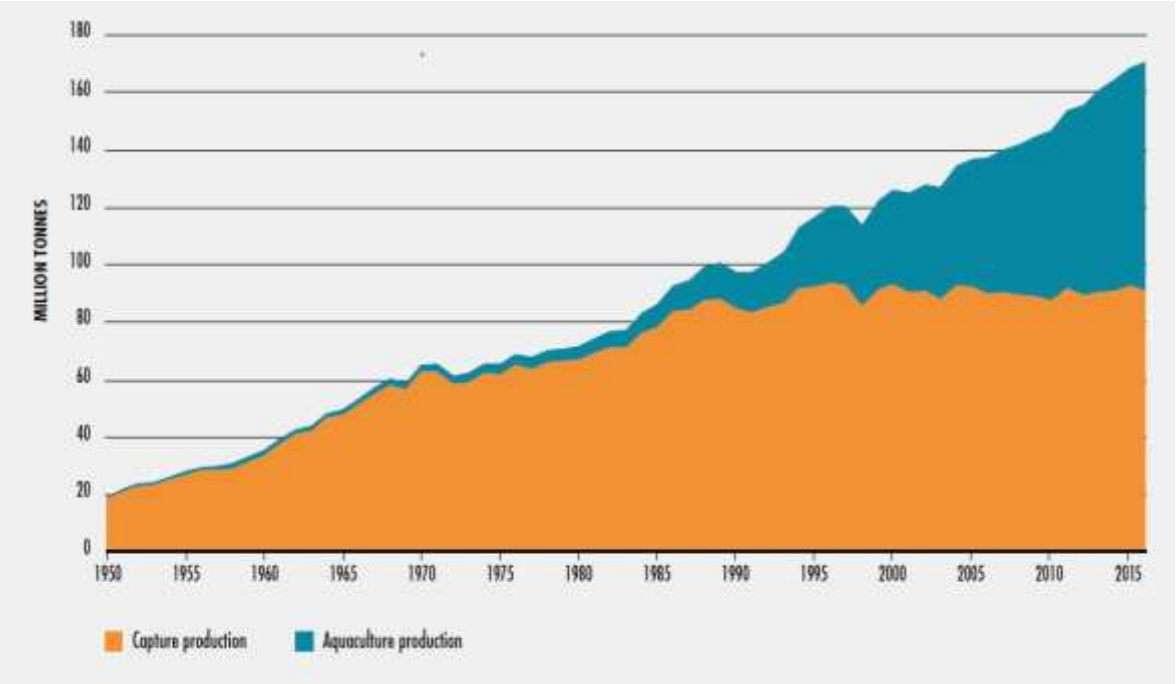

Figure 2.1: World capture fisheries and aquaculture production (Source: FAO (2018)

According to Machena and Moehl (2000), aquaculture for food production in Africa is relatively new since it was introduced in less than 100 years ago and has come a long way. Contrary to terrestrial agriculture, which dominates in most countries in the region, aquaculture has little traditional knowledge in existence among farmers. According to Landau (1992), first tilapia culture experiments were done in Kenya in 1924 and later in Congo in 1937 with around 300,000 ponds being established by end of the 1950s. However, in the early 1960s, aquaculture development slowed down to the end of colonial regimes that saw ponds being deserted because of little produce and absence of government support. This trend changed in the late 1960s to 1980s in that aquaculture development increased due to technical and financial help from donors (Machena and Moehl, 2000). Although the aquaculture sector in Africa is

growing at a very fast rate than the rest of the world, it still provides the smallest quantity of fish produced and eaten. In 2016, aquaculture in Africa provided 17% of the total fish, which translates to a fish production of 2.5% globally (Obiero *et al.*, 2019). However, rapid population growth in sub-Saharan Africa will result in the demand for food fish consumption to grow by 30% between the years 2010 and 2030 (Msangi *et al.*, 2013). Bhujel (2014) noted that Egypt, Uganda and Nigeria were the top countries in aquaculture production. In addition, African countries have acknowledged the importance of aquaculture which has seen countries like Kenya investing in it. Freshwater production dominates in Africa with Nile tilapia, Flathead grey mullet, African catfish and native carps being the most cultured and produced in Africa. Nile tilapia is produced in over 20 countries (Brummett and Williams, 2000; Bhujel, 2014) with the most common farming systems being ponds, cages, recirculation systems, pens and raceways. Ponds range from 500 m^2 to 25000 m^2 with levels of production ranging from 3 to 10 tonnes /ha/year. Pens and cages vary from 15m^3-1,600 m^3 and generally used for culture of Nile tilapia (*O. niloticus*), trout (*Oncorhynchus mykiss),* clariids (*Clarias)* and bagrid catfish.

Raceways are primarily used for trout and tilapia while fingerlings and table size fish production occurs under high-density water recirculation systems (Brummett and Williams, 2000; Hecht, 2005). Aquaculture in Africa is still slow and this can be attributed to various factors such as poor policies for aquaculture development, inadequate fish farming traditions, over exploitation on marine fisheries, retarded economic growth, substandard fish seed and fish feeds, poor infrastructure and limited collaboration between research and development sectors (Machena and Moehl, 2001; Hecht, 2005). The cost of fish feeds in East Africa, remain a critical limitation to the

growth of aquaculture which in turn results in Africa's contribution to global aquaculture production to be just approximately 17% (Obiero *et al.,* 2019).

Fish farming in Kenya began in the early 20th century when trout was introduced for sport fishing in rivers. However, its progress has been slow (Ngugi *et al.,* 2007). In 2003, the government of Kenya launched the "Eat More Fish Campaigns" which led to a rise in aquaculture production from 1000 metric tonnes (MT) to 4000 MT (Opiyo *et al.,* 2018). In 2009, the Kenyan government introduced the economic stimulus programme (ESP) to stimulate economic growth and the Fish Farming Enterprise Productivity Program was launched with an objective of commercializing aquaculture sector under the ESP (Munguti *et al.,* 2014). By 2010, 12,153 MT had been realized through the ESP (Opiyo *et al.,* 2018) (Figure 2.2). This was attributed to the construction of over 28,000 fish ponds in the first phase of the economic stimulus programme and the supply of *O. niloticus* and *C. gariepinus* fingerlings (Fisheries department, 2012). This growth led to an increased demand for processed fish feeds leading to increased fish feeds prices. The increased feed prices were due to the existence of only one fish feed company during the introduction of the economic stimulus program (Opiyo *et al.,* 2014). Therefore, despite this milestone in Kenya's aquaculture development, significant constraints like lack of sufficient supply of quality feeds and quality fingerlings continue to be a hindrance to the growth of aquaculture (Fisheries department, 2012; Munguti *et al.,* 2014).

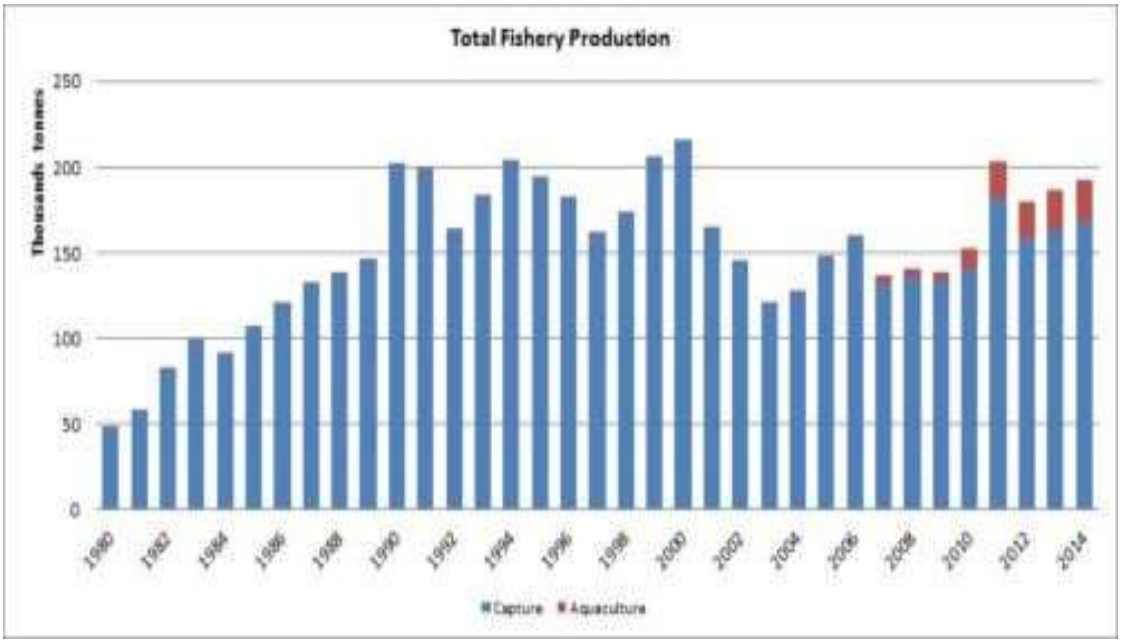

Figure 2.2: Fisheries and aquaculture production trends in Kenya from 1980 to 2014 (Source: FAO (2016))

2.2 Commonly farmed fish species in Kenya

Fresh water aquaculture in Kenya is mainly classified as either warm water or cold-water culture, with warm water culture being dominated by Nile tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus).* Cold water species comprise mainly rainbow trout (*Oncorhynchus mykiss*) (Opiyo *et al.*, 2018). The bulk of cultured fish is made up of *O.niloticus* (75%), followed by African catfish *(Clarias gariepinus)* (18%), common carp (*Cyprinus carpio*) (6%) and lastly rainbow trout (<1%) (Munguti *et al.*, 2014; Opiyo *et al.*, 2018).

2.2.1 Common carp

Common carp *(Cyprinus carpio),* (Figure 2.3) belongs to the order, cypriniformes and family, cyprinidae. It is among the oldest fish species domesticated for food and it is native to countries in Asia and Europe which are associated with Danube river basin and is regarded as an invasive fish species (Yousafzai *et al.,* 2012).

The common carp is found within the middle and lower course of rivers and in shallow areas. It grows best at a water temperature of 23- 30°C. Its optimal pH is 6.5-9.0 and can tolerate a salinity of up to 5%. It is omnivorous, thereby feeding on various benthic organisms such as aquatic insects, molluscs and zooplankton (Flajshans and Hulata, 2007). It can grow up to a maximum length of 1.5 m and weigh 37.3 kg (Yousafzai *et al.,* 2012). Common carp was first introduced in Kenya during the colonial period but has not been favored by the market. Back in 2011, common carp production from aquaculture was 373 tons which equated to 8% of the total aquaculture production in Kenya (Munguti *et al.,* 2014).

Figure 2.3: Common carp (*Cyprinus carpio*) Source: FAO (2004)

2.2.2 Rainbow trout

Rainbow trout (*Oncorhynchus mykiss*) (Figure 2.4) belongs to the family of Salmonidae (Salmonids) and is among the oldest fish species that were successfully farmed in North America and Europe (FAO, 2005). *O. mykiss* is indigenous to the pacific drainages of North America and has been introduced to all continents (except the Antarctica) for either aquaculture or recreational activities. The fish spawns easily, grows fast and are highly in demand as food fish and can tolerate a wide range of environments with an optimum water temperature of below 21°C, although it requires water with high levels of dissolved oxygen. It has two strains; anadromous one which exhibits fast developmental growth attaining 7-10 kgs in 3 years while the fresh water type, reaches 4.5 kg in the same time span (FAO, 2005). Based on the diets fed, the cultured trout can either have red or white flesh (Hardy, 2002)

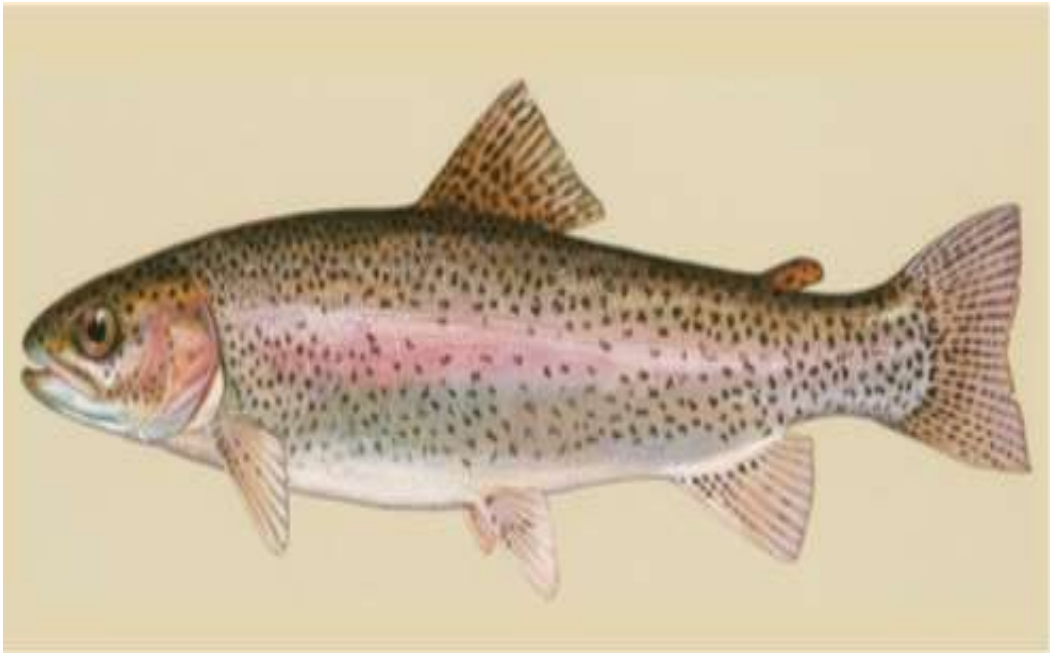

Figure 2.4: Rainbow trout (*Oncorhynchus mykiss*) Source: FAO (2004)

In aquaculture, rainbow trout feed intake depends on various factors such as the strain and size of fish, feeding frequencies and water temperature (NRC, 1993). In Kenya, rainbow trout was first introduced during the colonial period for sport fishing in rivers though its growth has been slow (Ngugi *et al.,* 2007). In 2011, rainbow trout contributed 1% in aquaculture production (Munguti *et al.,* 2014). This could be due to its temperature restrictions as it can only be cultured at 19°C and below, in the Mt. Kenya region.

2.2.3 African catfish

The African catfish *(Clarias gariepinus)* (Figure 2.5), belongs to the family Clariidae, within the Siluriformes order and is characterized by an elongated body with four pairs of barbles (Devaere *et al.,* 2007). Since 2004, the African catfish is among the most widely reared fish in sub-Saharan Africa with its leading producers are Nigeria and Uganda. However, in Kenya, its culture has never superseded Nile Tilapia (Ogello *et al.,* 2011). It is an omnivorous fish, feeding on insects, planktons and plant matter. Culturing African catfish is associated with many benefits which include provision of poly unsaturated fatty acids (PUFA) that help in prevention of cardiovascular diseases. This fish also has a high fecundity; it is used as bait for Nile perch fishery and as a predator for controlling the populations of tilapia in earthen ponds (Chepkirui-Boit *et al.,* 2011). In addition, it can withstand poor water quality and high stocking densities (Kasi *et al.,* 2015).

Figure 2.5: African catfish *(Clarias gariepinus)* Source: FAO (2004)

2.2.4 Nile tilapia

The Nile tilapia (*Oreochromis niloticus*) (Figure 2.6) remains one of the most frequently cultured fish species after salmonids and carp. It belongs to the family, Cichlidae, within the order Perciformes and has over 70 established species in the Tilapia and Oreochromis genera (Lovell, 1998). Tilapia is endemic to Africa and has been distributed globally, with a majority being cultured in sub-tropical and tropical countries. The major producers are China and the Philippines. *Oreochromis niloticus* is commonly referred to as the "aquatic chicken" because of its features such as; tolerance to varied environmental conditions, easy to culture, rapid growth, firm and tasty flesh with low fat levels and lack of intramuscular bones, omnivorous - feeding on low trophic levels on various materials such as phytoplanktons, zooplanktons and detritus and can utilize diets rich in fibre and carbohydrates. Because of its omnivorous feeding, the Nile tilapia is less expensive to culture as compared to salmon which needs high protein diets (Nguyen, 2008; Mjoun *et al.*, 2010).

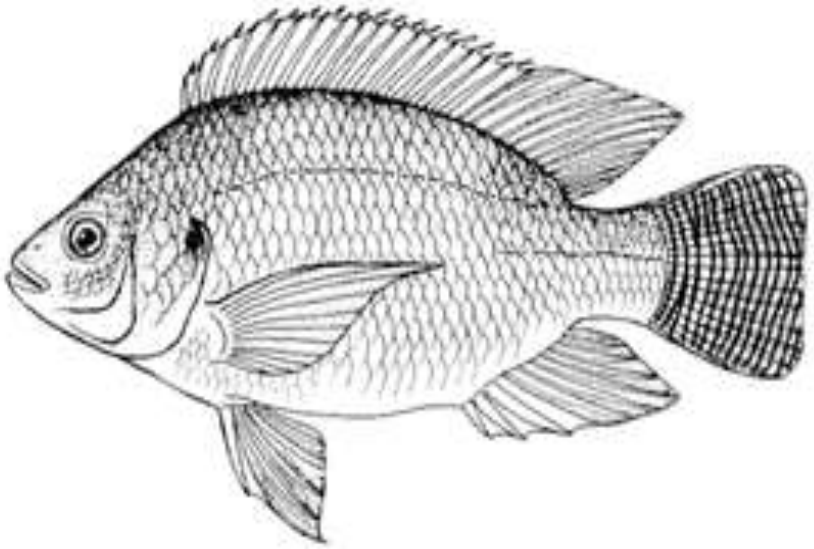

Figure 2.6: Nile tilapia (*Oreochromis niloticus*) Source: FAO (2005)

According to Fitzsimmons (1997), nutrition of Nile tilapia varies with the various life stages. Diets with high levels of protein, lipids, vitamins, minerals and lower carbohydrates are essential for the fish fry and fingerlings in order to aid in muscle, internal organs and bone development. The adult fish require more calories from carbohydrates and fats and a small protein percentage for growth. In addition, they need the ten essential amino acids namely arginine, lysine, histidine, threonine, valine, leucine, isoleucine, methionine phenylalanine and tryptophan (El Sayed, 2004). The Nile tilapia possesses a digestive tract that is at least six times the fish total length and is a site for nutrient digestion and absorption which usually are plant based (Opuszynski and Shireman, 1995).

In the commercial production of Nile tilapia, male monosex populations usually are used. This is because the male tilapia grows twice as fast as females. Although male and female fish consume the same quantity of food, the males' growth rate is higher because male monosex tilapia utilizes most energy for muscle and tissue development

while the mixed sex populations of fish especially female tilapia spend most energy on reproduction activities at the expense of tissue development (Gichukia *et al.*, 2015). In addition, female tilapia brings about uncontrolled reproduction which results to an excessive production of fingerlings, food competition and stunted growth due to reduced somatic growth in favour of sexual maturation which results in producing fish of different sizes which end up not reaching market size hence translating into losses (Gichukia *et al.*, 2015). Therefore, there is need to reverse female tilapia fry for low management requirements and increased production potential. This is done though the usage of a male sex hormone (17 α methyl testosterones, MT) in their feed which results in phenotypic males (Chakraborty *et al.*, 2011). According to Delong *et al*. (2009), for optimum growth in the culture of Nile tilapia, the ideal water quality conditions should be as follows; temperature 27-29°C, dissolved oxygen 5-7.5 mg/L, pH 6-9 and ammonia-nitrogen ≤ 2 mg/L.

2.3 Fish Nutrition

Nutrition remains the major high-cost component in fish farming production with feeds accounting for over 60% of operating costs with protein constituting a large part of the feed costs. Protein is a crucial nutrient as it provides the amino acids needed for synthesis of new and replacement of worn out tissues. Therefore, dietary protein is the most essential nutrient to be considered and should be optimized for synthesis of proteins and not for energy when formulating feeds so as to avoid poor growth and loss of weight in fish (Shiau, 1997; El-Sayed, 2004).

Fish meal usage as the primary protein source in feed formulation in aquaculture is due to its attributes such as being nutritious; high crude protein content; balanced amino acid profile especially lysine, methionine and tryptophan which are deficient in some plant proteins; high digestibility and palatability which translates into high feed intake and nutrient utilization by fish and lack of anti-nutritional factors (Watanabe, 2002; Gatlin, 2007; Ginindza, 2012). The main sources of fishmeal are by-catch from fisheries, trimmings and offal left-overs from fish processing and fish harvested for sole purpose of fishmeal production such as anchovies, herring and horse mackerel among others with the best quality being obtained from raw fish (IFFOO, 2006; FIN, 2008). The significant fishmeal producers are Peru, China and Chile (FAO, 2014). According to Tacon *et al*. (2008), out of the available 6 million tons of fishmeal, 65% is incorporated in fish feeds and the level of inclusion ranges from 40 - 60% in marine fish feeds to < 5% in catfish, tilapia and carp feeds.

However, depletion of natural fish stocks has resulted in fishmeal supply being limited and expensive due to its increased demand (Tantikitti, 2014). This has in turn made the prices of fishmeal to increase (Watanabe, 2002). As fish feed constitutes a higher proportion of total operational costs, this negatively affects a fish farmer's profitability. Therefore, lowering the fishmeal content in formulated diets or identifying practically available, nutritionally balanced and cheaper alternative sources of proteins to replace fish meal can maintain a sustainable aquaculture sector (Tidwell *et al.,* 2005; Gaber, 2006). Usage of plant protein sources is considered economically viable because they are cost-effective and readily available as compared

to animal protein sources which cannot be incorporated in feeds due to a ban on their use in animal production by the European Union (Tantikitti, 2014).

2.3.1 Animal protein sources

Animal proteins are cheaper than fishmeal and are easily available. Examples of animal protein sources include meat and bone meal, blood meal, hydrolyzed feather meal, and poultry by-product meal. Their main attributes are well balanced amino acid profiles, high levels of phosphorus and lysine as compared to plant protein sources. Blood meal for example is rich in protein and lysine; however, it is deemed unpalatable. Bone meal is also rich in phosphorus, zinc and iron as compared to soybean meal. Due to improved processing techniques, their digestibility has shown an improvement of up to 80-90%. Even though the animal lipids are cheaper, they contain unsaturated fats which have a low digestibility and so they must be incorporated with polyunsaturated fats to ease digestion. Another challenge is that, they cannot be incorporated in feeds due to a ban on their use in animal production by the European Union (Tantikitti, 2014). This is because animal proteins in the 80s and 90s were vectors for bovine spongiform encephalopathy (BSE) epidemic (European community, 2002). In addition, during production of these meals, contamination can occur and disease-causing agents like salmonella can be carried forward to the feed. Therefore, since animal protein usage in aquaculture feeds is forbidden in Europe except for non-ruminant blood meal, more attention should be given to plant protein sources such as the oil seed cakes which are in large quantities in markets and are economically and nutritionally valuable sources of proteins (Médale *et al.,* 2009).

2.3.2 Plant protein sources

As aquaculture continues to grow worldwide, availability of fishmeal will be limited due to the declining wild fish stocks. This means alternative plant protein sources have to provide diets that are capable of producing high quality fish flesh with minimal environmental impacts (Gatlin *et al.,* 2007). Plant proteins are considered to be economically valuable because of their low cost and relative abundance. However, they must possess high protein levels, nutrient digestibility, good palatability, low fibre and anti-nutritional factors. Some plant proteins which have been investigated as potential fishmeal replacements include corn, cottonseed, soybeans, wheat, canola, peas and barley (Gatlin *et al.,* 2007; Naylor *et al.,* 2009). However, Soybean is one of the most studied plant protein source in aquaculture diets and is the most nutritive among the plant protein ingredients. It has high crude protein levels ranging from 45-70% depending on the processing method and well-balanced amino acid profile which matches the needs of the cultured organisms. However, it has low levels of methionine. In addition, it is largely available in the market and it is cheaper than fishmeal (Hardy 2000; Dersjant-Li, 2002; El-Sayed, 2004; Nguyen, T.N. 2008; Phumee *et al.,* 2011; Tantikitti, 2014). In aquaculture, the commonly used soybean products are soybean meals which contain soybean from which oil has been removed. They are essential in providing the high-quality protein needed by tilapia and other farmed fish species (Nguyen, 2008).

