Oilseed Meals as Dietary Protein Sources for Juvenile Nile Tilapia (*Oreochromis niloticus* L.)

Thesis submitted for the degree of Doctor of Philosophy

By

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Dedication

Dedicated

to

My wife and son

Declaration

I hereby declare that this thesis has been achieved by myself and is the result of my own investigations. It has neither been accepted nor submitted for any other degree. All sources of information have been duly acknowledged.

Nelson Winston Agbo

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Abstract

One of the major problems facing aquaculture in Ghana is the non-availability of quality and affordable fish feeds. The present study investigated the nutritional suitability and cost-effectiveness of some Ghanaian oilseed by-products, soybean meal (*Glycine spp*), cottonseed meal (*Gossypium spp*), groundnut cake (*Arachis hypogaea* L.) and groundnut husk, as alternative protein sources to fishmeal (FM) in the diet of Nile tilapia (*Oreochromis niloticus* L.*)*. The oilseed meals were used individually, as mixtures, as mixtures enriched with methionine and mixtures detoxified by heat processing (autoclaving) and/or addition of supplements (viz. phytase and ferrous sulphate) intended to reduce levels of the most important antinutritional factors (ANFs). Diets, containing the oilseed meals at inclusion levels from 25% to 75% dietary protein, were formulated to be isonitrogenous (320 $g.kg^{-1}$), isolipidic (100 $g.kg^{-1}$) and isoenergetic (18 $KJ.g^{-1}$) and fed to juvenile Nile tilapia at 4-10% of their body weight for a period of eight weeks.

Proximate analysis showed that soybean meal (SBM), cottonseed meal (CSM), groundnut cake (GNC) and groundnut husk (GNH) had 500.3, 441.4, 430.5 and 205.6 $g.kg^{-1}$ crude protein, 38.2, 89.5, 12.8 and 89.2 $g.kg^{-1}$ crude fibre and 20.19, 19.61, 23.17 and 22.18 $kJ.g^{-1}$ gross energy respectively. Generally the oilseed meals had good essential amino acid (EAA) profiles with the exception of GNH. The EAA profile of SBM compared very well with FM but methionine and threonine were low (0.73 and 1.50 % of protein respectively) and the same was true for CSM and GNC with even lower levels. Analyzed ANFs in SBM, CSM, GNC and GNH were 17.54, 31.64, 14.86 and 3.99 $g.kg^{-1}$ phytic acid, 14.09, 1.24 and 2.34 $g.kg^{-1}$ trypsin inhibitors and 5.80, 6.50, 8.01 and 10.08 $g.kg^{-1}$ saponin respectively and in CSM 5.6 $g.kg^{-1}$ gossypol. Nutrient digestibility of these oilseed proteins suggested that Nile tilapia may be able to utilize SBM, CSM and GNC efficiently as dietary protein sources due to high apparent protein digestibility of 94.50%, 84.93% and 90.01% respectively. However, GNH may not be suitable because of very low apparent protein digestibility (27.67%).

These protein sources when used individually were shown to cause depressed growth and feed efficiency when substituting more than 50% of the FM protein in diets. This may be attributed to high levels of ANFs, high fibre content and poor EAA profile. However, the use of mixtures of these meals was found to be marginally more effective than that of single sources. This may have been as a result of lower levels of ANFs and improvement in essential amino acid profile due to mixing. Supplementing the mixtures with methionine led to improvement in feed utilization but without significantly improving the nutritive value compared with FM. Heat processing was effective in reducing heat labile trypsin inhibitors in SBM, CSM and GNC by almost 80%, but not phytic acid and saponins, which remained virtually unaffected. Use of meals detoxified by heat processing with/without supplements at 50% inclusion improved growth and feed utilization compared to the unprocessed meals and performance was generally not significantly different from FM.

Cost effectiveness analysis revealed that diets containing single feedstuffs or mixtures, particularly those containing equal proportions of oilseed meals and higher proportion of CSM replacing between 50% - 75% FM protein, were more profitable than FM diet. Similarly, the use of heat processed meals at 50% replacement of FM protein yielded greater profit than all other diets including the FM diet. However, essential amino acid supplementation of the meals was less profitable compared to the control. Generally, fish fed diets with oilseed meals would take longer to attain harvest size compared with FM and this could lead to an increase in production costs or a decrease in the number of production cycles which could be achieved within a year.

It can be concluded that there is nutritional and economic justification for using SBM, CSM and GNC as partial replacement for FM in diets of Nile tilapia. Based on growth performance, nutrient utilization and economic benefits the diet with heat processed oilseed meal mixtures (containing equal proportions of 16.67% each) at 50% inclusion has the best prospects for replacing FM protein in diets of *O. niloticus*.

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Chapter 1 - General Introduction

1.1 Global Overview of Aquaculture

Fish has long been valued as a source of protein for human nutrition. Consumption of fish generally cuts across ecological, socio-economic, cultural and religious boundaries, leading to its predominant role as an animal protein. Presently fish accounts for over 50% of total animal protein consumed in most developing countries and global estimate stands at 15.5% in 2003 (FAO, 2007b). Fish is a first class high-quality animal protein and relatively the cheapest source (Tidwell and Allan, 2001).

Global per capita fish consumption has increased over the past four decades, rising from 9.0 kg in 1961 to an estimated 16.5 kg in 2003 (FAO, 2007b). 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 (Tidwell and Allan, 2001). Global capture fisheries production especially marine fisheries resources are being exploited to their maximum or beyond the level of sustainability, the worldwide demand for fish is increasing, raising the question of whether in the future the global demand for fish products can be met (ICTSD, 2003). In order to breach the gap between demand and supply aquaculture is seen as the best solution.

Aquaculture is defined by FAO (1990) 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). It is now the fastest growing animal producing sector with an average annual growth rate for the world of 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO, 2007b). 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 (with a production of 67 mmt), there is still a negative balance between demand (110 mmt) and supply of fishery products (FAO, 2007a). 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 mmt 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 aquacualture 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).

1.1.1 Aquaculture Production

Global aquaculture production in 2006 was reported to be 67 mmt with a value of slightly over USD 86 million (FAO, 2007a) (Figure 1.1). Of the world total, China accounted for nearly 70% of the quantity and over half the global value of aquaculture production. Aquaculture production in developing countries increased at an annual rate of 11%, compared with 5% for China and about 2% for the developed countries (FAO, 2007b). 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 (Halwart *et al*., 2003; Hasan, 2001).

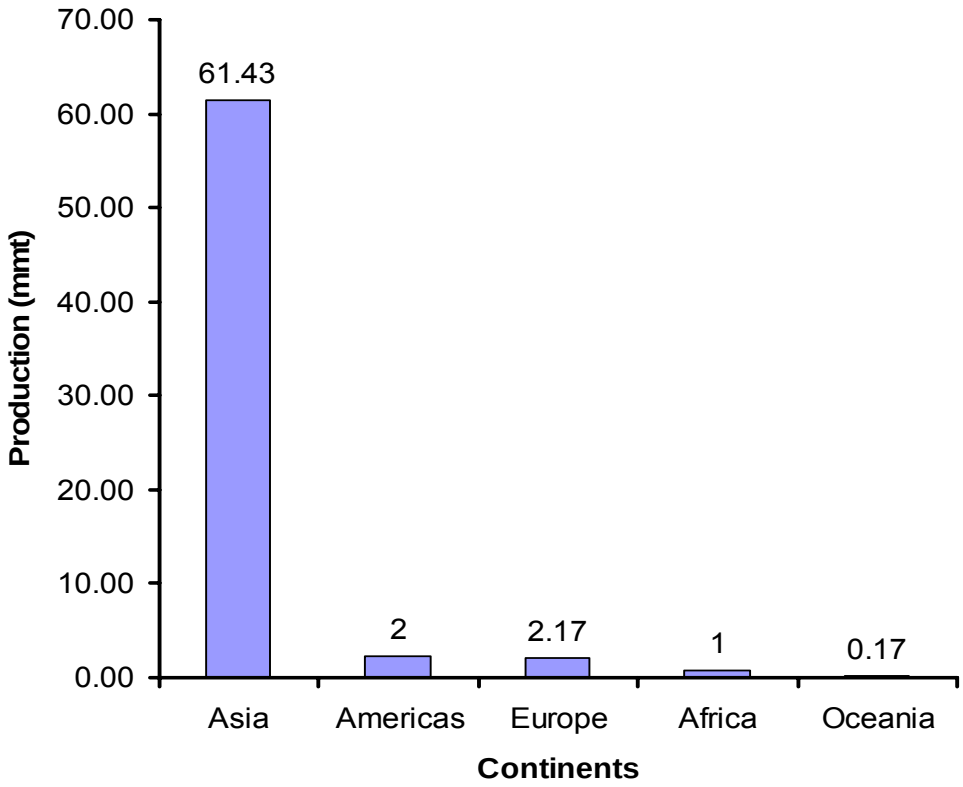

Figure 1.1 Global aquaculture production by continents for 2006 (FAO, 2007a)

1.1.2 Aquaculture in Africa

Total aquaculture production in Africa in 2006 was estimated to be 760,036 mt which is about 81.5% increase in the last decade (Figure 1.2). Egypt alone contributed about 595,030 mt (78.3%). Total aquaculture production in sub-Saharan Africa (SSA) is 160,302 mt accounting for only 21.1% of African production (FAO, 2007a). 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 1997 and 2006, overall aquaculture production in SSA has increased by 68.4% from 50,609 mt to 160,302 mt (FAO, 2007a)(Figure 1.3).

