Nutritional evaluation of some Ethiopian oilseed cakes in the diets of juvenile Nile tilapia, *Oreochromis niloticus* L.

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY in BIOLOGY (Fisheries and Aquatic Sciences)

By

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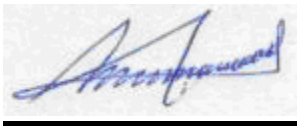
Declaration

I hereby declare that this thesis has been composed entirely by myself and has not been previously submitted for any other degree or qualification.

The work of which it is a record has been performed by myself, and all sources of information have been specifically acknowledged.

Akewake Geremew 

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Abstract

This study evaluated the suitability of Niger seed cake (NSC) and linseed cake (LSC) inclusion as potential plant protein sources in Nile tilapia, *Oreochromis niloticus*, diets. A series of four experiments were conducted in a recirculation system using juvenile *O. niloticus*. The fish were fed diets containing the oilseed cakes at dietary inclusion levels ranging from 20% to 50%. Diets were formulated to be isonitrogenous (32g 100 g^{-1}), isolipidic (10g 100 g^{-1}) and isoenergetic (18 kJ g^{-1}) and fed to juvenile Nile tilapia at 4-10% of their body weight per day for a period of 8 weeks. Niger seed cake (NSC) and linseed cake (LSC) were used individually or as mixtures to test for their effect on Nile tilapia growth and feed utilization. Also of interest was to compare digestibility and feeding values of NSC and LSC with that of soybean cake (SBC), the best plant protein source.

Proximate analysis showed that Niger seed cake (NSC) and linseed cake (LSC) had 324.2 and 310.0 g kg^{-1} crude protein, 201.1 and 136.3 g kg^{-1} crude fibre and 18.1 and 18.6 kJ g^{-1} gross energy, respectively. Nutrient digestibility of these oilseed cakes suggested that Nile tilapia may be able to utilize NSC better as dietary protein source due to a reasonably high protein digestibility coefficient (72.6%) than linseed cake (62.4%). Of the three oilseed cakes (NSC, LSC and SBC) tested soybean cake (SBC) produced significantly ($P< 0.05$) the highest nutrient digestibility coefficients.

When Niger seed cake (NSC) and linseed cake (LSC) were used individually as protein sources in Nile tilapia diets, depressed growth and feed efficiency were observed at dietary inclusion levels above 20%. This may be attributed to high levels of antinutritional factors (ANFs), high crude fibre content and poor essential amino acid profile. However, the use of mixtures of these two oilseed cakes (formulation 1 (F1): 50% NSC:50% LSC, Formulation 2 (F2): 33% NSC:67% LSC and Formulation 3 (F3) 67% NSC:33% LSC) was found to be marginally suitable than that of single sources, especially when the proportion of NSC in the mixture is 50% to 67%. The oilseed cake formulations with higher proportion of NSC could be included at 25% dietary inclusion without significantly reducing performance. This may have been as a result of lower levels of ANFs and improvements in the amino acid profile due to mixing.

It can be concluded that there is nutritional and economic justification for using NSC and LSC as protein sources in Nile tilapia diets. Based on growth performance, nutrient utilization and economic benefits the diet with F3 formulation at 25% level of inclusion has the best prospects for use in Nile tilapia diets.

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Chapter 1 - General Introduction

1.1 Introduction

Today, aquaculture is the fastest growing food producing sector in the world, with an average annual growth rate of 8.6% since 1980, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (FAO, 2014). The most recent statistics indicate that the sector reached aquatic production of 6.2% annual percentage growth rate (APGR) compared with meat production of farmed terrestrial animals such as pigs (APGR of 3.1%), poultry (APGR 5.1%), beef and meal (APGR 1.2%), and mutton and lamb (APGR 1.0%) (FAO, 2014). It is remarkable that one out of every three fish consumed in the world is now farm raised (Gatlin III *et al.*, 2007).

Despite the high growth rate of aquaculture, there is still a negative balance between demand and supply of fishery products (FAO, 2014). Aquaculture already supplies nearly 42.2% or nearly 66.6 million tonnes of fish consumed globally, and with production from wild fish stocks levelling off, it will fall to fish farmers to supply the estimated 50 million additional tonnes required to feed the rising world population by 2030 (Lem *et al.*, 2014). Increasing production capacity of aquacultural resources through intensification seems to be the way forward to meet the ever increasing demand for fish. This entails increasing primary, intermediate and terminal productivity capacities of our natural aquatic ecosystem and creation of productive artificial aquatic ecosystems through proper planning, development and management (Sadiku and Jauncey, 1995).

A major determinant of successful growth and intensification of aquaculture production depends on aquafeed. It accounts for a major part (30-70%) of the total operation cost of an average fish

farm (Rumsey, 1993; El-Sayed, 2004). Traditionally, animal protein sources, particularly fishmeal have been the major ingredients of aquafeeds (Glencross *et al.,* 2007). Ironically, fishmeal is one of the most expensive ingredients in formulated fish feeds. Although, fishmeal production has remained relatively stable averaging 6.07 million tonnes over the past two decades (Tacon *et al.*, 2006) its decline is likely and can no longer meet the demand from the expanding aquafeed industry. The challenge facing the aquaculture industry is to reduce inclusion rate of fishmeal and fish oil in aquafeeds (especially for farmed carnivorous finfish and marine shrimp) and identify economically viable and environmentally friendly alternatives to fish meal and fish oil on which many present aquafeeds are largely based (Gatlin III *et al.*, 2007). Replacement of fishmeal and fish oil with available and cheaper plant feedstuffs has been identified as an essential requirement for the future development of aquaculture (Tacon *et al.*, 2006).

Herbivorous fish species are well suited to the consumption of plant-based diets in aquaculture. One of the most important herbivorous fish species reared in aquaculture systems is the tilapia. Tilapias are naturally accustomed to eating plant ingredients, and are typically considered strict herbivores once they reach maturity (El-Sayed, 2006). A substantial amount of research is already underway, testing potential protein sources that can replace fish meal in tilapia diets. These plant protein sources include cereals (including their by-products), oil seed meals and pulses (including lupins and peas). Soybean meal, cottonseed meal, sunflower meal, rapeseed meal, wheat bran, corn gluten meal, cassava leaf meal and barley were reported to have a good potential in tilapia diets (El-Saidy and Gaber, 2002; Maina et al., 2002; Sklan *et al.*, 2004; Soltan, 2005; Guimaraes *et al.*, 2008a; Agbo *et al.*, 2011b; Madalla *et al.*, 2013).

Ethiopia has diverse agro-climatic conditions favouring production of many different kinds of crops, providing a wide range of ingredients and alternative feedstuffs suitable for fish feeding (Kapetsky, 1994). Ethiopia is among the top producers in the world for products such as sorghum, dry peas (Tacon *et al.*, 2011) and some oilseeds (such as Niger seed, linseed and sesame) (Wijnands *et al.*, 2007). Making use of these resources to complement the natural resource base promises a considerable potential for success. It is, therefore, imperative to address the role of locally available feed resources in reducing production cost and ultimately make aquaculture an attractive production system to farmers in Ethiopia.

Commercially formulated diet for fish production is expensive in world market and it is not profitable for most African entrepreneurs and farmers. Expensive diets in common production units limit the extent of acceptability by entrepreneurs to adopt commercial fish farming (Rana *et al.,* 2009). Therefore, research in fish farming should be geared among others, towards formulating high quality diets based on locally available feedstuffs. Research that develops and improves existing on-farm aquafeeds together with identification and utilization of alternative potential feed ingredients is required to sustain and expand tilapia production in Ethiopia. Published scientific works indicated that ingredients that have good potential use in Nile tilapia farming in Ethiopia include Niger seed cake, brewery waste and wheat bran as supplementary feed ingredients (Zenebe Tadesse *et al.*, 2012), *Jatropha curcas* kernel meal as protein source (Kassaye Balkew *et al.*, 2013) were few among many others.

Table 1.1 Chemical composition (% composition) of mechanically pressed oilseed cakes available in Ethiopia: Dry matter-DM; Crude Protein-CP; Lipid or Ether Extract-EE; Crude Fibre-CF; Acid Detergent Fibre-ADF; Neutral Detergent Fibre-NDF; Na= not available (Source: Tacon, 1987; Tadelle Dessie *et al.*, 2002; ILRI, 2010)

Oilseed cakes	DM	CP	EE	CF	ADF	NDF
Groundnut cake *(Arachis hypogaea)*	93.06	54.17	9.32	7.5	10.37	17.41
Cotton seed cake (*Gossypium* sp.)	93.3	38.34	5.9	16.6	Na	Na
Linseed cake (*Linum* sp.)	94.26	26.77	9.86	9.5	34.11	36.86
Mustard cake (*Brassica carinata*)	92.81	39.16	12.73	7.9	43.79	47.37
Niger seed cake (*Guizotia abyssinica*)	93.22	32.89	7.72	21.6	28.36	37.45
Sunflower cake (*Helianthus* sp.)	92.2	29.0	8.81	21.37	Na	Na
Safflower cake (*Carthamus* sp.)	91.9	21.7	5.9	30.7	Na	Na
Sesame seed meal (*Sesamum* sp.)	95.30	43.28	16.61	6.4	29.8	34.4
Rapeseed cake (*Brassica napus*)	94.33	37.80	8.99	12.8	20.05	25.7

Of the feed ingredients available in Ethiopia, and based on the ingredients identified in Table 1.1, their quality, availability, affordability and supply, the most promising alternatives to fish meal in (juvenile) tilapia diets are the oilseed cakes namely; Niger seed cake (*Guizotia sp.*) and linseed cake (*Linum sp*.).

1.2 Objectives of this research

It is clear from the preceding sections that one of the main constraints in Ethiopia is non-availability of quality and affordable fish feeds. The aim of the present research was, therefore, to develop cost effective diets for juvenile Nile tilapia using locally available oil seed cakes in Ethiopia as protein sources.

The main objective of this study was, therefore, to evaluate the suitability of Niger seed cake and linseed cake as fish feed ingredients in the diets of *O. niloticus*. The specific objectives were to evaluate the:

- Apparent nutrient and energy digestibility of Soybean cake, Niger seed cake, and Linseed cake in Juvenile Nile tilapia.
- Effect of dietary inclusions of Niger seed cake (a novel feed ingredient for aquaculture) on growth performance and feed utilization of Juvenile Nile tilapia
- Effect of dietary inclusions of linseed cake on growth performance and feed utilization of Juvenile Nile tilapia
- Incorporation and cost effectiveness of two oilseed cakes in various combinations and levels in diets of juvenile Nile tilapia

1.3 Thesis Structure

This dissertation is structured into seven chapters as shown in Figure 1.1 below. Chapters 1 – 3 are the general introduction, literature review and methodology, Chapter 4 (short-term feed trial, 2-3 weeks), chapters 5 to 7 (medium-term feed trials, 8 weeks) and chapter 8 the general conclusions and recommendations.

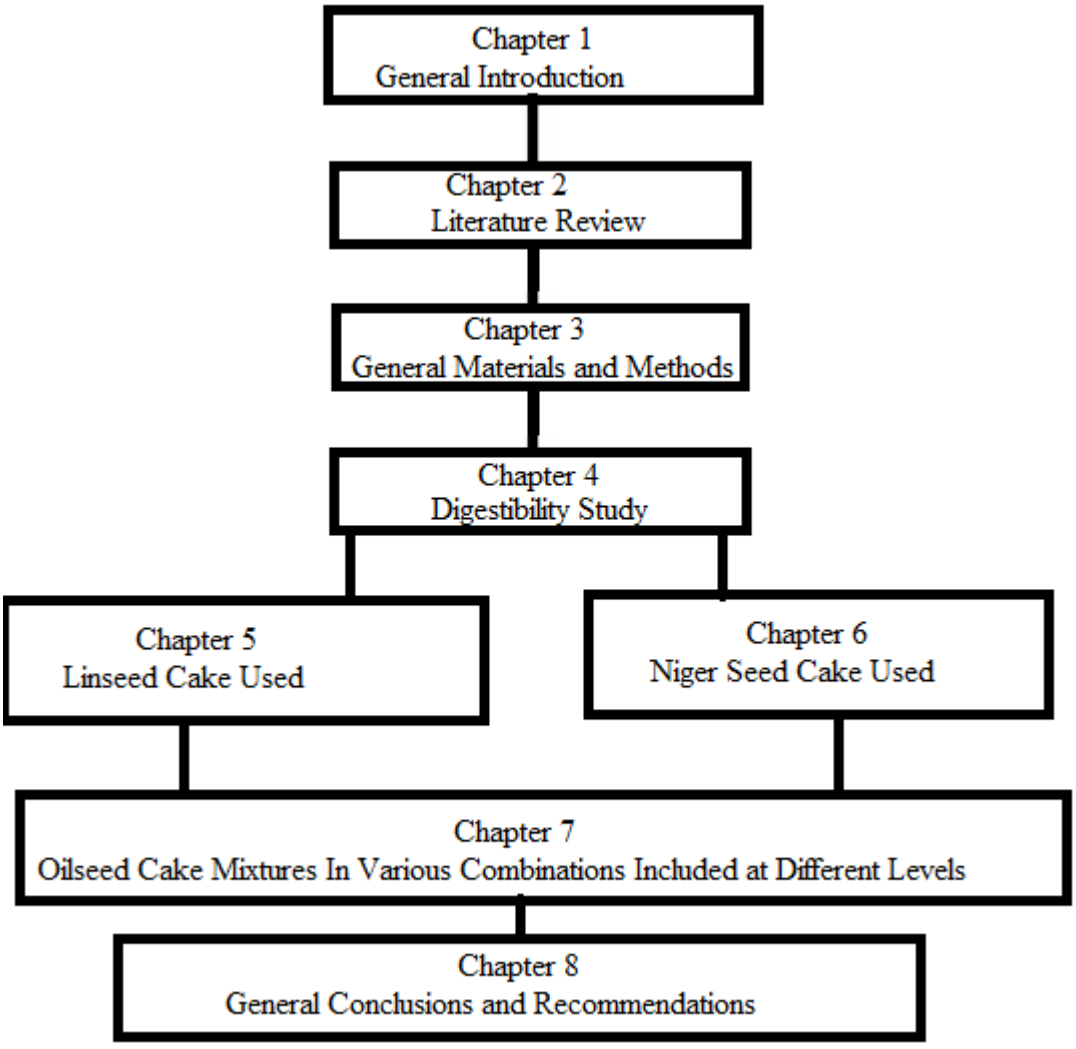

Figure 1.1 Structure of the dissertation

Chapter 2 – Literature Review

2.1 Global Overview of Aquaculture

Fish has long been valued as a highly nutritious food source for humans. Presently, fish accounts for over 50% of total animal protein consumed in most developing countries and global estimate stands at 16.7% in 2010 (FAO, 2014). Fish is high-quality animal protein and relatively the cheapest source (Tidwell and Allan, 2001). Tacon (2001) pointed out that ‗food fish' has a nutritional profile superior to all terrestrial meat, being an excellent source of high quality animal protein and highly digestible energy, as well as an extremely rich source of omega-3 polyunsaturated fatty acids (PUFAs), fat-soluble vitamins (A, D and E) and water-soluble vitamins (B complex) and minerals (calcium, phosphorus, iron, iodine and selenium). At present, ‗food fish' contributes for more than 2.9 billion people 20% of the total animal protein, and 4.3 billion people with 15% of such protein (FAO, 2013). Consumption of omega-3 fatty acids from seafood products (including those from aquaculture) has been shown to prevent or ameliorate certain types of diseases (e.g., coronary heart disease and stroke; autoimmune disorders; cancers of the breast, colon and prostate; hypertension and rheumatoid arthritis) (Kris-Etherton *et al.*, 2002).

Global per capita fish consumption has grown from 10 kg (live weight equivalent) in the 60s to an estimated 19.2 kg in 2012, which means that production used by humans for food is at a record high of more than 136 million tonnes in 2012 (FAO, 2014). Historically, the oceans were considered limitless and thought to harbour enough fish to feed an ever-increasing human population. However, the demands of a growing population, particularly in poorer countries, now far outstrip the sustainable yield of the seas (Lem *et al.*, 2014). The global capture fisheries are

now stabilized at approximately 93.7 million tonnes per year and this figure is not expected to increase since at least half of the world‘s recognized fish stocks are fully exploited and 32% overexploited or depleted (FAO, 2014). After a noticeable increase in the number of stocks that were overexploited or depleted during the 1970s and 1980s, the figures have since stayed at the current level (FAO, 2013).

Recent consumption and demand for seafood primarily driven by technological fishing development and market demand (arising from rapid income growth particularly in Asia and recent knowledge of the health benefits from fish and increasing populations) has led to a mismanaged system (Rana *et al.*, 2009). There are multiple reasons for the mismanagement of fisheries. First, increases in consumer demand has skyrocketed past what natural fish stocks can support. Second, pollution, climate change and the lack of global enforcement in fisheries management has led to severely depleted fish stocks. Some predict commercial fish stocks to disappear within the next decade or even sooner if problems are not addressed (FAO, 2007).

In order to bridge the gap between demand and supply aquaculture is seen as the best solution.

Aquaculture is defined by FAO (2013) as ―the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate ownership of the stock being cultivated”. Aquaculture has been conducted since pre-historic times and from a humble beginning has spread all over the world gradually transforming from a traditional practice into science (FAO, 1990).

Aquaculture has a long history. In the People‘s Republic of China (PR China), common carp were raised for food in freshwater ponds as early as 1100 B.C., while oyster farming was

recorded as early as the Han Dynasty (206 B.C.–220 A.D.) (Hishamunda and Subasinghe, 2003). Other examples of early aquaculture practices include the Japanese culturing oysters for pearls; ancient Egyptians stocking ponds with fish; the Greeks and Romans raising eels; the Europeans cultivating oysters (Pillay and Kutty, 2005).

2.1.1 Aquaculture Production

In 2012, the total world aquaculture production (including aquatic plants) was reported to be an all-time high of 90.4 million tonnes by volume and US$ 144.4 billion by value (FAO, 2014). This represents an annual increase of 6.2% in volume for world food fish aquaculture production over the period 2000–2012 (9.5% in 1990–2000) from 32.4 million to 66.6 million tonnes (Figure 2.1). In the same period, growth was relatively faster in Africa (11.7%) and Latin America and the Caribbean (10%). Asia produced 88.4% (by volume) of global aquaculture production. Of the world total, the PR China produced 61.7% of the total volume and 54.7% of the total value of aquaculture production. Most of the production came from extensive /semi-intensive systems in developing countries, particularly Asia, rearing mostly organisms low on the feed chain such as omnivores and herbivores (Lazard *et al.*, 2010). In 2012, the other nine top producers were India, Vietnam, Indonesia, Bangladesh, Norway, Thailand, Chile, Egypt and Myanmar (FAO, 2014). The majority of aquaculture production of fish, crustaceans and molluscs continues to come from the freshwater environment (63% by volume). Mariculture contributed about 9.7% of the total production (FAO, 2014).

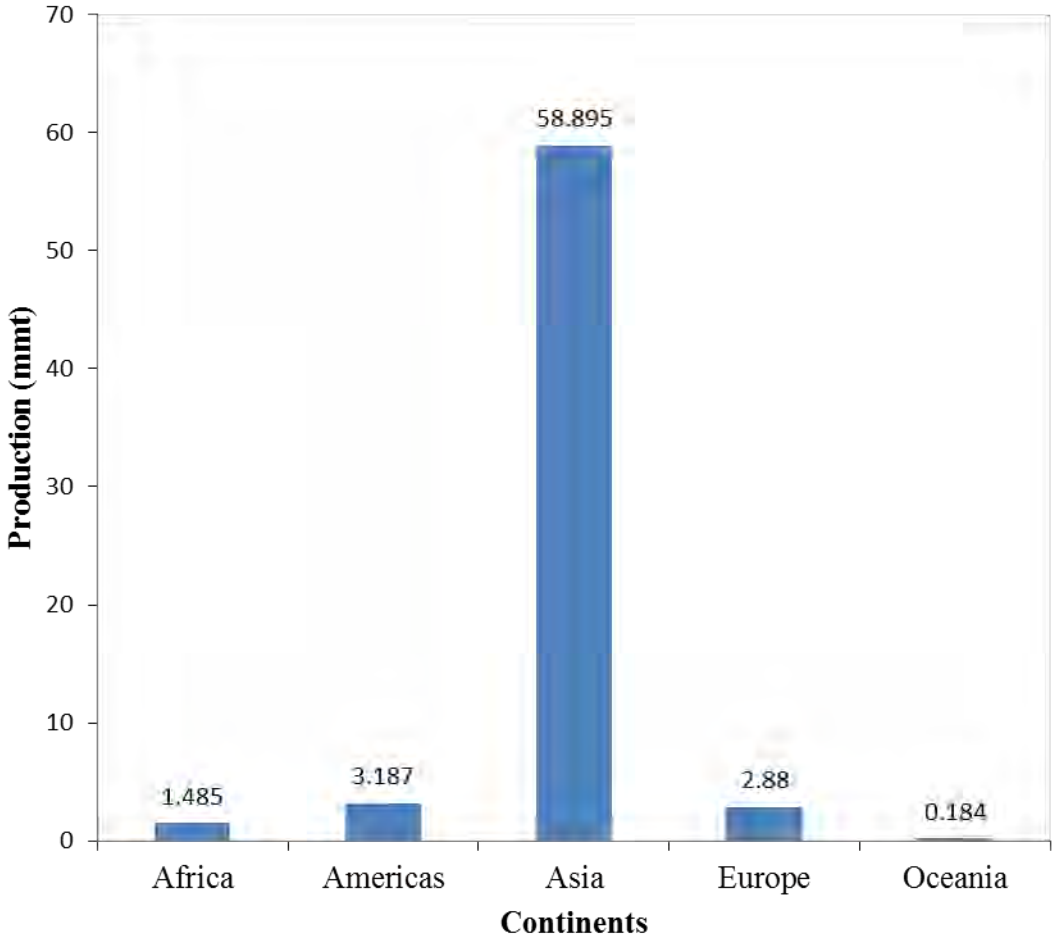

Figure 2.1 Global aquaculture productions in million tonnes by continents for 2012 (Data exclude aquatic plants and non-food products) (FAO, 2014)

Aquaculture is a significant socio-economic activity, especially for rural communities, contributing to livelihoods, food security and through such mechanisms as employment, services, use of local resources, diversified farming practices, domestic and international trade and other economic investments serving the sector (Edwards *et al.*, 2002; Belton, 2013).

2.1.2 Aquaculture in Africa

Unlike Asia, Africa has little aquaculture tradition and has been affected by a number of external problems that have prevented proper management and development despite investment. Total aquaculture production in Africa in 2012 was estimated to be 1, 539, 907 tonnes which is about 94.0% increase in the last two decades (Figure 2.2). Egypt alone contributed about 1,017,738 tonnes (67%). Egyptian aquaculture took off in the 1980s and now has a turnover of at least $1.96 billion, employing over 100,000 people and providing each Egyptian around one fish per week (Shaheen, 2013). Despite such faster growth of aquaculture in Africa (one of the fastest in the world with 11.7% growth rate in the first 12 years of the new millennium), the per capita fish supply was the lowest in the world with 9.7 kg/year (FAO, 2014). Total aquaculture production in sub-Saharan Africa (SSA) is 454,691 tonnes accounting for only 30.6% of African production (FAO, 2014). Aquaculture in SSA is dominated by Nigeria contributing about 52.8% with the other four top producers together contributing about 34.3% of SSA production. Between 1990 and 2012, overall aquaculture production in SSA has increased by 96.2% from 17,184 tonnes to 454,691tonnes (FAO, 2014) (Figure. 2.3).

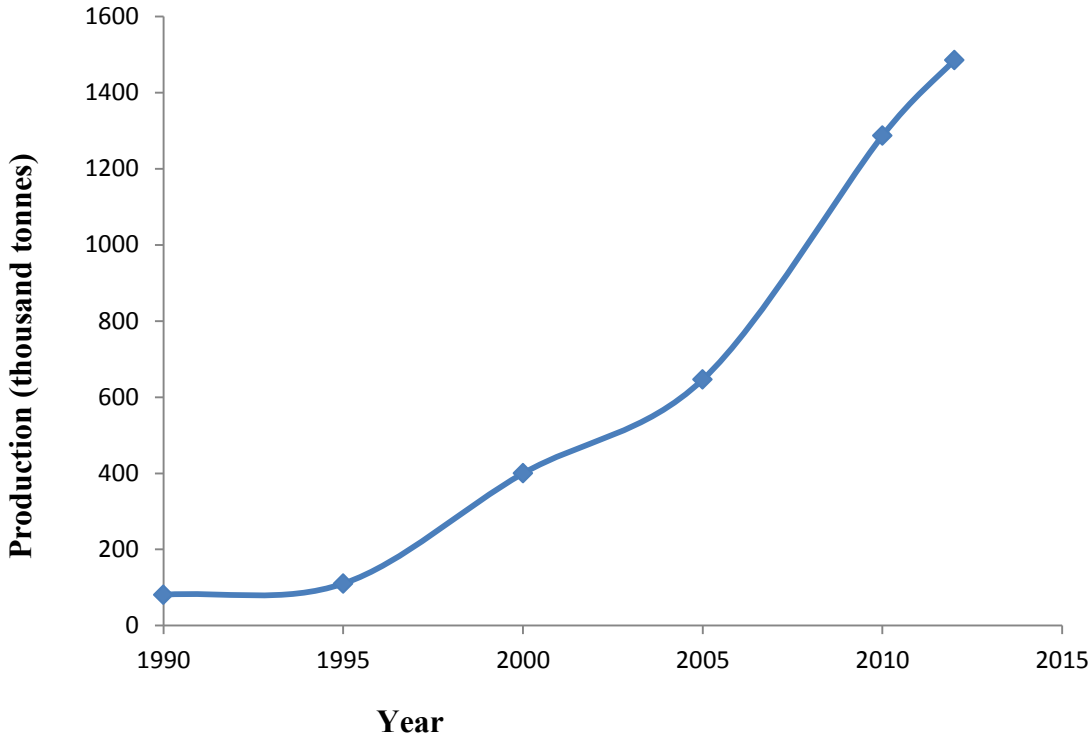

Figure 2.2 Aquaculture production in Africa for 2002-2012 (FAO, 2014)

Though, ”traditional” low-input/low-output small-holder fish farmers still dominate the scene in SSA, the greater proportion of production comes from commercial enterprises that range from small-scale semi-intensive to large industrial scale operations (Hecht, 2007). The conventional aquafeed formulations in SSA rely heavily on imported fishmeal to provide much of the dietary protein

If Africa is to satisfy its current demand, let alone the expected rise in aquaculture production, and therefore realize its potential, there will be a need to increase feed availability to support intensification. If investments are not forthcoming in establishing aquafeed processors then farms may increasingly rely on imported feeds (Hecht, 2007). Imported feeds will likely be an expensive option that will maintain high production costs and, therefore, the price of fish to end consumers (Rana *et al.*, 2009). The greatest input cost to most aquafeed production is raw

materials which typically fall in the range 80-90% of production costs (Henry, 2010). Therefore, any savings on raw materials will have a greater impact on production cost than most likely anywhere else in the process. If locally sourced raw ingredients and feedstuffs can be utilized and more efficiently processed, then small and medium-sized farms will benefit greatly. Production of feeds using locally sourced ingredients are usually cheaper, have sound ecological and economic benefits and sustainable supply of ingredients would assure future expansion of the aquaculture industry (Weimin and Mengqing, 2007). As a prerequisite, testing the suitability of locally sourced ingredient inclusion in aquafeeds is highly required in Africa.