Most studies have shown that fishmeal replacement levels by soybean meal vary significantly depending on fish species, feeding behavior, age and habitat. According to Chou *et al.* (2004), the right inclusion level for marine species is 20-60%, such that it is 40% for juvenile Cobia (*Rachycentron canadum) and* 45% for Japanese flounder.

In addition, studies have shown that soybean meal supplemented with methionine can completely replace fishmeal without affecting growth and feed utilization in Rohu, (*Labeo rohita)* (Khan *et al.,* 2003).

Cottonseed meal is highly palatable, cheaper than soybean, easily available and has a good protein level of 26-54% depending on the source and processing method. These attributes make it important in tilapia culture in most developing countries. In addition, it contains arginine, an essential amino acid of great importance to most aquatic organisms. Its arginine levels are also higher than those found in fishmeal and soybean meal. However, it lacks some essential amino acids like cysteine, lysine, and methionine but contains high fibre and gossypol which limit its inclusion in tilapia feeds (El-Sayed, 2004; Jiang *et al.,* 2018). Addition of lysine and methionine supplements can improve the protein quality of cottonseed meal (Li and Robinson 2006). Cottonseed meal can reduce growth, feed intake and efficiency in fish and its recommended levels should not exceed 5-15% particularly in salmonids. However, this limit is dependent on the species of fish, level of gossypol and the type of cottonseed meal (Hertrampf *et al.,* 2000).

Sunflower meal is another common plant protein source and is a byproduct of oil extraction in sunflower seeds. It has a great potential in replacing fishmeal in the production of fish feeds (Ogello *et al.,* 2017). It is rich in sulphur amino acids for instance methionine and cysteine. Studies done on poultry and cattle have shown good results at minimal levels of inclusion but growth reduces with increased inclusion levels (Olvera-Novoa *et al.,* 2002). The reduction in growth could be attributed to either low levels of lysine, anti-nutritional factors such as protease and arginase inhibitor and fibre content which has a negative effect on pellet quality and

digestibility of feeds if incorporated at higher inclusion rates (Abou *et al.,* 2008; Dayal e*t al.,* 2011).

Fish diet formulation incorporates non protein sources to provide the energy required while the more expensive protein in the diet which is costly is redirected towards protein synthesis (De Silva and Anderson, 1998). In addition, they can supply lipids and protein contents of the feeds. Energy supplements in crude protein content is less than 20% and include brans of wheat, maize, rice bran, wheat grain and corn among others (Robinson *et al.,* 2001).

Wheat bran is commonly used because it is thought to be palatable and has a mild laxative effect. Its crude protein level is 16.4% and its fibre content rarely exceeds 10%. Rice bran which is a byproduct of rice milling contains 9-18% protein and 10-14% crude fibre. It also contains a laxative effect in the gut and its high fibre level enables it to be utilized as nutrient dilutants in monogastric animals (Medugu *et al.,* 2011). Tilapia farming in sub-Saharan Africa utilizes rice bran as one of the feed ingredients and studies conducted in Kenya comparing rice, maize and wheat bran, show that rice bran performs poorly and is less profitable as compared to wheat and maize bran (Liti *et al.,* 2006). Although these individual plant sources are rich in proteins, some studies have emphasized usage of a mixture of plant protein sources in order to get well balanced amino acid profiles as compared to the usage of single plant protein sources. In addition, use of a mixture of plant proteins in formulating diets prevents high inclusion levels of any particular anti-nutritional substances (Francis *et al.,* 2001; Soltan *et al.,* 2008).

2.3.2.1 Limitations of using plant sources in fish feeds

According to Tantikitti (2014), plant protein feedstuffs possess several challenges which range from anti–nutritional factors, insufficient amino acid levels, poor digestibility and poor palatability. Palatability plays a vital role in the development of feeds as it ascertains the acceptability of the feed ingredients. Texture and palatability can be altered due to incorporation of high levels of plant protein ingredients (Ogunji, 2004). Houlihan *et al.* (2001), reported that feed acceptance is vital in feed formulation as it determines the feed intake regardless of how the feed ingredients are digestible.

Even though oilseeds and pulses are readily available in the market at a lower cost, their incorporation in formulated aqua feeds is often restricted by the presence of anti-nutritional substances (Tacon *et al.*, 1997).

Anti-nutritional factors (ANFs) are those substances produced by normal metabolism which interfere with food utilization, health and reproduction in animals (Akande *et al.*, 2010). According to Francis *et al.* (2002), ANFs can be classified into four groups: (i) those affecting utilization of protein and digestion (protease inhibitors, tannins and lectins); (ii) those that affect mineral use, for example, phytates, gossypol and oxalates; (iii) antivitamins and (iv) ANFs such as mycotoxins, mimosine, saponins and phytoestrogens. Soybean meal for example contains protease (trypsin) inhibitor which inhibits the activity of proteolytic enzymes within the gastrointestinal tract of animals, phytohaemagglutinin and anti-vitamins which can be destroyed during thermal processing. Cottonseed meal comprises elevated of gossypol and lacks lysine and methionine amino acids limiting its utilization in tilapia feeds (Tacon *et al.*, 1997; El-Sayed, 2004). In addition, studies have shown that, usage of soybean at

higher inclusion levels have resulted in lower fish yields. This can be attributed to the low methionine levels and incomplete denaturing of trypsin inhibitor (Dersjant-Li, 2002). Chen *et al.* (2011) reported that saponins resulted in adverse effects in Japanese flounder when soybean was used to replace fishmeal. Furthermore, morphological changes in intestines of common carp and Atlantic salmon have been linked to small intestine inflammation due to saponins found in soybean (Knudsen *et al.,* 2008). Various processing techniques to reduce ANFs include addition of feed supplements, water extraction and dry-wet heating. However, this should be done with caution as some treatment methods can affect the feed ingredients nutritional value. For instance, use of heat changes the chemical nature and reduces nutritional quality of carbohydrates and proteins (Francis *et al.,* 2001).

Another limitation of plant sources is the presence of indigestible organic matter such as insoluble carbohydrates and fibre which results in high amounts of fish excretion and waste (Naylor *et al.,* 2009). Bureau *et al.* (1999) reported that, monogastric animals including fish are unable to digest fibre as they do not secrete cellulase. A fibre content beyond 8-12% in fish is not desirable because it may cause a reduction of the quantity of usable nutrients in the diet. In addition, excessive fibre content can result in decreased nutrient digestibility and total dry matter which results in poor fish performance. Water quality is also affected due to increased faecal waste (De Silva and Anderson, 1995). According to Glencross *et al.* (2007), a feed ingredient is of limited use if it is digestible, has available nutrients but lowers the feed intake. Amino acids (AA) are the most basic structural components of proteins with around 25 distinct amino acids occurring in proteins which may be utilized by tilapia (Jauncey, 1998). Amino acids can be divided into two nutritional groups, essential and

nonessential. Essential amino acids (EAA) cannot be synthesized in sufficient amounts to support maximum growth hence must be included in the diet. These essential amino acids required by fish include isoleucine, leucine, lysine, arginine, histidine, methionine, phenylalanine, threonine, tryptophan and valine (De Silva and Anderson, 1995). Non-essential amino acids can be synthesized by the organism in sufficient quantities to support maximum growth. The non-essential amino acids are only nonessential in the dietary context, but they still carry out critical functions at the cellular and metabolic levels (Jauncey, 1998; Lovell, 1998).

2.4 Digestibility of fish feeds

2.4.1 Nutrient digestion

Digestibility refers to the estimation of the amount of energy and nutrients which an animal can acquire from a specific feed ingredient through the process of digestion and absorption. In addition, nutrients in faeces can never be available for growth and maintenance hence represent key losses at the expense of tissue growth (Glencross *et al.,* 2007). Fish feeds digestibility is mostly determined by the feed ingredients chemical composition and the characteristics of the cultured fish (Yang *et al.,* 2009). For feeds quality to improve, feed manufacturers require substantive information on physiological effects of formulated diets on nutrient digestibility and histological responses on the digestive system. This is because most effects on the histology of gastrointestinal tract are due to the type of feed given to fish, with the intestine being the primary site for nutrient absorption (Aanyu *et al.,* 2014). According to Dimitroglou *et al.* (2010), fish growth can be analyzed by studying the intestinal folds length, number and width where nutrient absorption needed for fish growth occurs.

2.4.1.1 Proteins

Proteins in well processed feedstuffs are normally digestible in fish with digestion coefficients of proteins being 75-95%. However, proteins digestibility can be depressed with increased dietary carbohydrates. Phytates, one of the ANFS, contained in soybean, forms complexes with proteins thus reducing amino acids availability. Tilapia lacks the intestinal enzyme phytase which hydrolyses phytates thus increasing nutrients digestibility. Inhibition of protein digestibility of either of the amino acids results in reduced growth and feed utilization. Studies have shown that, in rainbow trout and Atlantic salmon, phytase pre-treated diets resulted in increased growth due to enhanced protein utilization (Richie and Garling, 2004). In addition, insufficient heating of soybean meal results to a decrease in protein availability. Studies have shown that soybean protein digestibility increased from 45-75% when the heating temperature was raised from 127°C – 204°C (NRC, 1993).

2.4.1.2 Lipids

Fish require lipids for energy. Lipids also act as structural components of bio-membranes, and as carriers of fat-soluble vitamins and as enzyme co-factors. They are a preferred source of nutrients compared to carbohydrates because they are highly digestible. Some studies have shown that lipid digestibility increases with increased protein levels (Ginindza, 2012). Digestibility in lipids ranges from 85-95% in fish (NRC, 1993). According to Krogdahl *et al.* (2005), lipids tend to influence the speed of all nutrients passing through the gastrointestinal tract with high lipid levels reducing the passage speed to give enzymes additional time for hydrolysis.

2.4.1.3 Carbohydrates

Generally, dietary carbohydrates are poorly utilized by fish and different types of carbohydrates are used differently depending on the size and age of the fish. Warm water fishes tend to efficiently use higher levels of dietary carbohydrates than marine and cold-water fish. In tilapia for example, several factors tend to be associated with its carbohydrate utilization; carbohydrate absorption in the intestines is poor when diets contain fiber regardless of the source. Feeding frequency also affects carbohydrate utilization. Carbohydrates should be provided in fish diet as their absence results in proteins and lipids being catabolized for energy to offer metabolic intermediates for synthesis of other biologically relevant compounds (Wilson, 1994; Shiau, 1997). In addition, herbivorous fish and carnivorous fish exhibit varied digestible efficiency of digestible and non-digestible carbohydrates (Krogdahl *et al.*, 2005). Starch digestibility can also be affected by dietary lipids. Studies carried out on Atlantic salmon showed that starch was less digested in diets with high fat levels (240–300 g kg^{-1}) than low fat diets (160 g kg^{-1}) (Krogdahl *et al.*, 2005).

2.5 Factors that influence feed digestibility

Fish feed quality, is determined by the extent to which a fish can digest, absorb and assimilate nutrients. Several factors influence digestibility. These include the fish species under culture, physiological condition of the fish, age, size, temperature, the quality and quantity of the feed, fishmeal replacement levels and feeding frequency among others (Ginindza, 2012).

2.5.1 Physiological condition of fish, age and size

Stress in fish associated with handling, or diseases may affect hormonal profiles which in turn affect enzymatic secretions. Furthermore, the age of the fish influences nutrient digestibility with young fish preferring live food which is easily digestible by the underdeveloped digestive tracts. In addition, digestibility increases with size in omnivorous fish due to increased intestinal length which increases digestion and assimilation time (Nguyen, 2010; Ginindza, 2012).

2.5.2 Feed ingredients quality and quantity

Amino acids composition tends to influence the quality of dietary protein ingredients used in the formulation of feeds and with lack of essential amino acids affecting nutrient utilization (Rahman *et al.,* 2016). For example, fishmeal and levels of its replacement with plant-based proteins such as oil seed cakes and leaf meals affect fish performance, nutrient digestibility due to presence of anti-nutritional substances which can initiate pathogenesis of the gastrointestinal tract and weaken nutrients digestion and absorption (Francis *et al.,* 2001; Ginindza, 2012). Moreover, complete replacement of fishmeal with individual plant proteins lowers nutrient utilization due to high fiber levels and presence of anti-nutritional factors (Borgeson *et al.,* 2006). Incorporating increased levels of lipids affects quality of fish flesh due to increased levels of lipids stored in the edible muscle (Turchini *et al.,* 2009).

2.5.3 Increased water temperature

The body temperature and metabolic rate of fish is dependent of the water temperature as they are poikilothermic which in turn affects their nutrition, feeding behaviour and health (Lall and Tibbetts, 2009). Studies have shown that low temperature results to sluggishness by reducing the speed of digestion as has been noted in studies done on

carp and trout. However, increased temperature increases enzymatic secretions which intensify nutrient digestibility. Studies carried out on rainbow trout to evaluate effects of varying temperatures on digestibility showed a reduction in protein, starch, and energy as temperatures dropped from 15-6°C (Halver and Hardy, 2002; Yamamoto *et al.*, 2007; Mizanur *et al.*, 2014). Halver and Hardy (2002) reported that an increase in temperature increases the metabolic rate which increases rate of passage of ingesta through the gut without complete digestion hence affecting nutrient digestibility.

2.5.4 Feeding frequency and feed ratio

Feeding frequency refers to the number of times fish are fed in a day and it's important because it influences feed intake which in turn affects growth, quality of water which can deteriorate affecting growth of fish, survival and net returns (Eriegha and Ekokotu, 2017). Changes in water temperature can cause changes to feeding levels which may alter the quantity of entire nutrients digested and absorbed from feed intake (Halver and Hardy, 2002). Studies carried out on trout fed 1-3 times /day, resulted in decreased starch digestibility as the feeding frequency increased (Yamamoto *et al.*, 2007).

2.6 Methods used to determine feed digestibility

2.6.1 Direct assessment method

The direct assessment method entails measuring all the feed eaten by fish and the resulting excreta. The fish are force-fed on calculated quantities of feed; after that, the varying excrements are collected and subjected to analysis of nutrient content. To determine the amount of nutrients retained, the amounts of nutrients in excrements are

directly subtracted from those in the feed. To get rid of the fecal leaching problem, all water in the chamber is incorporated in the analyses. A significant shortcoming of this method occurs when fish are immobilized, then force-fed and because of stress, this can affect feed utilization. Also, it is difficult to collect data on feed intake and fecal production that is accurate (NRC, 1993; Glencross *et al.*, 2007).

2.6.2 Indirect assessment method

The indirect assessment method utilizes a non-digestible marker such as chromium (III) oxide (Cr_2O_3), which is included in the diet at a concentration of 0.5 to 1.0% and has proven to be a good indicator for digestibility studies in fish (Nose, 1960; Inaba *et al.*, 1962; Cho *et al.*, 1974; De Silva and Anderson, 1995). This method determines apparent digestibility (Glencross *et al.*, 2007). It is assumed that, throughout the experimental time, the amount of marker will remain the same in the feed and faeces and all the ingested marker by the fish will emerge in the faeces. To determine the nutrient digestibility, an assessment of the difference between the feed and fecal concentrations of the marker, and the nutrient or energy is done. The advantage of using this method is that it gets rid of the need to quantitatively collect all of the excreta and experimental fish can eat voluntarily (NRC, 1993).

2.7 Fish farming economics

Fish farming provides animal proteins as well as creating job opportunities to alleviate poverty in many developing countries (Iruo *et al.*, 2018). The cost of production in aquaculture comprises fixed and variable costs. Fixed costs include land, depreciation cost and they are non-recurring while variable or operational costs are directly involved in the production season and include fish species, culture system, labour,

harvesting, marketing and feed (Shang, 1985; Ahmed *et al.*, 2010). Feed is very instrumental in fish farming enterprises because the growth of fish depends on the amount and quantity of feed administered. Herbivorous fish are cheaper to culture compared to omnivorous and carnivorous fish because the type of feed administered reflects the position of the fish species in the food chain and the intensity of the culture system. Therefore, the more intensive fish production is, the higher the cost of feeds (Shang, 1985; El-Naggar *et al.*, 2008; Iruo *et al*., 2018).

Marketing, which is a component of fish farming economics, entails all the activities involved, such as processing, packaging, transporting and storing to ensure customers get fish in the desired form (Njagi *et al.*, 2013). Ngugi *et al*. (2007), advices farmers to conduct market surveys in order to determine the types and sizes of fish preferred by consumers; for example, whether whole or fillet and the best time to market the fish and the selling price. Prices can be determined by a number of factors such as the size of fish, with bigger fish fetching higher prices than smaller fish (Asche and Guttormsen, 2001), the species and freshness of the fish. A kilo of farmed Nile tilapia in Kenya can fetch around USD 3.93-4.91.

2.8 Water quality

Good water quality is very important for growth and survival of fish. Different physico- chemical and biological factors are key determinants of water quality as they can affect fish production either directly or indirectly. Organisms perform optimally with certain tolerable limits (Bhatnagar *et al.,* 2013). The physico–chemical parameters mostly measured are turbidity, temperature, dissolved oxygen, ammonia, pH, and alkalinity (Swann, 1992). Increased temperature brings about an increase in

oxygen demand whereas a further increase results in decreased oxygen solubility and rising levels of ammonia. Moreover, water clarity needs to be maintained in order to allow light penetration needed for growth of phytoplankton and hence the need to reduce turbidity levels. Levels of dissolved oxygen affect the growth, survival, distribution, behaviour and physiology of aquatic organisms (Bhatnagar *et al.*, 2013). Therefore, for optimum fish production, fish farmers ought to know the important water quality parameters that influence the health of a fish pond and control them to maximize fish yields.

2.9 The future of fish feeds

Based on the literature review, it is clear that, for a long time and at the moment, fishmeal is mainly derived from fish products to supply the protein required in feeds. However, the prices of fishmeal and fish oil are high and are expected to rise due to a decline in their global production and increased demand for usage in livestock and poultry feeds (El-Sayed, 2004). The solution to this problem seems to point to a need to reduce or eliminate the over-reliance of fishmeal and fish oil in aquaculture diets and shift focus to utilizing protein and oil sources from other sustainable resources. Furthermore, it is clear that plant-based protein sources and other nutrients, are cheap and can be made readily available and sustainable, supporting fish farming with economies of scale while also taking care of nature and the environment. Some studies have stressed the need for using a mixture of plant protein sources as more suitable in getting amino acid profiles that are well-balanced amino acid profile as opposed to usage of individual plant protein source. This is because individual plant sources such as soybean, cottonseed meal, sunflower meal, linseed meal, and canola

lack key amino acids and also contain ANFs which should be destroyed to meet nutritional requirements of fish (NRC, 1993; Soltan *et al.*, 2008).

CHAPTER THREE : MATERIALS AND METHODS

3.1 Study area

The study was conducted at the National Aquaculture Research Development & Training Centre (NARDTC), Sagana, Kirinyaga County (Figure 3.1), which lies at latitude 0°39'S and longitude 37°12'E at an altitude of 1230 m above mean sea level. The farm is located 105 km Northeast of Nairobi and has coverage of 59 ha, of which 20 ha are under fish ponds, including 0.02 ha utilized for research ponds. Proximate analyses of feed ingredients, formulated feeds and carcass were carried out at the Kenya bureau of standards laboratory (KEBS) while digestibility analysis was carried out at the department of animal production, Kabete campus, University of Nairobi.

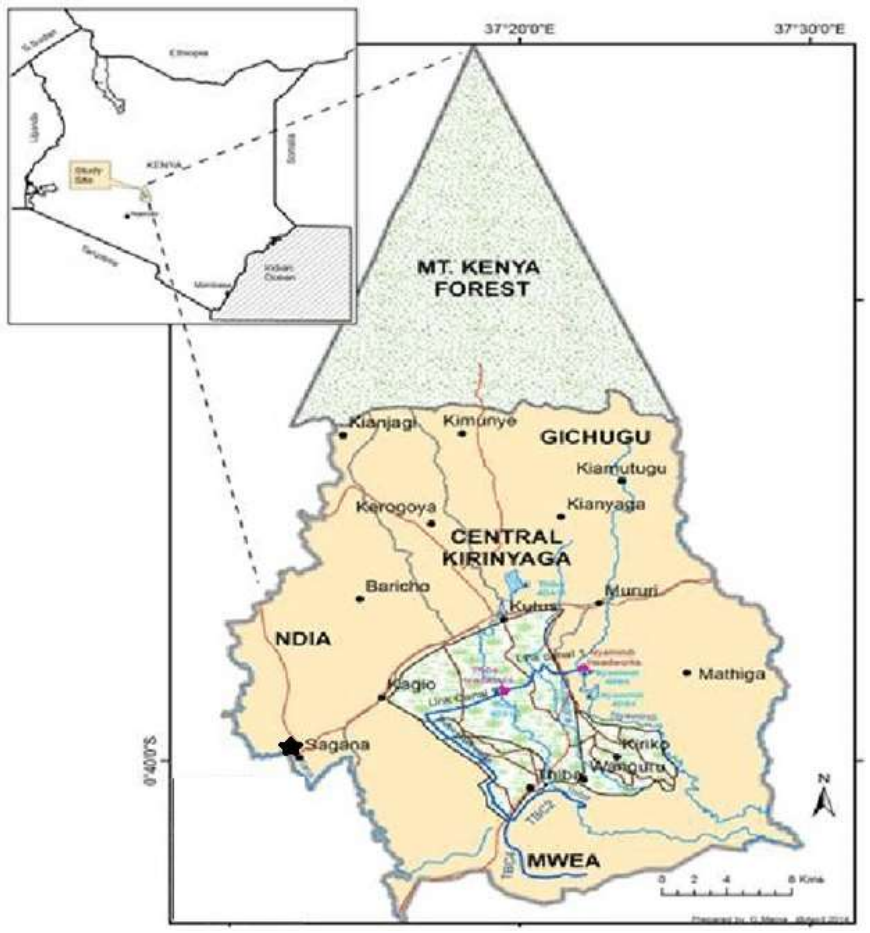

Figure 3.1:Map of Kenya (inset) Sagana, Kirinyaga County (Source**:** Neema and Fredrick, 2019**)**

3.2 Sources of feed ingredients

Soybean meal, cottonseed and sunflower meal, maize, wheat and rice bran were obtained from a local feed manufacturer in Thika town, Kiambu County, while freshwater shrimp meal was obtained from Kisumu County. All the feed ingredients were ground into a fine powder before being subjected to proximate analysis (Table 3.1).

Table 3.1: Proximate composition of fish feed ingredients used in diet formulation (g kg^{-1})

	Proximate composition parameters					
Ingredients	**MC**	**CP**	**EE**	**CF**	**NFE**	**Ash**
Freshwater shrimp meal	9.1	62.7	5.7	3.7	8.1	19.8
Soybean meal	8.5	46.4	0.4	8	38.2	7
Cottonseed meal	10.1	24.7	6.7	16.7	50.4	1.5
Sunflower meal	8.9	19.5	7.1	12.1	46.3	15
Wheat bran	11.5	14.2	4.9	8.4	67.2	5.3
Rice bran	7.7	14.9	16.8	4.6	44.7	19
Maize bran	9.5	11.2	7	2.6	70.3	8.9

Key: MC=Moisture content, CP=Crude protein, EE=Ether extracts, CF=Crude fiber, NFE=Nitrogen Free Extracts.

3.3 Diet preparation and formulation

Five experimental diets, each with 30% crude protein (CP) for each of three trials, were formulated from the following ingredients; soybean meal, cottonseed and sunflower meal, maize, wheat and rice bran. Cottonseed meal, sunflower meal and soybean meal were mixed in varying inclusion levels of (0%, 25%, 50%, 75% and

100%) to form the plant protein supplement mixture (PPSM) (Appendix VII). The ingredients were ground, mixed, moistened and extruded through a motor driven meat mincer. The resulting strands were sun dried and broken into appropriate sizes before being administered to the fish. The three trials were conducted with an emphasis of soybean efficiency. In the first trial, soybean was used as the only PPSM. In the second trial, soybean contributed 50% in the PPSM while cottonseed and sunflower meal formed the remaining 50% of the PPSM. In the third trial, the PPSM was made up of sunflower and cottonseed meal each contributing 50%. The PPSM acted as a single ingredient in the experimental diets. In the control diet, fishmeal was substituted by the PPSM at 0, 25, 50, 75 and 100% levels. Wheat, rice and maize bran mixture were also used as a single ingredient forming energy supplements. Thereafter, the formulated diets were subjected to proximate analysis in order to determine their nutritional composition.The composition of experimental diets for the three trials and their biochemical proximate composition are shown in Tables 3.2, 3.3 and 3.4. For digestibility experiment, Chromic oxide (Cr_2O_3) was used as an inert marker in the formulated diets for Trial I, II and III. Formulated diets (Appendix III, IV and V) were mechanically mixed with warm water to make dough which was later used to produce pellets. The resultant moist pellets were dried under a shade for approximately 12 hours. After that, the diets were reduced in size and sieved into 2–3 mm pellet sizes.