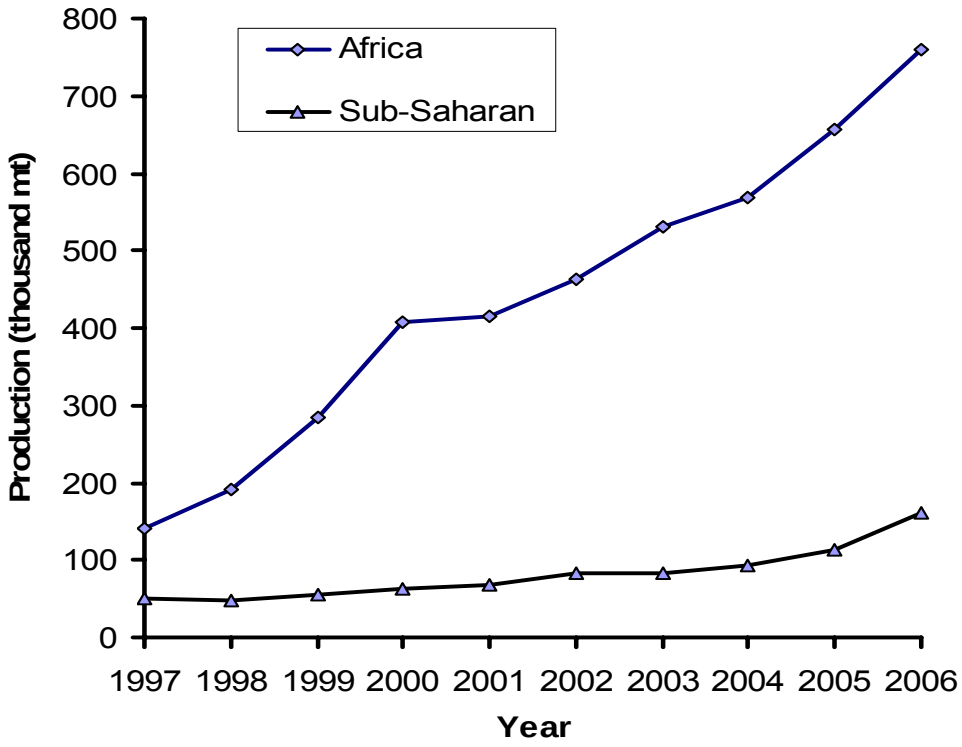

Figure 1.2 Aquaculture production in Africa and Sub-Saharan Africa for 1997-2006 (FAO, 2007a)

Although Africa has enormous potential for fish farming with 37% of its surface area suitable for artisanal and 43% suitable for commercial fish production (Aguilar-Manjarrez and Narth, 1998) it continues to be a minor player in global aquaculture.

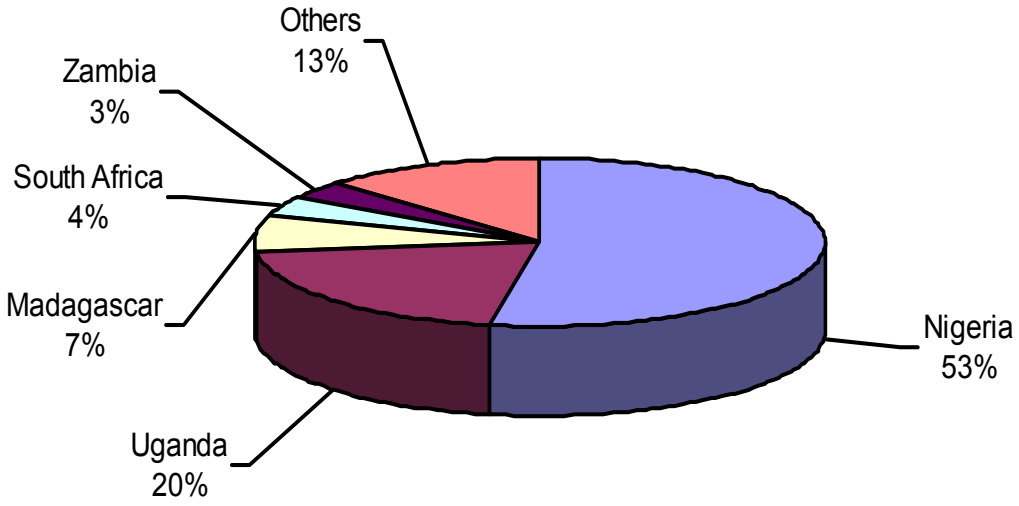

Figure 1.3 Aquaculture production by the top five countries in Sub-Saharan Africa (Total production 160,302 mt, 2006; FAO, 2007a)

FAO estimates that fish provides 22% of the protein intake in sub-Saharan Africa. This can, however, exceed 50% in the poorest countries, especially where other sources of animal protein are scarce or expensive (WorldFish Center, 2005). Per capita consumption of fish in sub-Saharan Africa is the lowest in all regions and continues to decline. This has been attributed to levelling off in capture fish production and increasing population growth (World Bank, 2004). In order to maintain the current level of per capita supply of fish in Sub-Saharan Africa (6.6 $kg.yr^{-1}$) up to 2015, fish production (capture fisheries and aquaculture) must increase by 27.7% over this period. This assumes an average annual population growth of 1.9% over the period 2002–2015 (World Bank, 2004). FAO projections show that with just 5% of the suitable areas used, Africa could meet its fish production target but it would need a lot of work. Based on 1997 levels of production, aquaculture would have to increase by 267% by 2020 to maintain the current fish consumption level in Africa (Delgado *et al*., 2003).

According to Hecht (2007) > 70% of total aquaculture production in SSA comes from commercial farms, produced by less than 20% of farmers, while the remaining < 30% is produced by small-scale farmers that represent over 80% of all farmers. The systems used by the commercial sector range from semi-intensive to intensive pond, cage and tank culture of catfish (*Clarias spp*.) and tilapia (*Oreochromis spp*.) and high value products such as shrimp (Madagascar and Mozambique) and abalone (South Africa) while non-commercial subsistence aquaculture primarily consists of small-scale pond culture of tilapia, catfish and common carp, *Cyprinus carpio* (Hecht, 2007).

Machena and Moehl (2001) reported that the major aquaculture products in Africa are mainly fresh- and brackish-water finfish.

According to Machena and Moehl (2001) the attributes of SSA include under-utilized water and land resources, available and inexpensive labour, high demand for fish and a climate that favours all year-round production. However, optimal use of these resources has frequently been curtailed by poor infrastructure and lack of production inputs. The potential for expansion is nevertheless considerable, but requires several enabling factors that include; a positive perception of aquaculture, sound policies at the national level, strong public institutions, availability of nutrient inputs, conducive investment policies to attract increased private-sector participation, and access to credit for commercial-scale enterprises (Machena and Moehl, 2001). Increasing demand for fish coupled with rapidly dwindling catches from capture fisheries, the implementation of new participatory approaches to technology development and transfer, and the emergence of a few successful large-scale tilapia culture operations directed at the export market offer opportunities for further expansion in both the small-scale and commercial sectors (Jamu and Brummet, 2004).

1.2 Aquaculture in Ghana

1.2.1 Historical Background

Modern forms of aquaculture were first introduced into Ghana by the Department of Fisheries in the 1950s when pond construction began on an experimental scale and farmed fish were used to stock reservoirs. Before then there were traditional practices such as ‘atidjas’ (brush parks in lagoons and reservoirs), ‘hatsis’ (fish holes), ‘whedos’ (mini dams in coastal lagoons) and the

culture of fresh water clams (*Egeria radiata*) in the Lower Volta (Balarin, 1988; FAO, 2004).

Although aquaculture started in the 1950s it was only from the 1980s that the private sector began to show real interest. This was as a result of a massive nationwide campaign launched by the government to encourage pond fish culture in the early 1980s. This campaign lacked technical support in terms of pond design, construction and management, provision of inputs such as fingerlings, feeds and fertilizers as well as harvesting and marketing strategies and therefore led to very poor harvests and subsequent mass failures. Most private entrepreneurs were so disappointed that they lost interest completely in fish farming (Manu, 2004; Prein and Ofori, 1996). This could be said to be the main problem that has resulted in the present state of aquaculture and the loss of interest by many prominent private farmers and entrepreneurs in fish farming. Systematic development of aquaculture was then reinitiated in the mid 1990's through the Fisheries Sub-Sector Capacity Building Project. Under that project, aquaculture hatcheries to produce and supply fingerlings to farmers were constructed and Fisheries Officers were also empowered through training to become better extension agents and provide the needed technology backup to fish farmers (Manu, 2004).

1.2.2 Need for Aquaculture Development in Ghana

Capture fisheries in Ghana are broadly categorized into marine and inland capture fisheries. Inland capture fisheries mainly occur in the Volta Lake, which is one of the largest man-made lakes in the world. Capture fisheries contributes over 90% of local fish catch and the rest comes from aquaculture (Nyanteng and Asuming-Brempong, 2003). Annual fish production from capture fisheries

for the period 1996 to 2006 was on average 425,325 mt (FAO, 2007a). Production trends in Figure 1.4 show that it peaked in 1999 and started declining since then. Directly or indirectly, capture fisheries provide livelihood for more than 2.2 million people and account for about 5% of agriculture gross domestic production (GDP) of the country (FAO, 2006; ISSER, 1997). According to Amevenku (1999) fish as a commodity is a major source of animal protein contributing about 60-70% of animal protein intake in the diet of many Ghanaians. Thus, the industry made up of fishing, processing, distribution and marketing is a vital socioeconomic activity for many families and communities. Therefore, any situation which adversely affects fishing activity and eventually the availability of fish would be expected to have an adverse effect on the entire population

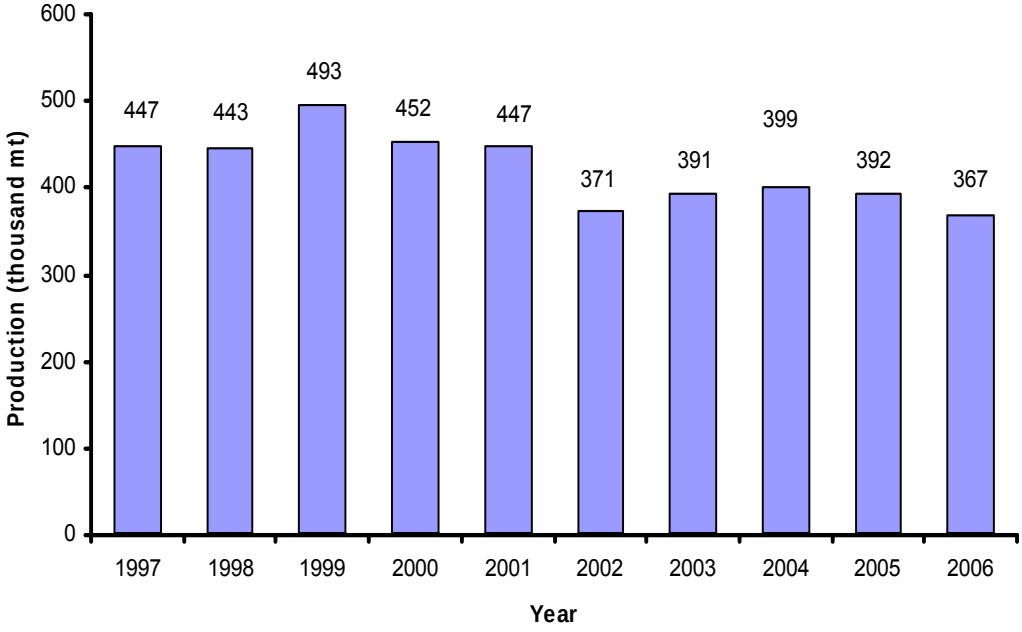

Figure 1.4 Fish production from capture fisheries in Ghana for 1997-2006 (FAO, 2007a)

In 2003, Ghana produced only 51.7% of its fisheries requirements from domestic sources and in 2004, achieved 68.1% of its fish requirement through domestic production and imports (FAO, 2006). With a shortfall of about 400,000

mt in domestic fish production from capture fisheries, resulting in an annual fish import of 200 million USD, the government of Ghana is relying on aquaculture development to make up for the shortfall (Ministry of Fisheries, 2005). The shortfall in fish supply illustrated in Table 1.1 forms a basis for the need for an alternative means of increasing fish production in Ghana. This situation, coupled with the fact that other sources of animal protein (mutton, pork, poultry, small ruminants, beef etc.) are relatively more expensive, make it imperative that measures be taken to increase fish supply so as to bridge the widening gap between supply and demand. Aquaculture and culture-based fisheries now provide the best hope for Ghana to supply sufficient fish to a growing population which has a clear preference for fish as its principal source of animal protein (Manu, 2004).