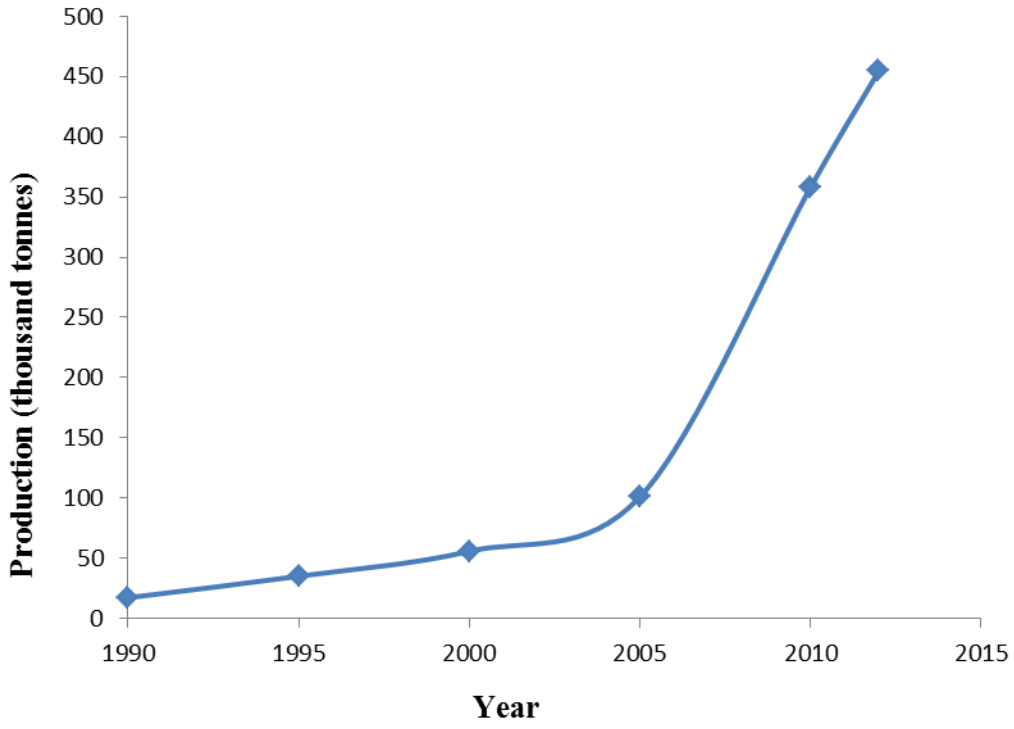

Figure 2.3 Aquaculture productions in sub-Saharan Africa for 1990-2012 (FAO, 2014)

2.2 Aquaculture in Ethiopia

2.2.1 Historical Background

Aquaculture in Ethiopia started in the form of pond fish culture in the early 1970s with the establishment of experimental ponds by the Sebeta Fish Breeding and Research Centre for production of tilapia and other non–indigenous fish (FAO, 2003). However, emphasis shifted from pond culture to reservoir stocking with this research centre becoming important fingerling distribution centre. Over 2.5 million fingerlings were released into the small water bodies and reservoirs throughout the country by this research centre (FAO, 2003).

Aquaculture operations other than culture-based fisheries in Ethiopia also include the extensive aquaculture operations in several small rural-based fishponds with sizes of between 100 and 300 m^2 (MoARD, 2009). The productions from such aquaculture operations in Ethiopia were insignificant (MoARD, 2009) despite the country's physical and socio-economic conditions that favour its development (Rothuis *et al.*, 2012). A more deliberate attempt to improve aquaculture took place in 2009 after the Ministry of Agriculture and Rural Development prepared "National Aquaculture Development Strategy Framework of Ethiopia" with a financial and technical support of FAO, Sub Regional Office for Eastern Africa. This was so, because the government through its ministry considered aquaculture as an important economic activity that supports diversification, integration and improvement in rural livelihoods. The overall objective of this strategy was to define a regulatory framework and to build a strong basis for the development of aquaculture in the country (MoARD, 2009).

More recently, the different regional research centres and higher education institutes started with optimism to revamp aquaculture development activities using local initiatives. One of the important undertakings by research centres in Ziway and Bahir Dar was integration of aquaculture with existing agricultural activities on farmers own land to realize its intended objectives (Alayu Yalew *et al.*, 2009; Lemma Abera, 2013). With the aim of supporting sustainable aquaculture developments through the smart use of water, the EU-funded FAO Smart-Fish Project together with Addis Ababa University launched a project to demonstrate aquaponics technology and assist beneficiary farmers in Shewarobit and Ziway (FAO, 2013). In efforts to alleviate personnel shortages there has been an increase in the number of higher education institutes offering full-fledged post-graduate fisheries and aquaculture programs, incorporating fisheries and aquaculture courses and that are also engaged in research (Rothuis *et*

al., 2012). Perhaps the most notable project was the DelpHE/DFID initiative launched in collaboration with Addis Ababa University (AAU) and Stirling University to improve the curriculum in the fisheries and aquatic sciences stream of AAU and develop pond and cage cultures in Ethiopia (Abebe Getahun, 2012). In this project 4 MSc and two PhD students, including this particular PhD study on oilseed cake evaluation, were involved under the supervisions of the two Principal Investigators of this project.

2.2.2 The need for aquaculture

Ethiopia has an estimated annual total exploitable capture fisheries potential of 51,000 tonnes from lakes and streams (LFDP, 1996). Official figures for 2010 indicate that the total fisheries and aquaculture production in Ethiopia is around 18,000 tonnes. An additional 1287 tonnes of seafood products were imported in 2010, while about 460 tonnes of fisheries products were exported. This means that an estimated 18,900 tonnes of fisheries and aquaculture products were consumed domestically. With this figure the per capita fish production is less than 240g (FAO, 2010), which is less than previously reported (FAO, 2003). This indicates that there is an unmet demand or shortage of fish and fisheries products in Ethiopia. This corroborates well with the suggestion of Gordon *et al.* (2007) that the price of fish and fisheries products in the country has increased as a result of shortages in supply. Contrary to the increasing demand the supply from the currently exploited natural stocks has already shown signs of stock decline due to overfishing (Reyntjens and Tesfaye Wudneh, 1998). Furthermore, the growing hydroelectric projects together with the irrigation projects will potentially aggravate the threat on the riverine stocks (Abebe Getahun and Stiassny, 1998). This, therefore, calls for an increasing attention to be given for aquaculture development in Ethiopia from the point of view that it can contribute to the conservation of biodiversity and economic development. While capture fisheries based on

species that are currently exploited in some water bodies seem to have reached their natural limits (FAO, 2003), there is considerable potential to expand aquaculture in Ethiopia in order to improve food security (Balarin, 1986; Kapetsky, 1994; Rothuis *et al.*, 2012).

Despite the huge water resource potentials in Ethiopia, the contribution of aquaculture is well below its potential due to various problems. These problems are compounded by inefficiencies in the input (feed, fingerlings, fertilizers and supporting services) (Breuil, 1995) and output (fish and fish products) marketing, including poor market infrastructure, lack of marketing support services and limited market information (Gordon *et al.*, 2007). Similarly, Balarin (1986) and Rothuis *et al.* (2012) attributed poor aquaculture development in Ethiopia to such factors as lack of development of commercial, cost effective feeds using locally available, cheap and unconventional resources.

2.3 Tilapia Aquaculture

2.3.1 Tilapia Production

Tilapia is the generic name given to a group of fish belonging to the class of Actinopterygii under the order Perciformes of Cichlidae family. These fishes are native to Africa but have become one of the most widely farmed fish with an increasing production output worldwide (Fitzsimmons *et al.*, 2011; FAO, 2014). Tilapia production is expanding in Asia, South America and Africa, with new supply targeting domestic and regional consumers rather than international markets (FAO, 2014). African producers are also now seeing tilapia‘s potential for domestic consumption as well as for export.

World tilapia production has boomed, output increasing two fold, from 1.9 million tonnes in 2005 to more than 3.9 million tonnes in 2011 and they constituted the second largest group of farmed finfish next to carps (FAO, 2013) (Figure 2.4). This production is widely distributed; with in over 130 tropical and sub-tropical countries (FAO, 2014). Asia is the major contributor with almost 60% in 2011, Africa 30% and the remainder mostly from central and North America (5.1%) and South America (4.5%). Almost half of the output comes from only two countries, China (excluding Taiwan) and Egypt, at 33% (1.3 million tonnes) and 15.3% (600,000 tonnes), respectively. Other major producing countries are Philippines (7%), Indonesia (6.2%), Uganda (5.5%), Thailand (4.8%), Brazil (3.6%), Taiwan (3.5%), Mexico (2.5%) and Tanzania (2.3%). The strong increase in global production has mostly been driven by China‘s dramatic increase from 106,000 tonnes in 1990 to almost 1.3 million tonnes in 2011 (FAO, 2013).

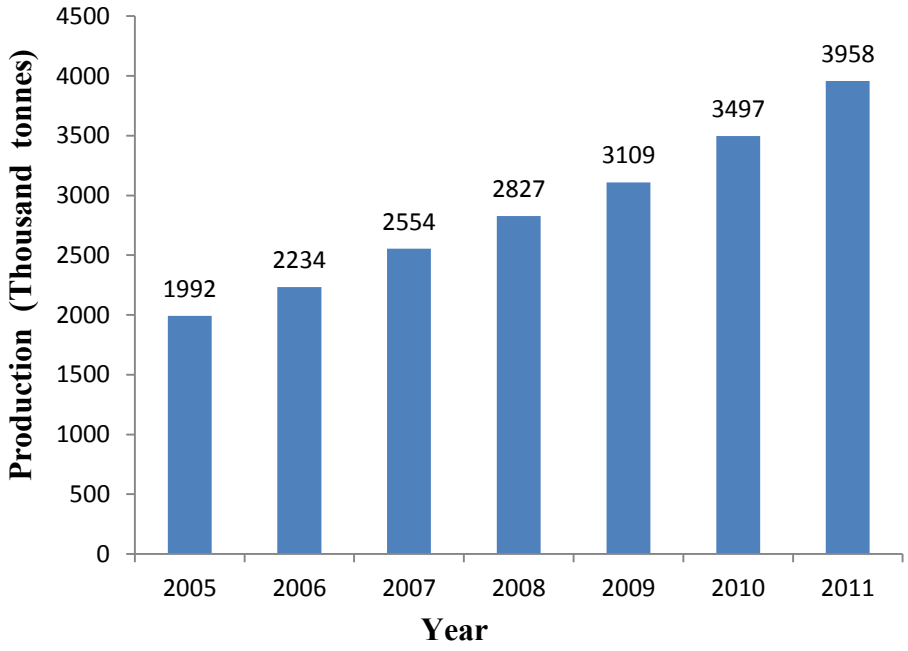

Figure 2.4 Global production of tilapia in aquaculture for 2005-2011 (FAO, 2013)

During the last half century fish farmers throughout the tropical and semi-tropical world have begun farming tilapia. Almost 83% of the production in 2011 was produced through aquaculture (FAO, 2013), with all commercially important tilapia belonging to the genus *Oreochromis* and their hybrids (Ng and Romano, 2013). More than 90% of all commercially farmed tilapia are Nile tilapia (*O. niloticus*). Less commonly farmed species are Blue tilapia (*O. aureus*), Mozambique tilapia (*O. Mossambicus*) and the Zanzibar tilapia (*O. urolepis hornorum*) (Popma and Masser, 1999).

Ng and Romano (2013) highlighted the attributes of tilapia and the reasons for its success as a warm water aquaculture candidate species. This is largely due to their robustness, tolerance, flexibility and overall plasticity. This plasticity is evident from their diversification and radiation into available niches, and characterized by a remarkable physiological hardiness, adaptability and general levels of tolerance to most potentially limiting environmental variables. Many tilapias are euryhaline and can be cultured in fresh, brackish or salt water. While they are not cold tolerant, they are eurythermal over a wide range, and this only limits their distribution to tropical, subtropical and warm temperate climates. They also have a good tolerance of low dissolved oxygen (DO) and are quite resistant to reasonable physical handling (Morales, 1991; Popma and Masser, 1999; Ross, 2000; Watanabe *et al.*, 2002). According to Lovell (1998), some of the cultured species have shown to survive DO as low as 0.1mg l^{-1} and tolerated a concentration level of 2.4mg l^{-1}unionized ammonia. The suitable ranges of water quality parameters for tilapia are shown in Table 2.1. Most tilapias are omnivorous with a preference for soft aquatic vegetation, fresh and decaying plant material and periphyton (Fitzsimmons *et al.*, 2011). The ability to accept lower cost diets from terrestrial based ingredients (El-Sayed, 2006; Kang'ombe *et al.*, 2007; Mzengereza *et al.*, 2014) and the ability of tilapia to thrive in biofloc

systems (Hargreaves, 2013) is yet another benefit that tilapia have over many of the other common aquaculture species. In consequence of their large size, good flavour, and rapid growth rate, many tilapias are at the focus of major aquaculture efforts (Ng and Romano, 2013).

Table 2.1 Water quality parameters suitable for tilapia. (Source: Suresh, 2003; Hussain, 2004; El-Sayed, 2006)

Parameters	Tolerance range	Desirable level
Water temperature, ^{o}C	12-35	26-32
Salinity, ppt	3-25	0-20
pH	5-10	6.5-8.5
Dissolved Oxygen, mg l^{-1}	2.0-8.0	>3.0-5.0
Ammonia, mg l^{-1}	0.0125	<0.1
Nitrite, mg l^{-1}	0.1-0.2	-
Nitrate, mg l^{-1}	0.0-3.0	-
Alkalinity, mg l^{-1}	>20	>20
Hardness, mg l^{-1}	20-50	<50

2.3.2 Nutritional Requirements of Tilapia

Proper nutrition is considered as a critical issue that plays a role in maintaining normal growth and health of fish. Good nutrition can help mitigate the effects of stress, decrease the susceptibility to disease, and serve as a primary method of boosting the immune system (Hixson, 2014). Fish diet contains nutrients and energy sources essential for maintaining normal growth and health. Deficiencies of these substances can reduce growth rates or lead to diseases, and in some cases, excesses can cause a reduction in growth rate. Dietary requirements can be established for energy, amino acids, protein, lipids, minerals, and vitamins (NRC, 1993). In intensive systems, tilapias have the advantage that they can be fed a prepared diet that includes a high percentage of plant proteins. Carnivorous fish require fish meal or other animal proteins in their diets, which in general are more expensive than plant proteins. Nutritional studies which substitute plant proteins supplemented with specific amino acid supplements may lower costs, but still not to the level that can be achieved with tilapia diets (Ng and Romano, 2013). Complete diets are used in systems that cannot provide any dependable nutrition. Tilapia exhibits the best growth rate when it is fed a balanced diet that provides a proper mix of protein, carbohydrates, lipids, vitamins, mineral, and fibre.

Nutritional requirements of fish differ for different species and more importantly vary with life stage. Protein is the most expensive dietary component that can represent 50% of the total feed cost in intensive aquaculture (Webster and Lim, 2002). Moreover, it is difficult to set a level of protein that is best for all situations as there are many factors that affect the dietary protein requirements; such as water temperature, feed allowance, amount of non-protein energy in the diet, protein quality, natural food available and management practices (Robinson *et al.*, 2001; Webster and Lim, 2002; El-Sayed, 2006; Ng and Romano, 2013). According to El-Sayed (2006)

fry and fingerlings of fish require diets with higher protein, lipids, vitamins and minerals and lower carbohydrates as they are developing muscle, internal organs and bones with rapid growth.

Sub-adult fish need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth (Table 2.2). In aquaculture, special attention is paid to protein requirements, because the dietary protein requirements of fish for maximum performance are generally higher than terrestrial animals. From various studies the protein requirements of larval, juvenile and adult stage tilapia have been reported to range between 35-40, 30-40 and 20-30%, respectively (El-Sayed, 2006). Tilapia broodstock require 30-40% dietary protein for optimum reproduction, spawning efficiency, and larval growth and survival (Ng and Romano, 2013).

Table 2.2 Dietary Protein requirements for Tilapia. (Source: Jauncey, 1998)

Whole body weight	Optimum dietary Protein Content (%)
First feeding fry to 0.5g	30-56, recommended 40-45
0.5-10g	30-40, recommended 30-35
10-30g	Recommended 25-30
30-harvest	Recommended 25-30

Fish do not have true protein requirement rather they require the 20 essential and non-essential amino acids that make up proteins (Webster and Lim, 2002). Protein in fish tissue is formed from all 20 amino acids. The fish can synthesize some of these amino acids in their body but some others cannot be synthesized and these, therefore, must be consumed. The ten amino acids that cannot be synthesized are the _essential amino acids' that must be provided in proper amounts in their diet. The essential amino acids required by Nile tilapia are shown in Table 2.3.

Table 2.3 The essential and semi-essential amino acid requirements of Nile tilapia as % of dietary protein. (Source: Ng and Romano, 2013)

Amino acids	% protein
Essential amino acids	
Arginine	4.2
Histidine	1.72
Isoleucine	3.11
Leucine	3.39
Lysine	5.12
Methionine	2.68
Phenylalanine	3.75
Threonine	3.75
Tryptophan	1.0
Valine	2.8
Semi-essential amino acids	
Cystine	0.53
Tyrosine	1.79

In general, suggested dietary lipid levels for tilapias range from 5% to 12% (Suresh, 2003). Lipids are essential nutrients in fish diets because they liberate approximately 9.4 kcal of gross energy g^{-1} which makes them the best sources of energy in terms of kcal g^{-1} compared with

carbohydrates (4.1 kcal of GE g^{-1}) and proteins (5.6 kcal of GE g^{-1}) (Webster and Lim, 2002). Free fatty acids are the sources of immediate energy for fish. In addition, fatty acids are the key components of all lipids, especially the two classes of essential fatty acids; omega-3 (n-3) and omega-6 (n-6) fatty acids that cannot be synthesized in animal body are very important in fish diet. In general, it appears that cold-water fish require highly unsaturated fatty acids (HUFA) of the n-3 class of lipids, while warm-water fish require HUFA from either the n-3 or n-6 classes, or a mixture of both (Webster and Lim, 2002). This suggests that tilapia utilizes plant oils (rich in n-6 fatty acids) more efficiently than fish oils (rich in n-3 fatty acids) (El-Sayed, 2006; Ng and Romano, 2013). The requirement to these essential fatty acids can be met by supplying their diet with marine fish oil, soybean oil, rapeseed oil, linseed oil or from other natural food organisms, such as zooplankton, found in the pond.

Older tilapia fish seem to cope with higher dietary fibre content, a maximum of 8-10% (Jauncey, 1998), than younger ones at about 6-8% (El-Sayed, 2006).

Carbohydrates usually represent less than 25% of the diet for fish less than 1.0g and increases to 25 - 30% for fish greater than 1.0g up to harvest (Shiau, 1997). Many fish appear to be able to utilize simple carbohydrates, such as sugars, more effectively than complex starches. However, warm-water species like tilapia have been reported to utilize complex sugars (starch) more efficiently than disaccharides and monosaccharides (El-Sayed, 2006; Ng and Romano, 2013). Starch which is richly found in cereals is less digested than fats and proteins, unless it is passed through heat and moisture involved treatments. Metabolizable energy (Metabolizable energy = food intake gross energy - faecal energy - energy in gaseous products of digestion - gill energy - urinary energy.) that can be provided to fish from fats, proteins and carbohydrates is estimated to be 8.5, 4.5 and 1.2-2.0 kcal/g, respectively (Webster and Lim, 2002). Lipid and, to a lesser

extent, carbohydrate can be used in diets of fish to 'spare' protein for growth. Higher levels of lipid and carbohydrate enable a reduction in the protein level necessary for optimum growth (Ng and Romano, 2013).

Vitamins and minerals are essential in fish feeds. However, these nutrients are only required in small quantities. Vitamins are organic substances that are essential for growth, health, reproduction and maintenance. There are two groups of vitamins: fat soluble which includes vitamins A, D, E and K, and water soluble e.g., biotin, choline, folic acid, riboflavin, thiamine and vitamin C. Vitamins are important since they facilitate the absorption of minerals, protect cells, and act as co-factors that are required for the metabolism of macro-nutrients. Also, vitamins are necessary for cell respiration (Webster and Lim, 2002).

Unlike the situation in terrestrial animals, aquatic species easily absorb minerals from their surrounding aquatic environment. Since tilapias are mostly farmed in freshwater/ low salinity waters, dietary supplementation of minerals is essential for optimal health and productivity (Ng and Romano, 2013). Minerals are important for the fish skeletal structure, electron transfer, regulation of acid-base equilibrium, osmoregulation and are important reaction cofactors (NRC, 1993). The aqueous environment can provide most minerals, but the phosphates and sulphates are often limiting and need to be provided in the diet (NRC, 1993). Mineral deficiencies often occur not because of lack of adequate amounts in the diet, but due to their reduced bioavailability especially when the diets are strongly based upon plant protein sources that contribute substantial amounts of fibre and phytic acid (Ng and Romano, 2013). Mineral deficiencies can lead to problems such as: anaemia, poor growth, skin and fin lesions, bilateral lenticular cataract development, erosion of fins, dwarfism (short body), anorexia, dark coloration, bone mineralization (softening), and deformities of the head, vertebrae and ribs (NRC, 1993). When

diets are supplemented with minerals, their uptake from the water must be considered because toxicity can also result from excessive dietary concentrations of minerals such as copper, iron, selenium and calcium (Roberts, 2002).

In tilapia, it has been evident from several studies (Clark *et al.*, 1990; Tung and Shiau, 1991) that feeding rate and meal frequency can influence the production performance of tilapia. Feeding rate (allowance) in practical feeding of fish involves either feeding to satiation or feeding with restricted ration. A study with red tilapia showed that best growth can be achieved near satiation feeding rate (Clark *et al.*, 1990). But satiation levels are not necessarily economical ways of feeding, because the food conversion at satiation level is often poor. In addition, it is difficult to determine the exact satiation level in fish because feeding takes place in the water environment. This may lead to overfeeding, which is wasteful and represent an economic loss besides having a deleterious effect on the water quality. Therefore, restricted feeding is recommended for feeding fish (Suresh, 2003). Recommended tilapia feeding rates and feeding frequencies are given in Table 2.4.

Table 2.4 Daily Feeding Allowances and Frequencies for Various Sizes of Tilapias at 28°C. (Source: NRC, 1993)

Size	Daily feeding (% of fish weight)	Times fed daily
2 days old to 1g	30-10	8
1-5g	10-6	6
5-20	6-4	4
20-100	4-3	3-4
>100g	3	3

2.4 Fish Meal as a Protein Source in Aquaculture Feeds

The fish feed industry is still dependent on fishmeal and fish oil from the industrial fishing operations. This important ingredient provides a highly digestible protein with balanced amino acid, trace minerals (calcium, phosphorus, iron, zinc, selenium and iodine), residual lipids with long chain omega-3 fatty acids (eicosapentaenoic acid; EPA and docosahexaenoic acid; DHA) and primary energy sources that satisfy most nutritional requirements of fish (El-Sayed, 1999; Halver and Hardy, 2002; Gatlin III *et al.*, 2007; Olsen and Hasan, 2012; Ng and Romano, 2013). Fishmeals are attractive materials for inclusion in a wide range of aquafeed pelleted products. The quality of fishmeal varies considerably from different countries due to seasonal fluctuations in species landed and changes in their compositional characteristics. These species can include anchovy, menhaden, and capelin, all of which are oily pelagic shoaling fish species (Miles and Chapman, 2006). On the basis of their origin, fishmeal can be of two types: white fishmeal

produced from non-oily whole fish, partly eviscerated fish and post-filleting residues, and brown fishmeal made from oily whole fish from which a large proportion of the oil has been extracted.

Almost 15% of the world fish harvest is not used for direct human consumption, but is converted into fish meal or fish oil for further application in animal feed (FAO, 2014). Of the 158 million tonnes of fish about 21.7 million tonnes is, therefore, handled and processed in other ways (non-food use) than fresh, frozen, smoked or canned (FAO, 2014). Out of the total fish production that is destined for non-food use in 2012, 75% (16.3 million tonnes) was reduced to fishmeal and fish oil. The supply of huge volumes of high quality fish meal is necessary to supply the aquaculture industry, which has been growing with around 8.8% annually since the 1950s (FAO, 2007). The major proportion of larger pelagic species which traditionally were used for fishmeal and oil production are now increasingly sold as human food (Olsen and Hasan, 2012). On the other hand the percentage of high quality fish meal of the total amount of fish meal is expected to grow from 8% to 50% during the next 30 years (Hydro Norway, 2000). The high demand for this limited resource available together with natural variations in the supply is pushing prices to historic highs with an increase of 206% between 2005 and 2013 to US$1919/tonne (FAO, 2014). Besides high prices of fish meal, the aquaculture industry is in competition for fishmeal, because it is used for animal production and is a primary protein source in the diets of cattle, poultry and pigs (Hardy, 2010).

It was recently estimated that about one-third of the raw material used for producing fishmeal and fish oil is based on by-products and waste rather than whole fish (FAO, 2014). This share is growing, replacing rather than adding to the volumes of small pelagic fish used for feed purposes. In recent years more fishmeal is being produced from fish by-products, which previously were often discarded. This can affect the composition and quality of the fishmeal

with, in general, more ash (minerals), an increased level of small amino acids (such as glycine, proline, hydroxyproline) and less protein, which may affect its share in feeds used in aquaculture (Olsen and Hasan, 2012). According to recent estimates, about 35% of world fishmeal production was obtained from fish residues in 2012 (FAO, 2014). The productivity of fish by-products from fish processing plants, which is inadequately handled in Ethiopia, will surely be on the decline as a result of fishery stock decline in some of the major lakes in the country. Fish offal (fish carcass remaining) is sometimes used as food in human diets in those places where active fishery is taking place (personal observ.).

The use of fish meal as a major protein component in formulated diets has become a significant cost factor in many aquaculture operations (El-Sayed and Tacon, 1997; Tacon, 1997; Hassan, 2001; Tacon *et al.*, 2006; Hardy, 2010). In addition, its unreliable quality and limited supply have stimulated research efforts to identify alternative sources of protein for aquaculture diets. Substantial progress has been made in replacing FM with alternative non-fish ingredients (Naylor *et al.* 2009; Hardy 2010); the percentage of FM incorporated in diets of omnivorous fish species such as carps and tilapia (the largest fed species group among cultured finfish) has declined by 25-50% depending on species and life stage (Tacon *et al.*, 2011). In total usage terms, it is expected that the total use of fishmeal by the aquaculture sector will decrease in the long term. Reports indicated a decrease from 4.23 million tonnes in 2005 to 3.72 million tonnes in 2008 (or 12.8% of total aquafeeds by weight), and expected to decrease further to 3.49 million tonnes by 2020 (or 4.9% of total aquafeeds for that year) (Tacon *et al.* 2011). The reasons for this decrease were attributed to the increasing market demand and prices, decreased supplies from tighter quota setting and more controls on unregulated fishing, and increased use of more cost-effective dietary fishmeal replacers.

Importation of fishmeal for use in fish feed makes the aquaculture sector economically unattractive in countries like Ethiopia where aquaculture is at its infancy. Therefore, searching for locally available low cost alternative feed ingredients that can be used as protein sources in fish diets is highly sought for Ethiopia.

2.5 The Need for Low Cost Fish Feeds

Although farming aquatic animals gives higher return, farmers in Ethiopia give more attention to crop farming than fish farming. According to Hecht (2007), poor financial circumstances, lack of capacity in regards to technology, poor supply and distribution services for inputs and, more importantly, lack of training opportunities for farmers within sub-Saharan Africa are main constraints impeding aquaculture development. This shows the need for information to be made available on technologies and resources such as feeds on which aquaculture mainly depends on their availability and accessibility. Often feed is the most expensive operating cost item accounting for over 50% of costs in semi–intensive aquaculture (De Silva, 1993) and up to 70% in intensive aquaculture (Thompson *et al.*, 2005). In Africa it is one of the major challenges facing the development of aquaculture (Jamu and Ayinla, 2003). Lack of quality feed is such an issue that nutrition research was given highest overall priority in the synthesis of national reviews and indicative action plans for sub-Saharan African aquaculture (Hecht, 2007). In view of this, the future development of small-scale aquaculture in Ethiopia depends on available feed resources, as feeding constitutes a significant portion of the operation cost. For the development of intensive aquaculture in Ethiopia, the major bottleneck is the lack of quality fish feed coupled with the high import tax on imported feeds and feed ingredients (with 41% or more than 70% tax rate) (Rothuis *et al.*, 2012).