Table 3.2: Feed formulation and proximate analysis of experimental diets for trial I (Replacing fishmeal with soybean meal only)

Ingredients	Experimental diets Content (%) Control (0%)	25%	50%	75%	100%
Freshwater shrimp meal	37.5	28	19	9	0
Soybean meal	0	13	25	39	51.5
Sunflower meal	0	0	0	0	0
Cottonseed meal	0	0	0	0	0
Rice bran	23.1	21.8	20.7	19.2	17.9
Wheat bran	22.0	20.8	19.7	18.3	17.1
Maize bran	17.4	16.4	15.6	14.5	13.5
Total	100	100	100	100	100
Proximate analysis					
Moisture (%)	8.6	9.9	10	9.9	10
Protein (%)	30.1	30.3	30.2	30.0	29.8
Ether extract (%)	9.2	5.3	8.7	10.5	5.5
Crude fibre (%)	8.5	10.2	10	10.2	7.7
NFE (%)	44.6	44.8	43.6	39.9	48.5
Ash (%	7.5	9.4	7.5	9.4	8.3
DE (kcal g^{-1})	3.5	3.3	3.5	3.5	3.4

Key: DE, digestible energy; NFE, Nitrogen Free Extracts

Table 3.3: Feed formulation and proximate analysis of experimental diets for trial II (Replacing fishmeal with a mixture of soybean meal, sunflower meal and cottonseed meal)

	Experimental diets Content (%)				
Ingredients	**Control (0%)**	**25%**	**50%**	**75%**	**100%**
Freshwater shrimp meal	37.5	28	19	9	0
Soybean meal	0	6.5	12.5	19.5	25.7
Sunflower meal	0	2.9	5.5	8.6	11.4
Cottonseed meal	0	3.6	7	10.9	14.4
Rice bran	23.1	21.8	20.7	19.2	17.9
Wheat bran	22.0	20.8	19.7	18.3	17.1
Maize bran	17.4	16.4	15.6	14.5	13.5
Total	100	100	100	100	100
Proximate analysis					
Moisture (%)	8.6	8.6	8.5	8.5	8.4
Protein (%)	30.1	30.2	30.1	30.0	30.2
Ether extract (%)	9.2	8.6	8.1	7.9	7.4
Crude fibre (%)	8.5	8.8	10.0	11.5	12.1
NFE (%)	44.6	45.7	45.4	44.6	44.4
Ash (%	7.5	6.6	6.2	5.8	5.8
DE (kcal g^{-1})	3.5	3.5	3.4	3.4	3.4

Key: DE, digestible energy; NFE, Nitrogen Free Extracts

Table 3.4: Feed formulation and proximate analysis of experimental diets for trial III (Replacing fishmeal with a mixture of cottonseed meal and sunflower meal)

	Experimental diets				
			Content (%)		
Ingredients	**Control (0%)**	**25%**	**50%**	**75%**	**100%**
Freshwater shrimp meal	37.5	28	19	9	0
Soybean meal	0	0	0	0	0
Sunflower meal	0	5.7	11	17.2	22.7
Cottonseed meal	0	7.3	14	21.8	28.8
Rice bran	23.1	21.8	20.7	19.2	17.9
Wheat bran	22.0	20.8	19.7	18.3	17.1
Maize bran	17.4	16.4	15.6	14.5	13.5
Total	100	100	100	100	100
Proximate analysis					
Moisture (%)	8.6	8.5	8.0	8.1	8.1
Protein (%)	30.1	30.0	30.4	29.9	29.9
Ether extract (%)	9.2	8.9	7.6	8.7	8.6
Crude fibre (%)	8.5	11.2	12.1	12.6	14.1
NFE (%)	43.54	41.24	41.8	40.55	40.24
Ash (%	7.5	5.6	4.5	1.8	1.8
DE (kcal g^{-1})	3.5	3.4	3.4	3.4	3.4

Key: DE, digestible energy; NFE, Nitrogen Free Extracts

3.4 Experimental design

Oreochromis niloticus fingerlings with mean weights of 6g, 25g and 28g were obtained from National Aquaculture Research Development & Training Centre, Sagana. The experiments were designed as three trials. For the three trials, thirty *O. niloticus* fingerlings were stocked in three replicates for each of the five treatments in cages installed in 800m^2 ponds (Appendix VI). The fingerlings were acclimated for 15 days before the onset of the experiments and they were given a control diet throughout the conditioning period. In the 1st Trial, five isonitrogenous (30% CP) and

isocaloric (3.5 kcal g^{-1}) diets were formulated substituting fishmeal (Fresh water shrimp meal) with soybean meal (SBM) at rates of 0, 25, 50, 75 and 100%. In Trial II, Similar diets as above were formulated replacing (FSM) with a combination of SBM, CSM and SFM at rates 0, 25, 50, 75 and 100%. In Trial III, similar diets as in Trial II were formulated replacing FSM with a combination of CSM and SFM only at rates of 0, 25, 50, 75 and 100%. All the fish were fed twice daily (10:00 am and 4:00 pm) at 10% of body weight. Sampling of fish was done bi-weekly to monitor growth and adjust the fish feed rations. The experiment was conducted for six months.

Water quality parameters were measured weekly for the physical parameters such as dissolved oxygen, temperature, Total dissolved solutes (TDS), salinity, hardness and pH and biweekly for nutrients such as dissolved ammonia, nitrites, nitrates, phosphates, total nitrogen (TN) and total phosphates (TP) using the multi parameter water quality meter, modelH19828 (Hanna Instruments Limited., Chicago, IL., USA). The cost benefit analysis of the feeds used in feeding was evaluated through the market prices of the feeds, the quantity of the feeds used for one fish and the average weight gained for an individual fish. At the end of the study, fish from all the cages were harvested, weighed and counted.

Digestibility trials for the three trials was done and the study used a complete randomized design (CRD) in glass aquaria measuring (0.6m × 0.3m × 0.3m) with five treatments (control (D0), D1 (25% fishmeal replacement), D2 (50% fishmeal replacement), D3 (75% fishmeal replacement), D4 (100% fishmeal replacement). Each treatment had three replicates with 10 Nile tilapia fish (initial mean weight 25 ± 2 g*)* per replicate stocked in the individual aquariums.

3.5 Proximate analysis of formulated feeds

All the formulated experimental diets were subjected to biochemical analyses at Kenya bureau of standards laboratory (KEBS). The proximate analysis was done in triplicates as outlined in AOAC (1995). The feeds were analyzed for crude protein (CP), crude fibre (CF), ash, nitrogen free extracts (NFE) and ether extract (EE).

3.5.1 Determination of moisture and dry matter

Approximately 5g of each formulated feed sample was placed into a dry pre-weighed crucible. The weighed samples were then dried in an oven at 105^{o}C for 24 hours. The crucibles with samples were removed from the oven and cooled in a desiccator to room temperature and weighed afterwards. Percent dry matter was calculated as: -

$$\% \text{ Moisture} = \frac{\text{Weight of moist sample} - \text{Weight of dried sample} \times 100}{\text{Weight of moist sample}}$$

3.5.2 Determination of ash content

The dried sample in the crucible used for moisture content determination was ashed in a muffle furnace for four hours at 550^{o}C, then cooled in the desiccator and weighed. Ash content was calculated as: -

$$\% \text{ Ash} = \frac{(\text{Wgt of crucible} + \text{ash}) - \text{Wgt of crucible} \times 100}{\text{Weight of crucible} + \text{ash}}$$

3.5.3 Determination of crude protein

The micro-Kjeldahl method was used to determine the nitrogen content in the samples. A sample weighing 0.5g was placed in a test tube and a catalyst (90% K2SO4 and 10% CUSO4) weighing 5g was added in the test tube. Approximately 15ml of H2SO4 was added and the solution transferred into an acid hydrolyzer for

three hours. Thereafter, the samples were titrated after digestion and the crude protein content determined using the formula;

N* 6.25; where N represents Nitrogen. The assumption is that, all proteins have the same amount of nitrogen which is 16%. Therefore, the CP content is determined on the basis of 100/16 which gives 6.25.

3.5.4 Determination of crude lipid

Ether extracts were analyzed using the soxhlet extraction method. A 5g feed sample was placed in a soxhlet extractor containing petroleum ether (40- 60^{o}C as the solvent. Thereafter, the sample was weighed in a thimble and extraction of fat took three hours. The remaining sample contained in the thimble was dried at 60^{o}C for 5 hours in an oven, then cooled and weighed taken. The crude fat content was calculated as: -

$$\% \text{ Crude lipid} = \frac{(\text{original wt. of sample} - \text{wt. of dried sample after extraction}) \times 100}{\text{Original weight of sample}}$$

3.5.5 Determination of crude fibre

Crude fibre was determined by boiling 5 grams of the feed sample in a standard solution of 3.13% H_2SO_4 for ten minutes. The solution was then filtered through a previously heated and weighed glass sinter plate with the help of a vacuum pump. The filter with sample was then boiled in a solution of 2.25% NaOH for 10 minutes and the solution filtered through a glass sinter plate using a vacuum pump. The remaining sample was rinsed with hot water followed by acetone to wash out the remaining sample. Afterwards the glass sinter plate with the filtered residue was dried in an oven for 5 hours at 60^{o} C, cooled in a desiccator and weighed. The residue was ashed for 4 hours at 550^{o} C in a muffle furnace and weighed. The dried residue was ignited and

crude fibre was estimated as the loss in mass on ignition of the dried residue using the following formula;

$$\% \text{ Crude fibre} = \frac{(\text{Loss in weight on ignition}) \times 100}{\text{Initial weight of sample}}$$

3.5.6 Determination of nitrogen free extracts (NFEs)

The percent nitrogen free extracts (% NFE) which are represented by easily soluble carbohydrates such as sugars and starch were calculated as follows:

% NFE = 100% - (% CP + % Ash + % CL + % CF) (AOAC, 1995), where NFE=Nitrogen free extracts; CP=crude protein; CL; crude lipids; CF=crude fibre.

3.6 Analysis of chromic oxide

Chromic oxide content was determined according to method adopted from Furukawa and Tsukahara (1966). A sample of 0.1g of feed and faecal matter were weighed into a Kjeldahl flask. Approximately 5 ml of concentrated nitric acid was added in the flask and the mixture boiled for around 20 minutes, without boiling dry. After cooling the sample, 3 ml of 70 % perchloric acid was added to the flask. The mixture was then gently heated again until the solution turned from green to an orange colour after which it was left to boil for a further 10 minutes to ensure complete oxidation. The solution was transferred to a 100 ml volumetric flask and topped up to the mark using distilled water. Chromic oxide content of the oxidized solution was then measured against chromate (IV) known standards directly by use of an atomic absorption spectrophotometer (Bulk scientific, Model 210 VGP) at a wavelength of 357.9nm (Furukawa and Tsukahara, 1966).

3.7 Evaluation of dietary performance in *O. niloticus*

At the beginning of the experiment, *O. niloticus* fingerlings each weighing 6g, 28 and 25g were used for trial I, II and III respectively. Thirty fish were stocked in each of the 45 cages installed in three 800m^2 fertilized earthen ponds. Five experimental diets each with 30% CP for each of the three trials were formulated by substituting FSM with SBM, CSM and SFM at rates of 0, 25, 50, 75 and 100% respectively and randomly fed to fish at 10% of their body weight. Sampling of fish was done biweekly by use of a seine net (Appendix VIII and IX). A representative sample of 30 fish was taken randomly from each treatment and weight and length measurements taken for each dietary treatment. Thereafter, the new feeding rates were determined and adjusted according to the determined average weight from the fish after every sampling. At the end of the 6 months culture period, all fish were harvested and measured for weight and total length after withholding feed for 24 hours. Weight was measured with a sensitive weighing balance readability 0.01g and length was measured with a measuring board to the nearest 0.01 cm. Growth performance of the five dietary treatments of fish was evaluated using the parameters as follows; final mean weight and length, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Condition Factor (CF) and survival rate (%). The following formulae were used for the calculations, according to Ricker (1979); Weight Gain (WG); is the difference between the mean final body weight (FBW) and the initial mean body weight (IBW) of fish over a period of time.

$$WG = (FBW-IBW) / IBW *100$$

Specific growth rate; refers to the instantaneous change in fish weight expressed as the percentage increase in body weight per day over any given time interval. It was

determined by taking natural logarithms of body weight, and express growth as % per day^{-1} (Ricker, 1979):

$$\text{SGR (\%)} = \frac{(\text{LnWf} - \text{lnWi}) \times 100}{\text{Number of days (t)}}$$

Feed conversion ratio (FCR) is defined as the ratio of dry feed fed per unit live weight gain and serves as a measure of the diet's efficiency. If a diet is appropriate for fish growth, then less food would be needed for production of a unit weight (De Silva and Anderson, 1995). FCR was calculated as:

FCR = Total feed fed / Live weight gained by fish

3.8 Evaluation of length-weight relationship of fish

Linear transformation of length and weight of fish was determined using the natural logarithm at observed length and weight. The length weight relationship (LWR) was calculated using the formula by Ricker (1979). The length weight relationship was used to calculate the regression coefficients. The parameter b of the length weight relationship was determined using the equation $W=aL^b$. Where; W= weight of fish in grams (g); L= total length of fish in centimeters (cm); a = is a constant and b the exponent. The equation was log-transformed and expressed as follows: Log W = Log a + b Log L; where 'a' is a constant representing initial growth and 'b' is the growth coefficient. Fish exhibits isometric growth if length increases in equal proportion with the body weight. The regression coefficient for isometric growth is '3' and a value greater than '3' indicates allometric growth (Olurin and Aderibigbe, 2006)

3.9 Experimental procedure for apparent protein digestibility trials

The fish (each 25g) were acclimatized for 7 days prior to the beginning of fecal collection during which they were fed the formulated experimental diets (Appendix III, IV, V). During the 30 days experimental period, fish were fed twice at the daily rate of 10% of their body weight. One hour after the feed was administered; any feed and faeces present in the aquaria were removed to ensure no feed residues remained. Faecal matter was collected from the aquaria using a siphon and a small sieve and then placed into a beaker (Figure 3.2). Faecal collection was done within 2 hours of voiding during the day and the fecal material voided during the night was collected next morning at 0700 hours. Faecal collection was done for 30 days. Samples of faecal material from each treatment replicated thrice were pooled and kept in beakers to dry awaiting analysis of faecal matter (Figure 3.2). They were then analyzed for crude protein following the procedures adapted from the Association of Official Analytical Chemists (AOAC, 1995).

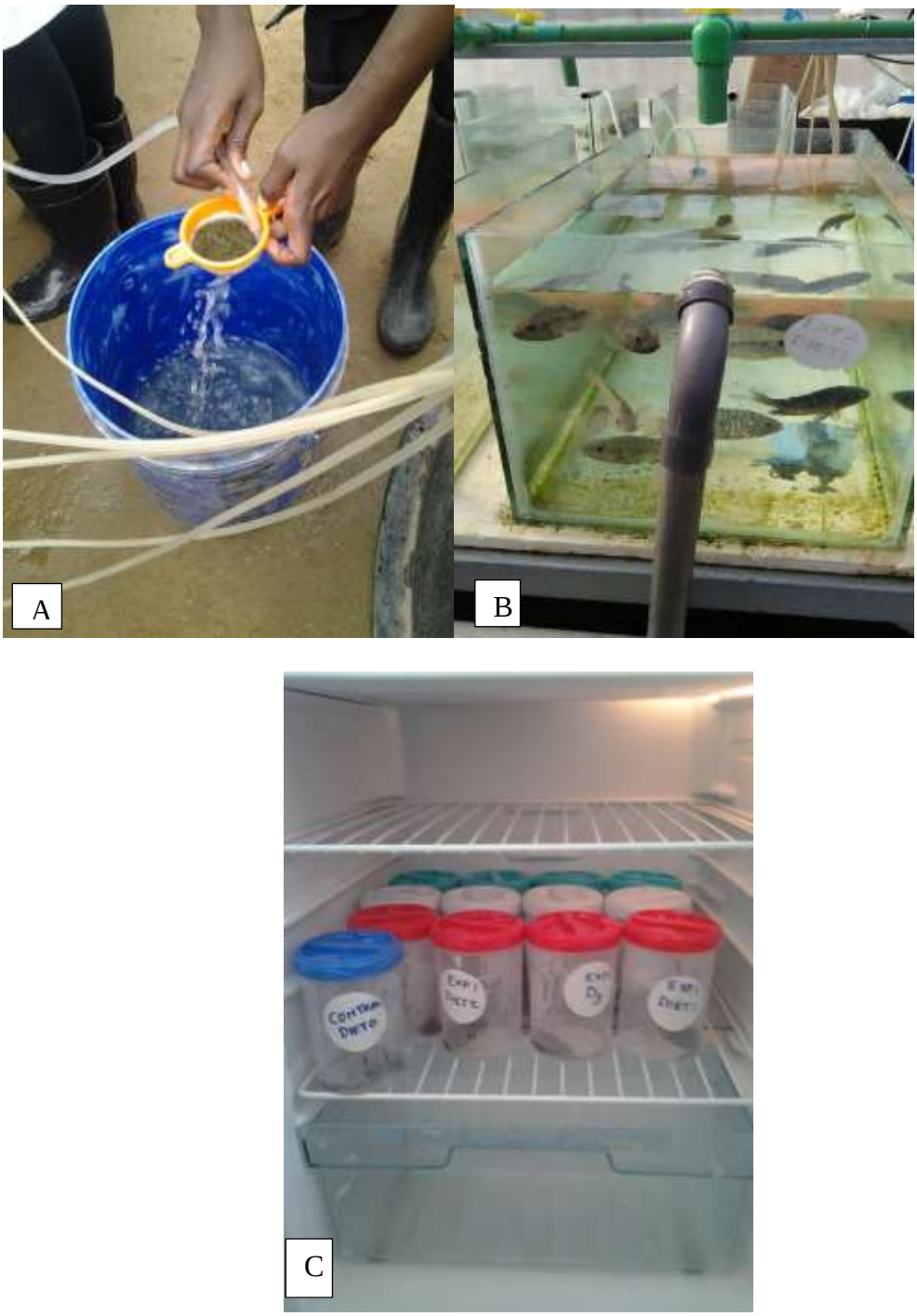

Figure 3.2: Photos showing experimental procedure for digestibility. A- collection of faeces: B- experimental fish after being fed: C- faecal samples awaiting bioanalysis

3.9.1 Determination of digestibility

Apparent digestibility coefficients (ADCs) of protein in the test diet ($ADCN_{diet}$) were calculated according to the formula given below:

ADC_p = 100- (100 * (% Cr_2O_3 in feed / % Cr_2O_3 in faeces) * (% nutrient in faeces / % nutrient in diet).

3.10 Whole fish body composition analysis

A random sample of ten fish was taken at the time of stocking to serve as an initial carcass sample and at harvest of the final carcasses for proximate analyses. The proximate analysis was carried out according to the standard methods by AOAC (1995) as described in section 3.5.

3.11 Cost benefit analysis

The cost-benefit analysis was conducted for the formulated diets. The cost of the feed ingredients was based on the existing market prices. The following parameters were used to analyse the cost-benefit for each treatment: Input expenditure included; *O. niloticus* fingerlings, cost of buying local fish feeds, cost of feeding fish and pond management per month per pond, cost of packaging fish harvested and cost of transporting fish harvested. Net fish yield was calculated as the difference between total weight of fish at harvest and total weight of fish at stocking. Income from fish yield; harvest from each pond with the same dietary treatment was sold at an estimated price from fish weight and price per kilo of fresh fish under existing market conditions. The net profit was determined from the difference between income and input expenditure.

3.12 Water quality sampling

The experimental ponds were fertilized weekly at a rate of 20 kg N ha^{-1} and 8 kg P $ha^{-1}$with urea and diammonium phosphate (DAP) respectively .Water quality parameters including dissolved oxygen, temperature, pH and electrical conductivity were measured weekly at 7am, 1pm and 6pm using a multi parameter water quality meter, (Model H19828,Hanna Instruments Limited., USA). Ammonia nitrates, nitrites, total alkalinity and phosphorus were measured weekly using standard methods (Boyd and Tucker, 1998).

3.13 Data analysis

Statistical analyses were done using Minitab version 16 software. The effects of experimental diets on growth, survival, feed conversion ratio, and carcass composition were analyzed using one-way analysis of variance (ANOVA). The Tukey's multiple range test was used as post hoc where ANOVA showed significant differences among treatment means. Significant differences were considered at $p < 0.05$.

CHAPTER FOUR : RESULTS

4.1 Effect of using a mixture of different plant protein sources in *Oreochromis niloticus* growth performance parameters

4.1.1 Effect of replacing freshwater shrimp meal with soya bean meal on growth performance of *O. niloticus*

The results on growth performance of *O. niloticus* fed on varying levels of soybean meal in cages for six months are shown in Table 4.1 below. There were significant differences ($p<0.05$) in final mean body weights among treatments. The control D0, that was fed on fishmeal only, had significantly ($p<0.05$) higher mean weights than the rest of the treatments while D4 that was fed on soybean meal only, had the least ($p<0.05$). Treatments D1, D2 and D3 that were fed on soybean and fishmeal at substitution levels of fishmeal (25%, 50% and 75%) respectively, had intermediate values which were significantly different ($p<0.05$) from D0 and D4. Mean weight gain, daily weight gain and specific growth rate followed a similar pattern to final mean body weights. However, survival rate was high and similar ($p>0.05$) among all treatments.

Table 4.1: Growth performance of *O.niloticus* fed on diets containing increasing levels of PPSM (Soybean meal only) in place of FSM

Experimental groups	**D0**	**D1**	**D2**	**D3**	**D4**
Rate of FSM substitution	**0%**	**25%**	**50%**	**75%**	**100%**
Initial length (cm)	7.58 ± 0.00^{a}	7.58 ± 0.00^{a}	7.58 ± 0.00^{a}	7.58 ± 0.00^{a}	7.58 ± 0.00^{a}
Initial body weight (g)	6.12 ± 0.00^{a}	6.12 ± 0.00^{a}	6.12 ± 0.00^{a}	6.12 ± 0.00^{a}	6.12 ± 0.00^{a}
Final mean body weight (g)	47.93 ± 1.68^{c}	38.13 ± 1.27^{b}	37.38 ± 1.32^{b}	33.71 ± 0.70^{b}	26.35 ± 0.85^{a}
Mean weight gain	41.81 ± 1.19^{c}	32.01 ± 1.14^{b}	31.26 ± 1.15^{b}	27.59 ± 0.69^{b}	20.23 ± 0.91^{a}
Daily weight gain (g)	0.25 ± 0.04^{c}	0.19 ± 0.01^{b}	0.19 ± 0.02^{b}	0.16 ± 0.02^{b}	0.12 ± 0.01^{a}
Specific growth rate (% day-1)	4.07 ± 0.29^{c}	3.11 ± 0.69^{b}	3.04 ± 0.36^{b}	2.68 ± 0.69^{b}	1.97 ± 0.31^{a}
FCR	1.64 ± 0.10^{a}	1.93 ± 0.08^{b}	2.06 ± 0.12^{b}	2.1 ± 0.08^{b}	3.06 ± 0.13^{c}
Survival (%)	97.8 ± 2.61^{a}	96.7 ± 1.48^{a}	94.4 ± 2.44^{a}	93.3 ± 1.66^{a}	96.7 ± 1.68^{a}

Key: values with the same superscript are not significantly different ($p<0.05$). Values are expressed as mean ± SEM). D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

Growth trend curves for *O. niloticus* in cages are presented in Figure 4.1. Diet D0 registered the highest growth, while D4 resulted in the lowest growth. The growths of fish that were fed on D1, D2 and D3 were intermediate and comparable ($p>0.05$) among the three dietary treatments.