Table 1.1 Projected fish supply and demand for Ghana to 2022

Year	Supply (mt)	Demand (mt)
2007	511,836	913,992
2012	584,767	1,044,226
2017	668,090	1,193,017
2022	763,286	1,363,010

Tradezone International, 2007

1.2.3 Aquaculture Production

Aquaculture production has more than doubled over the past ten years in Ghana from 550 mt in 1996 to 1,150 mt in 2006 (Figure 1.5)(FAO, 2007a). However, fish production was inconsistent from 1998 to 2002 as shown in Figure 1.5. Production levels were high with a drastic drop from 2003. According to Awity (personal communication) aquaculture production prior to 2003 used to be estimated and this could have led to overestimation, but in subsequent years a proper survey was conducted by the Directorate of Fisheries to ascertain actual production levels. This explains the drastic drop in

aquaculture production in 2003 after which there has been a gradual increase to 2006. Aquaculture production is very low compared with capture fisheries and was estimated as 1,150 mt in 2006 which was less than 1% of national fish production. The sub-sector comprises mainly small-scale and a few commercial operators. Average productivity from small-scale operators was less than 2.5 $mt.ha^{-1}yr^{-1}$ (Awity, 2005). Commercial aquaculture only emerged during the last five years but is already contributing between 30% and 35% of total production (Hecht, 2007).

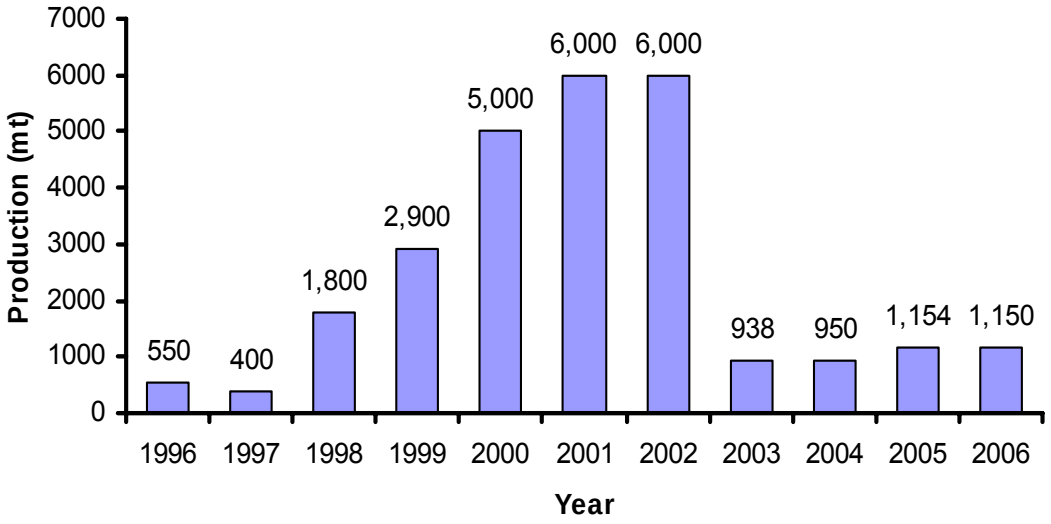

Figure 1.5 Aquaculture production (mt) in Ghana for 1996-2006 (FAO, 2007a)

Fish species cultured in Ghana are presented in Table 1.2. These include mostly tilapia species with *Oreochromis niloticus* being the most predominant single species constituting over 80% (950 mt in 2006) of aquaculture production (FAO, 2007a). Other species, particularly the catfishes (*Clarias sp., Heterobranchus sp*.), account for the remaining 20%.

Table 1.2 Fish species cultured presently and potential future candidates in Ghana

Species	Common names	Remarks
Oreochromis niloticus	Nile tilapia	Main culture species
Tilapia zillii	Redbelly tilapia	Cultured by small-scale
Sarotherodon galilaeus	Mango tilapia	farmers
Sarotherodon melanotheron	- Blackchin tilapia	" "
Hemichromis fasciatus	-	Often cultured in
Clarias gariepinus	North African catfish	polyculture with tilapia
Heterobranchus longifilis	Mudfish	"
Chrysichthys walkeri	Chrysichthys	"
Heterotis niloticus	Africa bonytongue	"
Parachanna obscura	Snake-head	Being considered for
Lates niloticus	African pike	aquaculture
Mugil sp.	Mullet	"

Source (FAO, 2006, Hecht (2007)

The Agriculture Sector, of which fisheries is part, contributed 35.3% and 37% to the GDP of the country in 2000 and 2005 respectively (Earthtrends, 2003; Tradezone International, 2007). Fisheries constitutes an important sector in national economic development and is estimated to contribute 3% of the total GDP and 5% of the GDP in agriculture (FAO, 2006). The contribution of aquaculture to the national economy has, however, not been disaggregated, so its importance is not fully recognized. There is lack of data and general information relating to aquaculture (Awity, 2005). For instance, information and data are not available on the exact contribution of aquaculture to food security, employment and poverty alleviation. It has, however, been estimated that production from ponds and culture-based fisheries is worth about USD 1.5 million a year (FAO, 2006).

Extensive, semi-intensive and intensive culture systems are practised in Ghana. Extensive aquaculture usually takes place in dams, dug-outs and small reservoirs, through restocking while semi-intensive culture of fish takes place in earthen ponds either as monoculture of tilapia or polyculture of tilapia and catfish (FAO, 1997). The aquaculture sub sector comprises largely small-scale

farmers who practise extensive or semi-intensive aquaculture in earthen ponds. The sector therefore lacks the capability to take up challenges of providing inputs such as fish seed and feed as viable commercial activities to support the development of the industry. Medium- to large-scale intensive (commercial) fish farming as a major farming activity is a recent development and has opened up avenues for employment (Awity, 2005). Most of the commercial establishments also produce fish from earthen ponds, however, there are few cage farms producing mainly tilapia in large quantities (FAO, 2006). Due to poor fish production from small scale farmers over the years, government's approach this time is to stimulate the development of aquaculture as a business-oriented enterprise and to uncover and create economic opportunities in the sector which is quite a deviation from the initial objective of developing it to provide food security for local subsistence (Ministry of Fisheries, 2005).

The potential for aquaculture development in Ghana is high in terms of land, water and other resources. Ghana is well-drained with water bodies (i.e. lagoons, estuaries, rivers, reservoirs and lakes; others are small water bodies, floodplains and swamps) the most important of which is the Volta Lake with a surface area of 8,730 km^2 and a shoreline of 4,880 km and a network of tributaries (Kapetsky, 1989; Mensah, 1979). There are more than 100 species of fish in Lake Volta system and most of these are exploited commercially and some serve as aquaculture species. Catches from the lake are dominated by *Tilapia sp., Chrysichtys sp., Synodontis sp., Mormyrids sp., Heterotis niloticus, Clarias sp., Schilbeide sp., Odaxothrissa mento, Bagrus sp. and Citharinus sp.* (Vanderpuye, 1984). Ghana also has vast land areas suitable for aquaculture development. With the assistance of the Food and Agriculture Organization

(FAO) of the United Nations, the Ministry of Food and Agriculture has mapped out areas in Ghana that are suitable for general aquaculture, including shrimp/prawn farming. At present only a small fraction of the area (less than 0.05%) is being used for pond culture. There is also high potential for cage culture in the Volta Lake system, dams and reservoirs in the country (FAO, 2006).

Despite the potential mentioned above, aquaculture in Ghana has not performed as expected due mainly to the following constraints: limited availability of good quality fish feed and seeds; inadequate extension and training services; limited direct domestic and foreign investment and credit facilities; undefined or poorly defined land rights, water rights and lack of legislation specifically for aquaculture (Ministry of Fisheries, 2005).

For comprehensive development of fisheries and aquaculture the government of Ghana has taken several steps to improve aquaculture. Some of the measures put in place include: the training of staff to provide good extension services; organizing short courses in fish farming techniques and study tours for fish farmers to expose them more to the industry; training of youth groups in pond construction so as to improve the ponds to the highest standard; setting up hatcheries for fingerling production for sale to fish farmers; strengthening the organizational capacity of fish farmers' associations through training in bookkeeping, group dynamics and the preparation of business plans; enacting several laws and policies to help regulate and govern the aquaculture sector (eg. Ghana's irrigation policy allows 5% of all irrigation sites to be used for aquaculture) and prohibition of farmed fish imports except with a permit from the

Ministry of Fisheries to ensure there is a good price for aquaculture products in the country (FAO, 2006).

1.3 Tilapia Culture

“Tilapia” is a generic term which is used to designate a group of commercially important food fish belonging to the family Cichlidae. Tilapia have been raised as food for human consumption for a long time; illustrations from Egyptian tombs suggest that the Nile tilapia, *Oreochromis niloticus*, was cultured more than 3000 years ago (Maar *et al.*, 1966). According to Pompa and Masser (1999) tilapia are referred to as “Saint Peter’s fish” in reference to biblical passages about the fish fed to the multitudes. Although endemic to Africa their distribution has been extended by introduction to include much of the tropics and subtropics. More than 100 species have been identified (Balarin, 1979). Currently, tilapia culture is widely practiced in many tropical and subtropical regions of the world. More than 22 tilapia species are being cultured worldwide. However, Nile tilapia (*Oreochromis niloticus), Mozambique tilapia (O. mossambicus), blue tilapia (O. aureus), O. hornorum, O. galilaeus, Tilapia zillii and T. rendalli* are the most commercially cultured tilapia species. Tilapia species are used in commercial farming systems in almost 100 countries and are developed to be one of the most important fish for aquaculture in this century (Fitzsimmons, 2000).