Future aquaculture expansion will depend on the continued development of sustainable protein alternatives; these alternatives must be readily available, cost friendly and contain correct nutritional properties (Gatlin III *et al.* 2007). For the sustainability of aquaculture development fish nutrition is the key knowledge. However, the major constraint of enhancing sustainable fish production is feed cost and reducing feed cost depends on efficient utilization of nutrients (Gabriel *et al.*, 2007).

Ingredients used in aquafeeds are either of plant or of animal origin. Plant feedstuffs are generally cheaper than animal protein feedstuffs. For instance, cereals (including their by-products), oil seed meals, pulses (including lupins and peas), are alternative plant protein sources that are increasingly being used in aquaculture feeds because of their nutritional quality, lower cost, and availability (Tacon *et al.*, 2011). Therefore, plant products tend to be the mainstay of farm made aquafeeds.

Feed resources are mostly based on agricultural by-products available in an area which may be of modest quality but need to be of a reliable quantity. This is because increasing aquaculture production requires corresponding increases in nutrition related inputs; i.e. intensifying culture practices by feeding more and better feedstuffs (New, 1987; Tacon, 1987). As a country based largely on agricultural production, Ethiopia is well positioned to increase its domestic output of animal feed ingredients, particularly feed grains and their by-products which, together, account for well over 14% of all feed ingredients consumed in the country (MoARD, 2007).

Farm-made aquafeeds allow farmers to adapt feed inputs to their own financial resources and requirements. They also facilitate the use of locally available agricultural by-products which

would otherwise have limited use within the community (Rana and Hasan, 2013). Farm-made aquafeeds are also potentially cheaper for farmers than commercial aquafeeds.

2.5.1 Utilization of plant feed ingredients in aquaculture feeds

Generally plant and their derived by-products have been widely assessed for inclusion in feeds for numerous fish species (El-Sayed, 1999). Although various legumes, pulses and cereals have been widely utilized, soybean has by far been the subject of most research interest (Ng and Romano, 2013). The success of soybean meal in replacing fishmeal in diets for a number of teleost fishes is primarily due to a fairly good amino acid balance and generally high nutritional value (Hardy, 2010). Other important factors are obviously market supply and cost.

Plant products have generally lower fat and ash contents than fishmeal, but their carbohydrate concentration is higher. However, the digestibility of carbohydrates is questionable as a portion of it is found usually in less digestible forms like raffinose, stachyose (Tacon, 1997; Gatlin III *et al.*, 2007; Sorensen *et al.*, 2011). Omnivorous fish such as *O. niloticus* can utilise much higher levels of dietary carbohydrates (Krogdahl *et al.*, 2005). Other nutritional components such as cationic minerals and phosphorus are less available to fish as they are found bound to phytic acid and fish lack the enzyme phytase in its digestive tract (Francis *et al.*, 2001; Makkar *et al.*, 2007).

Incorporation of plant materials in fish diets negatively affects texture and taste thus reducing palatability and consequently feed intake (El-Sayed, 1999; Francis *et al.*, 2001). Higher levels of plant products in fish diets increase the crude fibre content of the diet making it less digestible (El-Sayed, 1999). Fibre lowers digestion process by creating a barrier between nutrients and digestive enzymes. Fibre may also disrupt enzyme activities through adsorption or immobilization (Gaber, 2006).

Imbalances in macro and trace minerals, presence of antinutritional factors (ANFs) are regarded as nutritional concerns in all-plant feeds. Antinutritional factors (ANFs) are compounds plants produce as a defence mechanism to reduce chances of being eaten by animals. Antinutritional factors affect the health and production of animals by interfering with food utilization directly or through their metabolic products arising in living systems (Francis *et al.*, 2001; Makkar *et al.*, 2007). Antinutritional factors have the capacity to have significant deleterious effects on nutrient utilization by fish. Notably, substantial effects of a range of ANF have already been noted on fish using a wide range of experimental techniques (Bureau *et al.* 1998; Francis *et al.* 2001; Glencross *et al.*, 2003; 2006). Some of the effects of ANF in fish might include inhibition of growth, decreased food efficiency, structural and functional changes of the gut, goiterogenesis, pancreatic hypertrophy, hypoglycaemia, and liver damage (Francis *et al.* 2001; Krogdahl *et al.*, 2010). Although some plants are known to cause obvious signs of poisoning, much more subtle effects are produced only by prolonged ingestion of a given plant (Olsen *et al.*, 2007). The extent of negative effects of ANF on health of fish also depends on such factors as age, size, sex, and state of health and level of nutrition (Hemre *et al.*, 2009). The consequences/implications of ANFs in plants used as feedstuffs are not their direct toxicity to animals alone, but also the inconvenience and the economic loss associated with poisoning of animals and the cost of preventing or reducing such happenings (Francis *et al.*, 2001). It is worthy of note that plant poisons can either be accumulated in the animal or in certain organs or they are metabolized and excreted.

Fortunately, it was possible to augment the nutritional quality plant products and minimize the effect of antinutritional factors (ANFs) through processing (de-hulling and more refined processing methods) of these materials, genetic manipulation of plants and genetic selection of

fish (Rackis, 1974; Thiessen, 2004; Hardy, 2010; Krogdahl *et al.*, 2010). Prebiotic and probiotic supplementation of fish diets can also increase the utilization of plant feedstuffs through the direct or indirect modulation of the gut microbiota (Merrifield *et al.*, 2010). However, such improvements are frequently costly and require modern machinery and investment. This results in further elevating the cost of the ingredient and may limit scope for use.

2.5.2 Oil seed by-products as protein sources in aquafeeds

Many plants are grown specifically for the oil which the seeds or fruits produce, is utilized for human food and other purposes. Vast quantities of by-products from the vegetable oil industry are produced and these are the staple ingredients of animal feedstuffs, being high in protein and low in carbohydrate (Tacon, 1987). Oil extraction from seed or fruit is carried out by two methods: by pressing, or with chemical solvents. The product obtained by pressing is termed oilcake and that by solvent extraction, oil seed meal. All of these by-products are potential ingredients of aquaculture feeds.

Oilseeds are important agricultural commodities widely grown in Ethiopia. Major oilseeds are sesame seed, groundnuts, soybeans (partly used for oil extraction), rapeseed, Niger seed, linseed, sunflower seed, cottonseed and others. According to Central Statistical Agency (CSA, 2010) approx. 0.8 million hectares are presently cultivated with oil crops. Among the major oil crops, for instance, over 150,000 tonnes of Niger seed (*Guizotia abyssinica*), about 78,000 tonnes of cotton seed, 46, 425 tonnes of groundnut seed and 22,628 tonnes of rapeseed are produced annually (CSA, 2010) and used mainly by the oil processing industries. Oil seeds are partly used for domestic oil extraction and partly exported to international markets for animal feeds and oil extraction. Most of the sesame production among other oilseeds such as Niger seed, and

safflower seed is for export as they are regarded high-priority export crops by the government of Ethiopia (Wijnands *et al.*, 2007). All other oilseed crops (soybeans, groundnuts, cottonseed etc.) are almost entirely used domestically.

Production of rapeseed covers an area of 21,247 ha (CSA, 2010), with *Brassica carinata* representing 85 % of the total area of land devoted to the production of *Brassica species* in Ethiopia (Tadelle Dessie *et al.*, 2002). The production of rapeseed in Ethiopia is estimated at about 226,277 quintals (CSA, 2010) with large proportion of it processed for oil production, resulting in substantial quantity of rapeseed cake as a by-product (Tadelle Dessie *et al.*, 2002).

Currently, residues of oil extraction industries are providing a large quantity of oil seed cakes and meals to poultry and livestock producers at a relatively low cost. However, the type of seed cakes that are important to the different regions varies depending on the type of oilseed crop produced (Berhanu Gebremedhin *et al.*, 2009).

Several experiments were conducted on the use of these materials in low cost ration formulations for different animals, and on their nutritional/anti- nutritional qualities in Ethiopia (Ashenafi Mengistu, 2007 and references therein).

Chemical evaluation of the most important oil seed residues (cakes and meals) in Ethiopia showed that most of the oil seeds have reasonable nutritional values. However, Niger (*Guizotia abyssinica*) seed meal was found to be with the highest crude fibre (CF) (Alemu and Guenther, 1992 as cited in Tadelle Dessie *et al.*, 2002). The oil content of most of these residues is relatively high due to the inefficient mechanism of oil extraction practiced in Ethiopia.

Basically, the quality of seed cakes is affected by extraction method (mechanical method or Organic solvent method), number of extractions and adulteration (with other oil seed cakes). For

instance, modern and big edible oil mills in Ethiopia use organic solvent extraction method which squeezes most of the oil when other oil industries use mechanical pressing method (the residues are higher in oil content and lower in protein content), which is not efficient in oil extraction. This variation in the quality of the cakes created livestock farmers' preference to incline towards the one in which less oil is extracted (Berhanu Gebremedhin *et al.*, 2009). The number of extraction for example affects the quality of cotton seed cakes in that 2nd extraction gives more quality cake than the first.

Besides their use in animal feed locally, seed cakes in Ethiopia are exported. Availability which itself is determined by the presence of production of a given oil seed basically is an important factor that determines the type of oil seed cakes used in a given area (Berhanu Gebremedhin *et al.*, 2009). The country exported a total of 95,000 tonnes of oil seeds in 2003 (Wijnands *et al.*, 2007). Considering this export amount if the remaining 548,000 tonnes out of the 2009/2010 production of oil seeds are consumed domestically for oil extraction, the amount of oilseed cake production at an extraction rate of 70% (Devendra, 1988) will be more than 384,000 tonnes.

The most important factor limiting the use of oil seeds or their by-products as fishmeal replacers' at high dietary inclusion levels within compound aquafeeds is the presence of anti-nutritional factors; the inherent essential amino acid deficiencies like most plant proteins. This deficiency is readily rectified by dietary supplementation with the limiting free amino acids or by mixing complementary protein sources (Tacon, 1997).

A number of studies have evaluated a variety of oil seed residues for their suitability as replacements for fish meal (Tacon, 1997; Mbahinzireki *et al.*, 2001; El-Saidy and Gaber, 2004; Latif *et al.*, 2008). Among these, soybean is one of the most suitable oilseed plant protein

sources, has a good protein quality and EAA profile (Tacon, 1997). A large part of soybean production is used in the extraction of oil yielding a cake of high protein quality. This is processed to yield a wide array of soybean products, such as soy flour, soybean meal, soybean protein concentrate (SPC) and soybean protein isolate (SPI) that have been evaluated in fish (Gatlin III *et al.*, 2007). The limitations of soybean proteins as substitute for fishmeal are owing to sulphur-containing amino acids (Met, Lys, Cys) deficiency, antinutritional factors, lower essential amino acids digestibility and palatability (Hardy, 2010; Gatlin III *et al.*, 2007; Tacon, 1997; Francis *et al.*, 2001). Furthermore, studies reported that phosphorus and cationic minerals in soybean meal are less available due to their being bound in or by phytic acid (an anti-nutritional factor) (Tacon, 1997; Francis *et al.*, 2001; Gatlin III *et al.*, 2007). Despite this, soybeans have been widely used as aquafeed ingredients. Studies indicated that, soybean meal could be supplied as a dietary protein in fish feed between 67-100%, depending on species, size, culture system employed, dietary protein level, soybean meal source and processing methods (El-Sayed, 1999). Basically, different strategies have been devised to overcome the limitations of soybean meal. Such methods as dry and especially wet heating, extracting with water, addition of feed supplements (either addition of limiting essential amino acids, EAAs or other protein sources like animal protein sources) (Francis *et al.*, 2001), and addition of enzyme phytase (to degrade phytic acid) (Gatlin III *et al.*, 2007) have been suggested and successfully used to reduce the concentration of anti-nutrients. Hardy (2010) noted that soybeans are becoming increasingly expensive and less available because of higher production costs and their use in a growing number of applications.

Cotton seed cake is widely used as livestock feed in Ethiopia because of its high protein quality (Tadelle Dessie *et al.*, 2002) and ample availability (Berhanu Gebremedhin *et al.*, 2009).

Cottonseed is a by-product of cotton, a cash crop commonly grown in Ethiopia. Palatability and availability make it a very common protein supplement in fish feed. It has about 90% of the energy of soybean meal or linseed meal (Hossain, 1988). It can replace all soybean meal in ration when economics dictate. The nutritional value of cottonseed has been evaluated for several species of fish such as crucian carp (Cai *et al.*, 2011), common carp (Hossain, 1988), rainbow trout (*Oncorhynchus mykiss*) (Dadgar *et al.*, 2010), and tilapia (Mbahinzireki *et al.*, 2001; El-Saidy and Gaber, 2004). Cotton seed meals are among the most available plant protein sources in the world (Hardy, 2010). Besides being relatively cheap, it contains good protein contents (26-54%, depending on processing methods) and amino acid profile. Cotton seed cake contains anti-nutritional factors (phytic acid and gossypol), the most important of which being gossypol. Hence, the use of cotton seed meal in the diets of monogastric animals like fish is limited by the presence of this toxic chemical. It also contains relatively low levels of lysine, cystine and methionine and processing conditions may also have a negative effect on the amino acid content (El-Sayed, 1990). Crude fibre is a limiting factor in the use of cotton seed meal as feed. It is around 10% in decorticated meals and expellers while its content in corticated meal can exceed 20% (El-Sayed, 1990). The digestible energy of cotton seed meal is relatively low due to the high crude fibre content (Agbo *et al.*, 2011b). Further, a low availability of lysine limits the nutritional value of this protein source (Dadgar *et al.*, 2010). Protein degradability is similar to or slightly less than soybeans (80% versus 86%) (Agbo, 2008). Generally, it has been reported that the amount of cotton seed meal that can be included in feeds depends on the animal species, levels of free gossypol, dietary protein and available lysine (El-Sayed, 1990). It was reported to have 50-100% dietary protein inclusion level in different fish species. It was reported by El-

Sayed (1999) to have 50-80% inclusion level and even as a single ingredient (El-Sayed, 1990) in the diets of tilapiine species.

Linseed meal is another potential protein source obtained after grinding the flakes, cake, or chips which remain after removal of oil from flaxseed by mechanical or solvent extraction. It is mildly laxative, and must not contain more than 10% crude fibre to be more palatable (Thiessen, 2004). Its energy content is somewhat less than soybean meal but it is higher in fibre (El-Saidy and Gaber, 2004; Thiessen, 2004). Linseed meal is high in protein (30-45% CP) with good amino acid balance (El-Saidy and Gaber, 2004) and digestibility similar to soybean meal (Hossain, 1988). They are relatively high in EAA methionine among the oilseed cakes. Latif *et al.* (2008) reported that, linseed meal cannot be utilized at a level exceeding 20-30% of the diet in an unprocessed condition without compromising the growth of *Labeo rohita* fish. Limiting linseed meal incorporation at high levels are the presence of antinutritional factors (tannin and phytic acid) and amino acid imbalance. The inclusion level of linseed meal can be increased up to 30-40% by eliminating/reducing the amount of tannin and phytic acid by proper processing (fermentation by lactic acid bacteria) prior to their incorporation (Mukhopadhyay and Ray, 2001). However, it can be possible to replace up to 50% of fish meal provided that it is properly processed (fermented) and supplemented with deficient amino acids (Mukhopadhyay and Ray, 2005).

Other oilseed by-products may also have a good potential as they contain generally high crude protein levels but may be low in lysine, methionine and threonine. The oil content of oilseed cakes and meals varies according to the oil extraction method employed, usually ranging from below 1% within solvent extracted oilseeds to 8% within hydraulically pressed oilseed cakes. Oilseeds are generally poor sources of calcium, vitamin E and provitamin A (ie. carotenes), but

are good sources of phosphorus (mainly in the form of phytates) and B vitamins (Tacon, 1987). As with the cereals most oilseeds also contain a variety of endogenous antinutritional factors which, unless destroyed or deactivated, can seriously reduce their feed value to fish.

Various oilseed cakes are used in the production of other animal feeds in Ethiopia and hence aquaculture will be a direct competitor with other animal husbandry practices. In most cases, such oilseed cakes and other industrial by-products are essentially limited to the urban areas serving urban agriculture (Berhanu Gebremedhin *et al*., 2009). However, for Ethiopia as a whole the situation seems promising as its contribution to the world supply of oilseed cakes which are principal ingredients in animal feeds is minimal in comparison to the proportion that is used by local animal feed industry.

Chapter 3- General Materials and Methods

3.1. Experimental Facilities

All experiments were conducted at Ziway Fisheries Resources Research Centre (ZFRRC) of the Oromia Agricultural Research Institute, Ziway, Ethiopia, in an indoor facility with a re-circulating water system built by Addis Ababa University, Department of Zoological Sciences.

3.1.1. Experimental System for Growth Trial and Fish Husbandry

The re-circulatory water system used in this study consists of 21 tank units of 60 litres capacity each (Figure 3.1). These are connected to a plumbing system that supplies water continuously. Water supply to the tanks was using two centrifugal pumps from a sump tank through a common inflow pipe. Tanks were each fitted with inlets such that water flow (2 l min^{-1}) was almost in a spray fashion into the experimental tanks to enhance circular flow, which enabled self-cleaning of the tanks, as well as aeration (Figure 3.2). Fitted internally to each tank unit is an overflow and standing drain pipe (50mm diameter), onto which a screen is fixed. This maintains water level without letting out fish. Over the drain pipe (standpipe) a jacket (a guard pipe with 110mm diameter) is placed with a number of holes at the bottom, so as to suck faeces and uneaten food from the tank bottom into the drain pipe. Water from all experimental tanks drained through open gutters to the settling/biological filter tanks containing fine mesh nets, which filtered waste water. Tanks were mounted on a metal framework over the settling tanks that received waste water. Water from the settling tank flows in to a series of tanks filled with gravels which served both as mechanical and biological filters. These tanks were then in turn connected to a clean

water collecting sump from which water was pumped to both fish tanks and a header tank containing bio-balls (biological filter) where it was further treated and recirculated.

A small amount of water from the system was continuously replaced at a rate of 10 l hr^{-1} to avoid accumulation of excretory products, principally nitrate. The filters were cleaned once every three weeks to get rid of accumulated sludge resulting from faeces and uneaten feed.

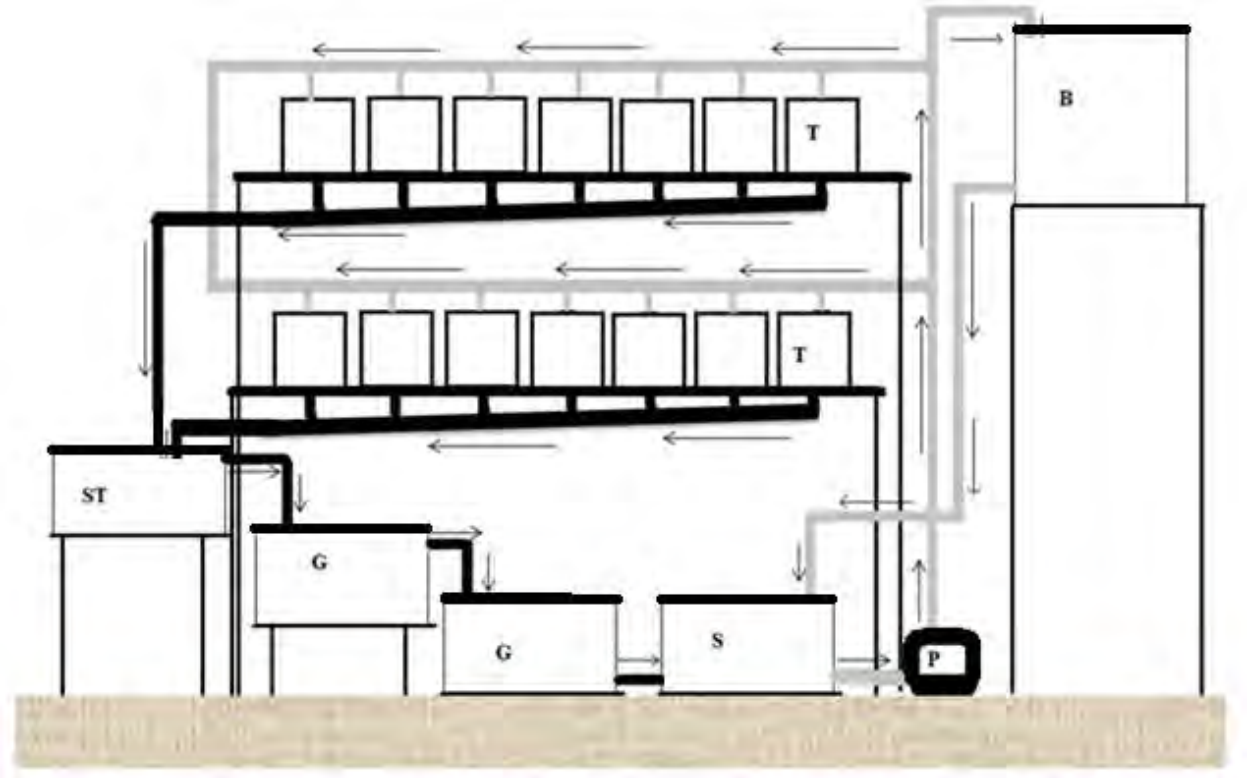

Figure 3.1 The recirculatory water system used for growth and digestibility studies.

Key: B=Biological filter, T=Fish tanks, G=Gravel filter, S=Sump, P=Pump, ST= solid settling tank Clean water inlet pipes, Dirty water outlet pipes, =direction of water flow

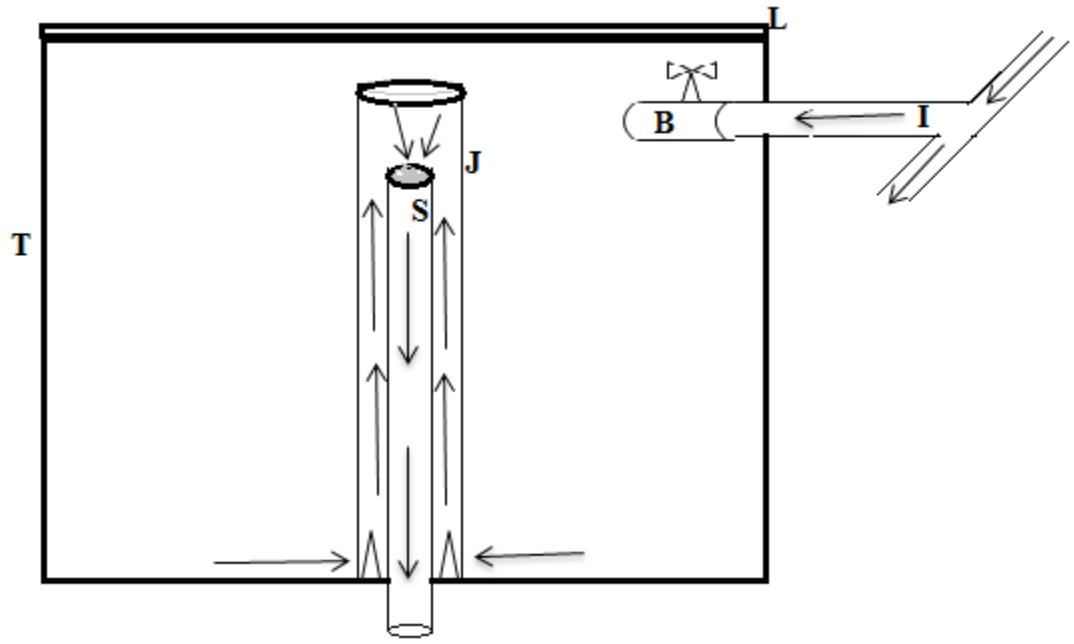

Figure 3.2 Fish tank used during growth trial

Key: L=lid, I=Inlet pipe, B= ball valve, J=Outer jacket for the standpipe, S=standpipe/drainage pipe, ⟶ =direction of water flow.

Temperature was maintained at 28 ± 1 ^{o}C with the aid of submerged heating elements (screw in water heating elements with thermostat, model 120-1500 low density 240V, 1500watt 5-1/8‘‘ length and threaded 1-1/2‘‘ outer nut for plumbing) in the sump tank. Air was supplied by an external compressor to maintain a dissolved oxygen concentration of approximately 5.5 mg l^{-1}. Water quality parameters including dissolved oxygen, pH, nitrite (NO_2), nitrate (NO_3) and ammonia (NH_3), were monitored weekly. Their average values during the study period were as

follows: temperature, 28.9 ^{o}C; pH, 7.3; ammonia, 0.17 mg l^{-1}; nitrite, 0.20 mg l^{-1}; nitrate, 50 mg l^{-1} and dissolved oxygen, 5.4 mg l^{-1}. A light:dark regime of 12 h:12 h was maintained using artificial light from fluorescent tube.

3.1.2 Faecal Collection System

In this study, a settling column system was employed for faeces collection, but it was adapted to the 60 l cylindrical tanks used (Figure 3.3). This collection system employed pipes fitted to the bottom of the rearing tanks with a vertical column and transparent hoses connected to a valve system at the bottom ends, where the faeces were deposited after settling. At the top end of the vertical column an overflow was provided to get rid of excess water flowing through the system. Deposited faeces were collected by opening the valve at the tip end and carefully draining the faeces into centrifuge bottles. The collectors were fixed to the rearing tanks the night before and faeces collected early the next morning. Faeces were immediately centrifuged at 4,300 x g for 10 min and the supernatant discarded. Wet settled solids of faeces were frozen at -20 ^{o}C to retard bacterial decomposition. Faecal samples were later defrosted and oven dried at 60^{o}C, ground and analysed for crude protein (CP), crude lipid (CL) and gross energy (GE).

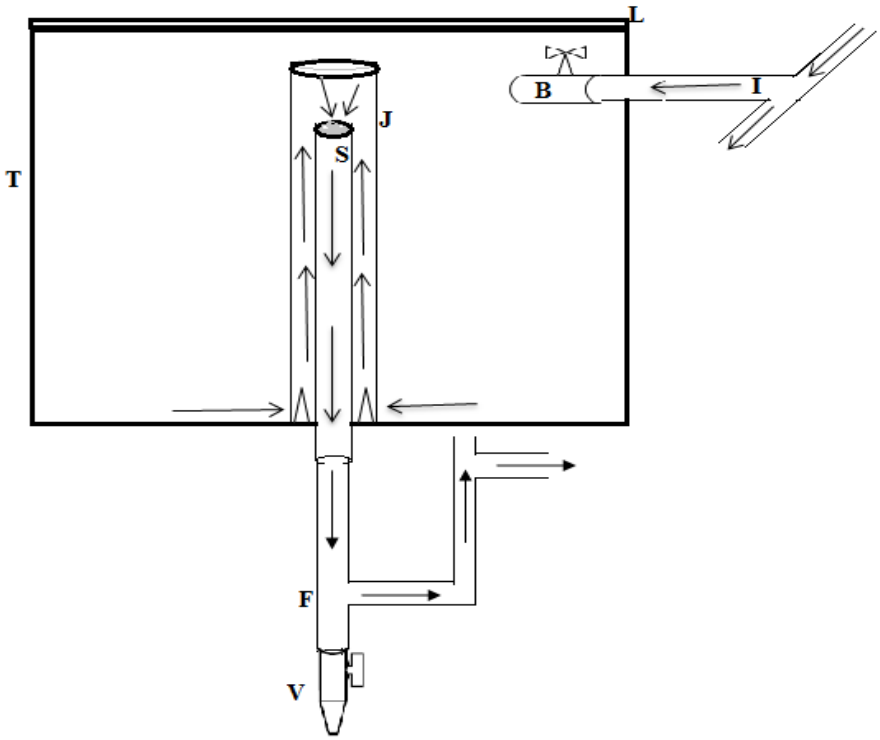

Figure 3.3 Faeces collection system used for the studies

Key: L =lid, I =Inlet pipe, B =ball valve, T =Rearing tank, J =Outer jacket of stand drainage pipe, S =Standing drainage pipe, F =Faeces collector, V =Valve to collect faeces,

→ =Direction of water flow

3.1.3. Experimental Fish

3.1.3.1. Broodstock management

The Lake Hora *strain* of ‗generation 2' *Oreochromis niloticus* broodstock were netted from a broodstock hapa in concrete ponds, manually selected, sexed and transferred into a 2 m^2 hapas in a greenhouse covered (to attain an average water temperature of 28^oC) 84m^2 concrete pond built in Ziway Fisheries Research Centre (Figure 3.4). A total of 30 females and 10 males were selected, weighed and stocked in each spawning hapa at a rate of 8 broodfish per spawning hapa

(4 fish/ m^2). Broodstock were fed diet containing 36% crude protein (formulated and prepared from soybean and fish meal used in this study) at a feeding rate of 2% of biomass in each hapa. Broodstock were fed two times a day at 9.00 am and 4.0 pm.