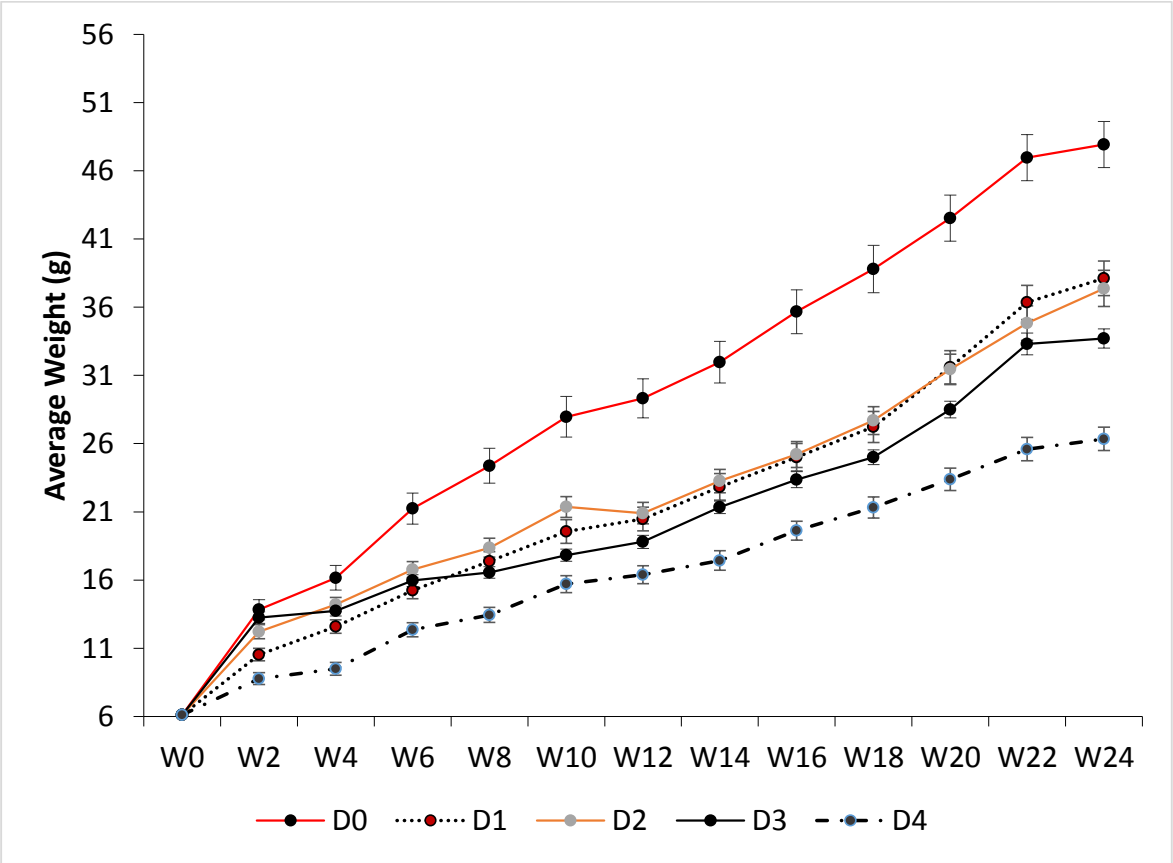

Figure 4.1: Growth curves for *O. niloticus* fed on formulated diets with varying levels of SBM in cages during 6 months culture period. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.1.2 Effect of replacing freshwater shrimp meal with a mixture of plant proteins (Soybean meal, cottonseed meal and sunflower meal) on growth performance of *O. niloticus*

The growth performance values for *O. niloticus* are summarized in Table 4.2 below. Generally, there were significant differences ($p<0.05$) in final mean body weights among treatments. D0 and D1, had significantly ($p<0.05$) higher mean weights than the rest of the treatments. Diets, D2, D3 and D4 had values which were significantly different ($p<0.05$) from D0 and D1. Mean weight gain, daily weight gain and specific growth rate followed a similar pattern to final mean body weights. Survival rate was high and similar ($p>0.05$) among all treatments.

Table 4.2: Growth performance of *O. niloticus* fed on diets containing increasing levels of PPSM (soybean meal, cottonseed meal and sunflower meal) in place of FSM. (Values are expressed as mean ± SEM)

Experimental groups	**D0**	**D1**	**D2**	**D3**	**D4**
Rate of FSM substitution	**0%**	**25%**	**50%**	**75%**	**100%**
Initial length (cm)	11.11 ± 0.00^{a}	11.11 ± 0.00^{a}	11.11 ± 0.00^{a}	11.11 ± 0.00^{a}	11.11 ± 0.00^{a}
Initial body weight (g)	28.11 ± 0.01^{a}	28.11 ± 0.01^{a}	28.11 ± 0.01^{a}	28.11 ± 0.01^{a}	28.11 ± 0.01^{a}
Final mean body weight (g)	140.33 ± 3.27^{b}	130.18 ± 2.89^{b}	103.18 ± 2.03^{a}	99.20 ± 4.12^{a}	89.91 ± 2.33^{a}
Mean weight gain	112.22± 1.79^{b}	102.07 ± 1.87^{b}	75.07 ± 1.51^{a}	71.09 ± 1.28^{a}	61.08 ± 1.45^{a}
Daily weight gain (g)	0.68 ± 0.04^{b}	0.61 ± 0.06^{b}	0.45 ± 0.04^{a}	0.42 ± 0.03^{a}	0.36 ± 0.07^{a}
Specific growth rate (% day-1)	2.38 ± 0.25^{b}	2.16 ± 0.27^{b}	1.59 ± 0.22^{a}	1.51 ± 0.21^{a}	1.31 ± 0.18^{a}
FCR	2.63 ± 0.14^{a}	2.64 ± 0.14^{a}	3.32 ± 0.23^{b}	3.50 ± 0.20^{b}	4.18 ± 0.48^{c}
Survival (%)	99 ± 1.12^{a}	97 ± 1.22^{a}	99 ± 0.98^{a}	98 ± 1.28^{a}	97 ± 1.14^{a}

Key: values with the same superscript are not significantly different ($p<0.05$). Values are expressed as mean ± SEM). D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

Growth trend curves for *O. niloticus* in cages are presented in Figure 4.2. The growth trend of *O. niloticus* was similar for all the dietary treatments during the first month. Thereafter, differential growth occurred until the experiment came to an end. The control, D0 registered the highest growth followed by D1 although the differences were not significant ($p>0.05$) while D4 resulted in the lowest growth.

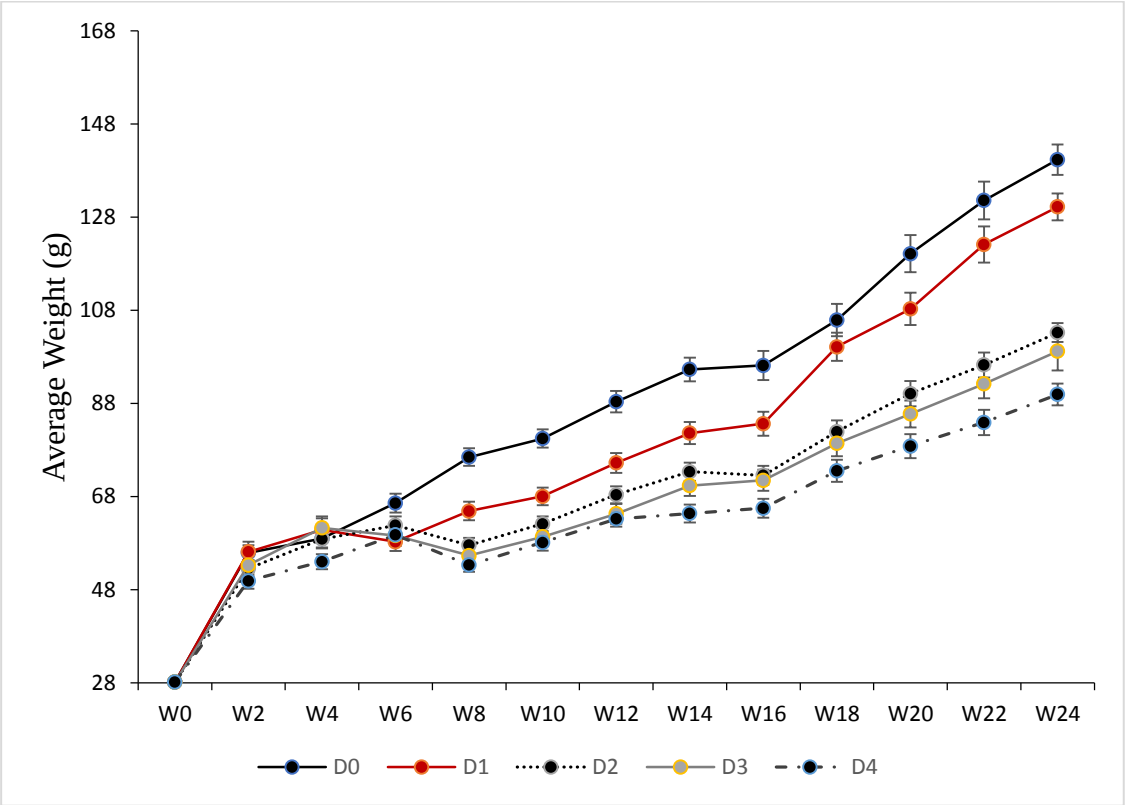

Figure 4.2: Growth curves for *O. niloticus* fed on formulated diets with varying levels of PPSM (SBM, CSM, SFM) in cages during 6 months culture period. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.1.3 Effect of replacing freshwater shrimp meal with a mixture of plant proteins (cottonseed meal and sunflower meal) on growth performance of *O. niloticus* in cages installed in fertilized earthen ponds

The Data on growth performance of *O. niloticus* fed on varying levels of cottonseed cake and sunflower cake in cages for six months indicated that, there were significant differences ($p<0.05$) in final mean body weights among treatments. Diets D0 and D1, had significantly ($p<0.05$) higher mean weights than the rest of the treatments while D4 had the least ($p<0.05$). Treatments D2, and D3 had intermediate values which were significantly higher ($p<0.05$) from D4. Mean weight gain, daily weight gain and specific growth rate followed a similar pattern to final mean body weights. Survival rate was high and similar ($p>0.05$) among all treatments (Table 4.3).

Table 4.3: Growth performance of *O. niloticus* fed on diets containing increasing levels of PPSM (cottonseed meal and sunflower meal) in place of FSM

Experimental groups	**D0**	**D1**	**D2**	**D3**	**D4**
Rate of FSM substitution	**0%**	**25%**	**50%**	**75%**	**100%**
Initial length (cm)	11.06 ± 0.01^{a}	11.06 ± 0.01^{a}	11.06 ± 0.01^{a}	11.06 ± 0.01^{a}	11.06 ± 0.01^{a}
Initial body weight (g)	25.15 ± 0.00^{a}	25.15 ± 0.00^{a}	25.15 ± 0.00^{a}	25.15 ± 0.00^{a}	25.15 ± 0.00^{a}
Final mean body weight (g)	107.12 ± 4.45^{c}	104.21 ± 3.89^{c}	89.05 ± 2.28^{b}	75.72 ± 1.49^{b}	66.41 ± 2.21^{a}
Mean weight gain	81.97 ± 1.64^{c}	79.06 ± 1.45^{c}	63.9 ± 1.57^{b}	50.57 ± 1.38^{b}	41.26 ± 1.29^{a}
Daily weight gain (g)	0.49 ± 0.03^{d}	0.47 ± 0.05^{d}	0.38 ± 0.01^{c}	0.30 ± 0.03^{b}	0.25 ± 0.01^{a}
Specific growth rate (% day-1)	1.94 ± 0.27^{c}	1.87 ± 0.32^{c}	1.51 ± 0.21^{b}	1.20 ± 0.19^{b}	0.98 ± 0.17^{a}
FCR	3.16 ± 0.21^{ab}	2.98 ± 0.22^{a}	3.26 ± 0.19^{b}	3.66 ± 0.21^{b}	4.69 ± 0.42^{c}
Survival (%)	96.7 ± 1.18^{a}	97.8 ± 1.43^{a}	95.6 ± 2.17^{a}	96.7 ± 1.33^{a}	95.6 ± 1.15^{a}

Key: values with the same superscript are not significantly different ($p<0.05$). Values are expressed as mean ± SEM). D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

Growth trend curves for *O. niloticus* in cages are presented in Figure 4.3. The control D0 registered the highest growth followed by D1 although the differences were not significant (p>0.05) while D4 resulted in the lowest growth. Diet D2 and D3 were intermediate and were not significantly different (p>0.05).

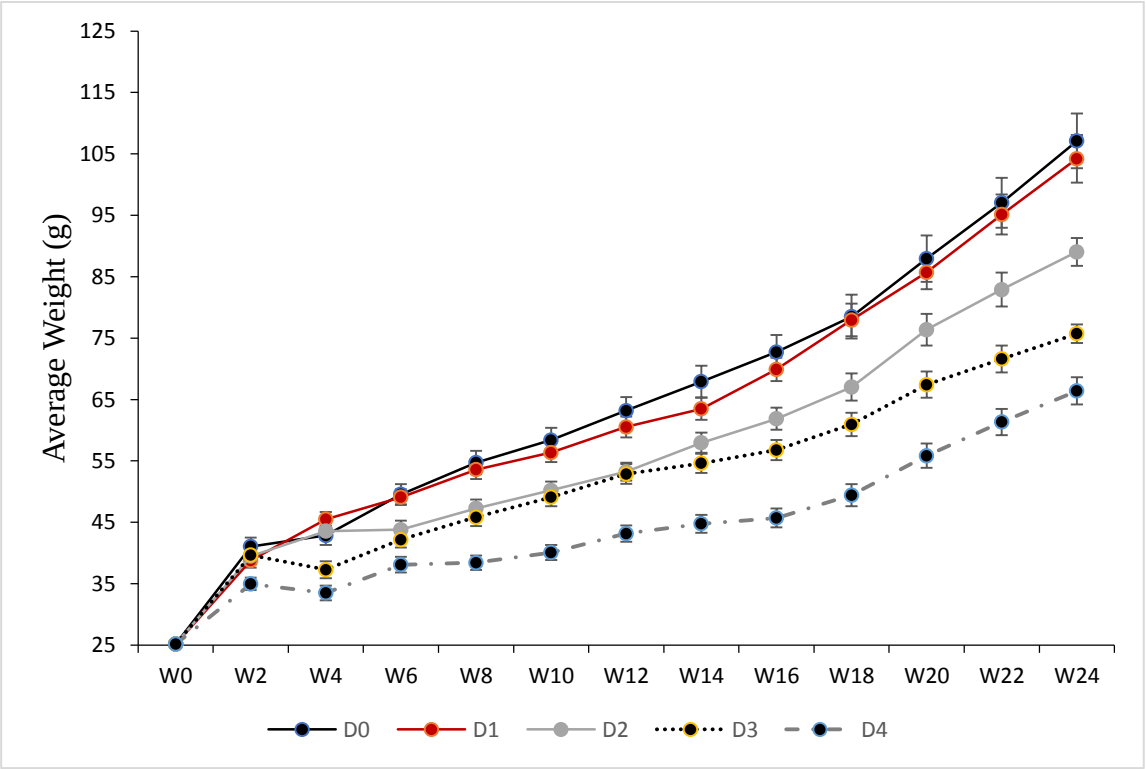

Figure 4.3: Growth curves for *O. niloticus* fed on formulated diets with varying levels of PPSM (CSM, SFM) in cages during 6 months culture period. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.1.4 Length-Weight Relationship of *O. niloticus*

The Length-Weight relationship of fish in trial one, are shown in Figure 4.4. The regression coefficient values obtained from the length weight relationship for diets D0, D2, and D4 were slightly higher than 3 (3.04, 3.08, and 3.1 respectively) whereas fish fed on diets D1 and D3 recorded regression coefficient values of 2.95 and 2.92 respectively. In all the diets, the log transformed data fitted well in the linear model with r^2 values all above 0.9. The relationships were also significant since all the p values were less than 0.05.

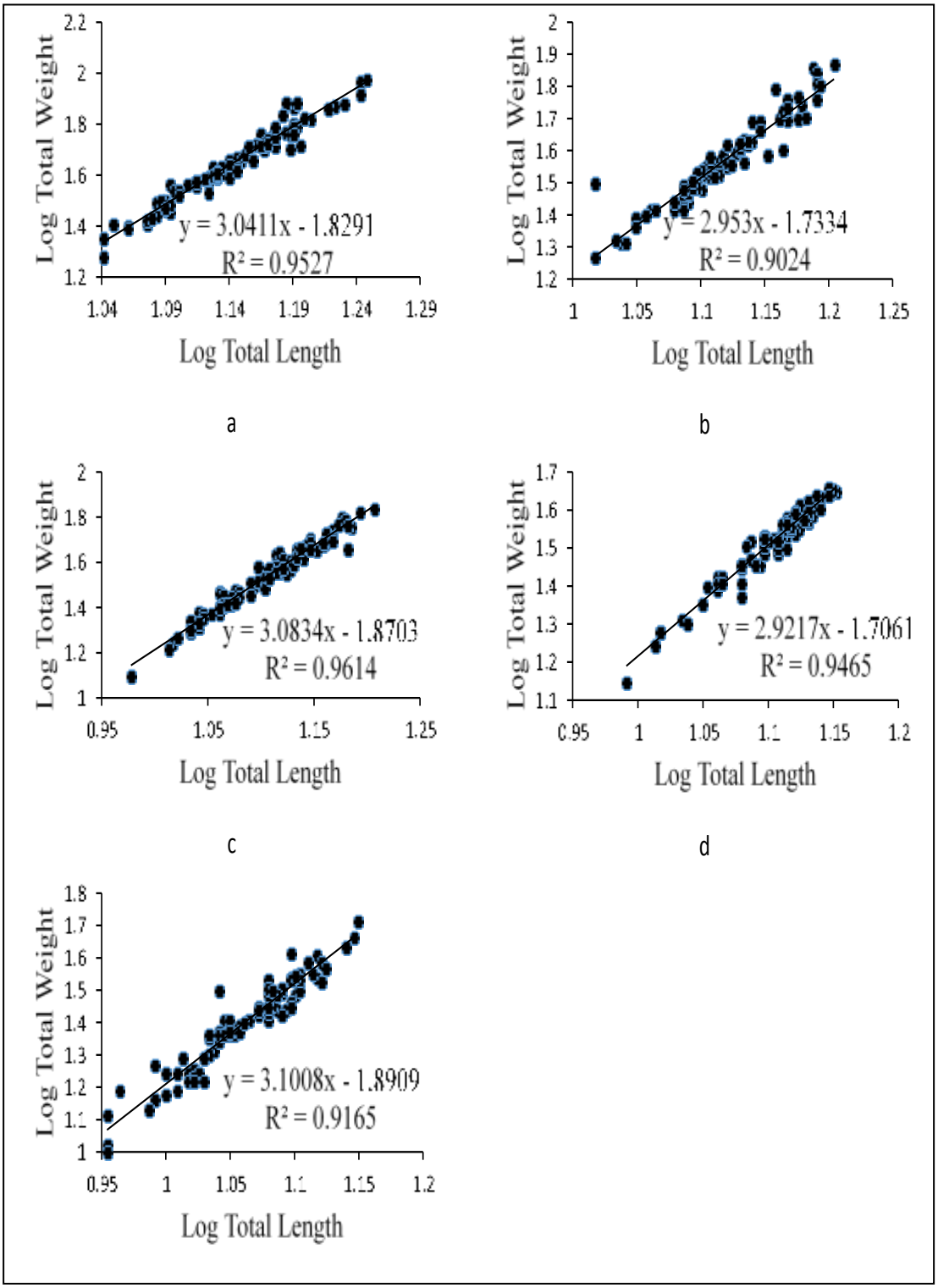

Figure 4.4: Length-Weight relationship, regression coefficients of *O. niloticus* fed on formulated diets with varying levels of SBM (a – D0, b – D1, c - D2, d - D3, and e - D4)

In the second trial, the regression coefficient values obtained from the length weight relationship for fish fed on diets D1 and D3 were above 3 (3.05, and 3.08 respectively) while the rest (D0, D2 and D4) were below 3. All the r^2 values were above 90% and the LWR was strong and significant ($P<0.05$). (Figure 4.5).

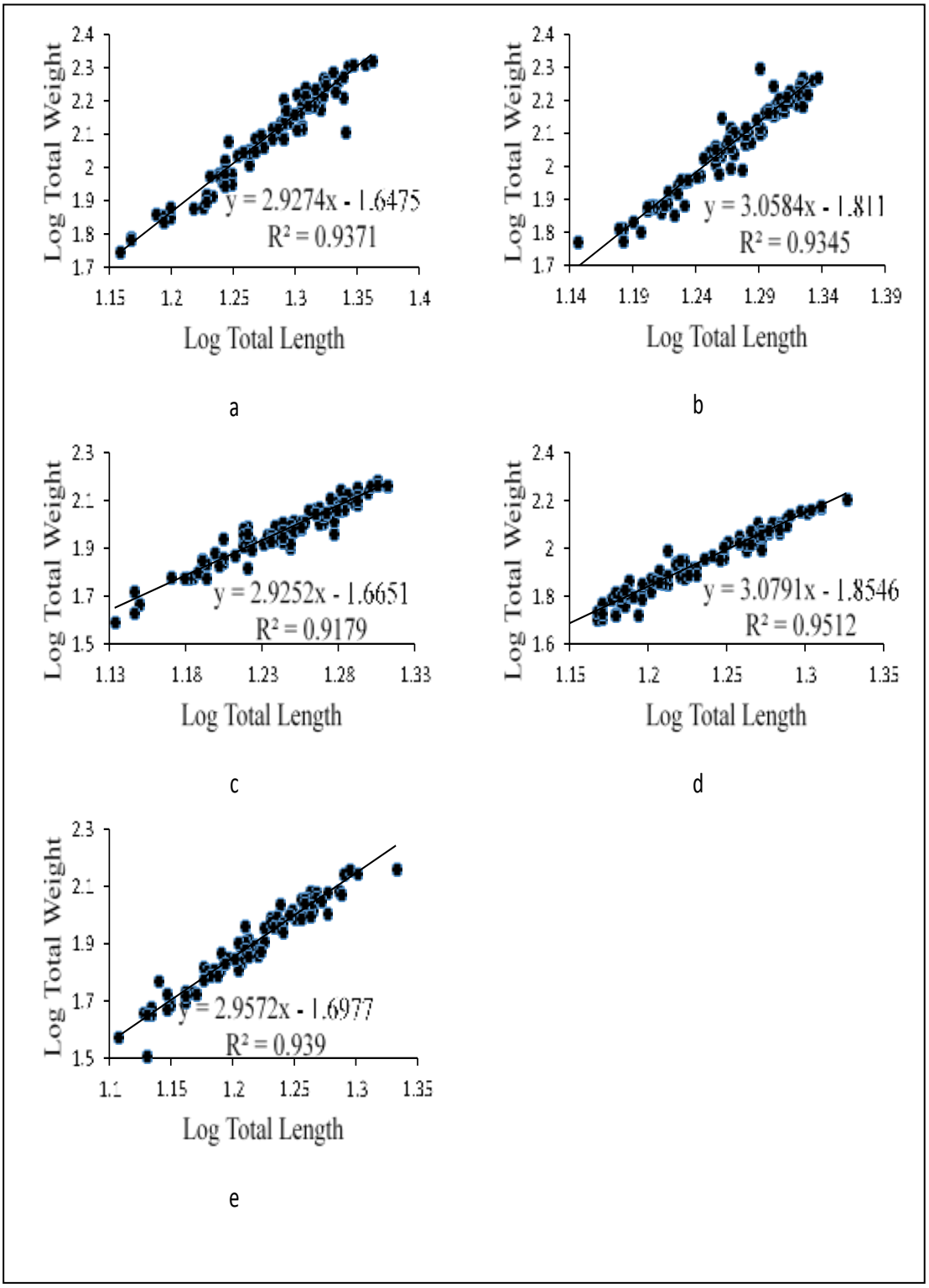

Figure 4.5: Length-Weight relationship, regression coefficients of *O. niloticus* fed on formulated diets with varying levels of PPSM (SBM, CSM, SFM) (a – D0, b – D1, c - D2, d - D3, and e - D4)

In the third trial, only fish under diet D3 recorded a regression coefficient value obtained from the length weight relationship below 3. All the rest were slightly above 3 (Figure 4.6). Just like in the previous two trials, the r^2 values were all above 90% and $p < 0.05$.