1.3.1 Tilapia Production

Tilapias are second only to carps as the most widely farmed freshwater fish in the world; three-quarters of the world's supply comes from aquaculture. World tilapia production has been increasing steadily during the last decade, with output more than doubling from 931,000 mt in 1997 to 2.3 million mt in 2006

(FAO, 2007a)(Figure 1.6). Tilapias are the third largest group of farmed finfish species, only after carps and salmonids with an average annual growth rate of about 11.5% (FAO, 1997). About 2.3 million mt. or 99% of farmed tilapia were produced in developing countries in 2006 with Africa producing about 13% of this amount (FAO, 2007b). Egypt is the second biggest tilapia producer (202,606 mt) after China and is the world's top producer of mullets (FAO, 2007b). In Ghana Tilapia (mainly *Oreochromis niloticus*) is the major species farmed and constitutes over 80% (950 mt in 2006) of aquaculture production (FAO, 2007a).

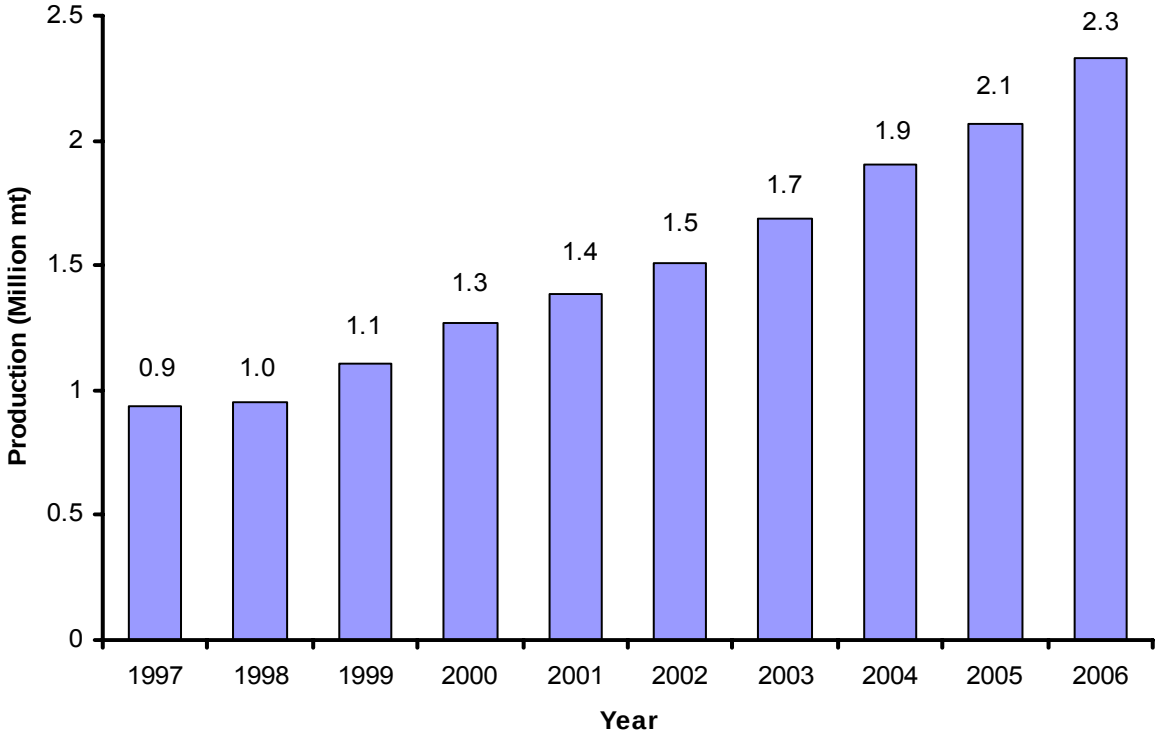

Figure 1.6 Global production of tilapias in aquaculture for 1997-2006 (FAO, 2007a)

The remarkable success of tilapias as a farmed fish can be attributed mainly to the following factors; they have desirable qualities as a food fish such as white flesh, neutral taste and firm texture, which has made them gain acceptance in a

wide variety of human cultures with differing tastes and food preferences (Shiau, 2002). They are also easily cultured fish in that they grow fast, are resistant to diseases and handling, easy to reproduce in captivity and able to tolerate a wide range of environmental conditions (Shiau, 2002; Suresh, 2003). According to Lovell (1998) some of the cultured species have been shown to survive dissolved oxygen concentration of 0.1 $mg.L^{-1}$ and tolerated unionized ammonia concentration of 2.4 $mg.L^{-1}$. Although tilapias are typically fresh water, they are euryhaline and able to grow well in saline water if properly acclimated. However, their activity and feeding become reduced below 20^{o}C and feeding stops around 16^{o}C (Lovell, 1998). Suitable ranges of water quality parameters for tilapia to survive are shown in Table 1.3. They eat food produced in culture systems as well as manufactured feeds composed of plant ingredients (Shiau, 2002; Suresh, 2003). Based on the above qualities, Pullin (1984) referred to tilapias as the 'aquatic chicken' i.e. an animal that can be farmed as easily and economically, and with the same broad market appeal, as chickens. Tilapia is the most cultured fish in Ghana for the reasons mentioned above and it is also considered a delicacy and therefore is in high demand both locally and abroad.

Table 1.3 Water quality parameters for tilapia

Water parameters	Tolerance range*	Desirable level^{+}
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	<1.0
Nitrite, $mg.L^{-1}$	0.1-0.2	-
Nitrate, $mg.L^{-1}$	0.0-3.0	-
Alkallinity, $mg.L^{-1}$	>20	>20
Hardness, $mg.L^{-1}$	>20	<50

*Adapted from (Hussain, 2004; Popma and Masser, 1999; Stickney R.R. 1979; Swann, 2007); $^{+}$(Suresh, 2003)

1.3.2 Nutritional Requirements of Tilapia

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

The best growth performance of tilapia is exhibited when they are fed a balanced diet that provides a proper balance of protein, carbohydrates, lipids, vitamins, minerals and fibre. Nutritional requirements of fish differ for different species and more importantly vary with life stage. According to Fitzsimmons (1997) fry and fingerlings require diets with higher protein, lipids, vitamins and minerals and lower carbohydrates as they are developing muscle, internal organs and bones with rapid growth. From various studies the protein requirements of juvenile tilapia have been reported to range between 30-56% (Jauncey, 1998; Suresh, 2003). The protein requirements of fish decrease with age and optimum dietary protein requirements for tilapia can be broadly generalised as shown in Table 1.4.

Table 1.4 Approximate dietary protein requirements for tilapia

Approximate wet body weight (g)	Optimum dietary protein content (%)
Fry to 0.5	30-56, recommend 40-45
0.5 to 10.0	30-40, recommend 30-35
10.0 to 30.0	recommend 25-30
30.0 to market size	recommend 25-30

Jauncey (1998)

According to Suresh (2003) there are limited and not entirely consistent data on the essential amino acid (EAA) requirements of fish. Recommended EAA for tilapia are shown in Table 1.5.

Table 1.5 Essential amino acid requirements of tilapia as % of dietary protein and of total diet (in parenthesis)

Amino Acid	*O. niloticus*[1]	O. *mossambicus*[2]
Arginine	4.20(1.18)	2.82(1.13)
Histidine	1.70(0.48)	1.05(0.42)
Isoleucine	3.10(0.87)	2.01(0.80)
Leucine	3.40(0.95)	3.40(1.35)
Lysine	5.10(1.43)	3.78(1.51)
Methionine + cystine	2.70(0.90)	0.99(0.40)
Phenylalanine + tyrosine	3.80(1.55)	2.50(1.00)
Threonine	3.80(1.05)	2.93(1.17)
Typtophan	1.00(0.28)	0.43(0.17)
Valine	2.80(0.78)	2.20(0.88)

[1](NRC, 1993) [2](Jauncey *et al.*, 1983)

In general, suggested dietary lipid levels for tilapias range from 5% to 12% (Suresh, 2003). It is recommended that dietary lipids contain both omega 3 and omega 6 fatty acids each representing 1% of the diet, although some reports suggest that fish grow better with a higher proportion of omega 6 to omega 3 (Fitzsimmons, 1997)(Table 1.6). Older fish seem to cope with higher dietary fibre content, a maximum of 8-10% (Jauncey, 1998), than younger ones at about 6-8% (Fitzsimmons, 1997). 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).

Table 1.6 Essential fatty acid requirement of *Oreochromis niloticus*

Fatty Acid Type	Level	Reference
18:2n-6	0.5-1.0%	Teshima *et al*. (1982)
20:4n-6	1.0%	Takeuchi *et al*. (1983)

Tacon (1987)

Minerals and vitamins are critical for good and balanced nutrition in tilapia and a lot of research has been conducted to determine these requirements (El-Sayed and Teshima, 1991; Jauncey and Ross, 1982; Roem *et al*., 1990; Watanabe *et al*., 1997).

Feeding rate (allowance) in practical feeding of fish involves two options. One is to feed the fish to satiation and the other is to feed a restricted ration (Suresh, 2003). Best growth is normally achieved by feeding to satiation. But satiation levels are not necessarily the most economic feeding levels, because food conversion at satiation levels is often poor. Also, it is difficult to determine satiation levels in fish because food consumption occurs in the water medium. This may lead to overfeeding, which is wasteful and deleterious to water quality. As a result, restricted rations are recommended for feeding fish (Suresh, 2003). It is also common practice to feed to satiety before determining the rate of feeding. Some recommended feeding rates for tilapia are given in Table 1.7.

Table 1.7 Feeding rates and frequencies for various sizes of Tilapias at 28 ^{o}C

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

Adapted from Juancey and Ross (1982), Coche (1982), Lovell (1998), Suresh (2003)

Measuring feed consumption of animals held in water is difficult. Food that is apparently fed to fish can be ignored by the animal or be delivered at an inappropriate time. To solve this problem several techniques have been

developed and perfected over the years. Some commonly used methods employed in investigating feed intake in fish include; x-radiography, self-feeding devices, direct observation, chemical markers and stomach contents analysis (Houlihan *et al.*, 2001; Jobling *et al.,* 2001). These methods are appropriate for particular study situation and may be used differently.