Figure 3.4 Spawning and conditioning hapa used for stocking broodfish in a greenhouse covered concrete pond.

3.1.3.2. Egg collection and incubation

Males and females were stocked into spawning hapas and the eggs from the females were removed, incubated, hatched (in a recirculating system) and the resulting swim-up fry were maintained for one week in a 200 litres rectangular tank provided with a continuous flow of dechlorinated filtered freshwater and aeration. The flow rate in each tank was approximately adjusted at a rate of 0.12 litre min^{-1}, to give about 1.4 turnovers per day. The swim-up fry were hand-fed daily 4-5 times with powdered fish meal feed (oven dried and ground fish offal). The fish were fed at 25% of the total body weight per day.

3.1.3.3 Fry harvest, preparation and stocking

Two hapas of size 2 m^2 were sewn using HDPE (high density polyethylene) netting and fixed in a concrete pond covered by a greenhouse structure. Fry of *O. niloticus* were kept in these hapas where they were fed with ground fish meal until ready for the growth trial.

3.1.3.4 Acclimation and Weighing Procedures

One week before the start of each experiment, fish were transferred to the experimental tanks from the greenhouse hapas for acclimation. In order to reduce variability in weight of fish within each tank fish were graded into similar sizes of ± 1 g before stocking randomly at a density of 20 fingerlings per 60-litre tank in triplicates per treatment. During this period fish were fed with pelleted reference diet used in the first digestibility experiment.

Prior to any experimental procedure all fish were anaesthetized using clove oil/ethanol (1:9, v/v) prepared and mixed with 20 litres of aerated clean water (Perdikaris *et al.*, 2010). For initial and final samples, all fish were individually weighed and measured under anaesthesia. Fish were netted, drained of water and gently blotted on a soft paper towel (in an attempt to reduce errors of fish weights recorded due to water adhering to each fish) before individual weighing to the nearest 0.01g on an electronic digital balance and their lengths measured to the nearest 0.1cm using a fish measuring board. Clean aerated water was used to recover the fish before being returned to the experimental tanks. Measurements of fish weight were made at the beginning and throughout growth experiments. For all intermediate weight measurements fish were bulk weighed, without anaesthesia, weekly. All fish in each tank were netted, using a fine mesh hand net. Excess water was then removed from the fish by blotting the net on a soft paper towel. Fish were then transferred to a tarred, water-filled, container and weighed collectively to the nearest

0.01g. The weekly mean weights of fish were used to calculate the daily food ration for the following week.

3.2 Diet Formulation and Preparation

Fish meal was prepared from a waste obtained from a local fish processing plant, ―Ziway fish processing plant". The filleting residues were purchased from the processing plant at a price of 0.15 birr kg^{-1}. The freshly collected filleting residues of tilapia were minced using an electrical meat mincer and then dried in an oven for 48 hours at 75^{o}C in order to prevent contamination by disease pathogens. The dried residue was ground into a fine powder using an electrical smashing machine, sieved (0.5mm mesh size sieve) and then stored in a plastic bag at -18 ^{o}C in a deep freeze.

The oilseed cakes and other grains used in this study were bought from commercial sources in Ziway, with the exception of soybean cake which was acquired from Addis Ababa oil processing factory outlet.The most commonly used oilseed cakes in animal feeds in Ethiopia are: linseed (*Linum spp)* cake (mechanically extracted) and Niger seed (*Guizotia* spp.) cake (mechanically extracted). Ingredients were used within 8 months and proximate composition was analysed before any diet formulation to check the nutritional quality.

Generally, all experimental diets were formulated to contain 320 g kg^{-1} protein, 100 g kg^{-1} lipid and 18 kJ g^{-1} energy. These levels were based on requirements for Nile tilapia (NRC, 1993).

The diets were formulated on as fed basis. Fish meal (of tilapia offal origin) as the main dietary protein source and wheat and corn grains (milled) main carbohydrate source were used in the experiments. A poultry grade Vitamin/ mineral premix (Table 3.1) at 50 g kg^{-1} and a binder (carboxymethyl cellulose, high viscosity) at 20 g kg^{-1} were added. The vitamin/mineral premix

was purchased from the local market in Addis Ababa. The premix is prepared for egg laying hens by an Israeli company called Koffolk Animal Health and Nutrition. Soybean oil was used as the source of lipid in the diets. Chromic oxide was added as an indigestible marker for digestibility study (Divakaran *et al.*, 2002).

Diets were prepared by wet extrusion using meat mincer (Model TJ 22). All ingredients were finely ground and sieved through a 500 μm sieve to obtain a homogenous mixture. The dry ingredients were then weighed out according to the formulation, placed in an aluminium bowl and mixed until uniformly blended using a modified mixer. The resulting homogenate was moistened after addition of water (20%-30%) slowly with continuous stirring until dough was formed before passing through an electrical meat mincer. The expeller like strands made by the meat mincer was dried in an oven with convector fan at 35-40 ^{o}C for 24 hours. They were then crushed in to crumbles and sieved with 1mm mesh size sieve. The resulting pellets were packaged in polythene bags and stored in a deep freeze at -18^{o}C. Prepared diet samples were analysed for proximate composition, energy and chromic oxide.

Table 3.1 Composition of poultry grade vitamin/mineral premix used in experimental diets (a product of Koffolk Animal Health and Nutrition)

Content	Amount (mg kg^{-1} of premix unless otherwise stated)
vitamin A (retinol)	2100
vitamin D_3 (chole-calciferol)	50
vitamin E (tocopheryl acetate)	10000 I.U
vitamin k_3	2000
Thiamine	1000
Riboflavin	4000
Niacin	10000
Pantothenic acid	5000
Pyridoxine	750
Folic acid	250
Vitamin B12	8
Vitamin H as Biotin	30
Betain	100,000
Antioxidant	125,000
Minerals	
Manganese	80000
Zinc	50000
Iron	20000
Copper	5000
Iodine	1200
Cobalt	200
Selenium	200

3.2.1. Methods of Proximate Analysis

Proximate analysis of dietary ingredients, diets, fish and faeces were carried out using the following procedures that broadly adhere to AOAC (1995) protocols:

Moisture: - Moisture content was determined by air-drying the samples in an oven at 105^{o}C for 24 hours till constant weight is achieved. It is a gravimetric measurement of water in the feedstuffs, diets and carcass expressed as a percentage of the initial sample weight.

Ash: – This measured the total inorganic matter by incineration. Approximately 5g of sample was weighed into a pre-weighed crucible and incinerated overnight at 600^{o}C using a muffle furnace. The increase in the final weight of crucible after incineration represented the ash and was expressed as percentage of the original sample.

Crude Protein: – The micro-kjeldahl method was used for the determination in triplicate as follows; 500mg sample was digested in concentrated sulphuric acid using a Labconco digestion apparatus. The resulting solution was distilled using Labconco distillation apparatus. Ammonia from the digest was released when reacted with 40% (W/W) sodium hydroxide and distilled, trapped in 4% boric acid and quantified by titration against 0.1M hydrochloric acid. Crude protein was estimated by multiplying the nitrogen content with a factor of 6.25. The result was expressed as a percentage of the original weight of the sample.

Crude Lipid: – The method was that of solvent extraction using Soxhlet extractor for 4 hours. Approximately 2g sample was weighed into a thimble and corked with cotton. 80ml petroleum-ether (40-60^{o}C) was added to a pre-weighed round bottle flask with ten glass balls. The thimbles were placed into the glass unit at the bottom of each condenser and the corresponding round bottles then fitted into the unit that holds the thimbles.

Extraction which involved boiling, rinsing and evaporation was conducted. After extraction, the thimble and sample and flask with the extract were oven dried at 105°C for 30 minutes, cooled in a desiccator for 30 minutes and weighed. Ether extracts were quantified by expressing the difference in weight as a percentage of the original sample weight.

Crude Fibre: –As CF is negligible in feedstuffs of animal origin, CF analysis was not conducted for feedstuffs of animal origin in the present study (CGIAR, 2009). One gram of defatted sample was boiled in a standard solution of 3.13% H_2SO_4 for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with hot water followed by acetone. The residue was oven dried at 60 ^oC for 4 hours, cooled in a desiccator and weighed. The residue was ashed at 600 ^oC in a muffle furnace overnight. CF was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample.

Nitrogen Free Extract (NFE) – This was estimated by subtracting the total of moisture, crude protein, crude lipid, ash and crude fibre from 100.

3.2.2. Chromic Oxide Analysis

Chromic oxide was determined according to the method of Divakaran *et al.* (2002) as this method gave the best prediction of chromic oxide when DPC (Diphenylcarbazide) colorimetry was used. The more precise results obtained in the study was attributed to the fact that DPC colorimetry does not directly measure absorbance of chromate ion but measures chromatophore resulting from the interaction between chromate ion and DPC. DPC colorimetry readings at 540nm were best when compared with the direct measurements without DPC at 350, 370 or

440nm. The imprecise readings obtained in the direct measurements were attributed to the interferences from impurities other than chromate ion in the sample.

The 200-250 mg of sample was weighed into a thick-walled pyrex boiling tube (20mm X 150mm) and 3 ml of molybdate reagent (prepared from 5g sodium molybdate, $Na_2Mo_4.2H_2O$, 70ml distilled water, and 75ml concentrated H_2SO_4 followed by cooling the mixture and addition of 100ml 70% perchloric acid) were added to the tube. One glass bead (2mm diameter) was added to prevent bumping during boiling. The tube was then clamped at 45^o angle in a fume hood and heated to a boil until fumes subsided to a minimum using an electric mantle for about 5-10 minutes (taking care not to boil dry). The solution becomes clear and turns yellow due to oxidation of chromic oxide to its monochromate (CrO_4^{2-}). After cooling the sample to ambient temperature, 3ml of 70% perchloric acid was added to the tube. The mixture was then gently heated again to boiling (for about 10minutes) to ensure completion of oxidation. The solution was transferred quantitatively and made up to 25 ml in a volumetric flask by rinsing repeatedly the boiling tube with distilled water. A known weight (2 - 4mg) of chromic oxide and a feed sample without chromic oxide were similarly treated. The oxidized chromic oxide was used as standard, and the feed without chromic oxide as blank.

Chromic oxide content was measured by DPC (Diphenylcarbazide) colorimetry. The standard oxidized chromic oxide solution was serially diluted with distilled water in the range of 10, 8, 6, 4 and 2ug/ml. All of the samples were then further diluted with distilled water to fall within the range of 10 – 2ug chromic oxide/ml (mostly done by adding to 1ml of diluted solution to 10ml distilled water).

To 1ml of sample placed in a test tube (16mm X 125mm) were added 1.5 ml of 3 N sulphuric acid and 3.5 ml of distilled water, and the mixture was mixed well in a vortex shaker. Then, 0.5ml of DPC (prepared by first dissolving 0.25g 1,5 DPC in 50ml of acetone and then diluting up to 100ml with distilled water) was added to each tube and immediately mixed in a vortex shaker. An additional 3.5 ml of distilled water was then added to make the final volume to 10ml. Absorbance of the coloured complex of serial dilutions and of the unknown feed and faecal sample concentrations was determined by spectrophotometer at 540nm. The absorbance for the known serial dilution was used to generate a regression equation to calculate unknown concentrations of chromic oxide in the samples.

3.2.3. Energy determination

Gross energy in diets and faeces was determined using Adiabatic Oxygen Bomb Calorimeter. The sample was combusted in a chamber pressurised with pure oxygen and resulting heat measured by increase in the temperature of the water surrounding the bomb. Benzoic acid was used as a standard.

3.3. Analysis of experimental data

Experimental data gathered during the growth trial and results from analysis of diets, faeces and carcasses were used to determine various biological parameters namely: growth performance; food conversion ratio; protein, lipid and energy utilization; and apparent digestibility of the ingredients and diets.

3.3.1. Growth performance

Parameters used to evaluate growth performance in this study were weight gain by fish and specific growth rate (SGR). SGR is the most commonly used expression of fish growth.

Weight Gain (WG): - Is the difference between the final body weight and the initial body weight of fish over a period of time expressed in percentage.

$$\mathrm{WG} = \frac{(\mathrm{FBW} - \mathrm{IBW})}{\mathrm{IBW}} \mathrm{X}\ 100$$

where FBW is final body weight (g), IBW is initial body weight (g). These weights are mean body weights.

Specific growth rate: - Is the instantaneous change in weight of fish expressed as the percentage increase in body weight per day over any given time interval.

It is calculated by taking natural logarithms of body weight, and expresses growth as % day^{-1}.

$$\mathrm{SGR} = \frac{(\ln\mathrm{FBW} - \ln\mathrm{IBW})}{\mathrm{D}} \mathrm{X}\ 100$$

where D is the number of days between weighings.

3.3.2 Feed conversion ratio (FCR)

FCR is defined as the amount of dry feed fed per unit live weight gain. It often serves as a measure of efficiency of the diet. The more suitable the diet for growth, the less food is required

to produce a unit weight gain, i.e. a lower FCR (De Silva and Anderson, 1995). It was calculated as:

$$\text{FCR} = \frac{\text{Feed fed (g)}}{\text{Live weight gain (g)}}$$

The main problem here is that FCR is usually given in wet weights, of both food and weight gain. Some foods, such as plants and or natural food, contain much more moisture than others, such as grains or dry pellets. This may cause bias not necessarily related to the nutrient content (Hepher, 1988).

3.3.3 Protein efficiency ratio

Protein efficiency ratio (PER) is defined as the ratio between the weight gain of fish and the amount of protein fed (De Silva and Anderson, 1995):

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Crude protein fed (g)}}$$

3.3.4 Productive Protein Value

Productive protein value (PPV) sometimes also called ‗efficiency of protein utilization' (Gerking, 1971), evaluates the protein in the diet by the ratio between the protein retained in fish tissues and the dietary protein fed. PPV is determined by carcass analysis of samples of fish taken before and after feeding with the evaluated protein, and generally expressed as a percentage of protein fed.

$$\text{PPV}(\%) = \frac{\text{Protein retained in tissues}}{\text{Dietary protein consumed}} \text{ X } 100$$

PPV is a more refined criterion for the evaluation of dietary protein compared to PER since it takes into account the transformation of the dietary protein into body protein rather than the overall increase in body weight (Hepher, 1988).

$$\text{Nutrient Deposition} = \left[\frac{(\text{FBW} \times \text{FBN}) - (\text{IBW} \times \text{IBN})}{(\text{feed intake} \times \text{feed nutrient})}\right] \times 100$$

Where FBW= final body weight (g), IBW= initial body weight (g), FBN= final body nutrient and IBN= initial body nutrient.

Due to practical constraints in experiments with fish, it was not possible to ensure that all food presented was ingested nor was it possible to collect uneaten food from the experimental tanks. Therefore, for calculation of FCR, PER and PPV (ANPU- Apparent Net Protein Utilization) the amount of feed fed (instead of feed consumed/intake) was used without correction being made for any wastage. This could actually lead to overestimation of feed and underestimation of the ratios.

3.3.5 Apparent Digestibility Coefficient

Digestibility of a diet or feed ingredient can be determined directly or indirectly. Unlike comparable studies with terrestrial animals, those with aquatic animals have an inherent difficulty because of the medium in which they live. Faecal traps, for example, are impossible to use, and the voided faeces lose nutrients immediately on discharge. Therefore, all digestibility estimations on aquatic animals, whichever method one chooses, are subject to some degree of error (Anderson and De Silva, 2003; Glencross *et al.*, 2007).

In the direct method, the quantity of food ingested and the quantity of faecal matter voided are determined. The ratio gives the percentage digestibility of the feed or the nutrient under consideration. The indirect method of estimating digestibility used in the present study relies on the use of markers. A marker is usually an indigestible material introduced in small quantities and distributed evenly in the test diet, or it may be an indigestible component of the diet itself.

These are known as external and internal markers respectively. Since it is indigestible, the marker will concentrate in the faeces relative to the digestible material and the relative quantities will provide a measure of the digestibility of the diet or its nutrient components (Anderson and De Silva, 2003). The apparent digestibility coefficients (ADC) for the nutrients of the diets were calculated as follows (Bureau *et al.*, 1999):

$$\mathrm{ADC} = 100 \times \left[1 - \left(\frac{\mathrm{F}}{\mathrm{D}}\right) \times \left(\frac{\mathrm{D}_i}{\mathrm{F}_i}\right)\right]$$

Where D=% nutrient of diet; F=% nutrient of faeces; Di=% Cr_2O_3 of diet; F_i=% Cr_2O_3 of faeces and ADC of ingredients as;

$$\mathrm{ADC}_{\text{test ingredient}} = \mathrm{ADC}_{\text{test diet}} + \left[(\mathrm{ADC}_{\text{test diet}} - \mathrm{ADC}_{\text{ref.diet}})\left(\frac{0.7 \times \mathrm{D}_{\mathrm{ref}}}{0.3 \times \mathrm{D}_{\mathrm{ingr}}}\right)\right]$$

Where D_{ref} = % nutrient (or kJ g^{-1} gross energy) of reference diet (as fed); D_{ingr} = % nutrient (or kJ g^{-1} gross energy) of test ingredient (as fed).

Digestible protein and energy were calculated as follows:

Digestible protein (DP, g kg^{-1}) = dietary crude protein (g kg^{-1}, dry weight basis) x $ADC_{protein}$

Digestible energy (DE, kJ g^{-1}) = gross energy (kJ g^{-1}, dry weight basis) x ADC_{energy}

3.3.6 Body Composition of Fish

Whole body proximate analysis and hepatosomatic index (HSI) was used to determine body composition of fish. The proximate analysis followed methods described in Section 3.2.1 and components such as moisture, crude protein, crude lipid and ash were analysed and expressed as percentage of fresh weight. At the end of each experiment 20 fish were randomly selected from each treatment, including the control, and euthanized by overdose of clove oil (Perdikaris *et al.*, 2010), dissected and livers removed, weighed and used to estimate the hepatosomatic index (HSI).

$$\text{HSI} = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

3.4. Cost Analysis of Diets

A simple economic analysis was conducted to assess the cost effectiveness of diets used in the feed trial. Only the cost of feed was used in the calculations with the assumption that all other operating costs remained constant. Costs of the feeds were calculated using market prices (Table 4.2) of ingredients in Ethiopia in 2014. El-Sayed (1990) proposed what he called Incidence Cost (IC), which is governed by the unit cost of the feed and its apparent FCR;

$$\text{Incidence Cost} = \frac{\text{cost of feeding}}{\text{weight of fish produced}}$$

IC is actually the cost of feed to produce a kg of fish (relative cost per unit weight gain), and the lower the value the more profitable using that particular feed is. El-Sayed (1990) also suggested another simple parameter called the Profit Index;

$$\text{Profit Index} = \frac{\text{Value of fish}}{\text{Cost of feeding}}$$

The value of fish was calculated using the sale price of birr 30.00 kg^{-1} fish.

3.5. Statistical Analysis

The experimental design used in this study was mainly completely randomized design (CRD) where different dietary treatments were randomly assigned to the experimental units (tanks). The null hypothesis tested in this study was; there is no significant difference between dietary treatment means. Statistical analyses in this study were conducted using Minitab Statistical Package (Version 15.0). Percentage data were arcsine square root-transformed to achieve normalized distribution of the data and homogeneity of variance before statistical analysis. Differences among dietary treatment means were tested by analysis of variance (ANOVA), and means compared using Tukey‘s Multiple Comparison Test (Steele and Torrie, 1960) to test for significance of variation between the means and differences were considered significant at $p < 0.05$.

Chapter 4- Digestibility of Soybean cake, Niger seed cake, and Linseed cake in Juvenile Nile tilapia, *Oreochromis niloticus* L.

Geremew A, Getahun A, Rana K (2015) Digestibility of Soybean Cake, Niger Seed Cake and Linseed Cake in Juvenile Nile Tilapia, Oreochromis niloticus L. J Aquac Res Development **6***: 333. doi:10.4172/2155-9546.1000333*

4.1 Introduction

With the increase in intensive aquaculture, demand for more efficient aquafeed is rising. Feed comprises the principal operating cost in fish production and the main protein source has traditionally been fish meal (Glencross *et al.*, 2007). Fishmeal, the conventional protein source in aquaculture feeds, supports good fish growth because of its protein quality and palatability (Ng and Romano, 2013). However, fish meal is often scarce and expensive, due to limited availability and high demand, which often leads to high fish production costs (El-Sayed, 2004; Hardy, 2010). According to Ng and Romano (2013), cost-effective, practical aquaculture feeds can be produced without the use of fish meal with no resulting or apparent loss in fish growth in some species. Hence, replacing fish meal with cheaper ingredients of either animal origin or protein-rich plant sources is a necessary priority for nutrition research (Glencross *et al.*, 2007; Ng and Romano, 2013). In view of this, oilseed meals have been found to have considerable economic potential (Tacon, 1997; Ng and Romano, 2013). While grain legumes have not been widely used within aquaculture feeds, oilseeds and their by-products frequently constitute a major source of dietary protein within aquaculture feeds for warm water fish species such as those commonly used in African aquaculture, including tilapias (*Oreochromis spp.*) and African catfish (Hecht, 2007).

A feed ingredient may appear from its chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be ingested, digested and absorbed in the target species. Only a proportion of ingested food is digested and its nutrients absorbed, the rest is voided as faeces. By definition, digestibility is a relative measure of the extent to which ingested food and its nutrient components have been digested and absorbed by the animal. Knowledge of nutrient digestibility is, therefore, important to establish the potential of an ingredient for use in diets of aquaculture species (Allan *et al.*, 2000; Ng and Romano, 2013). Determining the digestibility of nutrients in feedstuffs is important not only to enable formulation of diets that maximize the growth of cultured species, by providing appropriate amounts of available nutrients, but also to limit the wastes produced by the fish and reduce costs (Allan *et al.*, 2000; Glencross *et al.*, 2007; Zhou and Yue, 2012; Ng and Romano, 2013).

For tilapia feeds typical protein sources examined have included cereal grain products (Guimaraes *et al.*, 2008b), defatted soybean meal, full-fat toasted soybean, lupin seed meal and faba bean meal (Fontainhas-Fernandes *et al.*, 1999), cottonseed meal, sunflower meal (El-Saidy and Gaber, 2003), fish and poultry meals, corn gluten, rapeseed meal, sorghum, barley (Sklan *et al.*, 2004), anchovy meal, corn gluten meal, soybean meal, gammarid meal and crayfish exoskeleton meal (Köprücü and Őzdemir , 2005). Among the plant protein sources, soybean meal has been used most widely because it has a good amino acid profile, which, as the main source of protein, supports fish growth (El-Sayed, 1999). Soybeans, however, are not grown widely in Ethiopia; hence there is a need to evaluate soybeans together with other more locally available plant proteins. According to Lovell (1998) feed ingredients containing 20% or more crude protein are considered protein sources. In the present study soybean cake (SBC), linseed cake (LSC) and Niger seed cake (NSC) were selected as dietary protein sources on the basis of

their high protein content, availability and use in animal feeds in Ethiopia. Studies conducted on SBC, LSC and NSC showed they have good protein contents (30-40%), depending on processing methods (Tadelle Dessie *et al.*, 2002; Kassahun Assaminew *et al.*, 2012).

Niger seed is the most important oil crop of Ethiopia, providing 50–60% of the country‘s indigenous edible oil (Riley and Belayneh, 1989; Wijnands *et al.*, 2007). It is also minor oil crop in India, Kenya, Uganda, Sudan, Malawi and other African and Indian sub-continent countries (Getinet Alemaw and Sharma, 1996). Its seeds are inexpensive to process, and the cake remaining after oil extraction is used as a protein supplement in animal diets (Tadelle Dessie *et al.*, 2002). Niger seed cake contains few or no known antinutritional factors (Getinet Alemaw and Sharma, 1996).

Ethiopia ranks among the top five world producers of linseed which is the second most important oil crop in the country next to Niger seed (Wijnands *et al.*, 2007). The usefulness of linseed as an ingredient in the diets of fish has been studied by different authors (Hassan *et al.*, 1997; Mukhopadhyay and Ray, 2001; 2005). Nutrient and energy digestibility studies have been conducted more extensively on soybean for many fish species than on LSC. However, digestibility of NSC in fish diets has not been researched into at all, probably because it is restricted to Eastern Africa, mainly Ethiopia.

This study was conducted to evaluate the apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein (CP) and gross energy (GE) for SBC, LSC and NSC for Nile tilapia, *O. niloticus*.

4.2 Materials and Methods

4.2.1 Experimental System and Animals

The source of experimental fish and their breeding are described in Section 3.1.3. Fingerlings of Nile tilapia of an average weight of 8.9 ± 1.58 g were stocked at 10 per tank (60 litre tank) in a water recirculation system (described in Section 3.1.1, Figure 3.1). There were three replicates for each treatment. Fish were fed, by hand, twice a day (10:00, 16:00) at a rate of 6% of their body weight per day. The experiment took 2 - 3 weeks. The recirculation system was supplied with aerated water from a sump tank thermoregulated at 28 ± 1 ^{o}C and a constant photoperiod of 12 hours Light/12 hours Darkness was maintained (Section 3.1.1). Water quality parameters measured during the experiment averaged (± SD): temperature, 28.91 ± 0.36 ^{o}C; pH, 7.3 ± 0.1; ammonia, 0.17 ± 0.08 mg l^{-1}; nitrite, 0.20 ± 0.1 mg l^{-1}; Nitrate, 50 ± 23.21 mg l^{-1} and dissolved oxygen, 5.39 ± 0.38 mg l^{-1} and they were within acceptable ranges for tilapia.

4.2.2 Diet Formulation

A reference diet (Table 4.1) was formulated to satisfy the nutrient requirements of Nile tilapia (NRC, 1993). It contained 320 g kg^{-1} crude protein, 100 g kg^{-1} lipid and 18 kJ g^{-1}. The test ingredients for apparent digestibility were soybean cake (SBC), linseed cake (LSC) and Niger seed cake (NSC). All test feed ingredients were obtained from commercial sources in Ziway Ethiopia with the exception of soybean cake which was acquired from Addis Ababa Oil Processing Factory outlet.

Table 4.1 Composition of reference and test diets (g kg^{-1}) for the digestibility study

Ingredients	Reference diet	Test diets
Test ingredient	-	298.5
Fish waste meal	407.6	285.32
Soybean meal	100	70
Wheat grain	20	14
Corn grain	392.4	274.68
Soybean oil	5.0	3.5
Vitamin mineral premix[1]	50	35
Carboxymethyl cellulose	20	14
Chromic oxide	5.0	5.0

[1]As listed in Table 3.1

Three test diets were formulated using 70% reference diet and 30% of each of the test ingredients as described by Cho *et al.* (1985). This method assumes that there are no interactions among the components of the diet during digestion (Cho *et al.*, 1982). Chromic oxide was used as an inert marker at a concentration of 0.5% in the diets. Other supplements used in the diet are indicated in Table 4.1. Diet preparation is described in section 3.2. Faeces collection in the tanks was conducted using collectors as described in section 3.1.2.