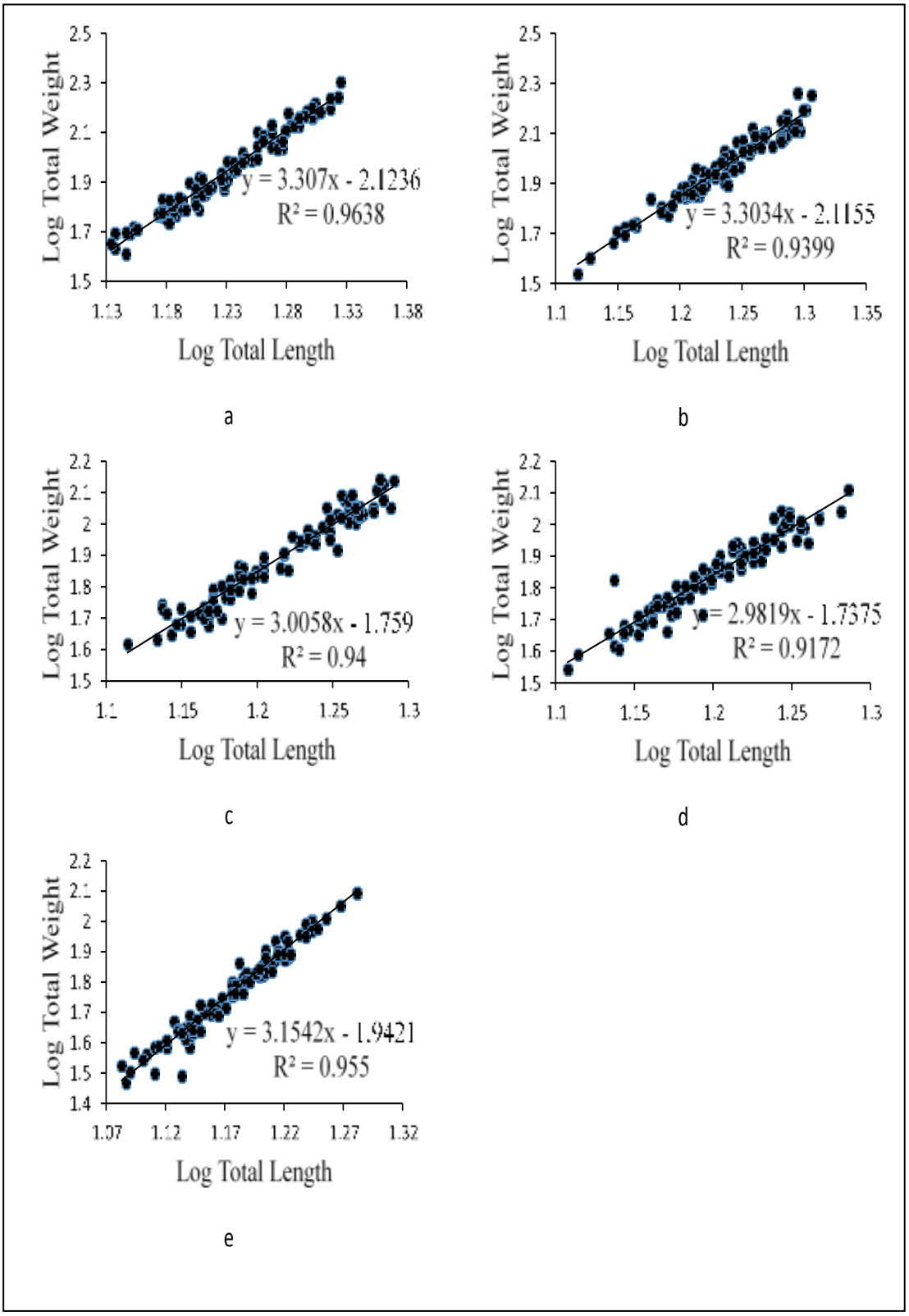

Figure 4.6: Length-Weight relationship, regression coefficients of *O. niloticus* fed on formulated diets with varying levels of PPSM (CSM, SFM) (a – D0, b – D1, c - D2, d - D3, and e - D4)

4.2 Apparent protein digestibility of the formulated diets fed to *Oreochromis niloticus*

4.2.1 Apparent protein digestibility of *O. niloticus* fed on soybean meal diets

Data on apparent protein digestibility (ADC_p) in cages are shown in Figure 4.7. The ADC_p values decreased with increasing levels of SBM. Diet D0 had the highest apparent protein digestibility (ADC_p) of 90.23 ± 0.12 followed by D1 (89.83 ± 0.17). Diet D2 had ADCp of 88.77±0.12 while D3 and D4 had significantly ($p<0.05$) lower ADCp of 87.46 ± 0.35 and 86.38 ± 0.25 respectively, as compared to the other diets.

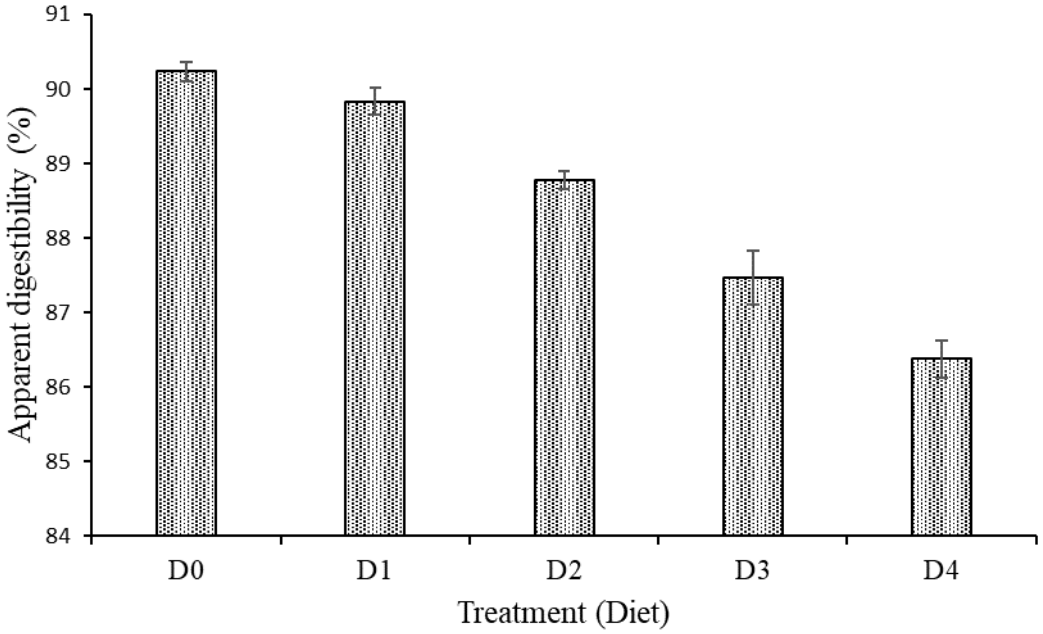

Figure 4.7: Apparent protein digestibility of *O. niloticus* fed on soybean meal diets. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.2.2 Apparent protein digestibility of *O. niloticus* fed on a mixture of soybean meal, cottonseed meal and sunflower meal diets

Results from trial II showed that the highest (90.23 ± 0.12) apparent protein digestibility was recorded in D0 followed by D1 and D2 (87.38 ± 0.37 and 86.99 ± 0.03) respectively, which were not significantly different from each other ($p>0.05$). The lowest (80.66 ± 0.35) apparent protein digestibility coefficient was recorded in D4 (Figure 4.8).

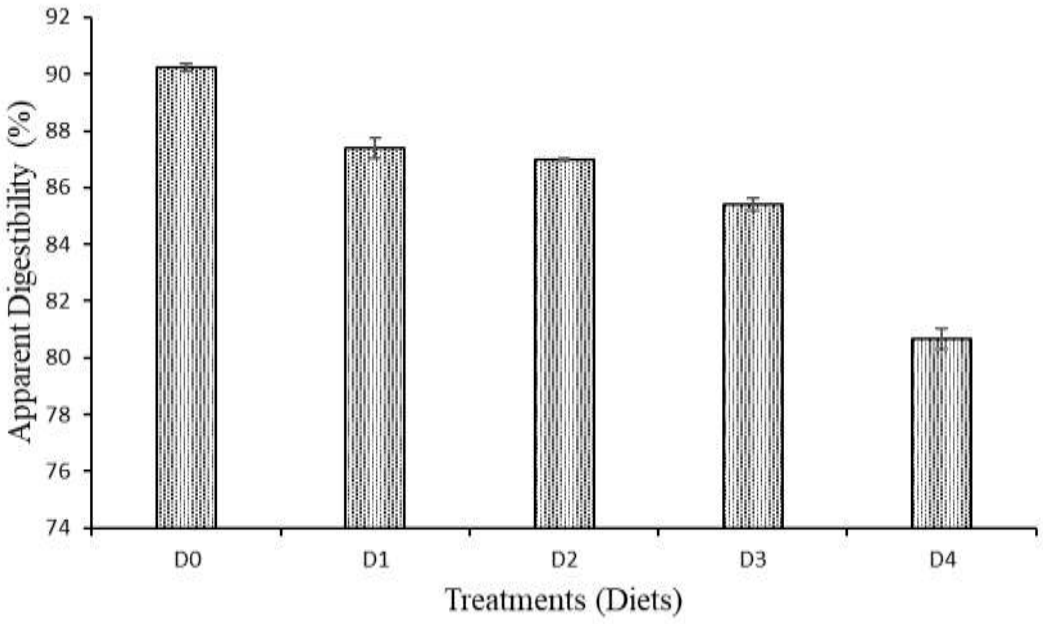

Figure 4.8: Apparent protein digestibility of *O. niloticus* fed on a mixture of soybean meal, cottonseed meal and sunflower meal diets. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.2.3 Apparent protein digestibility of *O. niloticus* fed on a mixture of cottonseed meal and sunflower meal diets

Unlike in the first two trials, in trial three, ADCp values were slightly low. The highest apparent protein digestibility was recorded in diet D0 (90.23 ± 0.12) followed by D1 (84.42 ± 0.25) whereas D4 recorded the lowest apparent protein digestibility (79.77± 020) which was significantly different ($p<0.05$) from the other diets (Figure 4.9).

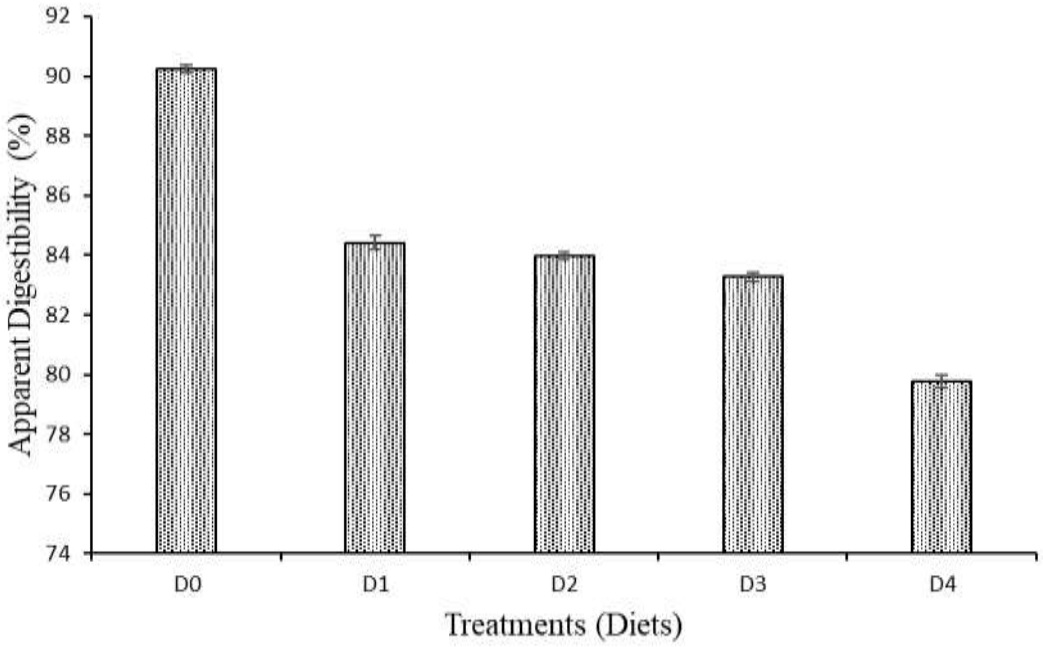

Figure 4.9: Apparent protein digestibility of *O. niloticus* fed on a mixture of cottonseed meal and sunflower meal diets. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.3 Effect of a mixture of plant protein sources on *Oreochromis niloticus* whole body composition

4.3.1 Effect of soybean meal diets on *O. niloticus* whole body composition

Table 4.4 shows the changes in crude protein, fat and ash in fish carcass fed on different experimental diets. Protein, fat and ash contents were significantly affected by the experimental diets ($p<0.05$). A significant increase in crude protein was observed in D0. Increasing soybean meal inclusion levels in the experimental diets, led to a significant ($p<0.05$) increase of fat content in the whole body of fish, with highest levels recorded in fish fed on D3 and the lowest in fish fed on D0. Ash content showed significantly decreasing trend with increasing inclusion levels of soybean meal in the experimental diets ($p<0.05$).

Table 4.4: Proximate composition of whole body of *O. niloticus* fed on formulated diets with varying levels of SBM

	Experimental diets					
Parameters	**Initial**	**D0**	**D1**	**D2**	**D3**	**D4**
Crude Protein	54.7 ±0.20	56.8 ±0.15^{a}	52.6 ±0.20^{b}	51.3 ±0.30^{c}	52.5 ±0.25^{b}	52.7 ±0.15^{b}
Crude Fat	4.4 ±0.05	4.1 ±0.15^{c}	8.3 ±0.15^{a}	8.3 ±0.10^{a}	8.7 ±0.10^{a}	7.5 ±0.15^{b}
Ash	19.3 ±0.12	17.4±0.15^{a}	15.9 ±0.05^{b}	13.6 ±0.10^{c}	12.3 ±0.20^{d}	10.4 ±0.20^{e}

Key: Values in the same row having different superscript letters are significantly different ($P<0.05$). Values are expressed as mean± SEM. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.3.2 Effect of a mixture of soybean meal, cottonseed and sunflower meal diets on *O. niloticus* whole body composition

In trial II, there were significant differences ($p<0.05$) for crude protein in the muscle tissue, among fish in all the experimental diets. Initial body composition for protein was 54.7%. Diet D2 had the highest increment in crude protein levels followed by D0, while the lowest was recorded in D1, D3 and D4. The fat content was significantly ($p<0.05$) higher in diet D3 and D4. Ash content also increased significantly across the dietary treatments with D4 having the highest levels of ash (Table 4.5).

Table 4.5: Proximate composition of whole body of *O. niloticus* fed on formulated diets with varying levels of PPSM (SBM, CSM, SFM)

	Experimental diets					
Parameters	**Initial**	**D0**	**D1**	**D2**	**D3**	**D4**
Crude Protein	54.7 ±0.15	56.4 ±0.27^{b}	53.4 ±0.27^{c}	59.6 ±0.30^{a}	53.6 ±0.12^{c}	54.6 ±0.25^{c}
Crude Fat	4.3 ±0.28	12.4 ±0.15^{b}	12.4 ±0.15^{b}	13.4 ±0.25^{b}	15.4 ±0.25^{a}	16.4 ±0.35^{a}
Ash	13.3 ±0.28	15.3±0.15^{c}	15.8±0.10^{c}	17.3±0.35^{b}	18.4±0.30^{ab}	19.6±0.10^{a}

Key: Values in the same row having different superscript letters are significantly different ($P<0.05$). Values are expressed as mean± SEM. D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.3.3 Effect of a mixture of cottonseed and sunflower meal diets on *O. niloticus* whole body composition

In trial III, the highest (59.3±0.20 and 59.3±0.10) crude protein levels were observed in D1and D2 respectively while the lowest crude protein levels were in D4. Crude fat content on the contrary, increased from a baseline of 4.3% at the beginning of the experiment to a highest value of 17% in fish under diet D4 and was significantly higher ($p<0.05$) than the other diet treatments. Total ash content decreased significantly ($p<0.05$) across all the diet treatments with D4 recording the lowest (10.8 ±0.15) value that was significantly different ($p<0.05$) from the other treatments (Table 4.6).

Table 4.6: Proximate composition of whole body of *O. niloticus* fed on formulated diets with varying levels of PPSM (CSM, SFM)

	Experimental diets					
Parameters	**Initial**	**D0**	**D1**	**D2**	**D3**	**D4**
Crude Protein	51.6 ±0.10	56.1 ±0.15^{d}	59.3 ±0.20^{a}	59.3 ±0.10^{a}	57.5 ±0.20^{b}	52.3 ±0.25^{c}
Crude Fat	4.3 ±0.10	7.9 ±0.050^{e}	9.1 ±0.10^{d}	12.3 ±0.15^{c}	14.7 ±0.15^{b}	17.0 ±0.10^{a}
Ash	19.5 ±0.25	17.3 ±0.17^{a}	15.8 ±0.12^{b}	13.4 ±0.15^{c}	12.1 ±0.15^{d}	10.8 ±0.15^{e}

Key: Values in the same row having different superscript letters are significantly different ($P<0.05$). Values are expressed as mean± SEM.D0=Control diet; D1=25% fishmeal replacement; D2=50% fishmeal replacement; D3=75% fishmeal replacement; D4=100% fishmeal replacement

4.4 Profitability and economic analysis of using a mixture of plant protein sources in *Oreochromis niloticus*

4.4.1 Profitability and economic analysis of using soybean meal diets in *O. niloticus*

Total fish yields, production costs, and revenue generated from different experimental diets are shown in Table 4.7. In trial I, diet D0 had the highest total cost of production with an average of USD 9.57 while diet D4 had the lowest (USD 9.36). In terms of total yield, diet D0 recorded the highest biomass at harvest of 4.3 kg. The lowest yield was attained in diet D4 with a biomass of 2.5 kg. The Break-even prices over total cost for all the diets were below the prevailing market price of USD 4.00. However, diet D0 had the lowest break-even price over total cost whereas diet D4 had the highest.

Table 4.7: Partial enterprise budget analysis for an aquaculture enterprise for *O. niloticus* fed on formulated diets with varying levels of PPSM (SBM)

Parameters	Experimental Diets				
	D0	D1	D2	D3	D4
Variable Cost (USD)	6.14	5.95	5.99	5.94	5.92
Fixed Cost (USD)	3.44	3.44	3.44	3.44	3.44
Total Cost (USD)	9.57	9.39	9.43	9.38	9.36
Total Yield (Kg)	4.25	3.36	3.23	2.94	2.45
Unit Selling price (USD)	4.00	4.00	4.00	4.00	4.00
Gross Revenue (USD)	17.02	13.44	12.92	11.76	9.82
Returns above Variable Cost (USD)	10.88	7.48	6.91	5.82	3.89
Returns above Total Cost (USD)	7.44	4.04	3.47	2.38	0.45
Break Even Price over variable cost (USD)	1.44	1.77	1.85	2.02	2.41

4.4.2 Profitability and economic analysis of using a mixture of plant protein sources (SBM, SFM, CSM) in *O. niloticus*

In the second trial, the highest cost of production was realized in fish fed on diet D1 (USD 14.87) while the lowest cost of production was in fish fed on diets D3 (USD 13.77) and D2 (USD 13.84). The total yield was highest in fish under diet D0 where the total biomass produced was 11.68 kg while the lowest was in fish fed on diet D4 (7.30 kg). Generally, the break-even price over total cost ranged from USD 1.26 in fish under diet D0 to USD 1.93 in fish fed on diet D4 whereas the prevailing market price was USD 4.00/kg (Table 4.8).

Table 4.8: Partial enterprise budget analysis for an aquaculture enterprise for *O. niloticus* fed on formulated diets with varying levels of PPSM (SBM, CSM, SFM)

Parameters	Experimental Diets				
	D0	D1	D2	D3	D4
Variable Cost (USD)	11.21	11.43	10.40	10.33	10.66
Fixed Cost (USD)	3.44	3.44	3.44	3.44	3.44
Total Cost (USD)	14.65	14.87	13.84	13.77	14.1
Total Yield (Kg)	11.68	10.59	8.55	8.09	7.30
Unit Selling price (USD)	4.00	4.00	4.00	4.00	4.00
Gross Revenue (USD)	46.72	42.36	34.2	32.36	29.20
Returns above Variable Cost (USD)	35.51	30.93	23.80	22.03	18.54
Returns above Total Cost (USD)	32.07	27.49	20.36	18.59	15.1
Break Even Price over variable cost (USD)	0.96	1.08	1.22	1.28	1.46
Break Even Price over total cost (USD)	1.26	1.40	1.62	1.70	1.93
Break Even Yield (total cost)	3.66	3.72	3.46	3.44	3.53

4.4.3 Profitability and economic analysis of using a mixture of plant protein sources (SFM and CSM) in *O. niloticus*

Results from trial III are shown in Table 4.9 below. The total cost of production and total yield was highest in diet D0 with USD 10.45 and 8.41 kg respectively whereas the lowest was in diet D4 where the total cost of production was USD 9.50 and total yield was 5.25 kg. Even though fish fed on diet D4 recorded the highest break-even price over total cost (USD 1.81), none of the diets yielded a break-even price above the prevailing market price of USD 4.0.

Table 4.9: Partial enterprise budget analysis for an aquaculture enterprise for *O. niloticus* fed on formulated diets with varying levels of PPSM (SFM, CSM)

Parameters	Experimental Diets				
	D0	D1	D2	D3	D4
Variable Cost (USD)	7.01	6.93	6.47	6.45	6.06
Fixed Cost (USD)	3.44	3.44	3.44	3.44	3.44
Total Cost (USD)	10.45	10.37	9.91	9.89	9.50
Total Yield (Kg)	8.41	8.33	7.1	6.2	5.25
Unit Selling price (USD)	4.00	4.00	4.00	4.00	4.00
Gross Revenue (USD)	33.64	33.32	28.4	24.8	21.00
Returns above Variable Cost (USD)	26.63	26.39	21.93	18.35	14.94
Returns above Total Cost (USD)	23.19	22.95	18.49	14.91	11.5
Break Even Price over variable cost (USD)	0.83	0.83	0.91	1.04	1.15
Break Even Price over total cost (USD)	1.24	1.24	1.40	1.60	1.81
Break Even Yield (total cost)	2.61	2.59	2.48	2.47	2.37

4.5 Water Quality

Water quality parameters did not vary significantly $p>0.05$ among treatments in the cages and in open pond over the experimental period. In trial I, dissolved oxygen values ranged between 4.16 mgL^{-1} and 5.24 mgL^{-1} (mean = 4.7±0.1). The average pH ranged between 8.64 to 9.57 (mean 9.1±0.1), water temperature ranged from 25.02˚C to 29.03˚C (mean 27.1±0.4) while conductivity values ranged from 91.50 µS/cm to 121.33 (mean 109.5±2.9)

In trial II, dissolved oxygen was within tolerable ranges of 3.71 mgL^{-1} to 5.93 mgL^{-1} (mean = 4.7±0.2). The average pH ranged between 8.27 and 8.59 (mean 8.4±0.02). Water temperature ranged from 24.95˚C to 29.61˚C (Mean 27.0±0.4). The range for conductivity was 85.50 µS/cm to 122.00 µS/cm (Mean 108.2±4.2).

In trial III, dissolved oxygen was within tolerable ranges of 3.76 mgL^{-1} to 6.45 mgL^{-1} (Mean = 4.8±0.05) while the average pH ranged between 8.30 and 9.19 (Mean 8.6±0.02). Water temperature ranged from 24.35˚C to 29.55˚C (Mean 27.2±0.4) and the range for conductivity was 76.0 µS/cm to 120.0 µS/cm (Mean 104.4±1.4).