1.4 Fish Meal as the Main Protein Source in Aquaculture

Nutrition is the most expensive component in aquaculture, particularly intensive culture, where it accounts for over 50% of operating costs (El-Sayed, 2004). Protein is an important nutrient which provides amino acids required for synthesising new tissue and/or replacing worn out tissues and also provides energy when other energy sources are limited. Dietary protein is, therefore, the most important nutrient considered when formulating fish feed to avoid any deficiency which may lead to poor growth and loss of weight. The protein component alone in fish diets represents about 50% of feed cost in intensive culture (El-Sayed, 2004). Therefore selection of dietary protein of the right quantity and quality is necessary for successful fish culture.

Fishmeal has traditionally been used as an important protein source in aquaculture feeds for both carnivorous and omnivorous species, and many feed formulations still have fishmeal included at levels in excess of 50% (Glencross *et al.,* 2007). Fishmeal is widely sought after because it is a rich source of essential amino acids, essential fatty acids, energy and minerals. It is also very palatable and highly digestible to most freshwater and marine fishes (Watanabe et al. 1997). According to Drew *et al.* (2007) fish meal is the “gold standard” to which plant proteins must be compared in terms of protein quality, fish growth

performance and health and cost. Fish meal is obtained from by- products of fish meant for human consumption or from fish that are harvested purposely for production of fish meal. It is produced by either drying raw/cooked fish or fish by-products followed by extraction of oil (Hardy and Tacon, 2002; Hertrampf and Piedad-Pascual, 2000).

There are two types of fish meal available; white fish meal produced from non-oily whole fish, partly eviscerated fish and post-filleting residues, and brown fish meal made from oily whole fish from which a large proportion of the oil has been extracted (Jauncey, 1998). Crude protein and ash contents of fish meal may vary from 500 to 720 $g.kg^{-1}$ and 100 to 210 $g.kg^{-1}$ respectively depending on fish species, the source and processing (Drew *et al.,* 2007). The fat content of fish meal is species specific and is normally extracted from the fish, however, fish meal from oily fish species may contain up to 9.0% oil (De Boer and Bickel, 1988). The residual oil in fish meal is rich in PUFA, predominantly of the omega 3 family (Hertrampf and Piedad-Pascual, 2000). Fish meal has high ash content and this is particularly high when made mainly from fish frames, which are predominantly fish bones. Generally, the higher the ash content of fish meal, the higher the calcium, phosphorus and magnesium content (De Boer and Bickel, 1988). According to Jauncey (1998) low protein fish meals, particularly those for local markets are occasionally adulterated with urea resulting in apparently high crude protein (N*6.25) fish meal.

1.4.1 Fish Meal Production and Consumption

Global fish meal production was estimated at 5.52 mmt in 2003 (Tacon *et al.*, 2006). For the past two decades fishmeal production has remained relatively stable, production fluctuated from as low as 4.57 mmt in 1977 to as high as 7.48

mmt in 1994 averaging to 6.07 mmt over the period. Low production of fishmeal was usually caused by the effect of El Niño events on catches of Peruvian anchovy (Tacon *et al.,* 2006). El Niño events normally occur in the fishing grounds of Peru and cause changes in ocean water temperatures. Increase in water temperatures lead to migration of Peruvian anchovies to cooler deeper waters where they become unavailable to fishing boats. Peruvian anchovies are exploited solely for fish meal and oil production and account for over 25% of the global production (Hardy, 2006). Today a number of other key fisheries such as North Atlantic capelin, Japanese sardine, US menhaden etc., have collapsed or underperformed leading to short supply, therefore high fish meal prices.

Price increases can also be attributed to high demand for fish meal due, among others, to the rapid growth of the aquaculture industry not only because of more facilities are being used, but also from increase in productivity of existing facilities resulting from intensification of production systems. Global aquafeed production in 2003 was estimated at approximately 19.5 mmt, and according to Barlow (2000) production is expected to increase to over 37 mmt by the end of the decade, which will be an increase of 17.5 mmt (Hardy, 2006). According to Tacon *et al.* (2006) < 10% of annual fish meal production was used in aquafeeds in the mid-1980s, but today that proportion is over 46%. Naylor *et al.* (2000) are of the opinion that considering the volumes of fishmeal and oil used in aquafeeds, especially for carnivorous species, the culture of these species should be perceived as a net fish consumer rather than producer, and this practice has raised concerns about the long-term sustainability of these industries. Tacon and Forster (2000) predicted that fishmeal use in aquafeeds will decrease from 2,190,000 mt in 2000 to 1,550,000 mt in 2010. They based

their prediction on the assumption that prices of fish meal will increase at the same time that market prices for farmed fish and shrimp decrease, forcing the fish feed industry to replace portions of fishmeal in aquafeeds with less expensive ingredients. Fish meal is still used in aquafeeds for both carnivorous and omnivorous species at levels in excess of 50% particularly in carnivorous species and being too reliant on one ingredient is risky, therefore, as a strategy to reduce the risk of over reliance on fish meal, the identification, development and use of alternatives to fish meal and oil remain a high priority (Glencross *et al.,* 2007).

In Ghana fish meal is normally produced from anchovies (*Engraulis spp.)*, which are caught in Ghanaian waters, especially from September to January. Annual landings averaged about 67,000 mt in the last seven years (Tradezone International, 2007). Trash fish and factory offal is recognised as a possible fish feed in Ghana, however, very little, if any, is used as most of it is sold as food for human consumption (Hecht, 2007). As stated in Section 1.2.2 the demand for fish by the Ghanaian populace is higher than the supply leading to competition, not only with aquaculture but also the poultry and livestock industries. The limited local production and high demand for fish meal has resulted in importation of fish meal into the country leading to very high prices on the market. Hecht (2007) reported that given the high price of fish meal in Sub-Saharan Africa (Ghana inclusive) it was fair to conclude that the use of alternative protein sources for fish feed in the region is a priority.

1.5 Utilisation of Plant Ingredients in Aquafeeds

The need to increase aquaculture production requires corresponding increases in nutrition related inputs; i.e. intensifying culture practices by feeding more and better feedstuffs (Machena and Moehl, 2001). Feeds are mostly based on agricultural by-products available in an area and may be of modest quality but of a reliable quantity. Commercially produced feeds require cost-effective inputs and the industrial means to manufacture feeds, preferably pelletized feeds. Therefore, countries that have expanding agricultural sectors and produce surpluses are often well placed for the economical production of commercial fish feeds (Machena and Moehl, 2001).

In terms of aquafeed manufacturing in SSA, Nigeria being the largest aquaculture producer also manufactures the largest amount of aquafeed. Production was estimated at 10,760 mt in 2000 and 2001 (Shipton and Hecht, 2005). This feed was, however, manufactured solely for the tilapia and catfish industries, accounting only for 30.3% of the country's aquatic feed production. The remainder, which represents the majority of the feeds used, were farm-made feeds. Dependence on farm-made products to satisfy feed requirements of aquaculture organisms is prevalent in all the SSA countries (Moehl and Halwart, 2005). To date, Ghana has not developed a formal aquafeed manufacturing sector. Feeds are still mostly produced at the farm level and in most cases only one, or a mixture of two or more feed ingredients (Table 1.10), are used as supplementary feed in pond culture. Farmers who were desperate to increase production have tried poultry feeds with little or no success (Amisah, personal communication). The few commercial farms in Ghana produce their own feed (Asmah, personal communication). In 2005 farm-made feeds were

estimated to be 547mt and feeds produced on a small pilot scale at 2 mt per month (Hecht, 2007). Ghana has a seemingly well established animal feed industry though no quantitative data is readily available. A report by Abban (2005) suggested that Ghana has adequate oilseed cake resources to supply present requirements and for future demand by aquaculture. Projections of aquaculture production and aquafeed requirements in Ghana for 2010 to 2020 have been made by Hecht (2007) based on the 2004 production of 950 mt (Table 1.8). Although poor aquaculture development has been attributed to many factors, the major challenge in Ghana is development of commercial, cost effective feeds (especially for tilapia) using locally available, cheap and unconventional resources.

Table 1.8 Projections of aquaculture production and aquafeed requirements in Ghana for 2010 and 2020

Period	Aquaculture production (mt)	Contribution of commercial aquaculture (%)	Commercial aquaculture production (mt)	Aquafeed demand (mt)
2010	1,529	35	535	802
2015	2,464	45	1,108	1,663
2020	3,968	60	2,380	3,571

Adapted from Hecht (2007)

Feed ingredients currently in use in aquafeed production and their availability in some West African countries are presented in Table 1.9. With respect to carbohydrate sources, rice bran, maize bran and wheat bran are the resources of choice and readily available (Hecht, 2007). Rice and maize production in Ghana in 2005 was 241,807 mt and 1,157,621 mt respectively. The total production of cereals in the same year accounted for 1,943,000mt (FAO, 2006).

Presently in Ghana fish meal, which is the main protein source, is in short supply and expensive as there is competition between livestock, poultry, aquaculture and humans as explained in Section 1.4.2 (Table 1.9). Fortunately,

however, oilseed crops whose by-products are good substitutes for fish meal are available in Ghana. The most important are groundnuts, cottonseed, soybean, copra and palm kernel among others. Groundnut production in Ghana has increased since 1961 from 47,000 mt to 520,000 mt in 2003 making it the ninth largest producer in the world (WGP, 2004). Part of this production is consumed locally as toasted nuts and processed for oil. Press-cake normally represents 50% of the shelled weight of the groundnut, which in turn constitutes 70% of the weight in shell (Agyenim-Boateng *et al.*, 1990). Groundnut cake is used as human food at the village level, however, industrially produced cake is sold for animal feed (Agyenim-Boateng *et al.*, 1990).

Cotton production in Ghana, since its cultivation in the early 1970s, has not been able to achieve its full potential although the country has excellent conditions for its development. Production expanded rapidly from 4% of the country's national requirement in 1970 to 50% in 1990 (Clark, 1994). The highest it achieved was 24,000 mt in 1998 and 24, 244 mt in 2003 (GMIS, 2008). Despite low production of cottonseed meal in Ghana it is readily available at cheap prices (Hecht, 2007) because of its influx from neighbouring countries, which depend on cotton as their main export commodity, providing valuable foreign currency earnings. For instance in Benin, Burkina Faso, Chad, Mali and Togo, cotton accounts for 5-10% of GDP, more than one-third of total export receipts and over two-thirds of the value of agricultural export (GMIS, 2008).