4.2.3 Analytical Techniques

Proximate analysis and gross energy of ingredients, diets, and faecal samples were conducted using the methods described in sections 3.2.1 and 3.2.3. Chromic oxide in diets and faecal samples was determined by acid digestion with molybdate reagent following the procedure described in section 3.2.2. Apparent digestibility coefficients of nutrients and energy of diets and ingredients were determined as described in section 3.3.5.

4.2.3 Statistical analysis

Each experimental diet was fed to three groups of fish in a completely randomised design. Data were analysed as described in section 3.5.

4.3 Results

4.3.1 Proximate composition, energy contents and prices of ingredients

Proximate composition and energy contents of the ingredients used in the study are given in Table 4.2. Crude protein for oilseed cakes ranged from 310-393.8 g kg^{-1} with SBC the highest and LSC the lowest. In contrast, crude lipid was highest for LSC (108.2 g kg^{-1}) and lowest for SBC (74.4 g kg^{-1}). NSC had the highest crude fibre (201.1 g kg^{-1}) level, about three times higher than SBC which had the lowest fibre content (64.8 g kg^{-1}). Gross energy values for ingredients ranged from 17.9 - 21.8 kJ g^{-1}.

Table 4.2 Proximate composition (g kg^{-1} as fed), energy content (kJ g^{-1}) and prices (birr kg^{-1}) of individual feed ingredients used in this study.

Ingredients	DM	CP	CL	CF	Ash	NFE	GE	Price
Linseed cake	908.8	310	108.2	136.3	82.7	233.4	18.6	7.5
Niger seed cake	928	324.2	92	201.1	90.7	220	18.1	4.0
Soybean cake	938	393.8	74.7	64.8	54	350.7	19.3	8.0
Fish waste meal	950	610.9	187.1	0	220.4	0	21.8	0.5
Wheat grain	875	96	16.5	57.9	13.6	690.9	17.9	8.0
Corn grain	882.9	78.1	42.6	27.1	13.0	722.2	19.0	5.0

*DM (dry matter), CP (crude protein), CL (crude lipid), NFE (nitrogen free extract) and GE (gross energy).

The prices of ingredients used in the study are shown in Table 4.2. Fish meal was the least expensive (0.5 birr kg^{-1}) ingredient as the cost for it is directly converted from the cost of fresh offal (0.15 birr kg^{-1}) and 3.33 kg of offal dried in an oven can make approximately 1kg of dried fish meal. SBC and wheat grain were the most expensive ingredients, about double the price (4 birr kg^{-1}) of NSC which was the least expensive among the oilseed cakes.

4.3.2 Proximate Composition and Energy Contents of Test Diets

Proximate and energy compositions of the reference and test diets used in the digestibility study are presented in Table 4.3. Analysed crude protein, crude lipid, NFE, dry matter, ash and energy contents of test diets showed little variation. However, crude fibre contents of diets varied

considerably. Crude fibre of test diets followed similar trend as the test ingredients. Energy contents of the diets ranged between 18.6 and 18.9 kJ g^{-1}.

Table 4.3 Proximate composition (g kg^{-1}) and energy of reference and test diets

		Test diets		
Components	Reference diet	SBC	NSC	LSC
Dry matter	922.8	927.8	924.6	918.9
Crude protein	321.0	341.0	320.3	316.1
Crude lipid	105.8	96.0	101.1	106.0
Crude fibre	18.6	32.2	72.8	53.5
Ash	126.1	98.8	109.8	107.4
NFE	351.5	359.5	320.6	336.0
Chromic oxide	5.2	4.8	4.7	4.9
Gross energy (kJ g^{-1})	18.9	18.9	18.6	18.7

SBC= soybean cake, NSC= Niger seed cake, LSC= Linseed cake

4.3.3 Nutrient and Energy Digestibility

Apparent digestibility coefficients (ADCs) of protein, lipid, dry matter and energy in selected test ingredients for Nile tilapia are shown in Table 4.4. The results indicated that ADCs of the nutrients and energy studied were significantly different between the test ingredients except for crude lipid digestibility. Generally SBC had the highest ADC coefficients followed by NSC with LSC having the least ADC for energy and nutrients.

Table 4.4 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy and digestible protein and energy (g kg^{-1} and kJ g^{-1} respectively, dry weight basis) in the test ingredients for Nile tilapia.

Components	Soybean	Niger seed	Linseed
Dry matter	78.0 ±2.6[a]	70.7 ±3.0[b]	59.0 ±0.5[c]
Crude protein	87.9 ±3.2[a]	72.6 ±2.0[b]	62.4 ±4.2[c]
Crude lipid	81.4 ±2.23[a]	78.9 ±2.8[a]	79.4 ±3.0[a]
GE (kJ g^{-1})	86.0 ±2.5[a]	72.9 ±1.8[b]	53.7 ±3.5[c]
DP	369	253	213
DE	17.7	14.2	11.0

***** GE (gross energy), DP (digestible protein) and DE (digestible energy)

4.4 Discussion

The suitability of three oilseed by-products (SBC, NSC and LSC) available in Ethiopia were evaluated for their proximate composition and ADC values with the aim of providing information that aids improved formulation of balanced diets for Nile tilapia. The ADC values for dry matter, protein and energy were significantly different between the three test ingredients. However, lipid digestibility was not significantly different between the test ingredients.

In this study the test ingredients used had a high crude protein content and their values were close to previously reported values by Kassahun Assaminew *et al.* (2012), except for relatively higher crude protein (32.4% Vs 28.1%) and lower crude fibre (25.3% Vs 20.1%) values reported for Niger seed cake in this study. This variation between nutrient compositions of NSC could be

due to differences in the origin, state and processing methods used to produce the cakes. The high crude fibre content of Niger seed cake could limit the inclusion of this ingredient at higher levels in the diets of fish. It has been reported that dietary fibre is not utilized by fish (Ng and Romano, 2013).

The digestibility of ingredients provides insight concerning nutrient utilization and should enable better ingredient substitutions in diets designed for target species. The nutrient digestibility will vary depending on the composition of ingredients used (Glencross *et al.*, 2007; Zhou and Yue, 2012; Ng and Romano, 2013). The results of this study showed that ADC for dry matter, crude protein and energy in test ingredients were affected by test ingredients. These differences can be explained by the differences in chemical composition, origin and processing of these feed ingredients. The results of the present study indicated that Nile tilapia fingerlings have the capacity to digest protein and lipid satisfactorily in the oilseed by-product ingredients tested.

The overall dry matter digestibility of the test ingredients in the present study ranging from 59% to 78% is in the range reported for plant protein-rich products (46-86.2%) in the diets of Nile tilapia (Fontainhas-Fernandes *et al.*, 1999; Guimaraes *et al.*, 2008a; Zhou and Yue, 2012). Dry matter digestibilities in this study were generally lower than those reported for Nile tilapia elsewhere (Köprücü and Őzdemir, 2005). For example, the lower dry matter ADC of soybean in the present study could be explained by the higher crude fibre content of the product evaluated in this study of 69 g/kg, compared with 39 g/kg in the study by Köprücü and Őzdemir (2005). Other studies on fish have also indicated the negative correlation between crude fibre content and dry matter ADC (Maina *et al.*, 2002; Guimaraes *et al.*, 2012; Asad *et al.*, 2013). In general, results of dry matter ADC can be used to estimate the amount of solid waste released to the

environment and to help determine the environmental impacts of aquaculture production (Allan *et al.*, 2000; Guimaraes *et al.*, 2012).

Generally, the protein quality of dietary ingredients is one of the leading factors (apart from palatability) affecting fish performance and protein digestibility (digestible protein) is the first measure of its availability to fish. Protein quality of dietary protein sources depends on the amino acid composition and their digestibility. In the present study, the values obtained for protein digestibility for SBC corroborate previous findings (87.4%-96.2%) for soybean meal in tilapia diets (NRC, 1993; Fontainhas-Fernandes *et al.*, 1999; Sklan *et al.*, 2004; Köprücü and Őzdemir, 2005; Guimaraes *et al.*, 2008a; Zhou and Yue, 2012). Protein digestibility of test ingredients LSC (62.4%) and NSC (72.6%) for Nile tilapia in this study was lower than the reported digestibility coefficients of various other oilseed meals for this species. For example, reported APD in tilapia were 78.5% for cottonseed meal (Guimaraes *et al.*, 2008a), 85% for rapeseed meal (Sklan *et al.*, 2004), 77.6% for peanut meal, 77.8% for canola meal, and 84% for de-gossypoled cottonseed meal (Zhou and Yue, 2012).

Lower values of LSC protein digestibility in this study could be explained by other dietary factors present in plant protein products such as: i) suboptimal amino acid balance (NRC, 1993); ii) presence of antinutritional factors (Liener, 1994); and iii) inadequate levels of energy in linseed meals (El-Saidy and Gaber, 2001). Plant products, especially oilseed cakes, usually have poor amino acid profiles and a certain amount of antinutritional factors (ANFs) which could affect nutrient utilization and, consequently, animal growth performance in different degrees depending on the type and amount of the compound (Tacon, 1997; Mukhopadhyay and Ray, 2001; 2005; Latif *et al.,* 2008). Although linseed meal has been reported to have one of the best amino acid profiles after soybean meal and the composition fulfills the requirements of amino

acid for Nile tilapia, the biological availability of amino acids in linseed to tilapia is less (Hanafy, 2006). Linseed contains mucilage (5-8%) which has a large capacity to bind to water and increases intestinal viscosity, thus reducing nutrient digestibility (Fedeniuk and Biliaderis, 1994). Major antinutritional factors known to be present in linseed include: cyanogens, phytic acid, tannins, estrogenic factors, antithiamine factor and antipyridoxine factor (Tacon, 1997). For example, the ANF phytic acid has the ability to non-selectively bind to proteins, carbohydrates and minerals (divalent cations such as Ca^{2+}, Fe^{2+} Mg^{2+}) and inhibit activities of a number of digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994).

The lower protein digestibility coefficients obtained for the two test ingredients (LSC and NSC) in the present study could not only be attributed to the ANFs but also to the higher levels of crude fibre that interfere in protein digestion of the diets that contain LSC and NSC. Previous studies (Maina *et al.*, 2002; Sklan *et al.*, 2004) indicated that feeds with high crude fibre contents have poor nutrient digestibility due to reduced enzymatic access to potential substrates or due to the direct interaction between crude fibre components and the digestive process. Fibre levels as high as 8–12% are tolerated by most fish, but such levels often result in growth depression (Leary and Lovell, 1975; Edwards *et al.*, 1977). Fish fed diets high in indigestible fibre increase their feed intake and gastric evacuation time, but the extent to which fish can compensate in this manner is limited (Leenhouwers *et al.*, 2007).

ADC values of fats in fish range from 85% to 95% when administered routinely either alone or in a mixed diet (Aksnes and Opstvedt, 1998). Reported fat digestibility in other species ranged from 70% to 90% (Lupatsch *et al.*, 1997; Sklan *et al.*, 2004) and similar values were found for tilapia in this study (78.8-81.3%). The ADCs of energy (53.6%-85.9%) in test ingredients for Nile tilapia in this study are generally in agreement with that reported (39-89%) and (54.8-

92.1%) by Sklan *et al.* (2004) and Köprücü and Őzdemir (2005), respectively. Variation in apparent GE digestibility coefficients of ingredients in this study followed the same trend as that of protein and DM digestibility.

In the present study all three oilseed cakes tested proved valuable as protein sources in the diets of Nile tilapia as indicated by their ADCs. However, best values were observed for SBC. NSC, which was less than half the cost of SBC, appeared to be a good protein feed ingredient for Nile tilapia diets on balance in terms of overall nutrient composition and acceptable digestibility coefficients despite the highest crude fibre content. The LSC generally performed poorly, although it contained 310 g/kg CP. The nutrient and energy digestibilities were very low except for lipid digestibility. It seems that the ANF present in linseed cake may be responsible for the low ADC values. However, further research is required to establish the effect of dietary inclusions of LSC and NSC on productivity and on the various potential methods of increasing their utilization in fish diets before considering these ingredients in production feeds. The results of this digestibility study should contribute towards a better understanding of the nutrition of this species, especially in the grow-out stages.

Chapter 5- Effect of dietary inclusions of linseed cake on growth performance and feed utilization of Juvenile Nile tilapia (*Oreochromis niloticus*)

5.1 Introduction

Intensification of aquaculture practices has contributed to a global increase in aquaculture production of about 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO, 2007). This increase has only been possible because of the increase in the production of formulated diets. Feed is a vital part of the operations cost (30-70%) in fish farming (Rumsey, 1993; El-Sayed, 2004). As a protein source of choice, fish meals are highly sought after for many formulated aquaculture diet (Glencross *et al.*, 2007). Fish meals provide high contents of essential amino acids, are low in carbohydrates, are usually well digested and induce good growth responses in cultivated species. However, fishmeal in world markets is not readily available and the price is ever increasing (Hardy, 2006).Therefore, in order to develop economically viable aquaculture systems it is necessary to look for cheap, locally available alternative protein sources, especially those unsuitable for direct human consumption.

A number of published reports are available regarding the suitability of animal products including poultry by-products, meat, bone and blood meals as substitutes for fish meal in fish diets (El-Sayed, 1998; Robaina *et al.*, 1997; Hernandez *et al.*, 2010). However, plant products offer a more suitable option as alternative protein sources in fish feeds although they often contain antinutritional factors, which can affect growth performance and fish health (El-Sayed, 1999; Francis *et al.*, 2001; Gatlin III *et al.*, 2007). The suitability of plant meals at a variety of different levels of fish meal substitution has been studied in several fish species (Tacon, 1993;

Hossain and Jauncey, 1989; Kaushik *et al.*, 1995; Thiessen *et al.*, 2003; Latif *et al.*, 2008; Dadgar *et al.*, 2010). Inclusion of plant protein above 25-50% of the total diet has frequently been reported to result in reduced growth and/or high mortalities attributed to an imbalance of indispensable amino acids, reduced digestibility of lipid and energy, presence of antinutritional factors and/or poor palatability (Tacon, 1993; Hasan *et al.*, 1997; Francis *et al.*, 2001; Latif *et al.*, 2008). Apart from legume seeds, oilseed and fruit by-products, such as groundnut, sunflower, linseed, rapeseed and cottonseed have been found potentially good sources of protein for farmed tilapia (Tacon, 1997; El-Saidy and Gaber, 2004; Mbahinzireki *et al.*, 2001; Latif *et al.*, 2008).

Ethiopia ranks among the top 5 world producers of sesame seed and linseed and is an important producer of Niger seed (Wijnands *et al.*, 2007). Linseed cake, a by-product after oil extraction from linseed (*Linum usitataissimum*), has a nutritional quality comparable with most oilseeds as it contains similar proportions of protein, lipid, minerals and other nutrients (Tadelle Dessie *et al.*, 2002; Kassahun Assaminew *et al.*, 2012), and its potential as a dietary protein source for animal feeds is well recognized particularly in Ethiopia (Nega Tolla *et al.*, 2001; Belay Duguma *et al.*, 2014).

Studies on the utilization of linseed meal as a dietary protein source have been conducted in a number of animals, both terrestrial and aquatic. In terrestrial animals different researchers have studied the suitability of dietary linseed cake incorporation (Nega Tolla *et al.*, 2001; Belay Duguma *et al.*, 2014). In aquatic animals linseed meal is often used as a dietary ingredient in aquafeeds. Hasan *et al.* (1991) used linseed meal as a feed ingredient successfully in the diet of *Labeo rohita*. Hossain and Jauncey (1989) studied the protein, energy content, and amino acid digestibility of linseed meal. The low apparent protein digestibility of linseed might be due to the presence of mucilage in it. In addition, Hossain and Jauncey (1989) investigated the nutritive

value of linseed meal of Bangladeshi origin and tested it in a diet of common carp, *Cyprinus carpio*. Mukhopadhyay and Ray (2005) studied the effect of fermentation on nutrient digestibility of linseed meal in the diets of Rohu carp, *L. rohita.* The availability of well-established information on the suitability of linseed meal as the alternative protein source in tilapia diet is scanty. The intensification of tilapia culture necessitates the development of biologically effective and acceptable feeds for complete and supplementary feeding. This, therefore, justifies investigating the use of linseed cake in tilapia feeding.

This study evaluates the nutrition potential of linseed cake (mechanically extracted) as alternative plant protein feedstuff in Nile tilapia diets (*O. niloticus*).

5.2 Materials and Methods

5.2.1 Experimental System and Animals

The experimental systems described in section 3.1.1 and section 3.1.2 were used for the growth trial and faecal collection, respectively. Each tank was supplied with a thermoregulated recirculating water at a flow rate of 2 litres min^{-1} and a photoperiod of 12 hours light/12 hours darkness (Section 3.1.1). Supplemental aeration was provided from a compressor using an air tube system and air stones. Water quality was monitored weekly during the experiment and parameters were averaged (± SD): temperature, 28.0 ± 0.6 ^{o}C; pH, 7.5 ± 0.2; ammonia, 0.19 ± 0.04 mg l^{-1}; nitrite, 0.2 ± 0.1 mg l^{-1}; nitrate, 20 ± 13.2 mg l^{-1} and dissolved oxygen, 5.94 ± 0.17 mg l^{-1} and they were within acceptable ranges for tilapia.

Mixed-sex Nile tilapia fingerlings with an average (± SD) weight of 3.33 ± 0.2 g were stocked at 20 fish per tank (60 litres tank) in triplicates. Fish were fed, by hand, four times a day (8:00,

11:00, 14:00, 17:00) at a rate of 6-10% of their body weight per day. Feeding rates were adjusted every week and the experiment lasted eight weeks (Figure 5.1). Each ration was dispensed over a period of 10 minutes in small portions in an attempt to minimise feed wastage. The quantity of food fed was recorded for subsequent determination of feed conversion ratios and feed utilization.

5.2.2 Diet formulation and preparation

Four isonitrogenous (320 g kg^{-1} protein), isolipidic (100 g kg^{-1} lipid) and isoenergetic (18 kJ g^{-1}) diets were formulated for the experiment (Table 5.1). The control diet was formulated with fishmeal as the major source of protein. Linseed cake was incorporated in the diet at inclusion levels of 20% (LSC20), 40% (LSC40) and 60% (LSC60). Diet preparation and other ingredients used were similar to those described in section 3.2.

Table 5.1 Composition of diets (g kg^{-1} as-fed) fed to juvenile Nile tilapia (*O. niloticus*) with varying inclusion levels of linseed cake

	Control	LSC20	LSC40	LSC60
Linseed cake	-	200.0	400.0	600.0
Fish waste meal	486.0	398.3	298	199.2
Wheat grain	156.4	135	100	50
Corn grain	270	160	126	74.8
Vitamin mineral premix[1]	50	50	50	50
Soybean oil	12.6	31.7	1	1
CMC[2]	20	20	20	20
Cr_2O_3	5	5	5	5

[1]As listed in Table 3.1 [2]Carboxymethyl cellulose (high viscosity)

5.2.3 Faeces Collection

At the end of the growth trial faecal collectors were fitted to rearing tanks and faeces were collected for two weeks (see section 3.1.2 for details). Faecal samples from each tank were pooled to represent respective treatments and immediately centrifuged, stored and later prepared for chemical analysis as described in section 3.1.2. Apparent digestibility coefficients of nutrients and energy of diets were determined as described in section 3.3.5.

5.2.4 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by methods described in sections 3.2.1. Energy contents of diets, faeces and carcass were analysed by methods described in section 3.2.3. Chromic oxide content of the diets and faecal samples were determined by the method given in section 3.2.2.

5.2.5 Analysis of Experimental Data

Growth performance and feed utilization were calculated as described in section 3.3.

5.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish in a completely randomised design. Data were analysed as described in section 3.5.

5.3 Results

5.3.1 Proximate Composition and Energy Contents of Diets

Proximate composition and energy contents of experimental diets are presented in Table 5.2. Crude protein contents varied little between the diets (318.31 - 333.0 g kg^{-1}) as did crude lipid (107.05 – 136.82 g kg^{-1}), ash (120.61-138.19 g kg^{-1}) and nitrogen free extract (275.3-309.19 g kg^{-1}). Energy levels in all experimental diets varied little within a narrow range (17.86-19.05 kJ g^{-1}). Crude fibre content in diet 2 (LSC20) was almost two times the level of crude fibre in the control diet (24.38 g kg^{-1}). The highest crude fibre content was found in diet 4 (LSC60) which is more than three times the crude fibre in the control diet.

Table 5.2 Proximate and energy composition of diets fed to Nile tilapia in this study.

Components (g kg^{-1})	Control	LSC20	LSC40	LSC60
	1	2	3	4
Dry matter	922.33	924.02	919.18	918.12
Crude protein	333.0	330.79	325.49	318.31
Crude lipid	117.57	136.82	107.05	107.19
Crude fibre	24.38	47.42	71.74	94.72
Ash	138.19	133.69	127.2	120.61
NFE	309.19	275.3	287.7	277.29
Gross energy (kJ g^{-1})	18.99	19.05	18.16	17.86

NFE= nitrogen free extract

5.3.2 Growth Performance

Growth responses of Nile tilapia fed the experimental diets are presented as initial and final mean weights, percentage weight gain (WG) and specific growth rate (SGR) in Table 5.3. There were significant differences in growth among treatments, with the exception of final body weights of LSC20 and the control diet. Growth performance tended to decrease with increasing levels of plant protein (Figure 5.1). The highest WG was recorded for the control diet (365.8%) and the least was for diet 4 (221.3%). SGR followed the same trend.

Table 5.3 Growth and feed utilization (per fish) of juvenile Nile tilapia fed linseed cake based diets.

Parameter	Control 1	LSC20 2	LSC40 3	LSC60 4
IBW	3.34±0.115^{a}	3.2±0.195^{a}	3.24±0.15^{a}	3.29+0.19^{a}
FBW	15.54±0.55^{a}	12.9±0.29^{b}	11.4±0.43^{c}	10.6+0.34^{c}
WG	365.8±24.63^{a}	303.4±32.5^{ab}	252.5±26.8^{bc}	221.3±20.1^{c}
SGR	2.76±0.09^{a}	2.5±0.14^{ab}	2.2±0.12^{bc}	2.08+0.1^{c}
S	95±5^{a}	98.33±2.9 ^{a}	95±5 ^{a}	98.33±2.9 ^{a}
FCR	2.44±0.09^{a}	2.6±0.09^{ab}	2.96±0.3^{bc}	3.3±0.17^{c}
FI	32.3±0.35^{a}	27.4±0.4^{b}	26.23±0.92^{b}	26.08±0.3^{b}
PER	1.13±0.04^{a}	1.06±0.04^{ab}	0.95±0.09^{bc}	0.87±0.04^{c}
PPV	20.57±1.2^{a}	16.73±0.9^{ab}	15.0±1.26^{bc}	13.3±0.82^{c}
ER	13.56±0.8^{a}	11.5±1.0^{ab}	9.13±1.0^{bc}	8.26±0.46^{c}

Values are means±SD (n= 3) and values within the same row with different letters are significantly different (P>0.05).

(IBW) initial body weight; (FBW) final body weight; (WG) weight gain; SGR (specific growth rate); (S) survival; (FCR) feed conversion ratio; (FI) feed intake; (PER) protein efficiency ratio; (PPV) Productive protein value; and (ER) energy retention.

5.3.3 Feed Utilization

Feed intake (FI) of the different diets ranged between 26.08 g and 32.3 g per fish at the end of the experiment, with the control diet having significantly ($P< 0.05$) higher FI than diets containing linseed cake. FI tended to decline with increasing levels of plant protein; however, no significant differences ($P> 0.05$) were found between diets containing linseed cake. The decline in feed intake was accompanied by the decrease in feed conversion ratio (FCR) and LSC40 and LSC60 had significantly ($P< 0.05$) lower FCR than the control. This was also true for protein and energy utilization.

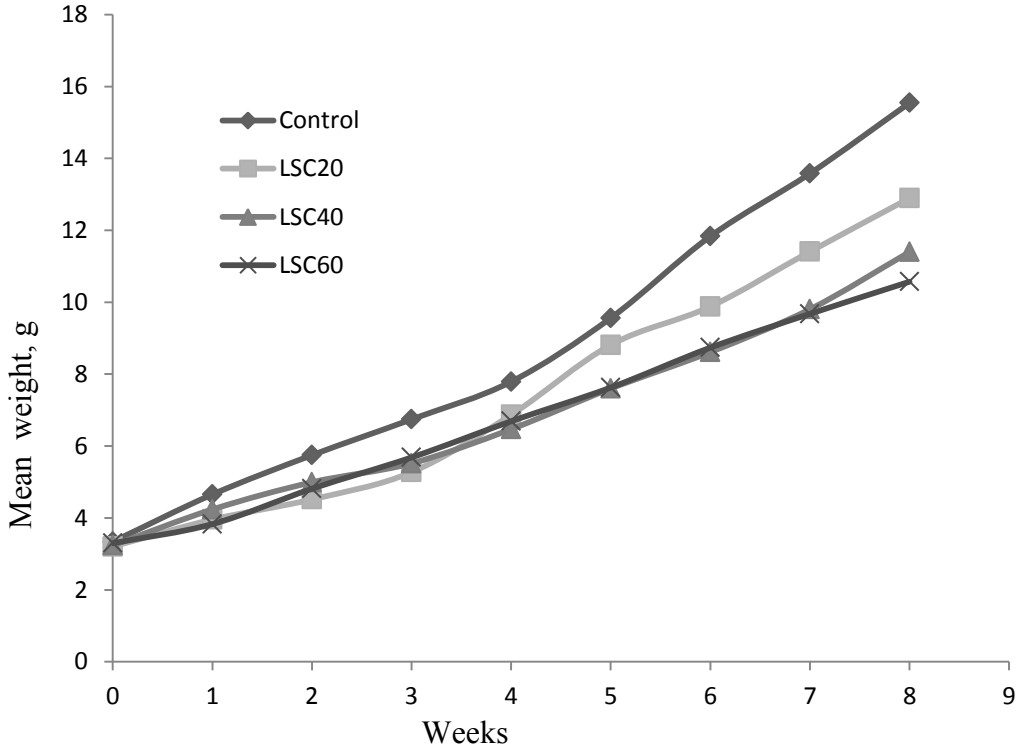

Figure 5.1 Growth response of Nile tilapia fed linseed cake based diets for eight weeks (56 days).

5.3.4 Apparent Nutrient Digestibility

Apparent nutrient digestibility is shown in Table 5.4. Apparent dry matter digestibility (ADMD) of diets ranged from 63% to 79.6%. ADMD generally decreased with increased levels of oilseed cake. Apparent protein digestibility (APD) for all diets was moderately high ranging from 65.4 to 89.8%. The control diet had the highest APD (89.8%) followed by diet with 20% level of linseed cake inclusion and diet 4 (LSC60) had the lowest APD with digestible protein also following the same trend. APD in general decreased with increasing plant protein inclusions in the diet. Apparent lipid digestibility (ALD) was higher than all the other nutrients studied except APD of the control diet. In this study, the values observed for ALD of diets 2 (LSC20) were higher than the control. ALD ranged from 75.92 to 88.83% with diet 4 having the lowest value. Apparent energy digestibility ranged from 72.0 to 84.6%. Similar trend in energy digestibility was observed as that of the lipid digestibility.

Table 5.4 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy and digestible protein and energy (g kg^{-1} and kJ g^{-1}, respectively, dry weight basis) in the linseed cake-based diets for Nile tilapia.