Table 4.10: Selected water quality parameters for *O. niloticus* reared in cages installed in ponds during the culture period. Values expressed (Mean ± SEM)

Parameter	Trial I	Trial II	Trial
Dissolved oxygen (mgL^{-1})	4.16 - 5.24	3.71 - 5.93	3.76–6.45
Temperature (^{o}C)	25.02 - 29.03	24.95 - 29.61	24.35 - 29.55
pH	8.64 - 9.57	8.27 - 8.59	8.30–9.19
Conductivity (µs)	91.50 - 121.33	85.50 - 122.00	76.0 - 120.0
Nitrates (mgL^{-1})	1.01- 0.031	0.21 - 1.19	0.03 - 1.27
Phosphates (mgL^{-1})	0.00	0.04 - 0.15	0.03 - 0.21
Ammonia (mgL^{-1})	0.00-0.08	0.05-0.20	0.02– 0.22

CHAPTER FIVE : DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Effect of using a mixture of plant protein sources on growth performance parameters of *Oreochromis niloticus*

The present study focused on substituting freshwater shrimp (*caridina nilotic*a) meal (FSM), with varying levels of soybean (*Glycine max*) meal (SBM), cottonseed (*Gossypium spp*) cake (CSM) and sunflower (*Helianthus annuus*) cake (SFM). Growth performance parameters results, from the three experimental studies indicated that the growth of *O. niloticus* is mainly influenced by the composition of the diets. This is because; initial body weights and lengths of the experimental fish were not significantly different. All three trials recorded a high survival indicating that the experimental diets were palatable and accepted to varying degrees by the cultured fish and these findings are similar to those reported by Muin *et al.* (2017).

5.1.1.1 Effect of replacing freshwater shrimp meal with soybean meal on growth performance of *O. niloticus*

Results from the present study showed that the growth performance of *O. niloticus* decreased significantly with an increase in plant proteins replacement in the diets. The significantly higher growth rate recorded in fish fed on D0 may be attributed to the usage of freshwater shrimp meal as the only protein source. Furthermore, freshwater shrimp meal has been reported to have balanced essential amino acids, vitamins, minerals, high digestibility and palatability which bring about good fish growth (Kirimi *et al.,* 2016; Ogello *et al.,* 2017). Diets D1, D2 and D3 had a similar growth pattern and these findings were not comparable to D0. This indicates that D0 performed better in terms of growth compared to the other diets, suggesting that

fishmeal may have provided the key amino acids required by fish such methionine, for their growth. On the contrary, Liti *et al.* (2006) reported that SBM could replace fishmeal completely without any adverse effects in the growth of Nile tilapia if the diets had a sub-optimal protein level of 24%. Therefore, based on the present study, SBM could not replace fishmeal beyond 75% and the variation in usage of SBM as a protein source may be attributed to fish species, size, methods of SBM processing and culture systems (Ogello *et al.,* 2014).

Diet D4 recorded the lowest growth performance when fishmeal was replaced entirely by soybean meal and these findings further agree with results of Shiau *et al*. (1989), who observed that male tilapia when fed with diets containing 100% SBM either with or without methionine supplements, recorded a significantly low weight gain and protein digestibility as compared to fish fed on pure fishmeal diets. In addition, the decreased growth performance in D4 may be attributed to various factors such as amino acid imbalances, especially methionine, which has been reported to have a negative effect on protein synthesis leading to poor growth and feed efficiency of fish (Wang *et al.,* 2017). Although Liti *et al.* (2005), Munguti *et al.* (2014) and Opiyo *et al.* (2014) have reported soybean meal to be superior to other plant sources; this may vary due to the source of soybean and methods used to destroy ANFs and presence of fibre. The processing methods include heat treatment, which may inactivate the inhibitors, proteinase and amylase. However, the thermal treatment process can also destroy other essential nutrients.

Furthermore, heating raises the cost and energy demands of soybean production. Removal of ANFs by heat-treatment has been attempted with varying degree of success. For all ANFs, fermentation or enzyme treatments directly focusing on

inactivation of a specific ANF may reduce content or activity in the feedstuff (Teimouri, 2018).

(FCR) is considered to be a key indicator of quality in fish feeds as it assesses feed utilization and absorption, with a low FCR indicating better utilization of fish feed hence minimizing feed wastage (Opiyo *et al.*,2014; Gichukia *et al.*, 2015). The low FCR of 1.64 ± 0.10 exhibited in fish fed on D0 in the present study showed that the fish utilized the feed well. The FCR values agreed with ranges reported for *O. niloticus* ranging from 1.43- 2.30 (Opiyo *et al.,* 2014*).* Soybean meal at 100% inclusion level altered nutrient utilization as seen in D4 which had the lowest feed efficiency and this could be attributed to lack of methionine supplementation, inadequate phosphorus and energy levels, poor feed intake and high levels of ANFs such as trypsin inhibitor which affects digestibility of protein, lectins changing morphology of intestine hence reducing nutrients absorption (Ogello *et al.*, 2014). In addition, De Silva and Anderson (1989) reported that fish feed acceptability could be influenced by elevated levels of plant material, which affects the texture and palatability of the diets. Fish survival in the present study, in all the treatments was not significantly different, which indicates that a lack of nutrition or the presence of ANFs did not influence mortality rates but adversely affected growth.

5.1.1.2 Effect of replacing freshwater shrimp meal with a mixture of plant proteins (Soybean meal, cottonseed meal and sunflower meal) on growth performance of *O. niloticus*

This study attempted to replace fishmeal with varying levels of mixture of different plant proteins in a bid to enhance utilization of plant protein sources in the diets of *O. niloticus.* The results showed that substituting fish meal with a mixture of plant protein sources at varying levels of inclusion brought about improved growth performance compared to usage of a single plant protein source (Soybean meal) used in trial I and this may be attributed to good amino acid profile and reduced levels of anti-nutritional factors which affect growth of fish.

Results on growth performance showed that the growth of *O. niloticus* fed on diets containing 25% fishmeal replacement were similar to that of the control diet an indication that 25% fishmeal substitution is possible using a mixture of plant proteins without adverse effects on growth performance and nutrient utilization. Results are similar to those reported by Olukunle (1982) and Richard (1983), who observed improved the growth performance of *O. mossambicus* when fed on diets comprising a mixture of plant protein sources namely; groundnut, sunflower seed and sesame meals compared with single ingredient diets. Borgesson *et al.* (2006), reported that Nile tilapia fed on diets containing a mixture of plant proteins namely canola and pea or flax and pea performed better than those fed on diets containing the individual plant proteins. Moreover, better growth performance was reported in carp fry fed diets containing a mixture of linseed, groundnut, mustard, and sesame meals (Hasan, 1986).

The higher growth performance recorded in D0 and D1 may be attributed to the complementary effects of blending plant protein mixtures which lowers the levels of

anti-nutritional compounds and brings about a balanced amino acid profile as compared to using individual plant protein sources, adequate availability of the essential amino acids (Soltan *et al.,* 2008; Ogello *et al.,* 2017). However, D2, D3 and D4 had similar growth, though it was a blend, and this manifestates plant protein inferiority which may be due to increased levels of crude fibre, poor palatability and presence of antinutritional factors (Soltan *et al.,* 2018). Although D4 had the lowest final mean body weight, it was not significantly different from D2 and D3, an indication that plant-based protein mixture is highly effective as compared to single ingredients. Besides, it can be concluded that soybean meal can be improved by blending it with other ingredients such as cottonseed cake and sunflower cake. Therefore, these results demonstrate that replacement of fish meal with a mixture of different plant protein sources in varying levels of inclusion brought about enhanced growth performance as compared to those of single ingredient (SBM) used in the first trial of the study which used soybean meal as the only single plant protein source to replace fishmeal. However, with increased inclusion levels of SBM, poor growth performance and feed utilization were observed as compared to D0, and this may have been due to high levels of ANFs and lack of methionine in soybean meal. Daily weight gain for fish in all the treatments was higher in trial II as compared to trial I, and this is because fish growth is exponential and exhibits lag phase, log and stationary phase. In trial I, the fish were in the lag phase, which is characterized by slow growth as compared to trial II which was in the log or exponential phase, which is characterized by rapid growth.

In the present study, D0 and D1 had a significantly higher weight gain as compared to the other dietary treatments and the decrease with reducing levels of fishmeal is an

indication that the fish could accept the alternative plant protein mixture only up to 25% inclusion level (Muin *et al.*, 2017). The lowest FCR values were recorded in D0 and D1 at 2.63 and 2.64, respectively, indicating that these values were slightly higher as compared to the recommended range of 1.43- 2.30, as reported by Opiyo *et al.* (2014). Also, the low FCR value indicates that the feeds were of high quality; therefore, a 25% fishmeal replacement with a mixture of plant proteins could be used to culture Nile tilapia with good feed utilization as reported by Muin *et al.* (2017). Diet D4 recorded the highest FCR value of 4.18, and the high FCR values recorded for fish in the present study could be directly attributed by dietary protein source, low digestible protein, and the energy in fish fed with cottonseed cake-based diets.

The growth curves for the present study were similar in the first month, likely attributed to application of fertilizer in the fertilization of the ponds providing extra nutrition from primary production in the pond. Charo-Karisa *et al.* (2013) reported that Inorganic fertilizer was preferred because it dissolves instantaneously as opposed to organic fertilizer which takes about 8 to 10 weeks to completely break down and release nutrients (Das and Jana, 1996). After the first month, fish utilization of the natural food may have reduced, as the fish consumed the experimental diets.

5.1.1.3 Effect of replacing freshwater shrimp meal with a mixture of plant proteins (cottonseed meal and sunflower meal) on growth performance of *O. niloticus*

The results obtained from this study showed that *O. niloticus* fed on diets with 25% fishmeal replacement exhibited similar growth performance to that of the control treatment (D0). Diet D0 and D1 recorded the highest final body weight, which was brought about by the blending of two plant proteins just to improve growth performance as compared to single ingredients. This improved performance may be attributed to several factors such as enhanced essential amino acids profile, which are deficient when used as individual ingredients, reduced exposure to ANFs, and improved digestibility and feed intake. Similar observations have been reported by Jackson *et al.* (1982), El-Saidy and Gaber (2003) and Borgeson *et al.* (2006).

The significantly lower growth rates in D4 as compared to other dietary treatments shows a positive relationship between growth suppression and increasing dietary levels. This can be attributed to presence of high fibre which is normally indigestible to most cichlids because they don't possess enzymes required for fibre digestion and imbalances of dietary amino acids such as phenylalanine and methionine which limit nutrient bioavailability (Obirikorang *et al.,* 2015; Ogello *et al.,* 2017). Furthermore, Anderson *et al.* (1984), recommended keeping maximum crude fibre levels for Nile tilapia diets not to exceed 5%. In the present study, the CSM and SFM diets had crude fibre levels at 8.5% to 14.1%. Inclusion of higher levels of CSM and SFM in D4, may have affected food intake, palatability, nutrient digestibility hence reducing growth (Aanyu *et al.,* 2014). Previous studies reported that, fish fed up to 100% cottonseed cake in dietary protein, resulted in depressed growth and inclusion level of up to 50% has been recommended (Agbo *et al.,* 2011). In addition, sunflower has a lot of fibre

which increases rate of evacuation thus reducing nutrient utilization and digestibility.

Daily weight gain, weight gain, specific growth rate, and final body weight gain values for D0 and D1 were significantly higher than for the other treatments and these findings indicate that fishmeal replacement (>25%) negatively affects growth parameters and this may be attributed to high crude fibre, poor digestibility, and presence of ANFs (El Saidy *et al.,* 2003).The lowest FCR was recorded in D1 at 2.98 while D4 recorded 4.69 which was higher than all other treatments depicting poor feed utilization.

5.1.1.4 Length-Weight Relationship of the Cultured *O. niloticus*

The length-weight relationship of fishes is an essential tool as it provides information on the condition and their growth patterns (Ighwela *et al.,* 2011; Silva *et al.,* 2015). In trial one, the diets exhibited isometric growth for D0, and D2, positive allometry in D4 and negative allometry in D1 and D3. In trial II, D1 and D3 showed isometric growth pattern, while the other three diets exhibited a negative allometric growth pattern. In trial III, only diet 3 had a negative allometry growth pattern. This shows that the fish which had a b value of less than 3 were slimmer with increasing length while those with b value greater than 3 became heavier with an increase in length (Jisr *et al.,* 2018). Generally, the mean value of 'b' in this study was in the range of 2.98-3.1 in all the diets. These values were within the range (2.5 – 3.5) recommended by Prasad and Anvar (2007) as ideal for many fish species. The high "r" values for regression equations in each dietary treatment indicated reasonable precision of these equations for Nile tilapia. Therefore, the length-weight equation, showed that the fish did not deviate from the general trend of such relationship for feed supplementation

(Mahboob, 2014). In addition, the results are in agreement with the findings of other researchers who recorded 2.9-3.4 for *Oreochromis niloticus* in fertilized earthen ponds and 2.7-3.2 in *Tilapia zillii* (Abdel and El-Marakby, 2004 and Anani and Nunoo 2016). The condition factor (K) is an indicator of fish health and it can be affected by feeds unavailability stress, season, and water quality (Igwehla, 2011). The condition factor of all the dietary treatments for the 3 trials in the present study was >1. A CF >1 indicates good fish health condition and an isometric growth pattern which is desirable in fish farming (Anani and Nunoo, 2011)

5.1.2 Apparent protein digestibility of the formulated diets in *Oreochromis niloticus*

The degree to which a fish can digest, absorb and assimilate nutrients is what defines how good a fish feed is. Therefore, evaluation of apparent digestibility coefficients of feed materials used in fish diets is one of the most crucial steps in formulating well balanced diets to meet the nutrient requirements of fish. Several factors influence digestibility and these include: fish species, physiological condition of the fish, age, size, temperature, feed quality and quantity, fishmeal replacement levels and feeding frequency (Gomes *et al.,* 1995; Ginindza, 2012).

5.1.2.1 Apparent protein digestibility of *O. niloticus* fed on soybean meal diets

Although the ADCp decreased with increasing levels of soybean meal (SBM), the values obtained in this study for *O. niloticus* (86.3% -90.2%) are higher than 81.44% which was reported by Hossain *et al*. (1992). This variation may be attributed to the ingredient's chemical composition, source, and mode of processing and method used for faeces collection as reported by El-husseiny *et al.* (2013). In addition, the high ADCp values for crude protein confirm the ability of Nile tilapia to digest plant

proteins (Ngugi *et al.,* 2017). Diet 4 (100% SBM) had the lowest ADCp of 86.3% which may be due to the presence of ANFs especially phytates, contained in soybean meal which form complexes with proteins thus reducing amino acids availability. Tilapia lacks the intestinal enzyme phytase which hydrolyses phytates thus increasing nutrient digestibility. Inhibition of protein digestibility of either of the amino acids results in reduced growth and feed utilization. Studies have shown that, in rainbow trout and Atlantic salmon, phytase pre-treated diets resulted in increased growth due to enhanced protein utilization (Richie and Garling, 2004). In addition, insufficient heating of soybean meal to destroy ANFs results in decreased protein availability. Studies have shown that soybean protein digestibility increased from 45-75% when the heating temperature was raised from 127°C – 204°C (NRC, 1993). Azaaza *et al.* (2012) also observed decreasing apparent protein digestibility values for Nile tilapia, with increasing inclusion levels of the green algae ulva (*Ulva rigada*) meal.

5.1.2.2 Apparent protein digestibility of *O. niloticus* fed on a mixture of soybean meal, cottonseed meal and sunflower meal diets

There was a declining trend in ADCp with increasing levels of dietary plant proteins across the treatments confirming similar results which have been found for other fish species such as rainbow trout *Oncorhynchus mykiss* (Luo *et al.,* 2006) and Japanese seabass *Lateolabrax japonicus* (Cheng *et al.,* 2010). The highest digestibility coefficients were recorded in D0 at 90.2% and the lowest in D4 at 80.6%. These values, were within the range of 75-95% for protein rich feedstuffs (Anani and Nortey, 2017). Moreover, these ADCp values obtained from the present study were slightly higher (80.6% - 90.2%) than the values (80.30% - 85.40%) for Nile tilapia obtained by El Saidy and Gaber (2003). The variation may be attributed to the mode of faecal collection which can affect the ADC values obtained (Cho *et al.,* 1982).

Moreso, digestibility estimations with fecal collection method from tanks have been shown to be 10% greater, compared with those obtained by stripping, indicating some nitrogen compounds are lost in water (Fenucci and Bolasina, 2005).

Diet D4 which had the highest replacing levels of fishmeal with the plant protein mixture, recorded the lowest ADCp. This reduced digestibility may be attributed to high inclusion levels of the plant protein feed ingredients which contain high levels of fibre, poor palatability and presence of ANFs which can initiate pathogenesis of the gastrointestinal tract and impair digestion and absorption of nutrients (Ginindza, 2012; Mzengereza *et al.*, 2016). Moreover, digestibility of a mixture of the three plant protein sources, is directly linked to chemical composition and digestive capacity of the individual plant protein sources used (Eggum and Christensen, 1974; Guimiraes, 2012).

5.1.2.3 Apparent protein digestibility of *O. niloticus* fed on a mixture of cottonseed meal and sunflower meal diets

There was a general reduction in digestibility as compared to the previous trial I and II. This observation is similar to what was reported by Olvera-Novoa *et al.* (2002) that there was a reduction in protein digestibility with increasing dietary contents of sunflower seed meals. The highest ADCp was reported in D0 which was made of purely fishmeal which is highly digestible and most preferred source of protein in fish diets.

Although trial III had higher crude fiber levels than trials I and II, apparent protein digestibility coefficients were high (84.42 -79.77) and within the range of protein rich feedstuffs (75-95%), as reported by NRC, (1993). Diet D4 recorded the lowest ADCp

of 79.7%. The reduced digestibility may have been due to the usage of plant protein sources only (Mzengereza *et al.,* 2016). This implies that, the apparent protein digestibility for PPSM (cottonseed meal and sunflower meal) was lower than that for fishmeal in Nile tilapia. This may have been attributed to the high inclusion levels of cottonseed meal and sunflower meal which led to reduced feed intake hence reduced feed utilization due to free gossypol in cottonseed cake, high saponin levels which bring about poor palatability and digestibility, and high fibre content which affects gastrointestinal transit time of feed and modifies nutrients digestibility (El-Saidy and Gaber, 2004; Soltan *et al.,* 2008; Agbo *et al.,* 2011; Mzengereza *et al.,* 2016). Diet D4 had the highest fibre level (14.1%) which was above the recommended value of 12%. This is not desirable in fish as the high fibre levels leads to a reduction in quantity of usable nutrients in diet and decreased nutrient digestibility (De Silva and Anderson, 1995).

Fibre which is found in feedstuffs comprises of the indigestible plant matter such as lignin, cellulose and hemicellulose and monogastric animals such as fish are not able to digest the fibre as they cannot secrete cellulase (NRC 1993; El- husseiny *et al.,* 2013). In addition, De Silva and Gunasekera (1989) reported that fish feed acceptability can be affected by increased plant protein levels since the taste and texture of diets will vary.

5.1.3 Effect of a mixture of plant protein sources on *Oreochromis niloticus* carcass composition

Chemical analysis at the end of an experimental feed trial helps in determining the influence of feed on the composition of fish. Various factors either endogenous or exogenous can affect the body composition of fish. During the study period, exogenous factors such as feeding frequency and temperature were kept uniform and therefore feed was the only exogenous factor that may have influenced the body composition of fish (Soltan *et al.*, 2001).

5.1.3.1 Effect of soybean meal diets on *O. niloticus* whole body composition

In the present study, crude protein, fats and ash were significantly influenced by dietary treatments. Diets with increased SBM inclusion levels produced higher lipid content and these findings agree with what was observed by Nyina-wamwiza *et al.* (2007) for *Clarias gariepinus.* In addition, the increasing fat content with increasing SBM inclusion levels may be attributed to rising lipogenesis with increased fishmeal replacement (Koumi *et al.*, 2011). Ash significantly reduced across the dietary treatments and these findings are similar with what was reported by Koumi *et al.* (2011), who observed a significant decrease in ash content with increased levels of fishmeal replacement. The fishmeal may have had high levels of phosphorus, which is associated with bone fraction. These findings are also similar with those reported by Goda *et al.* (2007) who observed a significant reduction in ash content in *Sarotherodon galileus* and *Oreochromis niloticus* fed on soybean meal which may be attributed to presence of phytic acid which tends to reduce availability of various minerals such as phosphorus, calcium magnesium and zinc.

5.1.3.2 Effect of a mixture of soybean meal, sunflower meal and cottonseed meal diets on *O. niloticus* whole body composition

Changes in chemical composition of Nile tilapia may have been due to the variation in dietary treatments. Diet D2 (50%) fishmeal replacement had a significant higher protein content than in the other diets, although the protein content had an irregular trend. These findings are similar with those of Winfree and Stickney (1981). Crude fat content was significantly higher among diets with higher inclusion of plant protein mixture. D4 recorded the highest fat levels. These results are similar to those reported by Agbo *et al.* (2011) who observed a similar trend in juvenile Nile tilapia. The significantly increased ash content observed across the dietary treatments may be attributed to the presence of various minerals which constitute around 1-2% of the edible portion of the fish, hence indicating Nile tilapia as a good source of minerals (Murray and Burt, 2001). Iluyemi *et al.* (2010) and Jabir *et al.* (2011) recorded similar ash content values of 14.1% and 14%, respectively.

5.1.3.3 Effect of a mixture of sunflower meal and cottonseed meal diets on *O. niloticus* whole body composition

Carcass composition was affected by dietary treatments. Diets with increased plant protein mixture inclusion levels produced higher lipid content that was significant across all treatments. Nile tilapia fed on D4 recorded the highest crude fat levels which may be due to the dietary lipid level (Goda *et al.*, 2007). Elsewhere, a similar trend of increased lipids has been observed in carps when fed plant-based diets (Kumar *et al.*, 2014).

5.1.4 Profitability and economic analysis of using a mixture of plant protein sources in *O. niloticus.*

5.1.4.1 Profitability and economic analysis of using soybean meal diets in *O. niloticus*

Economic analysis the incorporation of plant-based diets in fishmeal indicated positive net returns for all the diets. Although net returns were positive for all the diets, there were significant differences in economic returns among the diets with D0 being most profitable and this may have been attributed to the superiority of fishmeal due to their well-balanced nutritional content (Watanabe, 2002), resulting in high yield and higher net returns. Although D1 and D2 and were not significantly different in growth performance, D2 would be recommended because it reduced the costs of the diets by replacing fishmeal with higher inclusion levels of plant proteins with a fairly good growth performance. At increased inclusion levels of SBM D4, economic net returns above total variable costs and total costs declined probably because of poor growth response which may be attributed to poor palatability and presence of ANFs in the diet (Martínez-Llorens *et al.*, 2007).

5.1.4.2 Profitability and economic analysis of using a mixture of soybean meal, cottonseed and sunflower meal diets in *O. niloticus*

Profitability in the present study was affected by dietary treatments and at a price of US$ 4.0 kg^{-1} of fish, all test feeds posted positive returns above both variable and total costs indicating both short- and long-term economic viability. Net returns above Total Cost (TC) were highest in D0 followed by D1, with lower TCs recorded across the other treatments. Highest total fish yield and gross revenue was achieved in D0 and this is explained by the higher growth performance and better fish weight for treatments fed on fish meal diet only. In addition, higher nutrient digestibility of D0 may explain the increased yields when fish were fed fish meal diets. Although D0 did

not vary significantly from D1, diet D1 is much recommended than D0 because it's cheaper as compared to D0 due to usage of plant proteins at higher levels in D1 than D0. Similar findings were reported by Ngugi *et al.* (2016), who observed that formulated diets using a combination of rice bran and *C. nilotica* resulted in the best economic benefits; as compared to fishmeal-only diets. Resource poor farmers are more interested with lowering the cost of fish feeds even if it means the culture period would be longer (Middendorp and Verreth, 1991). However, the poor growth performance of plant protein feeds which can increase the culture period required to attain the desirable market size, can be countered through pond fertilization to enhance natural food production which will in turn reduce the entire cost of production and significantly enhance profitability (De Silva and Davy, 1992; Omondi *et al.,* 2001).