Soybean was first grown in Ghana in 1909 with the aim of making it an additional food item and as a possible cash crop for export to England (Shurtleff

and Aoyagi, 2007). Although conditions were good for its cultivation, production has been poor. In Hecht's (2007) report, he indicated that the most commonly and readily available plant product used as a protein source in Sub-Saharan Africa is soybean meal, which is generally imported and others are cottonseed meal and groundnut meal/cake reflecting, in many respects, worldwide trends (Figure 1.7) also reviewed in Chapter 4.

Table 1.9 Availability of the most common ingredients for the manufacture of animal feeds in West Africa

Commodity	Ghana	Nigeria	Cameroon
Plant origin			
Coffee pulp		+	++
Cacao husks		++	+++
Rice bran	++	+++	+
Wheat bran	+	++	++
Maize bran	++	+++	+++
Groundnut bran(husk)l	++		
Maize	+	++	++
Millet		+	+
Soybean	+	+	
Wheat		++	+
Oilseed meals/cakes			
Cotton	++	+++	++
Groundnut	++	++	+
Soybean*	+	+++	+
Sunflower		+	
Palm kernel		++	++
Copra	++		
Sesame		+	
Other seed cakes	++		
Brewery waste	++	++	++
Vegetable oils	++	+++	+++
Animal Origin			
Fish meal (local)	+	+	+
Fish meal (imported)	++	++	+
Blood meal		+	+++
Hydrolysed feather meal			++
Carcass and bone meal		+++	+
Crayfish meal		+	
Shrimp waste		++	
Chicken layer dropping meal			++
Fish oil		+	+
Rendered poultry oil		+	
Vitamin and mineral premix*	++	++	++

*=Imported; +=limited supply; ++=readily available, +++= abundant supply
Adapted from Hecht (2007)

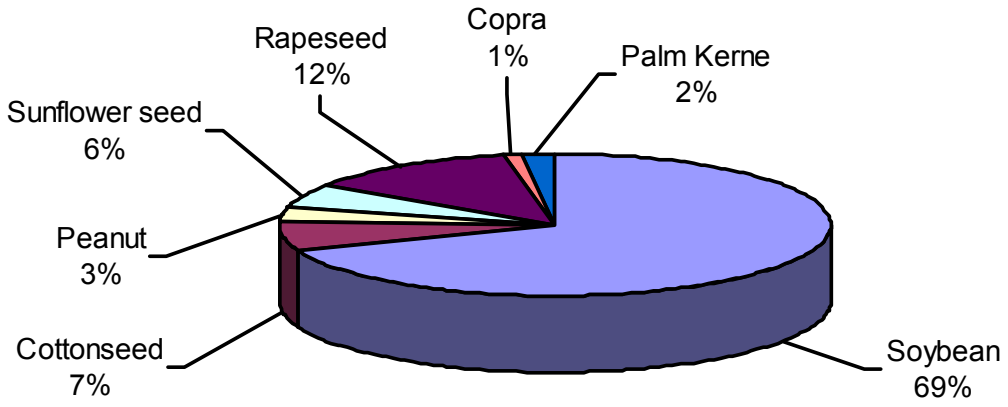

Figure 1.7 World oilseed meal consumption (total 171.35 million mt, USDA estimated 2002; (Bajjalieh, 2004)

According to Shipton and Hecht (2005) the vitamin and mineral premixes used for livestock and aquafeed industries are generally imported into sub-Saharan Africa, usually from Western Europe. They also reported that all the major ingredients that are normally used in the manufacture of livestock feeds are available in sub-Saharan Africa, and are therefore available to the aquafeed industry.

In recognition of the need to develop feeds for Ghanaian aquaculture production, an inventory was undertaken and a list of ingredients available for use in fish feeds was compiled, together with their proximate composition (Table 1.10). This survey was carried out in 1996 under the Freshwater Fisheries and Aquaculture Research Programme (FARP), a component of the National Agricultural Research Programme (NARP) funded by the World Bank. After this research there have been some attempts to use the ingredients identified to formulate commercial fish feed by individual researchers and

institutions without any particular outcome (Ofori, personal communication). A more recent (from the year 2000) attempt at improving aquaculture and particularly fish feed development was undertaken by the Agriculture Sub-Sector Improvement Programme (AgSSIP), also funded by the World Bank.

Table 1.10 Proximate composition (%) and gross energy ($KJ.g^{-1}$) of some agro-industrial by-products (AIBPs) in Ghana

AIBPs	DM	CP	EE	ASH	CF	AC	GE
Cottonseed cake	87.82	41.79	3.82	7.86	11.46	24.88	18.03
Copra cake	89.70	19.09	5.48	4.20	16.25	44.68	18.28
Groundnut cake	87.39	43.75	8.62	5.63	12.29	17.10	17.46
Palm kernel cake	89.11	12.78	11.19	3.70	22.39	39.05	19.33
Fish meal (Anchovy)	86.60	47.88	3.71	32.33	1.23	1.45	12.85
Wheat bran yeast	86.31	24.55	3.50	5.48	9.06	43.72	12.79
Wheat bran	89.67	16.76	5.22	5.36	12.93	49.40	13.09
Soybean meal	89.87	42.97	7.84	5.21	7.53	26.32	17.30
Brewers dried grain	89.85	19.65	5.27	7.74	8.48	48.71	13.48
Pito mash	80.81	13.41	1.88	5.66	21.0	38.80	15.09
Cowpea	91.40	26.96	2.69	2.85	0.77	58.13	15.31
Dried cocoa husk	86.31	6.40	1.09	8.11	21.66	49.12	9.73
Rice bran	89.18	5.23	4.59	12.73	22.28	44.43	10.05
Maize bran	87.73	10.13	8.58	2.71	4.23	62.08	15.38
Groundnut husk	90.59	13.58	21.04	9.50	15.07	31.46	15.51
Cocoa cake	88.77	21.89	18.64	2.90	11.96	30.61	18.41
Corn cob	94.66	1.72	0.14	1.46	39.28	52.06	9.09
Corn chaff	88.63	11.04	9.09	2.93	7.07	58.50	15.12
White maize	88.30	8.49	1.97	1.69	2.38	73.47	14.51
Sunflower seed meal	93.68	25.84	18.49	5.53	17.13	26.69	15.81
Cassava peel	88.15	3.93	1.29	7.78	11.16	63.91	11.89
Cassava peel/yeast	88.89	17.45	12.50	17.28	11.01	30.65	12.81
Maize leaves	92.14	2.91	0.07	6.64	36.52	46.00	8.24

Source: Nelson and Wallace (1998).
DM=Dry matter, CP=Crude protein, EE=Ether extract, CF=Crude fibre, AC=Available carbohydrate, GE=Gross energy

1.5.1 Alternative Protein Source to Fish Meal

Recent demand for fish, especially tilapia, has led to the need for intensification of fish production in Ghana and this situation has made it essential to develop suitable complete and supplementary diets for use in nurseries and grow-out facilities (Dassah, personal Communication). At present fish meal is the main source of protein in commercial fish feeds (Tacon *et al.*, 2006). Moreover, feed

for juvenile fish often requires good quality and high dietary protein levels, which may demand even higher dietary inclusion levels of fish meal up to 70% by weight (Tacon, 1981). However, fish meal use in diets of juvenile tilapia in Ghana is not feasible due to limited supply and high prices. Therefore, it is necessary to find alternative protein sources to develop suitable diets for tilapia, especially the juveniles, to increase aquaculture production.

Tilapia, such as Nile tilapia (*Oreochromis niloticus),* feed efficiently on natural fauna and flora and can utilize supplementary feed materials to achieve rapid growth and weight gain. However, intensification of tilapia culture requires development of suitable feeds both for complete and supplementary feeding in tanks and ponds. For tilapia feeds the typical protein sources examined have included poultry by-products (Gaber, 1996), cacao husks (Pouomogne *et al.*, 1997), legume seed meal (Fagbenro, 1998), defatted soybean meal, full-fat toasted soybean, lupin seed meal and faba bean meal (Fontainhas-Fernandes *et al*., 1999), sunflower cakes, fish (anchovy) meal and wheat bran (Maina et al. 2002), cottonseed meal, sunflower meal (El-Saidy and Gaber, 2003), fish, poultry meals, corn gluten, rapeseed meal, sorghum, barley (Sklan *et al*., 2004) and anchovy meal, corn gluten meal, soybean meal, gammarid meal and crayfish exoskeleton meal (Koprucu and Ozdemir, 2005). More recent researches in tilapia diets include meat and bone meal, soybean meal, cottonseed meal and corn gluten meal (Guimaraes *et al*., 2007) and peanut leaf meal (Garduno-Lugo and Olvera-Novoa, 2008). Similar work has also been conducted on other fish species including Atlantic salmon using protein sources such as; corn gluten, defatted soybean meal, defatted sunflower, dehulled lupin, defatted rapeseed, whole field pea, whole dehulled faba bean, whole wheat and

naked oat (Aslaksen *et al*., 2007), soy protein concentrate (Denstadli *et al.*, 2007), soy non-starch polysaccharides and defatted soybean meal (Kraugerud *et al.*, 2007) and genetically modified soybean and maize (Bakke-McKellep *et al.*, 2008). For Rainbow trout protein sources such as mixture of plant proteins (Vilhelmsson *et al.*, 2004), genetically modified defatted soybean meal and non-genetically modified soybean meal (Chainark *et al.*, 2006) and defatted soybean meal (Romarheim *et al.*, 2008) were investigated. In the case of Gilthead sea bream protein sources used were mixtures of plant proteins (corn gluten meal, wheat gluten, extruded peas, rapeseed meal) (Gomez-Requeni et al., 2004), (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin) (Sitja-Bobadilla *et al*., 2005), (corn gluten, wheat gluten, extruded peas, rapeseed meal and extruded whole wheat)(De Francesco *et al*., 2007) and for Red sea bream defatted soybean meal and corn gluten (Sarker *et al*., 2007). Other investigations on alternative protein sources for fish feed have been reviewed in the experimental chapters.