	1	2	3	4
Components	Control	LSC20	LSC40	LSC60
Dry matter	79.6	77.94	69.46	63.05
Crude protein	89.75	87.8	73.5	65.42
Crude lipid	85.55	88.83	80.04	75.92
Gross energy	82.98	84.6	79.5	72.04
Digestible protein	298.8	290.4	239.3	208.2
Digestible energy	15.75	16.1	14.43	12.86

5.3.5 Body Composition

Whole body proximate composition for initial and final fish samples is presented in Table 5.5. The MC of carcasses increased with an increase in the levels of plant in tilapia diet. Fish fed the control diet and diets with 20% linseed cake inclusions had significantly ($P< 0.05$) lower moisture contents. The CP, CL and GE contents of whole body of fish were significantly affected by the inclusion of linseed cake in Nile tilapia diets. Compared to that of the control diet the other diets did not affect the ash contents in the whole body composition of fish.

Table 5.5 Whole Body Proximate Composition (% wet Weight) and Energy of Nile Tilapia Fed linseed Cake-Based Diets

Components		1	2	3	4
	Initial carcass	Control	LSC20	LSC40	LSC60
MC	78.1	76.5±0.26[a]	76.4±0.1[a]	77.16±0.22b	77.48±0.18b
CP	15.33	16.42±0.42[a]	15.68±0.19[b]	14.68±0.2[c]	14.35±0.15[c]
CL	5.28	7.29±0.16[a]	5.33±0.26[b]	5.13±0.1[b]	5.14±0.12[b]
Ash	3.72	4.17±0.07[a]	4.17±0.11[a]	4.17±0.08[a]	4.28±0.11[a]
GE	5.7	6.16±0.15[a]	5.71±0.24[b]	5.12±0.1[c]	5.12±0.1[c]

(MC) moisture content; (CP) crude protein; (CL) crude lipid; and (GE) = gross energy. Values are mean ± SD of three replicates, and values within the same row with different letters are significantly different ($P < 0.05$).

5.4 Discussion

The study showed that increasing inclusion levels of linseed cake in the diets of Nile tilapia resulted in a significant decrease in feed utilization and growth parameters. Several authors (Mukhopadhyay and Ray, 2001; 2005; Davies *et al.*, 2000; Fagbenro and Davies, 2000; Mbahinzireki *et al.*, 2001; Agbo *et al.*, 2011a; b) have reported similar trends with plant proteins. Growth performance and feed utilization of fish fed diet with 20% level of linseed cake was found to be higher and no significant difference was observed between fish fed with this diet and that of the control diet. Results of growth performance and feed utilization in this study agree

well with that of Hasan *et al.* (1997) who reported low inclusion levels of linseed (25% protein) is possible in the diets of carp without any negative effect on feed intake and growth performance. Growth reduction observed at higher inclusion levels of linseed could be related not only to dietary amino acid profile but also to the presence of antinutritional factors (ANF). Davies *et al.* (2000) also reported higher inclusions of certain oilseed meal recorded poor growth and nutrient utilization by Nile tilapia. Although linseed meal has been reported to have one of the best amino acid profiles after soybean meal (Hanafy, 2006), the biological availability of amino acids in linseed to tilapia is less. Linseed contains a non-starch polysaccharide (NSP) called mucilage (5-8%) which has a large capacity to bind to water and increases intestinal viscosity (Fedeniuk and Biliaderis, 1994). High digesta viscosity, produced as a result of mucilage, delays gastric emptying and feed transit time, and decreases interaction of the intestinal enzymes with feed macromolecules, resulting in decreased nutrient availability (Leenhouwers *et al.*, 2007; Sinha *et al.*, 2011). Hossain *et al.* (2001) reported a reduction of growth and feed utilization in common carp fed diets containing *Sesbania* endosperm at 7.2% or more. They suggested that the NSP in Sesbania increased the viscosity of the digesta thereby affecting nutrient absorption and utilization. Diets containing soybean (from flaked soybean husk) fed to Atlantic salmon gave lower digestibility than those that were fed with diet containing soybean meal with reduced oligosaccharides (Refstie *et al.*, 1999). The effect of the former soybean product was associated with the antinutritional effects of NSPs fractions. This suggests that the NSP content of plant feed ingredients may also influence the nutritional value of its own protein. Major antinutritional factors known to be present in linseed include: Cyanogens, Phytic acid, Tannins and Estrogenic factors (Tacon, 1997). For example, the ANF

phytic acid has the ability to non-selectively bind to carbohydrates, protein and inhibit activities of a number of digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994).

Phytic acid also negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus (NRC, 1993; Liener, 1994; Tacon, 1997). It has the ability to chelate with not only proteins but also mineral cations such as K^{+}, Ca^{+2} , Mn^{+2}, Zn^{+2}, Mg^{2+}, Cu^{+2}, and Fe^{+3} at the pH occurring in the intestine of monogastric animals like fish (Makkar *et al.*, 2007) and therefore rendering them unavailable for absorption. Immature linseed contains a small amount of the cyanogenetic glucoside, linamarin, which in the presence of an associated enzyme, linase, liberates hydrogen cyanide (HCN) on hydrolysis. Unprocessed whole seeds, and linseed meal processed under low temperature, can be toxic to animals especially if wetted before being fed (Gohl, 1981; McDonald *et al.*, 1981). Normal processing involving high temperature treatment, however, destroys linase and most of the linamarin, and the resultant meals are quite safe (Gohl, 1981; McDonald *et al.*, 1981). Makkar *et al.* (2007) reported that tannins have an astringent bitter flavour and could also be the reason for reduced feed intake. In the present study, the low feed intake and feed utilization at higher levels of linseed cake inclusion could be attributed to the high fibre content, unbalanced amino acid as well as ANF present in the diets, which may have accounted for poor palatability. However, it seems that at a lower level of inclusion, there is a physiological mechanism in fish to compensate for the presence of ANFs hence their negative effects will not be felt (Francis *et al.*, 2001).

The observed higher body moisture content of fish fed high levels of linseed cake is in agreement with the results reported for carp fed diets containing high amounts of oilseed meals (Hossain, 1988). The observed significant reduction in body lipid, protein and energy content in those fish fed linseed containing diets was probably due to poor feed intake. For example, poor feed intake

has been associated with mobilization of body lipid reserves to meet energy requirements for vital body functions (Madalla *et al.*, 2013). The presence of mucilage and other ANFs may also contribute to inhibited enzymatic actions of protein and lipid digesting enzymes and hence delayed intestinal absorption of dietary nutrients (Han *et al.,* 2000). The characteristics of ANF tannin in forming complex with enzymes, as stated by Chung *et al.* (1998), seems to be one of the causes of lower body lipid content in fish carcasses in the group which were fed linseed cake containing diets. Experiments utilizing several plant protein sources containing tannin in fish feed demonstrated low lipid deposit in the carcasses and viscera (Hossain *et al.*, 2001a; 2001b).

Overall, protein digestibility was high for the control diet (89.75%) and LSC20 (87.8%). These results corroborate the findings of Agbo *et al.* (2011a) for groundnut by-product based diets in Nile tilapia. It is well known that plant proteins are highly digestible when effects from ANFs are avoided. For example, phytic acid often forms poorly digestible phytate-protein complexes. For this and other reasons, recommended levels for these compounds in feeds are below 5 mg g^{-1}. The present study supports previous evidence that this limit can be easily achieved at lower inclusion levels of plant products (Francis *et al.*, 2001). Digestible protein and energy values followed the same trend as the ADCs. Lipid digestibility decreased with the increase in plant protein inclusion levels except for LSC20, in which digestibility appeared to be slightly enhanced relative to the control. Such slight increase in lipid digestibility is likely in the present study, given that higher levels of soybean oil has been added to LSC20.

In the development of competitive feed formulas, considerations of cost are highly important. As with any new formulation involving raw materials whose market prices varies, meaningful assessment of cost is difficult. In the feed mixture used in this study, the most crucial component

with respect to cost were wheat grain and linseed cake, the price of which is determined by the seasonal availability of the ingredients (pers. Observ.).

Chapter 6- Effect of dietary inclusions of Niger seed cake on growth performance and feed utilization of Juvenile Nile tilapia (*Oreochromis niloticus*)

6.1 Introduction

Recent predictions by the United Nations Food and Agricultural Organisation indicate that aquaculture‘s contribution will supplant capture fisheries as the world‘s leading source of aquatic products by 2030 (FAO, 2014). Intrinsic to this rapid growth is the emergence of important tropical freshwater species including Nile tilapia (*Oreochromis niloticus*) as a significant new source of whitefish on the global market. This species becomes popular worldwide because of its easy reproduction, adaptability to intensive culture, acceptability of low input and sustainable feeds, resistance to impaired water quality, and widespread consumer acceptance (Fitzsimmons *et al.*, 2011; Ng and Romano, 2013). By production volume, tilapia is one of the largest freshwater aquaculture species worldwide and is mostly produced using semi-intensive systems in developing countries (FAO, 2014). A considerable improvement in the development of plant based aquafeeds is considered as one of the major driving forces responsible for the growth of tilapia culture in the world (Tacon *et al.*, 2011). The use of alternative plant proteins in fish feeds helped reduce feed cost without significantly reducing yield, hence reducing overall production costs. This has resulted in increased assessment of potential feed ingredients for future use in aquaculture (Ng and Romano, 2013). At present, plant protein choice and selection are based upon a combination of local market availability and cost, and the nutritional profile (including antinutrient content and level) of the plant meal in question (Gatlin III *et al.,* 2007; Krogdahl *et al.,* 2010). Plant protein is generally supplied through different cereals, their by-products and cakes/meals of oil seeds like cottonseed, sesame seed, sunflower seed, linseed, soybean and canola seed. Use of soybean and its products for the purpose of partial replacement of fish meal

in diets of tilapia with considerable success has been reported (El-Sayed, 1999; El-Saidy and Gaber, 2002). However, identifying other sustainable alternatives to the conventionally used plant based ingredients is highly envisaged for the growing aquaculture sector. One such feed ingredient that is novel for aquaculture is Niger seed cake.

Guizotia abyssinica (Niger) is an oilseed crop that is cultivated mainly in Ethiopia and India. Niger plant belongs to the same botanical family as sunflower and safflower (Getinet Alemaw and Sharma, 1996). Niger seed provides approximately 50-60% of Ethiopian edible oil supply (Riley and Belayneh, 1989). Its seed contains oil which makes up 42-44% of the seed weight and produces high quality oil due to the presence of about 70% linoleic acid (Kifle Dagne and Johnsson, 1997).

Niger seed cake is a by-product of oil seed crushing during industrial oil extraction. The cake has a relatively high crude protein content which varies from 30% (Getinet Alemaw and Sharma, 1996) to 34.5% (Ameha Sebsibe *et al.*, 2007). The protein in Niger seed cake contains high levels of sulphur containing amino acids and compares well with soybean meal and cotton seed meal (Tadelle Dessie *et al.*, 2002), which are usually regarded as a source of high quality plant proteins (El-Sayed, 1999; Ng and Romano, 2013). Niger seed cake contains few or no known antinutritional factors (Getinet Alemaw and Sharma, 1996).

Niger seed cake is the most widely used protein supplement in animal feed in Ethiopia (Bulcha Weyessa, 2007). There have been several studies on utilisation of Niger seed cake (NSC) as a protein source in terrestrial animals such as cattle (Nega Tolla *et al.*, 2001), goats (Ameha Sebsibe *et al.*, 2007), and poultry (Solomon Demeke, 2007). No studies, however, have yet examined the suitability of Niger seed cake as protein source in fish diets.

The objective of the present study, therefore, was to evaluate the suitability of Niger seed cake as a protein source for Nile tilapia.

6.2 Materials and Methods

6.2.1 Experimental System and Animals

The experimental systems described in sections 3.1.1 and 3.1.2 were used for the growth trial and faecal collection, respectively. Each tank was supplied with a thermoregulated recirculating water at a flow rate of 2 litres min^{-1} and a photoperiod of 12 hours light/12 hours darkness (Section 3.1.1). Supplemental aeration was provided from a compressor using an air tube system and air stones. Water quality was monitored weekly during the experiment and parameters were averaged (± SD): temperature, 28.0 ± 0.6 ^{o}C; pH, 7.5 ± 0.2; ammonia, 0.19 ± 0.04 mg l^{-1}; nitrite, 0.2 ± 0.1 mg l^{-1}; Nitrate, 20 ± 13.2 mg l^{-1} and dissolved oxygen, 5.94 ± 0.17 mg l^{-1} and they were within acceptable ranges for tilapia.

Mixed-sex Nile tilapia fingerlings with an average weight of 3.33 ± 0.2 g were stocked at 20 fish per tank (60 litres tank) in triplicates. Fish were fed, by hand, four times a day (8:00, 11:00, 14:00, 17:00) at a rate of 6-10% of their body weight per day. Feeding rates were adjusted every week and the experiment lasted eight weeks (Figure 6.1). Each ration was dispensed over a period of 10 minutes in small portions in an attempt to minimise feed wastage. The quantity of food fed was recorded for subsequent determination of feed conversion ratios and feed utilization.

6.2.2 Diet Formulation and preparation

Four isonitrogenous (320 g kg^{-1} protein), isolipidic (100 g kg^{-1} lipid) and isoenergetic (18 kJ g^{-1}) diets were formulated for the experiment (Table 6.1). The control diet was formulated with fishmeal as the major source of protein. Niger seed cake was incorporated in the diet at inclusion levels of 0% (NSC0), 20% (NSC20) and 40% (NSC40). Diet preparation and other ingredients used were similar to those described in section 3.2.

Table 6.1 Composition of diets (g kg^{-1} as-fed) fed to juvenile Nile tilapia (*O. niloticus*) with varying inclusion levels of Niger seed cake

	NSC0	NSC 20%	NSC 40%
NSC	-	200.0	400.0
FWM	486.0	398.3	298
WG	156.4	135	100
CG	270	160	126
PV/M premix[1]	50	50	50
SB oil	12.6	31.7	1
CMC[2]	20	20	20
Cr_2O_3	5	5	5

FWM = Fish waste meal, NSC = Niger seed cake, WG = Wheat grain, SB oil = Soybean oil, CG= Corn grain. PV/M premix = Poultry vitamin/ mineral premix, Cr_2O_3 = Chromic oxide, [2]Carboxymethyl cellulose (high viscosity), [1]As listed in Table 3.1

6.2.3 Faeces Collection

At the end of the growth trial faecal collectors were fitted to rearing tanks and faeces were collected for two weeks (see section 3.1.2 for details). Faecal samples from each tank were pooled to represent respective treatments and immediately centrifuged, stored and later prepared for chemical analysis as described in section 3.1.2. Apparent digestibility coefficients of nutrients and energy of diets were determined as described in section 3.3.5.

6.2.4 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by methods described in sections 3.2.1. Energy contents of diets, faeces and carcass were analysed by methods described in section 3.2.3. Chromic oxide content of the diets and faecal samples was determined by the method in section 3.2.2.

6.2.5 Analysis of Experimental Data

Growth performance and feed utilization were calculated as described in section 3.3.

6.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish in a completely randomised design. Data were analysed as described in section 3.5.

6.3 Results

6.3.1 Proximate Composition and Energy Contents of Diets

Proximate composition and energy contents of experimental diets are presented in Table 6.2. Crude protein contents varied little between the diets (331.2 - 333.6 g kg^{-1}) as did crude lipid

(100.6 – 133.6 g kg^{-1}), ash (130.4-138.2 g kg^{-1}) and nitrogen free extract (265.0-309.2 g kg^{-1}). Energy levels in all experimental diets varied little within a narrow range (18.0-19.0 kJ g^{-1}). Crude fibre contents in diet 2 (NSC20) and diet 3 (NSC40) were almost two times and four times the level of crude fibre in the control diet (24.4 g kg^{-1}), respectively.

Table 6.2 Proximate composition (g kg^{-1} as-fed) and energy (kJ g^{-1}) of diets used in the study.

	NSC0	NSC20	NSC40
Components	1	2	3
Dry matter	922.3	927.9	926.9
Crude protein	333.0	333.6	331.2
Crude lipid	117.6	133.6	100.6
Crude fibre	24.4	60.4	97.7
Ash	138.2	135.3	130.4
NFE	309.2	265.0	267.1
Chromic oxide	4.6	4.7	5.1
Gross energy (kJ g^{-1})	19.0	18.9	18.0

6.3.2 Growth performance

Tilapia was observed to be in good condition of health and survival was above 93% in all groups (Table 6.3). Growth response of Nile tilapia fed the experimental diets is presented as initial and final mean weights, percentage weight gain and specific growth rate (SGR) in Table 6.3 and

graphically in Figure 6.1. There was no significant (P> 0.05) difference in the mean final body weight (FBW) between fish fed the control diet (NSC0) and the other groups. The difference in body weight between control diet (NSC0) and diet 3(NSC40) was observed after the first week, but this difference was noticed in fish fed diet 2 (NSC20) after the fifth week (Fig. 6.1). This was also reflected in weight gain (WG) and specific growth rate (SGR), where fish fed diet 2 did not show any significant (P> 0.05) difference from that of the control when diet 3 showed a significantly (P< 0.05) lower body weight gain and specific growth rate. In general, fish fed the two Niger seed cake containing diets did not show any significant (P> 0.05) difference in growth performances.

Table 6.3. Growth and feed utilization of juvenile Nile tilapia fed Niger seed cake based diets for eight weeks.

Parameters	Diets		
	NSC0	NSC20	NSC40
	1	2	3
IBW	3.3±0.1[a]	3.4±0.1[a]	3.3±0.2[a]
FBW	15.5±0.55[a]	14.4±1.5[a]	12.6±2.2[a]
WG	365.8±24.6[a]	329.0±35.3[ab]	277.3±42.7[b]
SGR	2.74±0.09[a]	2.6±0.15[ab]	2.4±0.2[b]
S	95±5.0[a]	93.33±7.6[a]	95±8.7[a]
FCR	2.44±0.09[a]	2.63±0.2[a]	2.8±0.5[a]
FI	32.3±0.35[a]	31.3±1.7[a]	27.4±1.45[b]
PER	1.13±0.04[a]	1.05±0.08[a]	1.01±0.17[a]
PPV	20.6±1.2[a]	18.2±1.1[ab]	15.1±2.6[b]
ER	13.56±0.8[a]	12.68±1.0[a]	10.7±2.0[a]
HIS	3.23±0.25[a]	2.26±0.76[a]	2.54±0.44[a]

IBW (g): initial body weight; FBW(g): final body weight; BWG(%): body weight gain; SGR(% day^{-1}): specific growth rate; FI(G): feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; PPV(%):productive protein value; ER(%): energy retention; S (%): survival; HSI: hepatosomatic index. Values are means ± SD of three replicates, and values within the same row with different letters are significantly different ($P<0.05$)

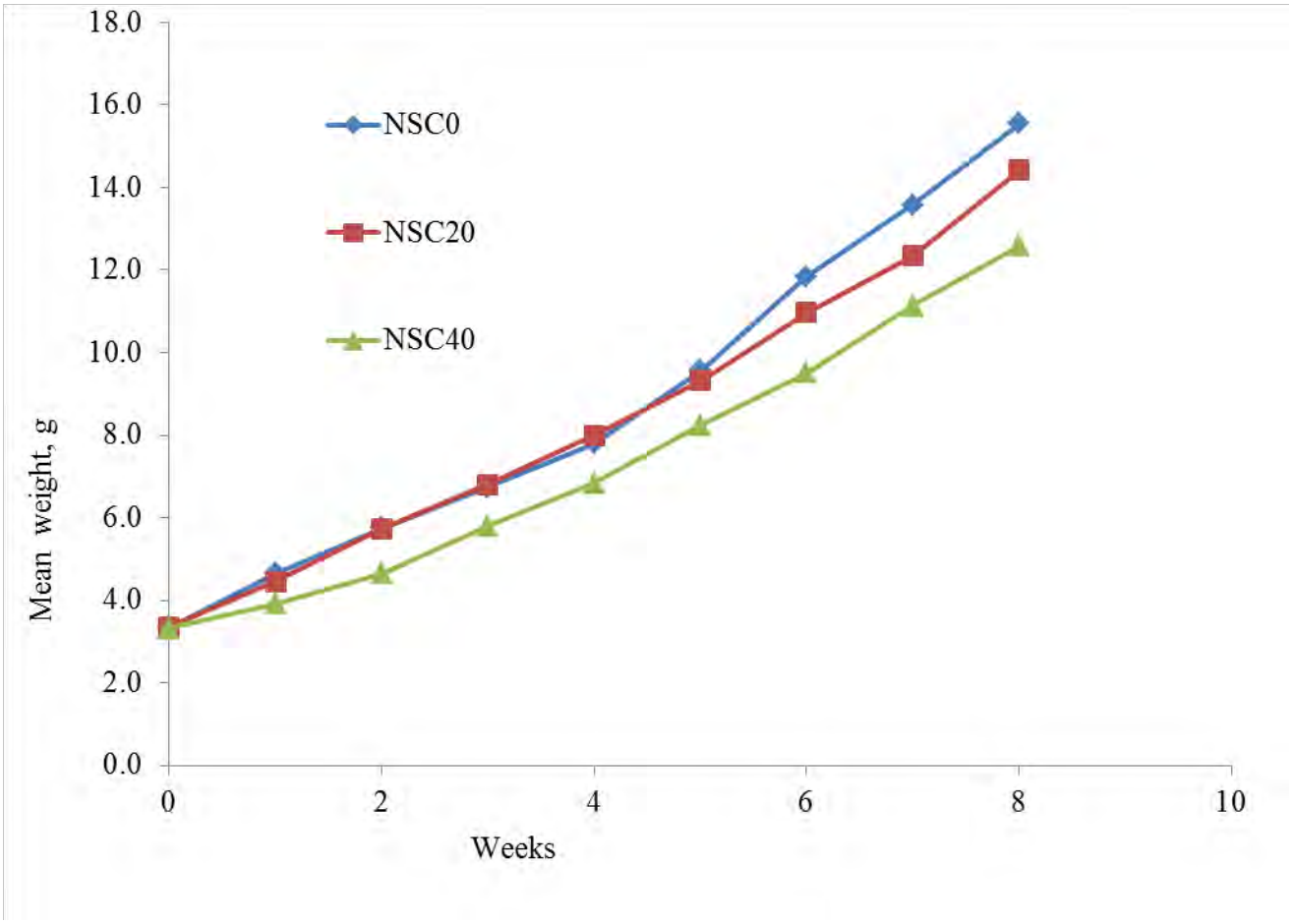

Figure 6.1 Growth response of fish (*O. niloticus*) fed Niger seed cake based diets for eight weeks

6.3.3 Feed Utilization

No significant difference was observed for feed intake (FI) between the fish fed on the control diet and diet 2 (NSC20) ($P > 0.05$). However, Nile tilapia fed on diet 3 (NSC40) showed a significantly lower FI than the control group as well as diet 2. Protein efficiency ratio (PER) and energy retention (ER) showed a decreasing tendency with increasing levels of oilseed cakes, though the observed differences in between the three dietary groups were not statistically significant ($P> 0.05$). Similar trend in the FCR was also observed. The net productive protein

value was calculated for all the dietary treatments and ranged from 15.1-20.6% (Table 6.3). The lowest value (15.1%) recorded for diet 3 was significantly lower than the control ($P< 0.05$). Since the carcass protein contents (Table 6.5) of fish groups fed diet 1 (NSC0) and diet 3 (NSC40) were significantly different, the PPV values did not fully reflect the PER values. Energy utilization followed similar trend as that of gross energy contents of whole body of fish. However, the difference was only statistically significant ($P< 0.05$) between the control and test diets. It ranged from 10.7 to 13.6% where the highest ER was observed for the control diet fish group and lowest for diet3 (NSC40).

6.3.4 Apparent Nutrient Digestibility

Apparent nutrient digestibility is shown in Table 6.4. Apparent dry matter digestibility (ADMD) of diets ranged from 74.3% to 79.6%. ADMD generally decreased with increased levels of Niger seed cake. Apparent protein digestibility (APD) for all diets was moderately high ranging from 79.02 to 89.8%. The control diet had the highest APD (89.8%) followed by diets with 20% level of Niger seed cake inclusion. APD in general decreased with increasing plant protein inclusions in the diet. Apparent lipid digestibility (ALD) was higher than all the other nutrients studied except APD of the control diet. In this study, the values observed for ALD of diets 2 was higher than the control, which has similar ALD values with diet 3 (NSC40). Apparent energy digestibility varied slightly between the different diets and ranged from 80.17 to 82.89% with diets 1 and 2 having almost similar values. Similar trend in energy digestibility was observed as that of the other nutrients where decreased values with increasing levels of Niger seed cake in the diets were observed.

Table 6.4 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy (GE) and digestible protein (DP) and energy (DE) (g kg^{-1} and kJ g^{-1} respectively, dry weight basis) in the Niger seed cake based diets for Nile tilapia.

	1	2	3
Components	NSC0	NSC20	NSC40
Dry matter	79.6	76.08	74.31
Crude protein	89.75	87.37	79.02
Crude lipid	85.55	89.2	85.52
GE (kJ g^{-1})	82.98	82.7	80.17
DP	298.8	291.5	261.7
DE	15.75	15.66	14.4

6.3.5 Body Composition

Whole fish body proximate composition for initial and final fish samples is presented in Table 6.5. Fish fed the control diet and diet 2 (NSC20) had significantly ($P< 0.05$) lower moisture contents and higher whole-body crude protein, crude lipid and gross energy contents. Although there is a tendency of increasing ash contents of whole body of fish with increasing plant protein levels in the diets, no significant differences in the whole body ash contents of fish were observed.

Table 6.5 Whole Body Proximate Composition (% wet Weight) and Energy of Nile Tilapia Fed Niger Seed Cake-Based Diets

Components		1	2	3
	Initial carcass	NSC0	NSC20	NSC40
Moisture content	78.1	76.5±0.26 [a]	76.46±0.25 [a]	77.22±0.13 [a]
Crude protein	15.33	16.42±0.42 [a]	15.81±0.16 [a]	14.15±0.21 [b]
Crude lipid	5.28	7.29±0.16 [a]	7.21±0.14 [a]	5.42±0.12 [b]
Ash	3.72	4.17±0.07 [a]	4.09±0.08 [a]	4.23±0.09 [a]
Gross energy	5.7	6.16±0.15 [a]	6.17±0.07 [a]	5.39±0.08 [b]

6.4 Discussion

The present study showed that it is possible to include Niger seed cake in the diets of Nile tilapia. In general, growth performance (SGR and WG) of fish decreased significantly with the increase of NSC above 20% level. This is in agreement with different studies (Jauncey, 1998; Mbahinzireki *et al.*, 2001; Olvera-Nova *et al.*, 2002) that showed reduction in fish growth as plant protein content increased beyond 20%. The efficiency at which the diets ingested are digested largely determines growth performance (Deganp and Yehuda, 1999). This is because the nutritive value of food depends not only on its nutrient content but also on the capacity of the animal to digest and absorb the nutrients (Cook *et al.*, 2000; Rust, 2003). In this study, the apparent digestibility of dry matter, crude protein, fat and energy for all the experimental diets

ranged between 74 and 89% despite the high fibre content in NSC (Table 6.4). This high level of nutrient digestibility is attributed to the fact that NSC was found to be fairly digestible for Nile tilapia (Chapter 4). Deganp and Yehuda (1999) also found high digestibility values for protein in sunflower seed meal (78%), rapeseed meal (86%) and cottonseed meal (79%). In this study, NSC20 had moderately similar digestibility with that of the control for dry matter, crude protein, fat and energy suggesting that the diet had more bio-available nutrients for the fish. The reduced growth rate, poorer feed intake and poorer protein utilization in fish fed diets with NSC inclusion level above 20% is probably attributable to higher (97.7 g kg^{-1}) crude fibre content in NSC40 (Table 6.2). In fish, fibre does not supply nutrients but instead may act as an anti-nutrient by reducing the utilization of other nutrients (Francis *et al.*, 2001). Because of its high water-holding capacity it can affect digesta viscosity in fish. High digesta viscosity, produced as a result of fibre, delays gastric emptying and feed transit time, and decreases interaction of the intestinal enzymes with feed macromolecules, resulting in decreased nutrient availability (Sinha *et al.*, 2011). In this study, PPV slightly decreased with an increase in dietary NSC levels, indicating that the final whole body protein of the experimental fish was lower for the diets with NSC, presumably as fibre increased with increasing NSC in the diet, which reduced diet digestibility and growth performance. Research has reported similar trends with high fibre containing plant proteins such as sunflower seed meal (El-Sayed, 1990; Maina *et al.*, 2002; Olvera-Novoa *et al.*, 2002) incorporated in the diets of tilapia. For example, decreased growth performance and feed utilization in Nile tilapia were related to high fibre content of sunflower seed meal, as dietary fibre increased from 2 to 17% (El-Sayed, 1990) and from 3.36 to 12.25% (Olvera-Novoa *et al.*, 2002) with increasing dietary levels of sunflower seed meal. Growth reduction at high inclusion levels of NSC may also be related to the dietary amino acid profile.