5.1.4.3 Profitability and economic analysis of using a mixture of cottonseed and sunflower meal diets in *O. niloticus*

The results of economic analysis indicate that all test-feeds posted positive returns above both Total variable (TVC) and Total Costs (TC) indicating both short- and long-term economic viability. Net returns above Total Cost (TC) were highest in D0 followed by D1 but were not significant. This may be attributed to the partial replacement of fishmeal with plant proteins. Therefore, D1 is equally profitable as D0 as growth performance was comparable. The D0 diet was most profitable among the test diets and it also recorded the highest yield which was not significantly different from D1. These findings are in agreement with those reported by Nyina-wamwiza *et al.* (2007) that, agricultural by products can be used partially and effectively as nutritionally balanced feeds for Nile tilapia. Diet D1 recorded reduced cost of the feeds as compared to D0 which was attributed to partial replacement of fishmeal with

plant proteins, hence more profitable for farmers. Therefore, use of plant protein sources could be more cost effective resulting in increased profits for fish farmers (Kirimi *et al.*, 2016).

5.2 Conclusions

i). This study indicates that a mixture of plant proteins comprising SBM, SFM and CSM at various inclusion levels, could partially replace fishmeal up to 25% without having adverse effects on growth and feed utilization. The improved growth performance was as a result of combining the plant proteins, which compensated for deficient amino acids and lowered high inclusion levels of ANFs in the diets. In all the three trials, 100% fishmeal substitution led to decreased growth performance, which could be due to high fibre levels, ANFs, and reduced palatability.

ii). From this study, SBM, CSM, and SFM were acceptable feed ingredients for *O. niloticus.* Most of the diet treatments were digested well except in diets containing 100% plant proteins.

iii). There was no trend in whole body composition of crude protein for all the experiments. However, increased inclusion levels of the oilseed meals resulted in high levels of crude fat.

iv). The economic analysis indicated that plant protein sources were cheaper than fishmeal, while SBM was the most expensive plant-based protein source. Diets containing elevated inclusion levels of plant proteins were economically better as compared to diets with more quantity of fishmeal. This is because cost of fishmeal was almost twice the cost of cottonseed meal and sunflower meal.

5.3 Recommendations

i). Oil seeds such as SBM, CSM and SFM should undergo some processing; for example, heat processing in order to destroy ANFs that contribute to poor fish growth.

ii). From an economic view, partial replacement of fishmeal at 25% for trial II and III had comparable results on the growth performance of Nile tilapia, although D1 was slightly cheaper than D0, hence it should be recommended to farmers because it is cost effective.

5.4 Recommendations for further studies

i. Digestibility studies in all the trials focused on crude protein only, but there is a need to study the other nutrients such as crude fats, crude fiber and ash. This is because; information on digestibility could aid the use of feed ingredient substitutions for least-cost formulated diets in *O. niloticus* for aquaculture.

ii. Studies should be conducted with the deficient essential amino acids supplements, especially methionine, which is vital in *O. niloticus* and would improve feed utilization.

REFERENCES

Aanyu, M., Ondhoro, C.C., Ganda, E., Kato, D.C. and Basiita, R.K. (2014). Intestine histology, nutrient digestibility and body composition of Nile tilapia (*Oreochromis niloticus*) fed on diets with both cotton and sunflower seed cakes. *African Journal of Biotechnology*, **13** (37): 3831–3839.

Abdel-Tawwab, M. and El-Marakby, H.I. (2004). Length-weight relationship, natural food and feeding selectivity of Nile tilapia; *Oreochromis niloticus* (L.) in fertilized earthen ponds. In *The 6th International Symposium of Tilapia in Aquaculture*, 500-509.

Abou Zead, M.Y., Soltan, M.A. and Ibrahim, M.S. (2008). Effect of replacing soybean meal by sunflower meal in the diets for Nile tilapia, *Oreochromis niloticus*. In: Elghobashy, H., Fitzsimmons, K., Diab, A.S. (eds.) Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 12-14 Oct 2008, **2**: 787-798

Agbo, N.W., Madalla, N. and Jauncey, K. (2011). Effects of dietary cottonseed meal protein levels on growth and feed utilization of Nile tilapia, *Oreochromis niloticus Journal of Applied Sciences and Environmental Management*,**15**: 235-239.

Ahmed, N., Alam, M.F. and Hasan, M.R. (2010). The economics of sutchi catfish (*Pangasianodon hypophthalmus*) aquaculture under three different farming systems in rural Bangladesh. *Aquaculture Research*, **41** (11): 1668-1682.

Akande, K.E., Doma, U.D., Agu, H.O. and Adamu, H.M. (2010). Major antinutrients found in plant protein sources: their effect on nutrition. *Pakistan Journal of Nutrition*, **9** (8): 827-832.

Anani, F.A. and Nunoo, F.K.E. (2016). Length-weight relationship and condition factor of Nile tilapia, *Oreochromis niloticus* fed farm-made and commercial tilapia diet. *International Journal of Fisheries and Aquatic Studies*, **4** (5): 647-650.

Anani, F.A. and Nortey, T.N.N. (2017). Apparent Nutrient Digestibility of Farm-made and Commercial Tilapia Diets in Nile Tilapia (*Oreochromis niloticus L.). Asian Research Journal of Agriculture*, **3** (3): 1-9.

Anderson, J., Jackson, A.J., Matty, A.J. and Capper, B.S. (1984). Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* L. Aquaculture **37**: 303–314. *Aquaculture Nutrition,* **17** (2): 214-222.

Asche, F. and Guttormsen, A.G. (2001). Patterns in the relative price for different sizes of farmed fish. *Marine Resource Economics*, **16** (3): 235-247.

Association of Official Analytical Chemists (AOAC) (2003). Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed. Washington DC, USA.

Azaza, M.S., Mensi, F., Ksouri, J., Dhraief, M.N., Brini, B., Abdelmouleh, A. and Kraiem, M.M. (2008). Growth of Nile tilapia (Oreochromis niloticus L.) fed with diets containing graded levels of green algae ulva meal (*Ulva rigida*) reared in geothermal waters of southern Tunisia. *Journal of Applied Ichthyology*, **24** (2): 202-207.

Bell, J.D., Kronen, M., Vunisea, A., Nash, W.J., Keeble, G., Demmke, A. and Andréfouët, S. (2009). Planning the use of fish for food security in the Pacific. *Marine Policy*, **33** (1): 64-76.

Bhatnagar, A. and Devi, P. (2013). Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Sciences*, **3** (6): 1980-2009.

Bhujel, R.C. (2014). Strategic review on sub Saharan African aquaculture seed supply. Partnership for African Fisheries (PAF) Aquaculture Working Group. NEPAD.

Borgeson, T.L., Racz, V.J., Wilkie, D.C., White, L.J. and Drew, M.D. (2006). Effect of replacing fishmeal and oil with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*, **12** (2):141-149.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K. and Corner, R. (2010). Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365** (1554): 2897-2912.

Boyd, C.E. and C.E. Tucker (1998). *Ecology of aquaculture ponds*.in pond Aquaculture water quality Management. Springer US. 8-86.

Brummett, R.E., and Williams, M.J. (2000). The evolution of aquaculture in African rural and economic development. *Ecological Economics*, **33** (2): 193–203.

Chakraborty, S.B., Mazumdar, D., Chatterji, U. and Banerjee, S. (2011). Growth of Mixed-Sex and Monosex Nile Tilapia in Different Culture Systems. *Turkish Journal of Fisheries and Aquatic Sciences*, **11** (1).

Charo-Karisa, H., Opiyo, M.A., Munguti, J.M., Marijani, E. and Nzayisenga, L. (2013). Cost-Benefit Analysis and Growth Effects of Pelleted and Unpelleted On-Farm Feed on African Catfish (*Clarias gariepinus* Burchell 1822) in Earthen Ponds. *African Journal of Food, Agriculture, Nutrition and Development*, ***13*** (4); 8019-8033.

Chen, W., Ai, Q., Mai, K., Xu, W., Liufu, Z., Zhang, W. and Cai, Y. (2011). Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, **318** :95-100.

Cheng, Z., Ai, Q., Mai, K., Xu, W., Ma, H., Li, Y. and Zhang, J. (2010). Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, **305** (1-4): 102-108.

Chepkirui-Boit, V., Ngugi, C.C., Bowman, J., Oyoo, E., Rasowo, J., Mugo, J. and Cherop, L. (2011). Growth performance, survival, feed utilization and nutrient utilization of African Catfish (*Clarias gariepinus*) larvae co-fed Artemia and micro-diet. *Aquaculture Nutrition,***17**: 82-89.

Cho, C.Y., Bayley, H.S. and Slinger, S.J. (1974). Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*). *Journal of Fisheries Resources and Biodiversity Canada*, **31:** 1523-1528.

Cho C.Y., Slinger, S.J. and Bayley, H.S. (1982). Bioenergetics of Salmonid Fishes: Energy Intake, Expenditure and Productivity. *Comparative Biochemistry and Physiology,***73**B: 25–41.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H. and Chen, H.Y. (2004). Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture,* **229**: 325-333.

Das., S.K. and Jana., B.B. (1996). Pond fertilization through inorganic sources: an overview. *Indian Journal of Fisheries*, **43** (2): 137-155.

Dayal, J.S., Rajaram, V., Ambasankar, K. and Ali, S.A. (2011). Sunflower oil cake as a replacement for fish meal in feeds of Tiger Shrimp, *Penaeus monodon* reared in tanks and in net cages. *Indian Journal of Geo-Marine Sciences*, **40** (3): 460-470.

De Silva, S.S., Gunasekera, R.M. and Atapattu, D. (1989). The dietary protein requirements of young tilapia and an evaluation of the least cost dietary protein levels. *Aquaculture,* **80**: 271-284.

De Silva, S.S. and Gunasekera, R.M. (1989). Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diets on consumption, growth performance and carcass composition in *Oreochromis niloticus* (L.) fry. *Aquaculture,* **80** (1-2): 121-133.

De Silva, S.S. and Davy, F.B. (1992). Fish nutrition research for semi-intensive culture systems in Asia. *Asian Fisheries Science*, **5**:129-144.

De Silva, S.S. and Anderson, T.A. (1995). Fish nutrition in aquaculture. 319 London: Chapman & Hall.

De Silva, S.S. and Anderson, T.A. (1998). Fish nutrition in aquaculture. London: Chapman & Hall.

DeLong, D.P, Losordo, T.M. and Rakocy, J. E. (2009). Tank culture of tilapia. The Southern Regional Aquaculture Center Publication **282**: 1-8

Dersjant-Li, Y. (2002). The use of soy protein in aquafeeds. *Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola*; **3**: 541-558.

Devaere, S., Jansen, G., Adriaens, D. and Weekers, P. (2007). Phylogeny of the African representatives of the catfish family Clariidae (Teleostei, Siluriformes) based on a combined analysis: independent evolution towards anguilliformity. *Journal of Zoological Systematics and Evolutionary Research*, **45**: 214-229.

Dimitroglou, A. Merrifield, D.L., Spring, P., Sweetman, J., Moate, R. and Davies, S.J. (2010). Effects of mannan oligosaccharide (MOS) supplementation on growth performance feed utilization, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*); *Aquaculture*, **300**:182-188.

Eggum, B.O. and Christensen, K.D. (1974). Protein digestibility of a feed mixture in a relation to the protein digestibility of the individual protein components. *British Journal of Nutrition*, **31** (2): 213-218.

El-Naggar, G.A.M.A.L., Nasr-Alla, A.H.M.E.D. and Kareem, R.O. (2008). Economic analysis of fish farming in Behera Governorate of Egypt. Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 12–14 Oct 2008.

El-Husseiny, O.M., Goda, A.M., Hassan, I. and Suloma, A. (2013). Modelling Digestibility Coefficients of Plant Protein Sources and Levels in Tilapia Diets.

El-Saidy, D.M.S.D. and Gaber, M.M.A. (2003). Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquaculture Research*, **34** (13):1119- 1127.

El-Sayed, A.F.M. (2004). Protein nutrition of farmed tilapia: searching for unconventional sources. In *New dimensions in farmed tilapia: proceedings of the Sixth International Symposium on Tilapia Aquaculture*, 364-378.

El-Sayed, A.F.M. (2006). Tilapia Culture. CABI Publishers, Oxfordshire, UK, 277 pp. reared in cold water. *Aquaculture*, **269**: 414-426.

Eriegha, O.J. and Ekokotu, P.A. (2017). Factors affecting feed intake in cultured fish species: A review. *Animal Research International*, **14** (2): 2697-2709.

European Community, (2002). Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. *Official Journal of the European Communities*, **45** (273): 1-95 EN 14.11.2009.

Filipski, M. and Belton, B. (2018). Give a man a fishpond: modeling the impacts of aquaculture in the rural economy. *World Development*, **110**: 205-223.

Food and Agriculture Organization (FAO) (2004). The State of World Fisheries and Aquaculture Production (SOFIA) 2004. Yearbook Fisheries Statistics. Rome, Italy: Food and Agriculture Organization of the United Nations.

Food and Agriculture Organization (FAO)2005a.Cultured Aquatic Species Information Programme. *Oncorhynchus mykiss.* Cultured aquatic species information programme. http://. Fao.org/fishery/cultured species/*Oncorhynchus mykiss*/en

Food and Agriculture Organization (FAO)*World aquaculture* 2010. Fisheries and Aquaculture Department *Technical Paper No.* 500/1. Rome, FAO. 2011**.** 105pp.

Food and Agriculture Organization (FAO) (2014). The State of World Fisheries and Aquaculture: Opportunities and challenges. 1st ed. [ebook] Rome: FAO.

Food and Agriculture Organization (FAO) (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Food and agriculture organization of the United nations, Rome, Italy.

Fenucci, J.L. and Bolasina, S.N. (2005). Apparent digestibility of crude protein and lipids in Brazilian codling, *Urophycis brasiliensis* (Kamp, 1858) (Pisces: Gadiformes), fed with partial replacements of soybean meal and meat meal diets. *Revista de biología marina y oceanografía*, **40** (2): 127-131.

FIN, (2008). Annual review of the feed grade fish stocks used to produce fishmeal and fish oil for the UK market. FIN: fishmeal information network

Fisheries Department (2012). Fisheries Annual Statistical Bulletin 2011. Nairobi: Ministry of Fisheries Development.

Fitzsimmons, K. (1997). Introduction to tilapia nutrition. In *Tilapia aquaculture. The International Symposium on Tilapia in aquaculture. ICLARM Conferences Proceedings, Florida* (pp. 9-12).

Flajshans, M. and Hulata, G. (2007). Common carp-Cyprinus carpio. Genetic impact of aquaculture activities on native populations (Editors D. Corosetti, E. Garcia-Vasquez & E. Veerspoor). Sixth Framework plan of the EC, final scientific report, 32-39.

Francis, G., Makkar, H.P.S. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**: 197-227.

Furukawa, A. and Tsukahara, H. (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bulletin of the Japanese Society of Scientific Fisheries,* **32:** 502-504.

Gaber, M.M. (2006). Partial and complete replacement of fish meal by broad bean meal in feeds for Nile tilapia, *Oreochromis niloticus*, L., fry. *Aquaculture Research*, **37** (10): 986-993.

Gabriel, U.U., Akinrotimi, O.A., Bekibele, D.O., Onunkwo, D.N. and Anyanwu, P.E. (2007). Locally produced fish feed, potentials for aquaculture development in sub-Saharan Africa. *Journal of Agricultural Research*, **297**: 287-295.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E. J., Stone, D., Wilson, R. and Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research,* **38** (6): 551-579.

Ginindza, J. (2012). Effect protein levels on nutrient and energy digestibility in diet of Arctic charr (*Salvilinus alpinus*). United Nations University Fisheries Training Programme, Iceland [final project].

Githukia, C.M., Ogello, E.O., Kembenya, E.M., Achieng, A.O., Obiero, K.O., Munguti, J.M. (2015): Comparative growth performance of male monosex and mixed sex Nile tilapia (*Oreochromis niloticus* L.) reared in earthen ponds. Croatian *Journal of Fisheries*, **73**: 20-25.

Glencross, B.D., Booth, M. and Allan, G.L. (2007). A feed is only as good as its ingredients–a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture nutrition*, **13** (1): 17-34.

Goda, A.M.A.S., Wafa, M.E., El- Haroun, E.R. and Kabir Chowdhury, M.A. (2007). Growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) and tilapia galilae Sarotherodon galilaeus (Linnaeus, 1758) fingerlings fed plant protein- based diets. *Aquaculture Research*, **38** (8): 827-837.

Gomes, E.F., Rema, P. and Kaushik, S.J. (1995). Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture*, **130:**177–186.

Guimarães, I.G., Pezzato, L.E., Barros, M.M. and Fernandes, R.D.N. (2012). Apparent nutrient digestibility and mineral availability of protein-rich ingredients in extruded diets for Nile tilapia. *Revista Brasileira de Zootecnia*, **41** (8): 1801-1808.

Halver, J.E. and Hardy, R.W. (Eds.). (2002). *Fish nutrition*. Academic press.

Halwart, M., Funge-Smith, S. and Moehl, J. (2003). The role of aquaculture in rural development. *Review of the State of World Aquaculture, FAO, Rome*, 47-58.

Hardy, R.W. (2000). New developments in aquatic feed ingredients, and potential of enzyme supplements. Pp. 216ñ226. Simposium Internacional de NutriciÛnAcuÌcola, 19ñ22 Noviembre 2000. MÈrida, Yucat·n, Me-xico.

Hardy, R.W. (2002). Rainbow trout, *Oncorhynchus mykiss*. *Nutrient requirements and feeding of finfish for aquaculture*, 184-202.

Hasan, M.R. (1986). Husbandry factors affecting survival and growth of carp (*Cyprinus carpio* L) fry and an evaluation of dietary ingredients available in

Bangladesh for the formulation of a carp fry diet. PhD Thesis. Stirling, UK: Stirling University.

Hecht, T. (2005). Regional review on aquaculture development. 4. Sub-Saharan Africa. FAO Fisheries Circular. No. 1017/4. Rome, FAO. 2006. 96 pp.

Hecht, T., Uys, W. and Britz, P.J. eds. (1988). The culture of sharptooth catfish, *Clarias gariepinus* in southern Africa. South African National Scientific Programmes Report No. 153, 133 pp. Pretoria, Council for Scientific and Industrial Research.

Hepher, B. (1988). *Nutrition of pond fishes*. Cambridge University Press.

Hertrampf, J.W. and Piedad-Pascual, F. (2000). Handbook on ingredients for aquaculture feeds. Kluwer Academic Publishers, 624 pp.

Hino, J. (2011). 'Uncharted Waters: Kenya takes dramatic leap in aquaculture' *Globa l Aquaculture Advocate*, July/August 2011 issue: 1921.

Hossain, M.A., Nahar, N., Kamal, M. and Islam, M.N. (1992). Nutrient digestibility coefficients of some plant and animal proteins for tilapia (*Oreochromis mossambicus*). *Journal of Aquaculture in the Tropics,* **7:** 257-266.

Houlihan, D., Boujard, T. and Jobling, M., (2001) *Food intake in fish*, Oxford, UK: Blackwell Science. p 417.

IFFOO, (2006). What is fishmeal and fish oil? International Fishmeal and Fish Oil Organisation.

Ighwela, K.A., Ahmed, A.B. and Abol-Munafi, A.B. (2011). Condition factor as an indicator of growth and feeding intensity of Nile tilapia fingerlings (*Oreochromis niloticus)* feed on different levels of maltose. *American-Eurasian Journal of Agricultural and Environmental Science*, **11** (4): 559-563.

Iluyemi, F.B., Hanafi, M.M., Radziah, O. and Kamarudin, M.S. (2010). Nutritional evaluation of fermented palm kernel cake using red tilapia. *African Journal of biotechnology*, **9** (4).

Inaba, D., Ogino, C., Takamatsu, C., Sugano, S. and Hata, H. (1962). Digestibility of dietary components in fishes: Digestibility of dietary proteins in rainbow trout. *Bulletin of the Japanese Society of Scientific Fisheries,* **28:** 367-371.

Iruo, F.A., Onyeneke, R.U., Eze, C.C., Uwadoka, C. and Igberi, C.O. (2018). Economics of Smallholder Fish Farming to Poverty Alleviation in the Niger Delta Region of Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences*, **19** (4): 313-329.

Jabir, M.A.R., Jabir, S.A.R. and Vikineswary, S. (2012). Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia

(Oreochromis niloticus) juvenile. *African Journal of Biotechnology*, **11** (24): 6592-6598.

Jackson, A.J., Capper, B.S. and Matty, A.J. (1982). Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. *Aquaculture,* **27** (2): 97-109.

Jauncey, K. (1998) Tilapia Feeds and Feeding. Stirling, Scotland: Pisces Press Ltd, p. 241.

Jiang, H.B., Chen, L.Q. and Qin, J. G. (2018). Fishmeal replacement by soybean, rapeseed and cottonseed meals in hybrid sturgeon *Acipenser baerii*♀× *Acipenser schrenckii*♂. *Aquaculture Nutrition*, **24** (4): 1369-1377.

Jisr, N., Younes, G., Sukhn, C. and El-Dakdouki, M.H. (2018). Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. *The Egyptian Journal of Aquatic Research*, **44** (4): 299-305.

Jonni, F.C.T. and Janice, A.R. (2014). The quest for indigenous aquafeed ingredients: a review. *Reviews in Aquaculture,* **8** *(2)*:154–171.

Kasi, M., Nirmell, S., Rahman, M.A., Arshad, Raj, M.G. and Arockiaraj, J. (2015). Induced ovulation and spawning of African catfish *Clarias gariepinus* (Bloch) using ovaprim**.** *Journal of Environment and Biotechnology Research*, **1** (1): 2-9.

Khan, M.A., Jafri, A.K., Chadha, N.K. and Usmani, N. (2003). Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. *Aquaculture Nutrition*, **9:** 391-396.

Kirimi, J.G., Musalia, L.M., Magana, A. and Munguti, J.M. (2016). Performance of Nile tilapia (*Oreochromis niloticus*) fed diets containing blood meal as a replacement of fish meal. *Journal of Agricultural Science*, **8** (8): 79-87.

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W. and Frøkiær, H. (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (Salmo salar L.). *British Journal of Nutrition*,**100**:120-9.

Koumi, A.R., Koffi, K.M., Atsé, B.C. and Kouame, L.P. (2011). Growth, feed efficiency and carcass mineral composition of *Heterobranchus longifilis*, *Oreochromis niloticus* and *Sarotherodon melanotheron* juveniles fed different dietary levels of soybean meal-based diets. *African Journal of Biotechnology*, **10** (66): 14990-14998.

Krogdahl, A., Hemre, G.I. and Mommsen, T.P. (2005). Carbohydrates in fish nutrition: digestion and absorption in post larval stages. *Aquaculture Nutrition*,**11** (2): 103-122.

Kumar, B.P., Ramudu, K.R. and Devi, B.C. (2014). Mini review on incorporation of cottonseed meal, an alternative to fish meal in aquaculture feeds. *International Journal of Biological Research*, **2:** 99-105.

Lall, S.P. and Tibbetts, S.M. (2009). Nutrition, feeding, and behavior of fish. *Veterinary Clinics of North America: Exotic Animal Practice*, **12** (2): 361-372.

Landau, M. (1992). Introduction to Aquaculture. Canada: John Wiley & Sons Press.
Li, M.H., Robinson, E.H. (2006). Use of cottonseed meal in aquatic animal diets: a review. North Am. *Journal of Aquaculture*, **68** (1): 14-22.

Liti, D.M., Mugo, R.M., Munguti, J.M. and Waidbacher, H. (2006). Growth and economic performance of Nile tilapia (*Oreochromis niloticus*, L.) fed on three brans (maize, wheat and rice) in fertilized ponds. *Aquaculture nutrition,* **12**: 239–245.

Lovell, T. (1998). Nutrition and feeding of Fish. second edn, Boston, MA; London: Kluwer Academic.

Lovshin L.L. (2000). Criteria for selecting Nile tilapia and red tilapia for culture. Department of fisheries and allied aquacultures. Pond dynamics/aquaculture collaborative research support program. USA

Luo, L., Xue, M., Wu, X., Cai, X., Cao, H. and Liang, Y. (2006). Partial or total replacement of fishmeal by solvent- extracted cottonseed meal in diets for juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, **12** (6): 418-424.