Of the feed ingredients available in Ghana, and based on the ingredients identified in Table 1.9 and Table 1.10, their quality, availability, affordability and supply, the most promising alternatives to fish meal in (juvenile) tilapia diets are the oilseed meals/cakes namely; soybean meal (*Glycine spp*), cottonseed meal (*Gossypium spp*.) and groundnut meal/cake (*Arachis hypogaea L.*). Oilseed meals are by-products after the removal of oil from oil-bearing seeds produced by annual plants and perennial trees. The latter includes oil palm and coconut palm trees. Most important annual plants belong to the botanical families of legumes and crucifers (Hertrampf and Piedad-Pascual, 2000). Meals from vegetable oil processing are protein feedstuffs whose protein content ranges

between 20 to 54% depending on processing methods and they generally have good amino acid profiles (Lovell, 1981; Tacon, 1987). Originally, vegetable oil producing plants were cultivated primarily for their oil for human consumption and industrial applications. The increasing demand for protein for animal production and aquaculture has made the by-products more important than the oil. Soybean meal (SBM) is a typical example of an oilseed meal which has become a more important feedstuff (important protein source) in animal feed than the oil, the primary product (De Boer and Bickel, 1988). Oilseeds constitute a readily, available source of dietary protein for use within compound aquafeeds. Due to their high oil content, oilseeds tend to be relatively high in nutritional energy. This can be an advantage in feed formulations which require ingredients that provide higher levels of energy. However, energy also serves as a 'cap' on the usage of whole oilseeds (Bajjalieh, 2004). Despite the advantages mentioned above, oilseed meals contain undesirable constituents such as high crude fibre, ash and antinutritional factors (ANFs), the high crude fibre content being caused by the protective cover of the seed's germ and nutrients in the form of hulls and shells (Hertrampf and Piedad-Pascual, 2000; Tacon, 1995a).

Soybean (*Glycine spp*) meal: - Soybean meal is the by-product after removal of oil from soybeans and it is the major protein source used in aquaculture feeds, not only because of its high protein content but also due to its worldwide availability (Hertrampf and Piedad-Pascual, 2000)(Figure 1.7). Solvent-extraction of the oil results in products containing 44% crude protein if the soybean hulls are included or 48% crude protein without the hulls (NRC, 1993). Soybean meal without hulls has a reduced fibre content (approximately 3.4%)

compared to the meal containing hulls (approximately 6.2%). Both of these meals have lipid contents around 1% due to the efficient solvent extraction process. Soybean products are considered as having one of the most balanced amino acid compositions among plant feedstuffs, and the relative amounts of essential amino acids in the various products are very similar when expressed as a percentage of crude protein (FAO, 1983; Lim and Akiyama, 1992). Amino acids such as methionine and cystine are generally considered to be most limiting in soybean products compared to the quantitative amino acid requirements of most fish species (FAO, 1983; NRC, 1993). It is highly palatable to most warmwater fish, but less palatable to salmonids (Lovell, 1998).

Another soybean product commonly referred to as full-fat soybean meal is produced by heat treatment of whole soybeans. This product has a crude protein content of approximately 38% (as-fed basis) and a lipid level of approximately 18% (Lim and Akiyama, 1992). Although soybean meal (SBM) is generally considered to be one of the best readily available plant protein sources in terms of its protein quality and EAA profile, like most other plant proteins it does contain a wide variety of endogenous ANFs, the most important being trypsin inhibitors (Table 1.11), which require removal or inactivation through processing prior to usage within aquafeeds (Tacon, 1995b).

Groundnut (*Arachis hypogaea L.*) Meal/Cake: - Groundnuts also known as peanuts, earthnuts, arachis nuts, monkey nuts, Manilla nuts, Chinese nuts, pindar or goober peas are grown mainly for human consumption of the seeds, however, all other parts of the plant can be easily utilized (Hertrampf and

Piedad-Pascual, 2000; WGP, 2004). Groundnuts can be used directly for food and/or processed to produce oil and a high protein meal. Nearly two thirds of all groundnuts produced are processed for oil (Bunting *et al.,* 1985). Groundnut meal/cake is the residue remaining after extraction of the oil from the groundnut. The groundnut consists of about 30% shell and 70% kernel (of which 36% is oil and 36% is meal) and the kernels contain 4.1% testa or skin (De Boer and Bickel, 1988). The groundnut skin which is usually removed after roasting is also called 'husk' in Ghana and it is used as supplementary feed (Hasan, 2007). Groundnut meal contains around 40% (corticated) to 50% (decorticated) crude protein and is highly palatable (Lovell, 1998; WGP, 2004). It is deficient in lysine and methionine but has good binding properties for pelleting (Lovell, 1981). Groundnut and its meal/cake are a reasonable source of dietary minerals especially potassium, phosphorus and magnesium, however, they are a poor source of fat soluble vitamins like A, D and K (WGP, 2004).

Groundnut oil is an excellent source of mono- and polyunsaturated fatty acids, exceeding the levels of these fatty acids in soybean and corn oil, but significantly lower than in sunflower and safflower oil. Groundnut oil contains about 1% palmitic acid and 80% oleic and linoleic acid (Nwokolo, 1996). Raw groundnuts have antinutritional factors like trypsin inhibitors, various lectins etc. (Table 1.11). They have very low concentrations of most of the antinutritional factors found in raw soybean. The testa or skins of groundnuts contain 16% to 20% tannins, which are known for their toxicity (Hertrampf and Piedad-Pascual, 2000). The principal contaminant of groundnut is aflatoxin, which is a group of highly toxic substances produced by moulds *Aspergillus flavus* and *Aspergillus parasiticus*. This contamination usually occurs under damp conditions. Aflatoxin

is a hepatotoxin and mortality among afflicted animals and fish invariably results from severe liver damage (FAO, 1983; Nwokolo, 1996).

Cottonseed (*Gossypium spp.*) Meal: - Cotton (*Gossypol hirsitum*) is grown for its fibre in the manufacture of textiles. For every 100 kg of cotton fibre produced, the cotton plant also yields about 160 kg cotton-seed, which is the by-product. Cottonseed meal is the residue produced after oil extraction from cotton-seed (Hertrampf and Piedad-Pascual, 2000). Cottonseed meals are among the most available plant protein sources in the world (Figure 1.7). Besides being relatively cheap, CSM contains good protein contents (26-54%, depending on processing methods) and amino acid profile. However, it contains relatively low levels of lysine, cystine and methionine and processing conditions may also have a negative effect on the amino acid content (Lovell, 1998). 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%. The digestible energy of cotton-seed meal is relatively low due to the high crude fibre content (Hertrampf and Piedad-Pascual, 2000). CSM contains ANFs the most important of which is gossypol (Table 1.11).

1.6 Limitations to the Utilization of Fish meal substitutes

High inclusions of plant protein in fish diets have frequently been reported to result in reduced growth and/or high mortalities attributed to poor palatability, high crude fibre, reduced digestibility of lipid and energy, imbalance of essential amino acids and presence of antinutritional factors (Balogun and Ologhobo, 1989; Fagbenro, 1999; Francis *et al.*, 2001; Houlihan *et al*., 2001; Mambrini *et al.,* 1999; Ogunji, 2004; Tacon, 1993).

1.6.1 Palatability/Acceptebility of Plant Ingredients

It is important in feed development to ascertain the acceptability of the feed ingredients, particularly as the texture and palatability or taste could change as increasing levels of especially plant ingredients are incorporated (Ogunji, 2004). According to Houlihan *et al.* (2001) palatability of feed is a major factor determining feed acceptance because, irrespective of how digestible the nutrients and energy from a particular ingredient are, if the ingredient reduces feed intake it is of limited use in a feed formulation. Feed palatability has been defined by Glencross *et al.* (2007) as acceptable to the taste or sufficiently agreeable in flavour to be eaten. Feed acceptance depends upon a variety of chemical, nutritional and physical characteristics, all of which can be influenced by the choice of feed ingredients and processing conditions used in the manufacture of feed (Jobling *et al.*, 2001). The ability of fish to detect and ingest a feed can be affected by physical and chemical properties, the former being pellet density (sinking rate), size (shape, diameter and length), colour (contrast), and texture (hardness) and the latter being the chemical composition of the feed which will largely depend upon the types of ingredients used (Jobling *et al*., 2001). It is known that many plants contain chemicals (secondary compounds/metabolites) that defend them against attack from herbivores (Hay and Fenical, 1988; Houlihan *et al*., 2001). Secondary compounds produced by plants include terpenes, polyphenolics, alkaloids, a range of aromatic compounds and amino acid derivatives and some ANFs (Becker and Makkar, 1999; Dreisbach, 1971; Hay and Fenical, 1988), therefore, including plants in fish feeds may give rise to palatability problems. For example, Ogunji and Wirth (2001) observed that incorporating high levels of soybean meal and blood meal in Nile tilapia diets led to unacceptability and consequently poor performance;

this was attributed to the unpalatability of the feed. Rainbow trout were reported to find feed less palatable when fish meal was partially substituted with a leaf protein concentrate, and there may also be acceptability problems when feeds are formulated to include high concentrations of soybean meal (Dias *et al*., 1997; Fowler, 1980; Papatryphon and Soares, 2000; Tacon and Jackson, 1985; Watanabe *et al.*, 1997).

The negative effects of feeding deterrents may to some extent be ameliorated by including exogenous dietary feeding stimulants or attractants (Kubitza and Lovshin, 1997; Papatryphon and Soares, 2000; Tacon and Jackson, 1985). There are four main types of low-molecular-weight compounds which seem to serve as stimulants of feeding behaviour in fish and may act alone or in combination. The most common stimulants reported to be effective are free amino acids, quaternary ammonium compounds, nucleotides, and organic acids, although other substances may evoke feeding behaviour in some fish species (Carr *et al.*, 1996; Mackie and Mitchell, 1985; Takeda and Takii, 1992; Xue and Cui, 2001).

1.6.2 Fibre Content

Fibre refers to indigestible plant matter such as lignin, cellulose, hemicellulose, pentosans, and other complex carbohydrates found in feedstuffs (NRC, 1993). Monogastric animals including fish are generally unable to digest fibre because they do not secrete cellulase (Bureau *et al*., 1999). Fibre provides physical bulk to feed and may improve pelletability (NRC, 1993). Cellulose and hemicellulose have been used as diluting agents and fillers especially in experimental fish diets (De Silva and Anderson, 1995; Jauncey, 1998). Small amounts of dietary fibre have been reported to improve efficiency of protein utilization in laboratory

diets (Buhler and Halver, 1961) and gastric evacuation time of rainbow trout (Hilton *et al.*, 1983). However, it is not desirable to have a fibre content exceeding 8-12% in diets for fish, as increase in fibre content would consequently lead to the decrease of the quantity of a usable nutrient in the diet. Excessive fibre content could also result in decrease in total dry matter and nutrient digestibility of the diet resulting in poor performance (De Silva and Anderson, 1995). Because fibre is indigestible, it adds to the faecal waste which affects the water quality and hence fish performance (Lovell, 1998).