Many authors reported sub-optimal amino acid balance as one of the reasons for reduced growth response and feed utilization in various warm-water aquaculture species fed diets with higher levels of oilseed meals (Lim and Dominy, 1991; Mbahinzireki *et al.*, 2001; Olvera-Novoa *et al.*, 2002). Olvera-Novoa *et al.* (2002) observed a reduction in methionine content in diets with higher than 20% sunflower meal inclusion levels. Sunflower and Niger seeds belong to the same Composite Family and their nutritional profile was found to show similar pattern of amino acid and fatty acid profile (Bhatnagar and Gopalakrishna, 2015). The recommended level of NSC inclusion in tilapia diets complies with the recommendations of different authors on the accepted levels of sunflower seed cake in tilapia diets. For example, Olvera-Novoa *et al.* (2002) recommended an inclusion of sunflower seed cake up to 20% in the diet of *Tilapia rendalli.* Jauncey (1998) recommended an inclusion level of sunflower seed cake of up to 25% for Nile tilapia while Maina *et al.* (2002) reported that it should not exceed 30% of the total crude protein. Sanz *et al.* (1994) observed better results when replacing 40% of fish meal with sunflower seed meal supplemented essential amino acids. These results seem to confirm that amino acid deficiency could be one of the reasons for limiting the growth of tilapia.

Fish fed diets higher in NSC level had higher body moisture content while the opposite was true for protein, lipid and gross energy. Similar results have been reported for carp fed diets containing high amounts of plant proteins such as mustard oilcake, linseed and sesame meal (Hossain, 1988). The decrease in lipid content is probably due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. Earlier studies, in Nile tilapia, have shown a decrease in carcass lipid following feeding diets in which SBM was replaced by other plant protein sources such as *Cassia fistula* meal (Adebayo *et al.*, 2004); Roquette seed, *Eruca sativa* (Fagbenro, 2004) and

green algae *Ulva rigida* (Azaza *et al.*, 2008), which reflect a reduction of lipid deposition and obviously affected the size of the liver. Quite surprisingly, no significant variation in HSI was found in this study among treatments, despite the fact that diet significantly affected lipid deposition at higher inclusion levels of NSC. These absences in HSI differences is accounted for, at least in part, by marked between individual differences in HSI, which largely reflected the growth heterogeneity in this study.

From the above discussion, Niger seed cake appears to be promising new feed ingredient for aquaculture industry in Ethiopia. Since the supply of fishmeal and fish oil is a major constraint on the growth of the aquaculture feed industry, the use of such processed oilseed cake provides long term sustainability to the industry. However, further studies are recommended to explore means of improving NSC digestibility by reducing crude fibre. The result of this study on the nutritional quality of NSC as an alternative protein source for Nile tilapia indicates that up to 20% of NSC could be incorporated in diets without adverse effect on either growth or feed efficiency, which corresponds to a reduction of 20% of fish meal of the control diet.

Chapter 7- Evaluation of dietary inclusions of Niger seed cake and linseed cake mixtures in the diets of juvenile Nile tilapia (*Oreochromis niloticus*)

7.1 Introduction

The ever increasing demand for fisheries products coupled with the decline in supply from capture fisheries is being balanced by the aquaculture industry that is producing intensively. In aquaculture, feed is the largest production cost accounting for about more than 50% of the operational cost (De Silva, 1993). In the diets of farmed fish protein ingredients are also the largest feed cost factors accounting for more than 50% of the feed cost (Webster and Lim, 2002). Because of its balanced amino acid profile that closely matches the fish‘s requirement patterns, fishmeal acts as the major protein source in aquafeeds on which the aquaculture industry heavily relies on. Despite such reliance on fish meal, the wild caught fish used in making fish meal have already been classified as fully exploited, over exploited or depleted resulting in scarcity of supply of fish meal as well as a rise in the price. The aforementioned factors coupled with increased competition from other animal farming industries necessitated the need to replace fish meal with less expensive protein rich sources. In view of this, several researches have been centred to explore feed ingredients that are inexpensive, readily available and nutritious protein sources which can supply the nutritional needs of fish. Thus research efforts are continuing in search of cheap and alternative protein sources to minimize feed expenditure, especially cost of dietary protein. One obvious approach which has given promising results involved the utilization of plant ingredients in aquafeeds.

The utility of plant protein sources to exclusively or partially replace the fish meal protein is being investigated in many countries. However, this often resulted in decreased apparent digestibility and impaired fish performance due to anti-nutritional factors such as protease inhibitors, tannin, lectins and others (Francis *et al.*, 2001; Gatlin III *et al.*, 2007; Krogdahl *et al.*, 2010).

Various oilseed cakes are produced in Ethiopia on a large scale as by-products of the edible oil industry. These include Niger seed, cottonseed, sunflower, soybean and linseed cakes. The efficiency of various alternative protein sources as partial or complete replacement for fish meal has been individually evaluated in fish diets, e.g. sunflower meal (El-Saidy and Gaber, 2002), soybean (El-Saidy and Gaber, 2002; Soltan, *et al.,* 2001), linseed meal (El-Saidy and Gaber, 2001), canola (Soltan, 2005) and cottonseed meal (Agbo *et al.*, 2011b). Individually, these plant by-product meals are fairly rich in protein and have favourable essential amino acid profiles, but they are deficient in one or more essential amino acids and contained various quantities of anti-nutritional factors (NRC, 1993, Francis *et al.*, 2001). Therefore, complete or partial replacement of fishmeal at higher levels with individual plant proteins has generally resulted in a decrease in fish growth performance (Mbahinzirek *et al.*, 2001; Sklan *et al.* 2004).

Some studies have also stressed that a mixture of plant protein sources is a more appropriate strategy to obtain adequate amino acid profile and reduce exposure to individual antinutritional factors compared to the incorporation of a single plant protein source (Watanabe *et al.,* 1995; Regost *et al.,* 1999; Borgeson *et al.*, 2006; Latif *et al.*, 2008; Soltan *et al.*, 2008). Recently, comparative studies conducted in rainbow trout, turbot, sea bass and sea bream attempted to completely substitute fish meal by a mixture of plant proteins. All diets were supplemented with L-amino acids to meet the amino acid needs estimated for rainbow trout (NRC, 1993). Results

were disappointing and compared to a control diet, growth retardation was observed even in rainbow trout. Beside the effects of known or unknown antinutritional factors, a deficiency of one or more amino acid was suspected, suggesting that supplementation of diet according to amino acids needs available in NRC (1993) was not sufficient. Mambrini and Kaushik (1995) suggested that amino acid profile of fish meal reflects well the fish amino acid needs which could imply to supplement plant protein based diets at higher levels than required by NRC (1993).

Most nutrition studies on alternative ingredients have focused on biological performance, with less attention given to economic performance. However, Meade (1989) points out that economic evaluation of new inputs in aquaculture has a vital influence on economic viability and hence long term sustainability. Moreover, such evaluation is important in decision making as some novel ingredients may display poor biological performance but prove to be cost effective nonetheless (El-Sayed and Tacon, 1997). Availability of a cost-effective diet can make a difference between a profitable and unprofitable operation and hence determine the economic viability of a fish farming operation. Therefore, success of aquaculture among rural communities will obviously largely depend on availability of cost-effective feeds compounded from inexpensive locally available ingredients. This approach was recently further emphasized by Kassahun Assaminew *et al.* (2012) in an economic analysis of Nile tilapia production in Ethiopia.

To ensure the sustainability and future growth of aquaculture in Ethiopia there is a need on developing alternative and cost effective feed formulations and appropriate feeding strategies. Niger seed cake and linseed cake were used in combined form to evaluate as dietary protein sources for juvenile Nile tilapia. Though numerous workers using individually or with other

ingredients evaluated linseed meal previously, the effects of combination of this oilseed cake with Niger seed cake was not studied previously. The present investigation was undertaken to evaluate the biological and economic performance of Nile tilapia fed practical diets containing blends of linseed cake and Niger seed cake in various combinations incorporated at different levels.

7.2 Materials and Methods

7.2.1 Experimental System and Animals

The experimental systems described in Sections 3.1.1 and Section 3.1.2 were used for the growth trial and faeces collection respectively. Each tank was supplied with thermo-regulated and re-circulating water at a flow rate of 2 litres min^{-1} and a constant photoperiod of 12 hours Light/12 hours Darkness (Section 3.1.1. Supplemental aeration was provided using air stones. The quality of water was monitored weekly during the experiment and parameters averaged (± SD): temperature, 26.93 ± 0.35 ^{o}C; pH, 7.8 ± 0.18; ammonia, 0.05 ± 0.02 mg l^{-1}; nitrite, 0.20 ± 0.0 mg l^{-1}; nitrate, 20 ± 0.0 mg l^{-1} and dissolved oxygen, 7.39 ± 0.57 mg l^{-1}.

Mixed-sex Nile tilapia fingerlings with an average weight of 2.44 ± 0.20 g were stocked in triplicate 60 litre tanks. Fish were hand-fed four times a day (8:00, 11:00, 14:00, 17:00) at a rate of 10% of their body weight per day for the first four weeks and reduced to 6% for subsequent weeks as an adjustment to the increase in fish weight in accordance to their feeding rate. Feeding rates were adjusted every week and the experiment lasted eight weeks (Figure 7.1). Each ration was dispensed over a period of 10 minutes in small portions in an attempt to minimize feed wastage. The quantity of food fed was recorded for subsequent determination of feed conversion ratios and feed utilization.

7.2.2 Diet formulation and preparation

Seven isonitrogenous (320 g kg^{-1} protein), isolipidic (100 g kg^{-1} lipid) and isoenergetic (18 kJ g^{-1}) diets were formulated for the experiment (Table 7.1). The oil seed cakes used in this study: Niger seed cake and linseed cake were blended in three different formulations such that one formulation (F1) contained 50% Niger seed cake and 50% linseed cake, a second formulation (F2) contained 33.3% Niger seed cake and 66.67% linseed cake, and a third formulation (F3) contained 66.67% Niger seed cake and 33.33% linseed cake. Six experimental diets were formulated by incorporating each of the three oilseed cake formulations at two levels of dietary inclusions, 25% and 50%. The control diet was formulated with fish meal as the main source of protein. The diets were tested in a two-factorial plus control experiment with three replicates, totalling 21 culture units (tanks). Diet preparation and other ingredients used were similar to those described in Section 3.2.

Table 7.1 Composition of diets fed to juvenile *O. niloticus* using different combinations of two oilseed cakes at two different levels of dietary inclusions (g kg^{-1} as-fed)

	Control	F1(25)	F2(25)	F3(25)	F1(50)	F2(50)	F3(50)
F1	-	250.0	-	-	500.0	-	-
F2	-	-	250.0	-	-	500.0	-
F3	-	-	-	250.0	-	-	500.0
FWM	470.7	359.3	360.0	358.2	240.0	240.0	240.0
WG	154.3	130.0	130.0	130.0	90.0	90.0	90.0
CG	290.0	175.7	175.0	176.8	85.0	85.0	85.0
PV/M premix[1]	50.0	50.0	50.0	50.0	50.0	50.0	50.0
SB oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0
CMC[2]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cr_2O_3	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cost[3]	5.3	5.75	5.85	5.65	6.2	6.4	6.0

FWM = Fish waste meal, F1= formulation 1, F2= formulation 2, F3=formulation 3, WG = Wheat grain, SB oil = Soybean oil, CG= Corn grain. PV/M premix = Poultry vitamin/ mineral premix, Cr_2O_3 = Chromic oxide, [1]As listed in Table 3.1, [2]Carboxymethyl cellulose (high viscosity), [3]Cost of diet in Ethiopian birr kg^{-1}

7.2.3 Faeces Collection

At the end of the growth trial faecal collectors were fitted to rearing tanks and faeces collected for two weeks (see Sections 3.1.2 for details). Faecal samples from each tank were pooled to represent respective treatments and immediately centrifuged, stored and later prepared for chemical analysis as described in Section 3.1.2. Apparent digestibility coefficients of nutrients and energy of diets were determined as described in Section 3.3.5.

7.2.4 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by the methods described in Section 3.2.1. Energy contents of diets, faeces and carcass were analysed by methods described in Sections 3.2.3. Chromic oxide content of the diets and faeces were determined by the method in Section 3.2.2.

7.2.5 Analysis of Experimental Data

Growth performance and feed utilization were calculated as described in section 3.3.

7.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish in a completely randomised design. The seven different diets formulated in this experiment were arranged in a 2 X 3 factorial design plus a control (augmented factorial design). Because of the hierarchical design of the experiment differences in continuous response variables were analysed by nested ANOVA (by nesting the two-factorial structure within the grouping of control versus treatment), which was applied with the variable replication as a random factor. Arcsine transformations were applied to proportions to meet assumptions (Sokal and Rohlf, 1995). The analysis of variance (ANOVA) and the

corresponding F-tests for the main and interaction effects covered only an overall decision concerning the presence of an interaction. But, information about the location of the difference(s) or comparisons of particular interest were made available by using a post-hoc multiple contrast procedures (Yossa and Verdegem, 2015). The procedure provided the feature of controlling the family wise error (FWE) for a certain user-defined set of comparisons formulated as multiple contrasts of the treatment means (Schaarschmidt and Vaas, 2009). For a single interaction contrast the null hypothesis that the user defined contrast is zero is rejected if the confidence interval does not include the value zero. Adjusted *P*-values for the individual hypotheses were provided, such that the significance can be inferred, while controlling the overall probability of a type I error (an error of declaring a false positive decision or an effect when none existent). For comparisons of means simultaneous multiple comparison procedures according to Hothorn *et al.* (2008) using the package *multcomp* within the statistical software R version 3.1.3 (R Development Core Team, 2015) and the methods described in Schaarschmidt and Vaas (2009) were performed.

7.3 Results

7.3.1 Proximate Composition and Energy Contents of Diets

Proximate composition and energy of experimental diets and oil seed cake formulations used in this study are given in Table 7.2. Protein, fibre and ash contents of oil seed cake formulations increased with increase in the proportion of Niger seed cake. Lipid and NFE contents of the oil cake formulations increased with the increase in the proportion of linseed cake. Crude protein, crude lipid, ash and gross energy contents of the different diets were almost similar. The NFE contents of the diets ranged between 265 to 323 g kg^{-1} with the highest and lowest values

obtained for the control diet and Diet 7 (F3(50)), respectively. However, crude fibre content varied ranging between 24.8 g kg^{-1} and 105.3 g kg^{-1} where the control diet had the lowest and F3 (50) the highest. Crude fibre content increased with increasing levels of oilseed cake inclusion.

Table 7.2 Proximate composition (g kg^{-1} as-fed) and gross energy (kJ g^{-1}) of oilseed cake formulations and diets used in the study.

	Oil cake Formulations			Control	F1(25)	F2(25)	F3(25)	F1(50)	F2(50)	F3(50)
Nutrients	F1	F2	F3	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
DM	918.4	915.2	921.6	921.0	922.6	921.9	923.3	923.8	922.2	925.4
CP	317.1	314.7	319.4	325.0	325.0	324.8	325.0	320.4	319.2	321.6
CL	100.1	102.8	97.4	112.9	111.9	112.6	111.0	110.0	111.4	108.7
CF	168.7	157.9	179.5	24.8	62.5	59.8	65.2	99.8	94.5	105.3
Ash	86.7	85.4	88.0	135.0	130.4	130.2	130.5	124.0	123.4	124.7
NFE	245.8	254.4	237.3	323.2	292.9	294.6	291.6	269.4	273.8	265.2
Cr_2O_3				4.6	4.7	4.44	5.0	4.7	5.1	5.2
GE	24.6	25.4	23.7	18.9	18.9	18.9	18.4	18.0	18.0	17.9

DM= dry matter; CP= crude protein; CL= crude lipid; CF= crude fibre; GE = gross energy; NFE= Nitrogen free extract; Cr_2O_3= chromic oxide

7.3.2 Growth Performance

Growth performance of Nile tilapia fed the experimental diets is presented as initial and final mean weights, percentage weight gain (WG) and specific growth rate (SGR). Data on growth

performance of Nile tilapia fed the different experimental diets is presented in Table 7.3 and growth responses are also shown in Figure 7.1. Results of the post-hoc analysis for user defined contrasts (Table 7.4) are also presented in the simultaneous confidence interval plot for user defined contrasts as shown Table 7.4. Results of the nested ANOVA (Table 7.5) indicated that significant interaction between the two factors (formulation and levels of inclusion) was observed only for the final mean weight (FW). All the six oil seed cake based treatment diets resulted in a significant decrease in all the growth parameters considered ($P< 0.05$), except F1(25) and F3(25) diets (Figure 7.2; contrasts 1-6). Comparisons of F1 and F3 formulations at each level of inclusion did not show significant differences in the growth performance of fish. In addition, significant better growth performance was found when F1 and F3 were used compared to F2 formulation. Results obtained in this study showed that increasing the level of dietary inclusions of oil seed cake formulations tended to decrease growth performances of Nile tilapia. However, the post-hoc analysis did not provide significant evidence ($P>0.05$) for the effect of increasing F3 on specific growth rate of fish (Figure 7.2; contrasts 13-15).

Table 7.3 Growth and food utilization of Nile tilapia fingerlings fed diets with oilseed cake formulations included at two different levels of inclusion.

	Control	F1(25)	F1(50)	F2(25)	F2(50)	F3(25)	F3(50)
IW	2.3±0.06	2.3±0.1	2.4±0.1	2.5±0.2	2.5±0.1	2.5±0.02	2.4±0.04
FW	13.6±0.2	13.4±0.4	12.0±0.08	11.1±0.3	9.0±0.4	13.2±0.4	11.3±0.4
WG	490.0±14	477.1±40	391.5±29	341.1±18	257.8±23	433.4±12	363.2±24
SGR	3.06±0.04	3.0±0.1	2.7±0.1	2.6±0.07	2.2±0.1	2.9±0.04	2.6±0.09
PER	1.0±0.03	1.06±0.07	1.04±0.1	0.9±0.02	0.8±0.03	1.1±0.8	0.9±0.02
FCR	2.8±0.1	2.7±0.2	2.8±0.07	3.2±0.1	3.8±0.1	2.6±0.2	3.1±0.1
PPV	16.3±0.6	16.0±0.8	15±0.7	12.7±0.4	10.4±0.5	16.6±1.2	13.1±0.6
FI	34.7±1.5	32.1±1.4	28.5±0.2	29.5±1.3	26.5±2.4	29.9±1.1	29.9±0.6
ER	11.8±0.6	10.2±0.4	10.2±0.8	8.7±0.1	7.7±0.3	11.2±0.1	9.2±0.3
S	98.33±2.9	96.7±2.9	96.7±2.9	96.7±2.9	93.33±2.9	95±0	95±0

IW(g)= initial weight, FW(g)= final weight, WG(%)= weight gain, SGR(% day^{-1})= specific growth rate, S(%)= survival, FCR= feed conversion ratio, FI(g)= feed intake, PER= protein efficiency ratio, PPV(%)= productive protein value, ER(%)= energy retention. Values are means ± SD of three experimental replicates

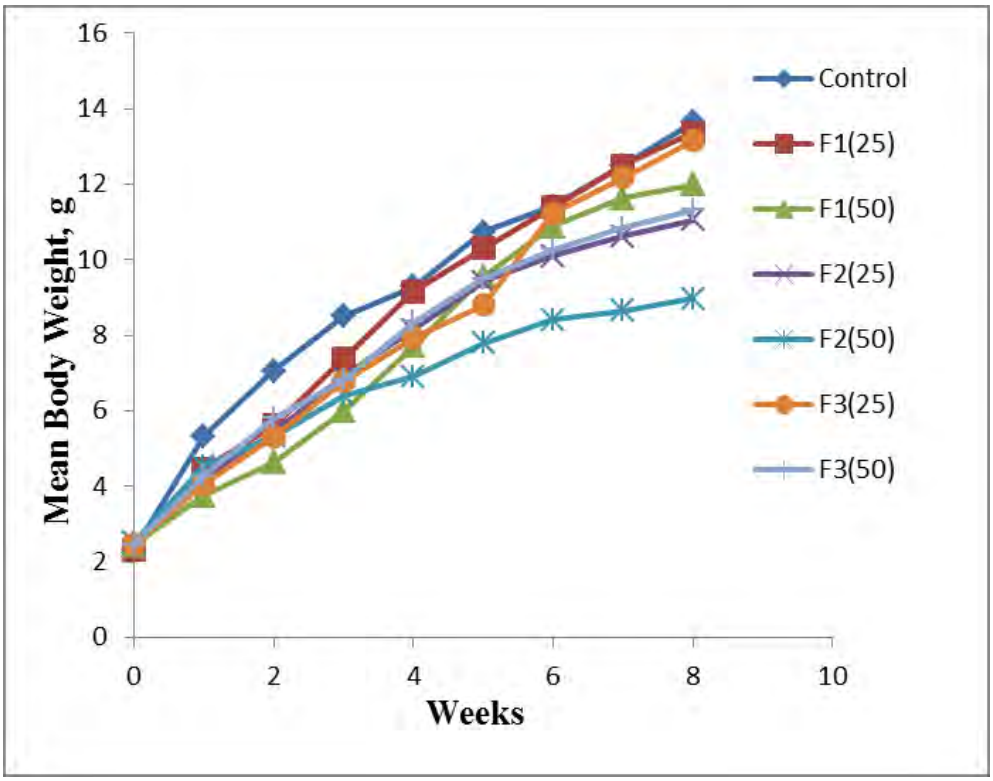

Figure 7.1 Growth response of fish fed mixtures of oilseed cake based diets at two levels of dietary inclusions for eight weeks

Table 7.4 The linear hypotheses of 15 user defined comparisons tested using simultaneous multiple contrast procedure used in the analysis of experimental data[1]

Contrast	Linear hypotheses
1.	F1(25) - CON = 0
2.	F1(50) - CON = 0
3.	F2(25) - CON = 0
4.	F2(50) - CON = 0
5.	F3(25) - CON = 0
6.	F3(50) - CON = 0
7.	F2(25) - F1(25) = 0
8.	F3(25) - F1(25) = 0
9.	F3(25) - F2(25) = 0
10.	F2(50) - F1(50) = 0
11.	F3(50) - F1(50) = 0
12.	F3(50) - F2(50) = 0
13.	F1(50) - F1(25) = 0
14.	F2(50) - F2(25) = 0
15.	F3(50) - F3(25) = 0

[1]Defined are the hypotheses for the differences of the single treatment means to the mean of the control (Contrasts 1–6), the comparisons of the formulations separate for each level of inclusion (Contrasts 7–12), and the comparisons between levels of inclusion 25% and 50% separate for each formulation (Contrasts 13–15).

Table 7.5 F values of growth parameters (FW: final weight, SGR: specific growth rate and WG: weight gain) analysed by nested ANOVA (the two factorial part of the design nested within the grouping of control Vs. treatments)

Variation Source	**Df**	**FW**	**SGR**	**WG**
Control Vs. Treatments	1	184.474***	130.568***	146.167***
Level of inclusion	1	90.697***	70.874***	58.658***
Formulation	2	93.785***	93.969***	74.669***
Formulation X Level of inclusion	2	5.895*	3.345^{ns}	1.095^{ns}

For F values the associated p values are indicated *P<0.05; **P<0.01; ***P<0.001 and ns= not significant (P>0.05); Df= degree of freedom

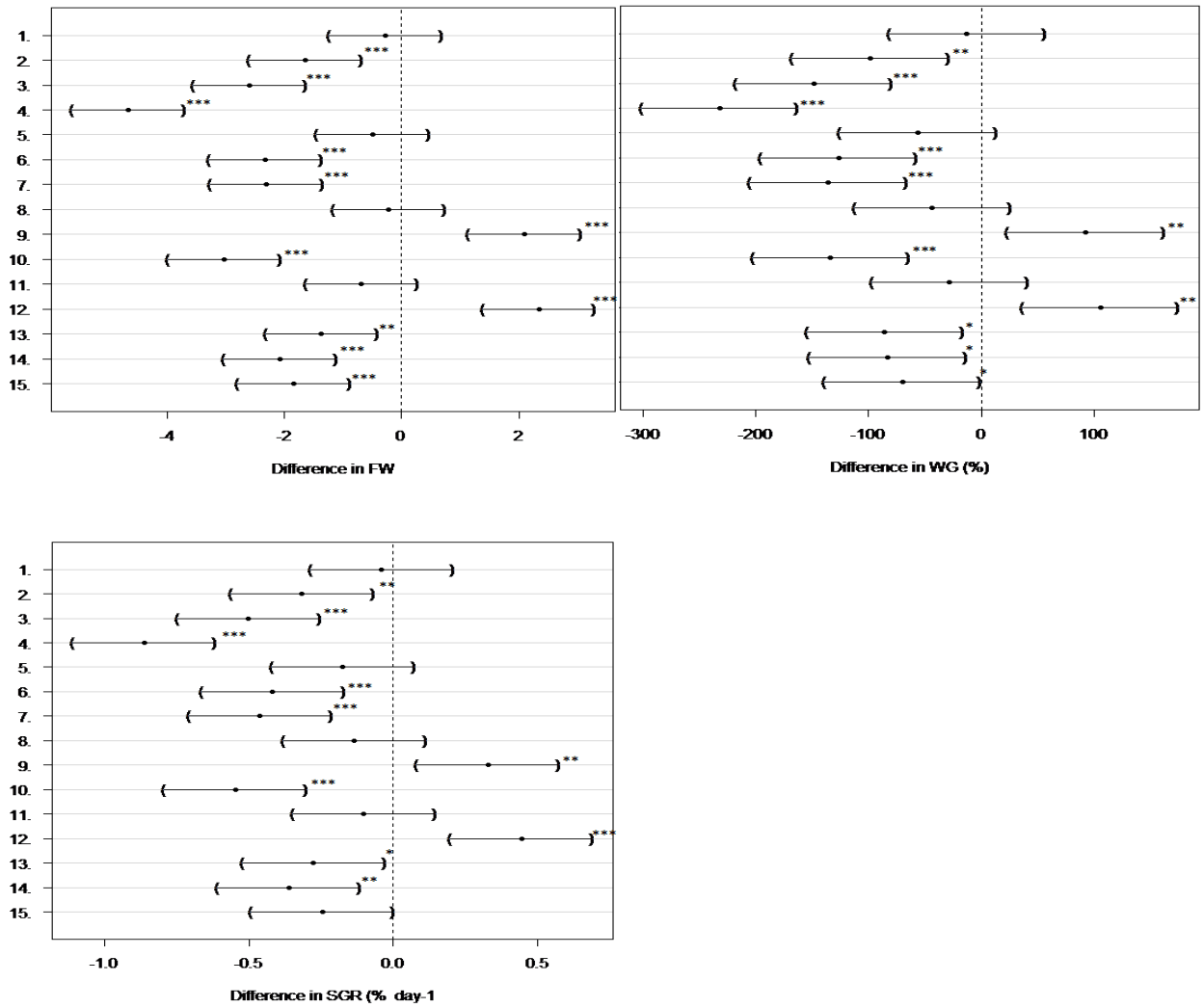

Figure 7.2 The effect of oil seed cake formulation and level of dietary inclusion on growth performance of juvenile Nile tilapia. Points in each chart represent estimates of the differences for the parameters stated (WG: % weight gain, FW: final mean weight (g) and SGR (% day^{-1}): specific growth rate) on the x-axis and parentheses mark the limits of the simultaneous 95% confidence regions for these differences. Calculated are differences of means for 15 user defined contrasts (1.F1(25)–CON, 2.F1(50)–CON, 3.F2(25)–CON, 4.F2(50)–CON, 5.F3(25)–CON, 6.F3(50)–CON, 7.F2(25)-F1(25), 8.F3(25)-F1(25), 9.F3(25)-F2(25), 10.F2(50)-F1(50), 11.F3(50)-F1(50), 12.F3(50)-F2(50), 13.F1(50)-F1(25), 14.F2(50)-F2(25) and 15.F3(50)-F3(25)).The null hypothesis is rejected if the confidence interval does not include the value zero.