Machena, C. and Moehl, J. (2000, February). Sub-Saharan African aquaculture: regional summary. In *Aquaculture in the third millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand* (pp. 341-355).

Machena, C. and Moehl, J. (2001). Sub-Saharan Africa aquaculture. In: Subasinghe, R.P., Bueno, P., Phillips, M.J., Hough, C., McGladdery, S.E. and Arthur, J.R., (Eds.) *Aquaculture in the Third Millennium, Technical Proceedings of the Conference on Aquaculture in the Third Millenium. Bangkok, Thailand. 20-25 February 2000*, pp. 341-355. Rome, Italy: NACA, Bangkok and FAO.

Mahboob, S., 2014**.** Replacing fish meal with a blend of alternative plant proteins and its effect on the growth performance of *Catla catla* and *Hypophthalmichthys molitrix*. *Pakistan Journal of Zoology*, **46**: 747-752.

Maina, J.G., Beames, R.M., Higgs, D., Mbugua, P.N., Iwama, G. and Kisia, S.M. (2003). Partial replacement of fishmeal with sunflower cake and corn oil in diets for tilapia *Oreochromis niloticus* (Linn): effect on whole body fatty acids. *Aquaculture Research*, **34**: 601–608.

Martínez- Llorens, S., Moñino, A.V., Tomás Vidal, A., Salvador, V.J.M., Pla Torres, M. and Jover Cerdá, M. (2007). Soybean meal as a protein source in gilthead sea bream (*Sparus aurata* L.) diets: effects on growth and nutrient utilization. *Aquaculture Research,* **38** (1), 82-90.

Mathiesen, A.M. (2012). The State of the World Fisheries and Aquaculture 2012.

Maynard, A.L. and Loosli, K.J. (1962). Animal nutrition, 5th edition, McGraw-Hill, New York, 613 p.

McheleLimbu, S. (2015). The effect of floating and sinking diets on growth performance, feed conversion efficiency, yield and cost effectiveness of African sharp tooth catfish, *Clarias gariepinus* reared in earthen ponds. *International Journal of Fisheries and Aquatic Studies*, **2** (5): 253-259.

Medale, F. and Kaushik, S. (2009). Protein sources in feed for farmed fish. *Cahiers Agricultures,* **18** (2): 103-111.

Medugu, C.I., Raji, A. O., Igwebuike, J.U. and Barwa, E. (2011). Alternative cereal grains and cereal by-products as sources of energy in poultry diets-A review. *Research Opinions in Animal & Veterinary Sciences*. Micropterus salmoides. *Journal of World Aquaculture Society,* **36**: 454–463.

Middendorp, H.A.J.and Verreth, J.A.J. (1991). The development of small-scale hapa culture of Tilapia (*Oreochromis niloticus*) in Northeast Thailand. 2. The feasibility of using low-cost compound feeds. *Asian Fisheries Science*, **4**: 317-327.

Mizanur, R.M., Yun, H., Moniruzzaman, M., Ferreira, F., Kim, K.W. and Bai, S.C. (2014**).** Effects of feeding rate and water temperature on growth and body composition of juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf 1880). *Asian-Australasian journal of animal sciences*, **27** (5): 690.

Mjoun, K., Rosentrater, K.A. and Brown, M.L. (2010). Tilapia: environmental biology and nutritional requirements. USDA-ARS Extension Publications.

Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M.M. and Anderson, J.L. (2013). Fish to 2030: Prospects for Fisheries and Aquaculture. World Bank Report, (83177-GLB).

Muin, H., Taufek, N.M., Kamarudin, M.S. and Razak, S.A. (2017). Growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus, 1758) maggot meal diet. *Iranian Journal of Fisheries Sciences*, **16** (2): 567-577.

Munguti, J.M., Waidbacher, H., Liti, D.M., Straif, M. and Zollitsch, W.J. (2009). Effects of substitution of freshwater shrimp meal (*Caridina nilotica* Roux) with hydrolyzed feather meal on growth performance and apparent digestibility in Nile tilapia (*Oreochromis niloticus* L.) under different culture conditions. *Livestock Research for Rural Development*, **21** *(129).*

Munguti, J., Charo-Karisa, H., Opiyo, M.A., Ogello, E.O., Marijani, E., Nzayisenga, L. and Liti, D. (2012). Nutritive value and availability of commonly used feed ingredients for farmed Nile Tilapia (Oreochromis niloticus L.) and African catfish

(*Clarias gariepinus*, Burchell) in Kenya, Rwanda and Tanzania. *African Journal of Food, Agriculture, Nutrition and Development*, **12**: 1-22.

Munguti, J.M., Musa, S., Orina, P.S., Kyule, D.N., Opiyo, M.A., Charo-Karisa, H. and Ogello, E.O. (2014). An overview of current status of Kenyan fish feed industry and feed management practices, challenges and opportunities. *International Journal of Fisheries and Aquatic Studies*, **1** (6): 128-137.

Murray, J. and Burt, J.R. (2001). The composition of fish. Ministry of technology Torry advisory note No. 38.

Musiba, M.J., Ngupula, G.W., Kashindye, B.B., Elison, M., Shoko1, A.P., Ndikumana, J., Zziwa, E. and Katunzi, E.F.B. (2014). Performance of locally formulated feeds for rearing of African catfish in Tanzania. *African Crop Science Journal*, **22** (4): 979-986.

Mustapha, M.K., Akinware, B.F., Faseyi, C.A. and Alade, A.A. (2014). Comparative effect of local and foreign commercial feeds on the growth and survival of *Clarias gariepinus* juveniles. *Journal of Fisheries*, **2** (2): 106-112.

Mzengereza, K., Msiska, O.V., Kapute, F., Kang'ombe, J. and Singini, W. (2014). Nutritional Value of Locally Available Plants with Potential for Diets of Tilapia Rendalli in Pond Aquaculture in Nkhata Bay, Malawi. *Journal of Aquatic Resources Development*, **5**: 265.

Mzengereza, K., Singini, W., Kapute, F., Kang'ombe, J. and Kamangira, A. (2016). Apparent nutrient digestibility of plant-based diets by Tilapia rendalli (Boulenger, 1896). *Journal of Aquaculture Research and Development*, **7** (2).

National Research Council (NRC) (1993). Nutrient Requirements of Fish. Washington, D.C., USA: National Academy Press.

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

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. and Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences U S A*,**106**: 15103-15110.

Neema, D. and Fredrick, O.O. (2019). Incidence and Severity of *Solanum lycopersicum* Bacterial leaf spot Caused by Xanthomonad species in Farms in Wanguru, Mwea, Kirinyaga County, Kenya. *International science and engineering journal*, **2** (10): 127-134.

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

Ngugi, C.C., Egna, H., Oyoo-Okoth, E. and Manyala, J.O. (2016). Growth, yields and economic benefit of Nile tilapia (*Oreochromis niloticus*) fed diets formulated from local ingredients in cages. *Cellulose*, **80** (80.0): 80-0.

Ngugi, C.C., Oyoo-Okoth, E., Manyala, J.O., Fitzsimmons, K., and Kimotho, A. (2017). Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds. *Aquaculture Reports*, **5**: 62-69.

Njagi, K.A., Njati, I.C. and Huka, G.S. (2013). Factors affecting profitability of fish farming under economic stimulus programme in Tigania East District, Meru County, Kenya. *IOSR Journal of Business and Management*, **15** (3): 25-36.

Nguyen Duy Quynh, T. (2010). Evaluation of local feed resources for hybrid catfish *(Clarias macrocephalus x C. gariepinus)* in smallholder fish farming systems in central Vietnam (Vol. 2010, No. 72).

Nguyen, T.N. (2008). The utilization of soybean products in tilapia feed-A review. In: H. Elghobashy, K. Fitzsimmons & A.S. Diab (eds.). Proceedings of the 8th International Symposium on Tilapia in Aqua-culture. The Central Laboratory for Aquaculture Research, Cairo, 53-65.

Nose, T. (1960). "On the digestion of food protein by goldfish (*Carassius auratus* L.) And rainbow trout (*Salmo irideus* G.)". *Bull. Freshwater Fisheries Research Laboratory*, **10:** 11-22.

Nyina-wamwiza, L., Wathelet, B. and Kestemont, P. (2007) Potential of local agricultural by-products for the rearing of African catfish *Clarias gariepinus* in Rwanda: effects on growth, feed utilization and body composition. *Aquaculture Research*, **38** (2): 206-214.

Obiero, K., Meulenbroek, P., Drexler, S., Dagne, A., Akoll, P., Odong, R. and Waidbacher, H. (2019). The contribution of fish to food and nutrition security in Eastern Africa: Emerging trends and future outlooks. *Sustainability*, **11**(6): 1636.

Obirikorang, K.A., Amisah, S., and Agbo, N.W. (2015). Evaluation of locally-available agro-industrial byproducts as partial replacements to fishmeal in diets for Nile Tilapia (*Oreochromis niloticus*) production in Ghana. *Journal of Animal Resource Nutrition*, **1**:1.

Oduho, G.W., Baker, D.H. and Tuitoek, J.K. (2005). Pelagic fish *Rastrineobola argentea* as a protein source for broiler chicken. *Agricultura tropicaetsubtropica*, **38**: 82-87.

Ogello, E.O. and Opiyo, M.A. (2011). A Review of the African Catfish Production in Kenya: Opportunities and Challenges. In: *Samaki News*: Aquaculture Development in Kenya towards Food Security, Poverty Alleviation and Wealth Creation, 7 (1): 18-20.

Ogello, E.O., Munguti, J.M., Sakakura, Y. and Hagiwara, A. (2014). Complete Replacement of Fish Meal in the Diet of Nile Tilapia (*Oreochromis niloticus* L.)

Grow-out with Alternative Protein Sources. A review. *International Journal*, **2** (8): 962-978.

Ogello, E.O., Kembenya, E.M., Githukia, C.M., Aera, C.N., Munguti, J.M., and Nyamweya, C.S. (2017). Substitution of fish meal with sunflower seed meal in diets for Nile tilapia (*Oreochromis niloticus* L.) reared in earthen ponds. *Journal of applied aquaculture*, **29** (1): 81-99.

Ogunji, J.O. (2004). Alternative protein sources in diets for farmed tilapia. *CABI International 2004. Nutrition Abstracts and Reviews, Series B* **74** (9): 23-32.

Okemwa, E. and Getabu, A. (1996). 'Fish Farming in Kenya with Particular Reference to Lake Victoria Fisheries'' Paper Presented at the Seminar on the Management of Integrated Freshwater Ecosystems in Tropical Areas'' May 16-19. Brussels.

Olurin K.B. and Aderibigbe O.A. (2006). Length-weight relationship and condition factor of pond reared *Oreochromis niloticus*. *World Journal of Zoology* 1 (2): 82-85.

Olagbemide, P.T. (2015). Nutritional Values of Smoked Clarias Gariepinus from Major Markets in Southwest, Nigeria. *Journal of Science Frontier Research: D Agriculture and Veterinary*, **15** (6).

Olvera-Novoa, M.A., Olivera-Castillo, L. and Martinez-Palacios, C.A. (2002). Sunflower seed meal as a protein source in diets for Tilapia rendalli (Boulanger, 1896) fingerlings. *Aquaculture Research*, **33** (3):223-229.

Olukunle, O.A. (1982). Partial plant protein substitution in diets of first feeding stage fry of tilapia, O*reochromis niloticus*. MSc Thesis, Institute of Aquaculture, University of Stirling. Stirling, Uk.

Omondi, J.G., Gichuri, W.M. and Veverica, K. (2001). A partial economic analysis for Nile tilapia *Oreochromis niloticus* L. and sharp toothed catfish *Clarias gariepinus* (Burchell 1822) polyculture in central Kenya. *Aquaculture Research,* **32**: 693–700.

Onyia, L., Kama, K.S., Michael, K.S and Adewuyi, A. (2015). Growth Performance and Cost Benefit Analysis of *Clarias gariepinus* Fed with Different Commercial and Compounded *Feeds Discourse Journal of Agriculture and Food Sciences;* www.resjournals.org/JAFS ISSN: 2346-7002; **3** (1): 1- 6.

Opiyo, M.A., Githukia, C.M., Munguti, J.M., and Charo-Karisa, H. (2014). Growth performance, carcass composition and profitability of Nile tilapia (*Oreochromis niloticus* L.*)* fed commercial and on-farm made fish feed in earthen ponds. *International Journal of Fisheries and Aquatic Studies*, **1**: 12-17.

Opiyo, M.A., Marijani, E., Muendo, P., Odede, R., Leschen, W. and Charo-Karisa, H. (2018). A review of aquaculture production and health management practices of farmed fish in Kenya. *International journal of veterinary science and medicine*, **6** (2): 141-148.

Opuszynski, K. and Shireman, J.V. (1995). Herbivorous fishes. Culture and use for weed management. CRC Press, London, Tokyo, 223 pp.

Phumee, W.Y., Wei, S., Ramachandran, R. Hashim. (2011). Evaluation of soybean meal in the formulated diets for juvenile *pangasianodon hypophthalmus* (Sauvage, 1878)

Pompa, T. and Masser M. (1999). Tilapia: Life history and biology. SRAC (Southern Regional Aquaculture Center) Publication No. 283.

Prasad, G. and Anvar, P.H. (2007). Length-weight relationship of a cyprinid fish puntius filamentosus from Chalakude River. *Kerala, Zoos' Print Journal,* **22** (3): 2637-2638.

Rahman, M.M., Han, H.S., Kim, K.W., Kim, K.D., Lee, B.J. and Lee, S.M. (2016). Apparent digestibility coefficients of the extruded pellet diets containing various fish meals for olive flounder, *Paralichthys olivaceus*. *Fisheries and Aquatic Sciences*, **19** (1): 27.

Riche, M. and Garling, D. L. (2004). Effect of phytic acid on growth and nitrogen retention in tilapia *Oreochromis niloticus* L. *Aquaculture Nutrition*, **10** (6): 389-400.

Richards, A. (1983). The potential of sunflower and sesame meals as alternative source of protein in diets for *Oreochromis niloticus.* M.Sc. Thesis, Stirling University, U.K. 82pp.

Ricker, W.E. (1979). Growth rates and models. *Fish physiology*. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. **8**: 673-743.

Robinson, E.H., Li, M.H. and Manning, B.E. (2001). *A practical guide to nutrition, feeds, and feeding of catfish* (Vol. 1113). Office of Agricultural Communications, Division of Agriculture, Forestry, and Veterinary Medicine, Mississippi State University.

Rosa, R., Bandarra, N.M. and Nunes, M.L. (2007). Nutritional quality of African catfish *Clarias gariepinus* (Burchell 1822): a positive criterion for the future development of the European production of Siluroidei. *International Journal of Food Science & Technology*, **42**: 342–351.

Rothuis, A.J., A.P. van Duijn, J.C.M., van Rijsingen, W. van der Pijl and Rurangwa, E. (2011). Business opportunities for aquaculture in Kenya; with special reference to food security. LEI report 2011-067/IMARES report C131/11, Wageningen UR, Wageningen, pp 28.

Shang, Y.C. (1985). Aquaculture economics: An overview. *GeoJournal*, **10** (3): 299-305.

Shiau, S.Y., Kwok, C.C., Hwang, J.Y., Chen, C.M. and Lee, S.L. (1989): Replacement of fish meal with soybean meal in male tilapia (*Oreochromis niloticus*

and *O. aureus*) fingerling diets at a suboptimal level. Journal of World Aquaculture Society, **20** (4): 230 – 235.

Shiau, S.Y. (1997). Utilization of carbohydrates in warm water fish—with particular reference to tilapia, *Oreochromis niloticus* X *O. aureus*. *Aquaculture*, **151** (1-4): 79-96.

Silva, T.S.D.C., Santos, L.D.D., Silva, L.C.R.D., Michelato, M., Furuya, V.R.B. and Furuya, W.M. (2015). Length-weight relationship and prediction equations of body composition for growing-finishing cage-farmed Nile tilapia. *Revista Brasileira de Zootecnia*, **44** (4): 133-137.

Soltan, M.A., Ibrahim, M.K., Fatma, A., and FathElbab, A.F. (2001). Effect of partial and total replacement of fishmeal by soybean meal on growth and proximate analysis of Nile tilapia. *Egyptian Journal of Nutrition and Feed*, *799*, 812.

Soltan, M.A., Hanafy, M.A. and Wafa, M.I.A. (2008). Effect of replacing fish meal by a mixture of different plant protein sources in Nile tilapia (*Oreochromis niloticus* L.) diets. *Global Veterinaria*, **2** (4): 157-164.

Swann, L. (1992). A Basic Overview of Aquaculture: History, Water Quality, Types of Aquaculture and Production Methods. Technical Bulletin Series 102.

Tacon A.G.J. (1997). Fishmeal replacers: Review of antinutrients within oilseeds and pulses - A limiting factor for the aquafeed Green Revolution? In: Tacon A.G.J. (ed.), Basurco B. (ed.). Feeding tomorrow's fish. Zaragoza: CIHEAM, p. 153-182 (Cahiers Options Méditerranéennes; n. 22).

Tantikitti, C. (2014). Review article: feed palatability and the alternative protein sources in shrimp feed. *Songklanakarin Journal of Science and Technology*, **36:** 51–55.

Teimouri, S. (2018). Removal of three proteinaceous antinutrients from a soybean cultivar and effects of inclusion in diets processed at two heat levels on nutritional value and gut health for Atlantic salmon *(Salmo salar L)* (Master's thesis, Norwegian University of Life Sciences, Ås).

Tidwell, J.H., Coyle, S.D., Bright, L.A. and Yasharian, D. (2005). Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass Micropterus salmoides. *Journal of the World Aquaculture Society*, **36** (4): 454-463.

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

Udo, I.U. and Umanah, S.I. (2017). Current Status of the Nigerian Aqua Feed Industry: a review. *International Journal of Innovative studies in Aquatic Biology and Fisheries*, **3** (1): 14-22.

Wang, Y.R., Wang, L., Zhang, C.X. and Song, K. (2017). Effects of substituting fishmeal with soybean meal on growth performance and intestinal morphology in orange-spotted grouper (*Epinephelus coioides*). *Aquaculture Reports*, **5:** 52-57.

Watanabe, T. (2002). Strategies for further development of aquatic feeds. *Fisheries Science*, **68**: 242–252.

Wilson, R.P. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*, **124** (1-4): 67- 80.

Winfree, R.A. and Stickney, R.R. (1981**).** Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. *The Journal of nutrition*, **111** (6): 1001-1012.

Yamamoto, T., Shima, T., Furuita, H., Sugita, T., and Suzuki, N. (2007). Effects of feeding time, water temperature, feeding frequency and dietary composition on apparent nutrient digestibility in rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio*. *Fisheries science*, **73** (1): 161-170.

Yang, Q., Zhou, X., Zhou, Q., Tan, B., Chi, S. and Dong, X. (2009). Apparent digestibility of selected feed ingredients for white shrimp *Litopenaeus vannamei*, Boone. *Aquaculture Research*, **41**: 78–86.

Yousafzai, A.M., Siraj, M., Ahmad, H. and Chivers D.P. (2012). Bioaccumulation of heavy metals in Common Carp: Implications for human health. *Pakistan Journal of Zoology*, **44** (2).

APPENDICES

APPENDIX I: APPROVAL OF RESEARCH PROPOSAL

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 4150

Internal Memo

FROM: Dean, Graduate School

DATE: 7th November, 2016

TO: Maundu M. Anne
C/o Zoological Sciences Dept.
Kenyatta University

REF: 184/29588/2014

SUBJECT: APPROVAL OF RESEARCH PROJECT PROPOSAL

This is to inform you that Graduate School Board at its meeting of 02nd November, 2016 approved your Research Project Proposal for the Ph.D Degree Entitled, **"Digestibility, Growth and Economic Returns of Nile Tilapia (Oreochromis niloticus) And African Catfish (Clarias gariepinus) Fed on a Mixture of Plant Proteins".**

You may now proceed with your Data Collection, Subject to Clearance with Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the registrar (Academic) is hereby requested to grant you Substantive registration for your Ph.D studies.

Thank you.

HARRIET ISABOKE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Zoological Sciences
Registrar (Academic) Att: Mr. Likam

Supervisor:

1. Dr. Rekha Sharma
   Department of Zoological Science
   Kenyatta University
2. Dr. Joshua Mutiso
   C/o Department of Zoological Science
   Kenyatta University
3. Dr. Jonathan Muuguti
   Head of Aquaculture Division
   Kenya Marine & Fisheries Research Institute
   C/o Department of Zoological Science
   Kenyatta University

EO/awn

APPENDIX II: NACOSTI RESEARCH PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **839884**

Date of Issue: **22/November/2019**

RESEARCH LICENSE

This is to Certify that Ms.. anne maundu of Kenyatta University, has been licensed to conduct research in Kirinyaga on the topic: DIGESTIBILITY, GROWTH AND ECONOMIC PERFORMANCE OF NILE TILAPIA (Oreochromis niloticus) AND AFRICAN CATFISH (Clarias gariepinus) FED ON A MIXTURE OF PLANT PROTEINS IN CAGES for the period ending : 22/November/2020.

License No: **NACOSTI/P/19/2747**

839884

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY &
INNOVATION

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APPENDIX III: COMPOSITION OF EXPERIMENTAL DIETS FOR *O. NILOTICUS* WITH VARYING PROPORTIONS OF SOYBEAN MEAL

Trial I (Soybean meal as PPSM) composition of diets					
	Experimental diets				
Ingredients	**D0**	**D1**	**D2**	**D3**	**D4**
FWS	37.5	28	19	9	0
ppsm (soybean meal only)	0	13	25	39	51.5
rice bran	23.1	21.8	20.7	19.2	17.9
wheat bran	22.0	20.8	19.7	18.3	17.1
maize bran	17.4	16.4	15.6	14.5	13.5
Cr_2O_3	0.1	0.1	0.1	0.1	0.1
Total	100.13	100.11	100.1	100.1	100.1

APPENDIX IV: COMPOSITION OF EXPERIMENTAL DIETS FOR *O. NILOTICUS* WITH VARYING PROPORTIONS OF SOYBEAN MEAL, SUNFLOWER AND COTTONSEED MEAL

Trial II (Soybean, sunflower and cottonseed meal as PPSM) composition of diets					
	Experimental diets				
Ingredients	**D0**	**D1**	**D2**	**D3**	**D4**
FWS	37.5	28	19	9	0
Soybean meal	0	6.5	12.5	19.5	25.75
sunflower meal	0	2.9	5.5	8.6	11.4
cottonseed meal	0	3.6	7	10.9	14.4
Rice bran	23.1	21.8	20.7	19.2	17.9
wheat bran	22.0	20.8	19.7	18.3	17.1
maize bran	17.4	16.4	15.6	14.5	13.5
Cr_2O_3	0.1	0.1	0.1	0.1	0.1
Total	100.03	100.01	100	100	100.05

APPENDIX V: COMPOSITION OF EXPERIMENTAL DIETS FOR *O. NILOTICUS* WITH VARYING PROPORTIONS OF SUNFLOWER AND COTTONSEED MEAL

Trial III (Cottonseed and sunflower meal as PPSM) composition of diets					
	Experimental diets				
Ingredients	**D0**	**D1**	**D2**	**D3**	**D4**
FWS	37.5	28	19	9	0
Soybean meal	0	0	0	0	0
sunflower meal	0	5.74	11	17.2	22.7
cottonseed meal	0	7.3	14	21.8	28.8
Rice bran	23.1	21.8	20.7	19.2	17.9
wheat bran	22.0	20.8	19.7	18.3	17.1
maize bran	17.4	16.4	15.6	14.5	13.5
Cr_2O_3	0.1	0.1	0.1	0.1	0.1
Total	100.03	100.05	100	100	100

APPENDIX VI: CAGE EXPERIMENTAL SET UP

Photo showing 15 cages installed in 800m^2 fertilized earthen pond

APPENDIX VII: FISH FEED PREPARATION

APPENDIX VIII: FISH SAMPLING

APPENDIX IX: LENGTH –WEIGHT MEASUREMENTS