Adjusted p-values are indicated for each contrast along with each confidence interval (***: P<0.0001, **: P<0.01; *: P< 0.05).

7.3.3 Feed utilization

There was significant interaction (Table 7.6) between oil seed cake formulation and dietary inclusion level for all feed utilization parameters (FCR, PER, PPV and ER) considered in this study, except feed intake (FI). The six oilseed cake-based diets used significantly lowered FI in Nile tilapia, except for diet 2 (F1(25)) (Fig 7.3; contrasts 1-6). Likewise the remaining parameters of feed utilization (FCR, PPV and PER) were negatively affected except for diet 2 (F1(25)), diet 3 (F1(50)) and diet 6 (F3(25)) as they all showed no significant difference from that of the control. The observed effect of diet 6 (F3(25)) on feed utilization was not statistically significant, except for its significant negative effect on feed intake. Comparisons of F1 and F3 formulations at each level of inclusion did not show significant differences in all of the feed utilization parameters, except for feed intake (Fig. 7.3; contrasts 7-12). But significantly better feed utilization was found for F1 and F3 formulations compared to F2 formulation. Increasing the level oilseed cake formulations tended to affect negatively feed utilization in Nile tilapia. However, increasing the level of F1 formulation did not significantly affect feed utilization of Nile tilapia (FCR, FI, PPV, ER, PER) (Fig. 7.3; contrasts 13-15).

Table 7.6 F values of feed utilization parameters analysed by nested ANOVA (the two factorial part of the design nested within the grouping of control Vs. treatments)

Source	Df	Feed intake	Feed conversion ratio	Protein efficiency ratio	Productive protein value	Energy retention
Control Vs. Treatments	1	60.42***	13.33**	8.607*	60.88***	39.623**
Level of inclusion	1	5.51*	63.24***	47.311***	73.67***	39.297**
Formulation	2	2.498^{ns}	94.91***	91.787***	98.1***	61.776**
Formulation X Level of inclusion	2	2.306^{ns}	14.1**	12.572**	9.22**	7.682**

For F values the associated P values are indicated as *P<0.05; **P<0.01; ***P<0.001; ns= not significant (P>0.05); Df= degree of freedom

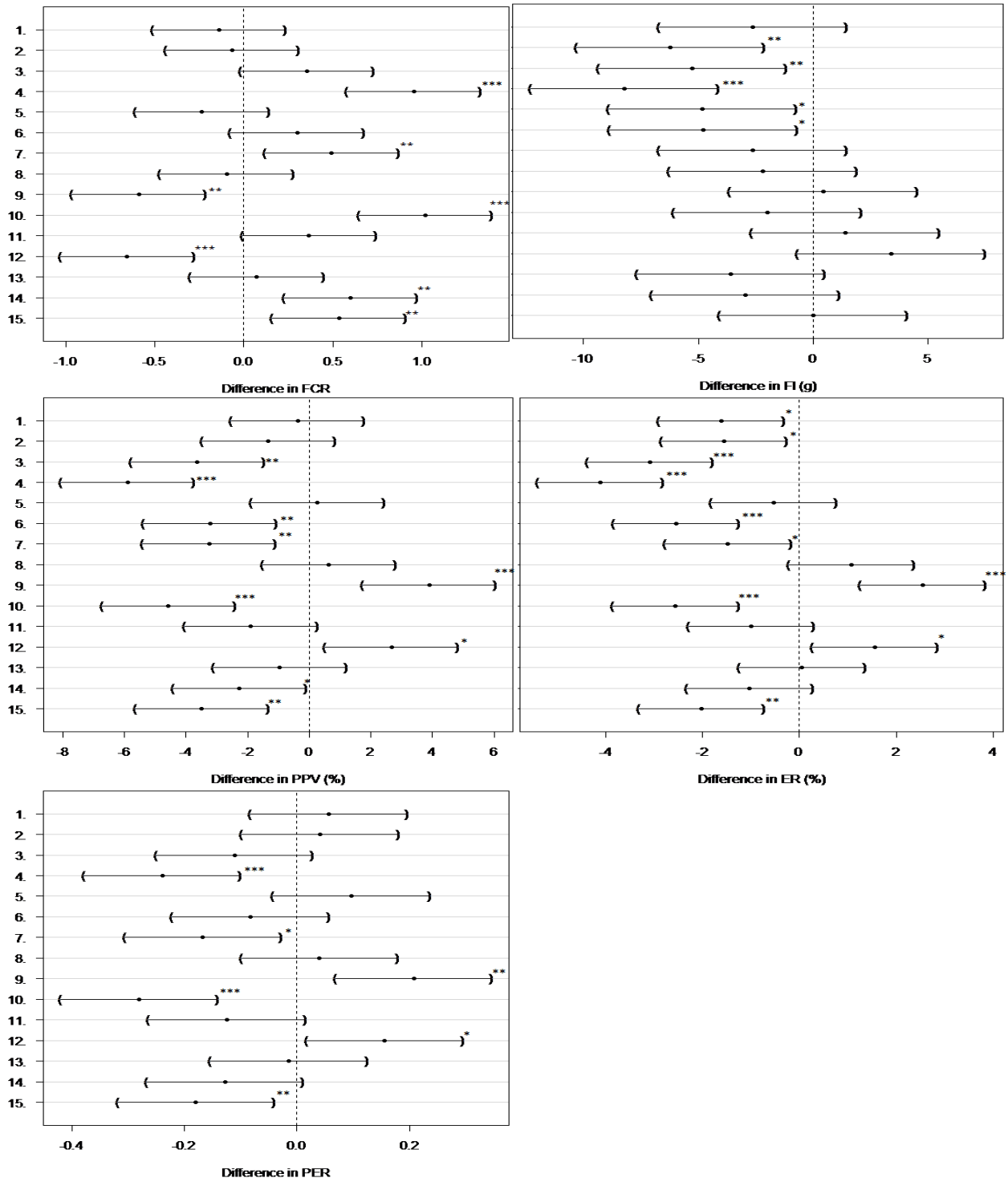

Figure 7.3 The effect of oil seed cake formulation and level of dietary inclusion on growth performance of juvenile Nile tilapia. Points in each chart represent estimates of the differences for the parameters stated (FI: Feed intake, PPV: Productive Protein value, PER: protein

efficiency ratio, ER: energy retention and FCR: feed conversion ratio) on the x-axis and parentheses mark the limits of the simultaneous 95% confidence regions for these differences. Calculated are differences of means for 15 user defined contrasts (1.F1(25)–CON, 2.F1(50)–CON, 3.F2(25)–CON, 4.F2(50)–CON, 5.F3(25)–CON, 6.F3(50)–CON, 7.F2(25)-F1(25), 8.F3(25)-F1(25), 9.F3(25)-F2(25), 10.F2(50)-F1(50), 11.F3(50)-F1(50), 12.F3(50)-F2(50), 13.F1(50)-F1(25), 14.F2(50)-F2(25) and 15.F3(50)-F3(25)).The null hypothesis is rejected if the confidence interval does not include the value zero. Adjusted p-values are indicated for each contrast along with each confidence interval (***: P<0.0001, **: P<0.01; *: P< 0.05).

7.3.4 Apparent Nutrient Digestibility

The control and F1(25) diets had the highest nutrient and energy digestibilities while F2(50) diet had the lowest digestibility coefficients (Table 7.7). Within the different oil seed cake formulations increasing the level of dietary inclusion resulted in a decrease in the nutrient and energy digestibility coefficients.

Table 7.7 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy and digestible protein and energy (g kg^{-1} and kJ g^{-1} respectively, dry weight basis) in the complex oil seed cake-based diets for Nile tilapia.

	1	2	3	4	5	6	7
Components	Control	F1(25)	F2(25)	F3(25)	F1(50)	F2(50)	F3(50)
Dry matter	82.1	81.2	76.5	81.1	76.5	71.2	78.3
Crude protein	92.1	92.9	85.7	87.7	89.6	81.8	87.4
Crude lipid	90.2	90.4	89.8	90.0	89.4	85.0	89.2
Gross energy	86.9	87.6	84.8	85.3	84.4	78.3	83.3
Digestible protein	299.2	302.0	278.5	284.9	287.0	261.1	281.0
Digestible energy	16.4	16.5	16.0	15.7	15.2	14.1	14.9

7.3.5 Body composition

No significant interactions in between the two factors (formulation and level of inclusion) were observed for whole body proximate composition and energy of Nile tilapia (Table 7.8). Significant differences ($P>0.05$) in whole body protein, lipid and energy contents between the control and experimental diets were observed, with the exception of whole body crude lipid contents of fish fed diets F1(25) and F3(25) (Table 7.8). All the experimental diets did not show significant differences in the whole body moisture and ash contents. The values for final ash content was higher than the initial ash content while the final moisture content was lower than

the initial whole body moisture. The 15 user defined contrast for final ash and moisture contents did not show any significant differences at all and the plots are not presented. Ash contents of fish fed the control diet were significantly higher than those fish fed oil seed cake based diets.

Table 7.8 Whole Body Proximate Composition (% wet Weight) and Energy of Nile Tilapia fed complex oil seed Cake-Based Diets. Results F values of nested ANOVA are presented for each source of variation. Mean ± SD are values for final nutrient and energy compositions.

Components		MC	CP	CL	Ash	GE
	Initial carcass	78.1	14.1	5.4	3.84	4.44
1	Control	75.2±0.7	15.9±0.4	5.1±0.1	4.2±0.1	6.1±0.1
2	F1(25)	74.2±0.2	14.9±0.3	5.6±0.4	4.2±0.2	5.6±0.1
3	F1(50)	75.0±0.2	14.3±0.2	5.7±0.09	4.2±0.1	5.3±0.2
4	F2(25)	74.0±0.15	14.2±0.5	5.4±0.3	4.2±0.1	5.4±0.1
5	F2(50)	75.3±0.7	13.8±0.2	5.7±0.1	4.3±0.1	5.3±0.03
6	F3(25)	74.1±0.25	14.9±0.1	5.4±0.2	4.2±0.2	5.6±0.04
7	F3(50)	74.1±0.53	14.2±0.3	5.6±0.05	4.2±0.1	5.3±0.2
Variation Source			F values of ANOVA			
Control Vs Treatment	F	7.218*	67.774***	12.31**	0.168^{ns}	71.801***
Formulation	F	10.807**	17.709***	3.3^{ns}	1.153^{ns}	16.734**
Level of inclusion	F	2.664^{ns}	8.375**	1.095^{ns}	0.58^{ns}	1.981^{ns}
Formulation X level of inclusion	F	3.32^{ns}	0.307^{ns}	0.604^{ns}	0.511^{ns}	2.058^{ns}

(MC) moisture content; (CP) crude protein; (CL) crude lipid; and (GE) = gross energy. Values are means ± SD of three experimental replicates. For F values the associated p values are indicated as ***: P<0.0001, **: P<0.01; *: P< 0.05.

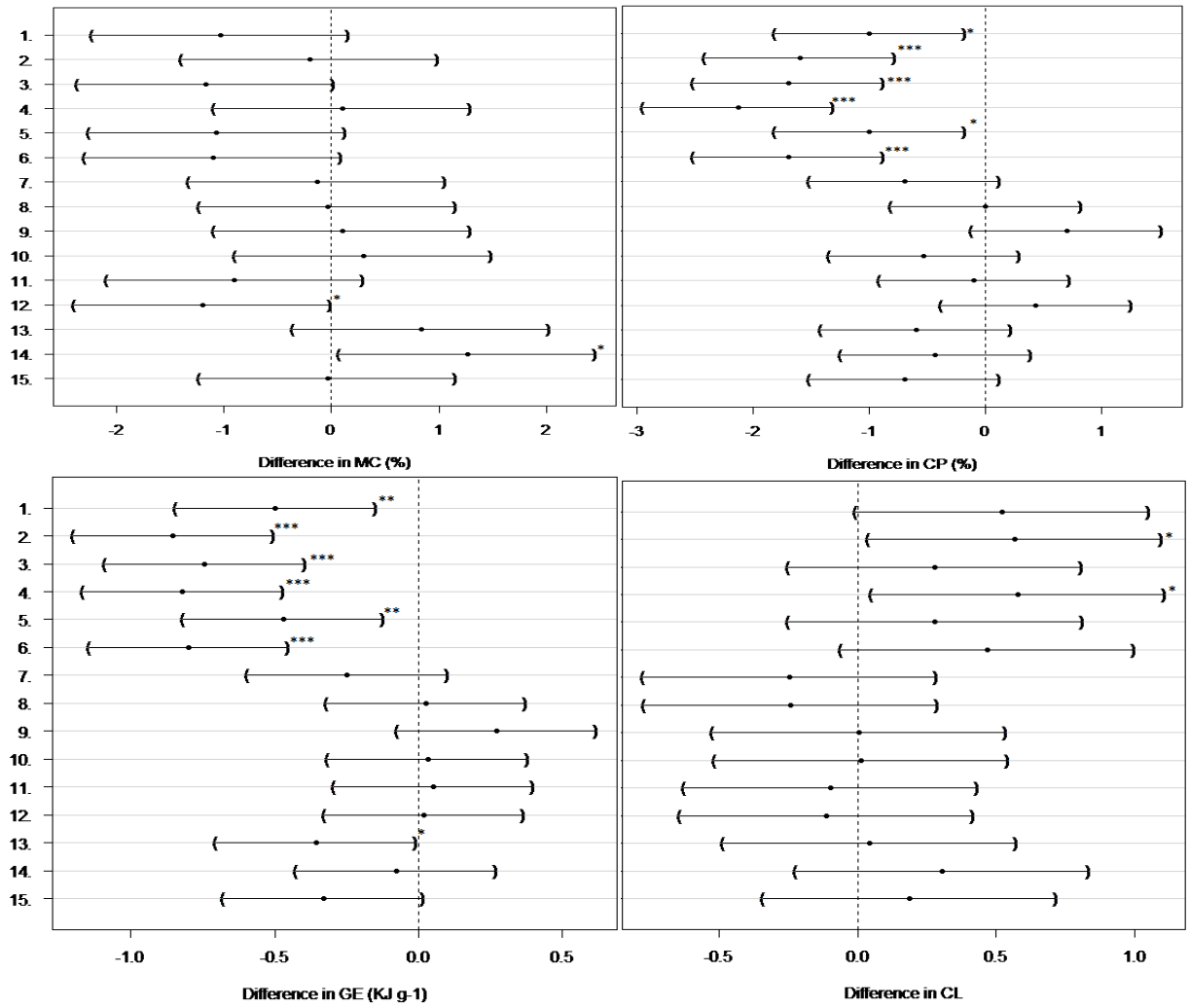

Figure 7.4 The effect of oil seed cake formulation and level of dietary inclusion on whole body nutrient and energy composition of juvenile Nile tilapia. Points in each chart represent estimates of the differences for the parameters stated (CP: Crude protein, CL: Crude lipid, MC: Moisture content and GE: Gross energy) on the x-axis and parentheses mark the limits of the simultaneous 95% confidence regions for these differences. Calculated are differences of means for 15 user defined contrasts (1.F1(25)–CON, 2.F1(50)–CON, 3.F2(25)–CON, 4.F2(50)–CON, 5.F3(25)–CON, 6.F3(50)–CON, 7.F2(25)-F1(25), 8.F3(25)-F1(25), 9.F3(25)-F2(25), 10.F2(50)-F1(50), 11.F3(50)-F1(50), 12.F3(50)-F2(50), 13.F1(50)-F1(25), 14.F2(50)-F2(25) and 15.F3(50)-F3(25)).The null hypothesis is rejected if the confidence interval does not include the value zero.

Adjusted p-values are indicated for each contrast along with each confidence interval (***: P<0.0001, **: P<0.01; *: P< 0.05).

7.3.6 Cost-benefit analysis of diets

The costs of ingredients used in this analysis are presented in Table 4.2. The costs per kilogram of experimental diets were calculated. The cost per kilogram of experimental diets varied little with the control having the least (5.3 birr) and diet 5 (F2(50)) the highest (6.4 birr) (Table 7.9). The cost analysis, however, showed that the highest profit was obtained by diet 6 (F3(25)) and the lowest by diet 5 (F2(50)).

Table 7.9 Cost analysis of diets fed to *O. niloticus* in the experiment

([1]Birr kg^{-1}, exchange rate 20.47 Ethiopian birr= USD 1.00 (2015), sale price of fish= 30.0 birr kg^{-1} fish)

Diet	Diet cost[1]	Incidence cost[1]	Profit index
1. Control	5.3	14.8	2.02
2. F1(25)	5.8	16.1	1.86
3. F1(50)	6.2	17.4	1.73
4. F2(25)	5.9	18.7	1.6
5. F2(50)	6.4	24.3	1.23
6. F3(25)	5.7	14.7	2.04
7. F3(50)	6.0	18.6	1.61

7.4 Discussion

Results of the present study indicated the potential of the three oil seed cake formulations made up of two locally available oil seed cakes (Niger seed cake and linseed cake) for inclusion in Nile tilapia feeds. The study also demonstrated the feasibility of incorporating plant protein mixtures (PPM) at higher levels with good success.

In the present study, feeding F1(a formulation with 50% NSC and 50% LSC) and F3 (a formulation with 67% NSC and 33% LSC) oil seed cake formulations at 25% level of dietary inclusion did not affect growth in Nile tilapia. Also increasing the level of F3 inclusion in the diet of Nile tilapia to 50% did not affect growth rate. This is an improvement compared to oil seed cakes fed individually (Chapters 5 and 6) to Nile tilapia, especially linseed cake in which inclusion level of 20% led to a significant reduction in final body weight (Chapter 5). These results corroborate the findings of Olukunle (1982) and Richards (1983) who found similarly improved growth performance in *O. mossambicus* fed mixtures of sunflower meal, groundnut meal and sesame meal rather than feeding individual plant protein. A significant amount of researches have demonstrated the potential of utilizing plant protein mixtures to replace high proportion of fish meal without the need for extra dietary supplementation (Fontainhas-Fernandes *et al.*, 1999; El-Saidy and Gaber, 2003; Borgeson *et al.*, 2006). In the study by El-Saidy and Gaber (2003), a mixture of soybean, cottonseed, sunflower and linseed meals (all in equal proportion of 25%) were used to demonstrate the potential of relying completely on mixtures of plant proteins. Borgeson *et al.* (2006) investigated simple (soybean meal and maize gluten meal) or complex (soybean meal, maize gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) plant protein mixtures and found that complete

replacement of fishmeal by complex plant mixture did not significantly affect the growth performance of Nile tilapia.

In the present study, growth performance (FW, WG and SGR) of fish fed the control diet was significantly higher than all the test diets with the exception of F1(25) and F3(25). Among the oil seed cake based diets, growth performance of fish fed diets containing F1 and F3 formulations at the same level of inclusion (25% or 50%) did not show significant differences, but significantly higher performances than fish fed diets with F2 formulation was observed. Poor growth performance of fish fed diets with F2 formulation that is high in the proportion of linseed cake reaffirmed the reduced body weight registered in fish fed diets containing this ingredient (Chapter 5). Therefore, the improved growth and feed utilization in diets containing 25% of F1 and F3 formulations with less linseed cake proportion, is probably due to reduced exposure to antinutritional factors responsible for suppressing feed intake. It has been suggested that combining several plant protein sources with inherent antinutritional factors may reduce exposure of fish to those antinutritional factors due to lower inclusion levels of each of the individual protein sources in the combined mixture (Fontainhas-Fernandes *et al.*, 1999; Borgeson *et al.*, 2006). Mixing plant proteins may also lead to interactions between various antinutritional factors or with other components in the diet resulting in the reduction of their deleterious effects (Francis *et al.*, 2001). For example, interaction between tannins and lectins removed the inhibitory action of tannins on digestive enzyme amylase (Fish and Thompson, 1991), and interactions between tannins and cyanogenic glycosides reduced the deleterious effects of the latter (Goldstein and Spencer, 1985). Plant products, especially oil seed cakes, usually have poor amino acid profiles (Tacon, 1997). When growth comparisons are made in between diets with mixtures of plant proteins, interpretation of the results is very difficult because the interaction

between nutrients may be involved. In this study no attempts were made to measure the amino acid contents of the ingredients and the diets used. Studies indicated that using different plant protein sources in combination results in protein complementation so that the resulting mixed complementary amino acid profiles make up the required optimal amounts of essential amino acids (EAA) (Hossain, 1988; Sadiku and Jauncey, 1995). Tadelle Dessie *et al.* (2002) reported that the protein in Niger seed cake contains high levels of sulphur containing amino acids that compares well with soybean meal and cotton seed meal. Although the biological availability of amino acids in linseed meal is questionable, Hanafy (2006) reported the amino acid profile of linseed meal as one of the best after soybean meal. It is, therefore, difficult to rule out improved amino acid balance in oil seed cake based diets as a possible explanation for improved growth in this study relative to feeding single plant protein ingredients.

In the present study significantly lower feed intake was found in oilseed cake based treatments, except for diet F1(25). Feed intake of fish fed the control diet and diet F1(25) were similar to the one reported by El-Saidy and Gaber (2003). The comparisons of feed intake for the different formulations separate for each level of inclusion and the comparisons between the levels of inclusion separate for each formulation indicated no statistically significant differences. FCRs of oilseed cake based diets were not significantly different from the control diet with the exception of diet 3 F2(50). No significant differences in FCR were observed in between F1 and F3 formulations at each inclusion levels. Also, increasing the level of F1 inclusion did not significantly affect FCR and similar patterns were observed for PER, PPV and FI. This suggests that mixtures of plant proteins in equal proportions are better utilized when incorporated in the diets of Nile tilapia. This could be due to the compensatory effect which led to some reduction of antinutritional factors and improved palatability as a result of mixing.

To some extent the digestibility coefficients were reflective of the digestibility of individual ingredients. Nutrient digestibility of linseed cake was found to be lower than Niger seed cake in Chapter 4. Results of this study showed that increasing the proportion of Niger seed cake, which is more digestible than linseed cake, up to 50% resulted in increased digestibility. This observation is in agreement with the suggestion that digestibility of a compound diet will depend on the digestibilities of individual ingredients (Fontainhas-Fernandes *et al.*, 1999). This is due to the fact that individual digestibilities are additive though a potential for interaction also exists. This was demonstrated in hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus* (Sklan *et al.*, 2004) as well as in gilthead sea bream, *Spaurus aurata* (Lupatsch *et al.*, 1997) in which individual nutrient digestibility values could be used for estimating digestibilities in compounded feeds.

Compared to the control group, the whole body CP and GE of fish fed oilseed cake based diets were significantly lower. Results of ash and moisture contents of whole body of fish were not significantly different between the control and dietary treatments. However, there was an increase in protein, lipid, ash and energy contents of fish in comparison with the initial carcass values in all the dietary treatments. In this study the diets containing higher levels of plant protein inclusion produced significantly lower carcass lipid. This observation is in agreement with Adebayo *et al.* (2004), Fagbenro (2004) and Azaza *et al*. (2008) who observed similar reduction in carcass lipid in fish fed diets containing plant proteins.

From the above discussion, it may be concluded that the reduced growth performance of fish fed F2 formulation may be related to the limiting level of essential amino acid in the diet, high antinutritional substances in linseed cake which depressed the feed intake and growth in fish fed F2 formulation with higher proportion of linseed cake. The result of this study on the nutritional

quality of the different formulations as an alternative protein source for Nile tilapia indicates that up to 50% of F1 and F3 could be incorporated in the diets of Nile tilapia, which corresponds to a reduction of 50% of fish meal of the control diet. The most efficient diet in terms of cost per unit weight gain of fish was obtained with the 25% F3 formulation inclusion level. This may be attributed to the low cost of procuring Niger seed cake, which is about 67% of the cost of formulation 3 (F3). The expected reduction in the cost of mixed oilseed cake based diets may justify the use of this formulation in Nile tilapia feed.

Chapter 8- General Conclusions and Recommendations

In Ethiopia, the physical and socio-economic conditions favour the development of aquaculture. However, the contribution of aquaculture is far below its potential due to various problems including lack of quality feeds. In view of this, a search for locally available low cost alternative feed ingredients that can be used as protein sources in fish diets is a key issue to be addressed for the development of aquaculture in Ethiopia. Therefore, this study evaluated suitability of Niger seed cake (NSC) and linseed cake (LSC) as potential feed ingredients in Nile tilapia diets. The choice of these ingredients was based on their relatively high nutritional content, local availability, abundance, and potential cost effectiveness.

Major conclusions from the study are as follows:

1. Nutrient digestibility study revealed that Nile tilapia may be able to utilize NSC better as dietary protein source due to fairly high protein digestibility coefficient (72.6%) than LSC (62.4%). Apparent energy digestibility also followed the same trend with NSC having higher coefficient (72.9%) than LSC (53.7%). Lipid digestibility was not significantly different between the two ingredients. Compared to the two oilseed cakes tested the apparent nutrient digestibility of soybean cake (SBC) was significantly the highest, except for lipid digestibility. The lower nutrient digestibility of LSC was attributed to antinutritional factors (ANFs) present in linseed cake.
2. Evaluation of linseed cake (LSC) inclusion individually as protein source at different levels (i.e. 0, 20, 40 and 60%) in Nile tilapia diets demonstrated that it can only be used up to 20% without any adverse effect on growth and feed efficiency. Growth depression

at higher levels may be attributed to high levels of ANFs, high fibre content and poor amino acid profile.

3. Evaluation of Niger seed cake (NSC) inclusion individually as protein source at different levels (i.e. 0, 20 and 40%) in Nile tilapia diets demonstrated that it can only be used up to 20% without any adverse effect on growth and feed utilization. Growth depression at higher level may be attributed to higher crude fibre content and poor amino acid profile.
4. Evaluation of NSC and LSC mixtures in various proportions were more effective than the single individual sources. Growth and feed utilization of fish fed F1 and F3 formulations showed that up to 50% inclusion could be more effective than a single source in Nile tilapia diets. This was particularly evident with equal proportions of NSC and LSC (F1), which did not significantly affect feed utilization at 50% level of dietary inclusion. This could be due to a compensatory effect which led to some reduction of ANFs and improved palatability as a result of mixing. The most efficient diet in terms of cost per unit weight gain of fish was diet 6 (F3(25)).

Further studies are recommended on:

1. Analysis of Nile tilapia diets formulated based on the amino acid and fatty acid profiles of linseed cake and Niger seed cake. In this study, the feed was formulated based on the proximate composition of the ingredients.
2. Dietary factors relating to the intestine morphology (histological analysis) also need to be assessed for the two oilseed cakes since the intestine is the key site for nutrient absorption.

3. Exploring different methods of improving the nutritional profile of ingredients such as removal of antinutritional factors as well as reducing fibre content using methods like ensiling to improve digestibility
4. Identifying and evaluating other locally available materials from both plant and animal sources that have a potential to serve as protein sources in formulating cost effective diets

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