1.6.3 Amino Acid profile

Amino acids are the structural components of proteins. Approximately 25 different amino acids (AAs) occur in proteins likely to be used by tilapia (Jauncey, 1998). AAs can be divided into two nutritional groups, essential and nonessential. Essential amino acids (EAA) are those that the animal cannot synthesise or cannot synthesise in sufficient quantity to support maximum growth and therefore must be provided in the diet. Fish require ten EAAs namely; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (De Silva and Anderson, 1995). The nonessential amino acids (NEAA) are those that can be synthesised by the animal in quantity to support maximum growth, provided that a suitable source of amino nitrogen is available. NEAA are only nonessential in the dietary context, they still perform many essential functions at the cellular and metabolic levels (Jauncey, 1998; Lovell, 1998).

The requirements for amino acids in animals are well defined in various sets of recommendations such as those of NRC (1993). Requirements vary depending on the species and age of animals. EAA requirements for tilapia are presented

in Table 1.5. According to De Silva and Anderson (1995) the EAA profiles of plant proteins used for feed formulation are usually poor, implying they are deficient of one or more EAAs, compared to the requirements of the animal. EAA requirements and supplementation in fish feed are reviewed further in Chapter 6.

1.7 Important Antinutritional Factors in the Selected Oilseed Meals

Most of the potential plant-derived nutrient sources are known to contain a wide variety of antinutritional substances. Antinutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilisation and affect the health and production of animals (Makkar, 1993). They could be broadly divided into four groups: (1) factors affecting protein utilisation and digestion, such as protease (trypsin) inhibitors, tannins, lectins; (2) factors affecting mineral utilisation, which include phytates, gossypol pigments, oxalates, glucosinolates; (3) antivitamins; (4) miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phytooestrogens and saponins (Francis *et al*., 2001). The ANFs in soybean meal, cottonseed meal and groundnut meal are summarised in Table 1.11.

Table 1.11 Important antinutritional factors present in selected oilseed ingredients

Plant-derived nutrient source	Antinutritional factors present
Soybean meal	Protease inhibitors (trypsin, chymotrypsin), lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens
Cottonseed meal	Protease inhibitors, phytic acid, phytoestrogens, gossypol, antivitamins, cyclopropenoic acid
Groundnut cake	Protease inhibitors, Phytic acid, phytohaemagglutinins, saponins, phytoestrogen

Adapted from Francis *et al*.(2001) and Tacon (1995b)

1.7.1 Trypsin Inhibitors

Trypsin inhibitors (TIs) are crystalline globular proteins that reduce the activity of trypsin and chymotrypsin which are pancreatic enzymes involved in protein digestion (Hertrampf and Piedad-Pascual, 2000; Liener and Kakade, 1980). TIs have a wide distribution in plants and are present in most legume seeds and cereals (Norton, 1991). TIs form irreversible complexes with trypsin and inhibit its activity (Liener and Kakade, 1980). Among plant products soybean products have the highest TI concentrations especially unprocessed ones.

TIs have been reported to cause growth depression, and reduced feed efficiency and survival of some fish species (Abel *et al*., 1984; Balogun and Ologhobo, 1989; Smith, 1977; Viola *et al*., 1983; Wilson and Poe, 1985). Furthermore, feeding of raw, unprocessed, soybeans which usually contain high TIs levels can cause adverse effects, such as pancreatic hypertrophy and depressed growth in pigs, chickens, rats, and fish (Dabrowski *et al*., 1989; Rackis, 1974) and interference with protein digestion. The common culture fish species differ in their ability to tolerate dietary TI. Research has shown that salmonids are more sensitive than either carp or catfish (NRC, 1993). The effects of TIs on tilapia are not well established, however some work by Wee and Shu (1989) indicated that dietary TI levels of 1.6 $mg.g^{-1}$ or higher retarded Nile tilapia growth but grew well at dietary levels of 0.6 $mg.g^{-1}$ and Mitchell *et al.* (1993) have also indicated that commercial salmonid feeds can exhibit up to 86% inhibition of trypsin. Rumsey (1991) found little effect on growth or feed intake at TI levels below 5 $mg.g^{-1}$, when feeding trout at levels of TI ranging from 2.6 - 51.0 $mg.g^{-1}$.

1.7.2 Phytic Acid

Phytic acid is a major component of all plant seed constituting 1-3% by weight of many cereals and oilseeds although as high as 3-6% has been reported for particular varieties (Cheryan, 1980; Graf, 1983). Phytic acid in most cereals (for example, corn, wheat and rice) is associated with specific parts of the seed such as the endosperm, germ and hull. Phytate (Ca-Mg salt of phytic acid) chelates with mineral cations like potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), copper (Cu) and forms poorly soluble complexes (Papatryphon *et al*., 1999; Rackis, 1974; Smith, 1977). These salts of phytic acid are known as phytins and their availability/digestibility to monogastric animals including fish is very limited due to lack of digestive enzyme phytase for efficient phytate hydrolysis during digestion (Hughes and Soares, 1998; Jackson *et al*., 1996; NRC, 1993). The majority of phosphorus in most protein-rich plant ingredients is bound in phytate therefore, limited in bioavailability to most fish because they lack the digestive enzyme phytase (Jobling *et al*., 2001). Similarly phytate forms complexes with proteins and amino acids (Liu *et al*., 1998; Spinelli *et al*., 1983; Sugiura *et al*., 2001) and their availability/digestibility to monogastric animals including fish such as tilapias become very limited (NRC, 1993). The inclusion of phytate containing ingredients in the diet has been reported to negatively affect growth and feed efficiency in commonly cultured fish species, such as carp, tilapia, trout and salmon (Francis *et al*., 2001; Portz and Liebert, 2004). Salmonids seem to be able to tolerate dietary levels of phytate in the range of 5 – 6 $g.kg^{-1}$, while carp appears to be sensitive to these levels. It seems to be advisable to maintain the level of phytates below 5 .0 $g.kg^{-1}$ in fish feeds (Francis *et al.,* 2001).

The inclusion of microbial phytase in feed is an approach to increase phytic phosphorus bioavailability and thereby reduce or fully replace the use of inorganic phosphorus supplements (Cao *et al*., 2008; Oliva-Teles *et al*., 1998; Rodehutscord *et al*., 1995b). Phytase is an enzyme chemically known as myo-inositol-hexaphosphate phosphohydrolase, produced either by microorganisms or present in some plant ingredients. Microbial phytase either as a dry powder or as a liquid is available commercially and Natuphos was the first commercially available phytase, from a genetically modified *Aspergillus niger* strain (Baruah *et al*., 2004). Optimum microbial phytase activity occurs at two pH values: the highest activity being at pH 5.0-5.5 and second highest at pH 2.5 (Simons *et al*., 1990). Phytase cannot withstand high temperature and should be applied by avoiding excess heat during extrusion and other steps in diet manufacture (Vielma *et al*., 2002). It is deactivated at temperatures above 65^{o}C (Cao *et al*., 2008). For instance, pelleting a diet at 70°C reduces phytase activity by 15-25% (Schwarz and Hoppe, 1992). Phytase can be utilized in fish feeds by pretreating feedstuffs (Cain and Garling, 1995), incorporating it during diet preparation (Portz and Liebert, 2004) or spraying onto pellets (Vielma *et al*., 2002).

1.7.3 Gossypol

Gossypols are polyphenols, contained in the pigment glands of plants, mostly confined to the genus *Gossypium* (FAO, 1983; Francis *et al*., 2001). It is the yellow pigment which constitutes 20% to 40% of the substances inside the glands of the seed kernel (FAO, 1983). Gossypol is either bound or free, the bound form being non-toxic and of little significance since it is unavailable and passes through the gastro-intestinal tract unabsorbed (Evans, 1985; Tanksley, 1990) but the free form is highly toxic (Ogunji, 2004). The amount of free

gossypol in cottonseed meal depends upon processing (Lovell, 1981). The use of cottonseed meal in fish feed is limited by its gossypol content, since high levels have been shown to be toxic to monogastric animals including fish (Herman, 1970). Feeding diets containing gossypols causes negative effects, such as growth depression and intestinal and other internal organ abnormalities (Berardi and Goldblatt, 1980). A dietary level of 0.03% free gossypol suppressed growth rate and a level as low as 0.01% caused liver damage in rainbow trout (Herman, 1970). Growth suppression also occurred in channel catfish fed diets containing more than 0.09% free gossypol (Robinson and Li, 1994). However, some fish species can tolerate higher gossypol concentrations, for instance tilapia has been fed up to 0.18% free gossypol with no observed effect (Lovell, 1998).

When cottonseed meal containing very low levels of gossypol was used excellent performance was recorded in *O. mossambicus* (Jackson *et al*., 1982). These authors explained that gossypol in cottonseed is concentrated in the pigment glands of the seed and during mechanical processing the gossypol is released and reacts with the amino groups of lysine, rendering it unavailable. According to Smith (1970) the use of solvent extraction methods during processing results in the glands with less binding to lysine. Lysine deficiencies, due to the lysine-binding capacity of free gossypol, can be avoided, if the amino acid is added to the feed (Hertrampf and Piedad-Pascual, 2000).

1.7.4 Saponins

Saponins are glycosides found in many plant products which can potentially be used in fish feed. Saponins contained in various plant families vary in nature and amount according to plant part, physiological age and environment

(Fenwick *et al*., 1991). Saponins are toxic to cold-blooded animals and are generally known for their bitter taste, foaming in aqueous solutions, and their ability to haemolyse red blood cells (Birk and Peri, 1980). Saponins in various legume seeds range between 18 and 41 $mg.kg^{-1}$ and defatted roasted soybean flour contain 67 $mg.kg^{-1}$ (Fenwick *et al*., 1991). Dietary saponins are known to have several adverse effects on fish performance, including reduction of feed intake due to their astringent taste (Guillaume and Metailler, 1999) and interference with digestibility and absorption of nutrients due to formation of sparingly digestible saponin-nutrient complexes (Ikedo *et al*., 1996; Potter *et al*., 1993). Saponins may also damage intestinal epithelium mucosa and repiratory epithelium (Bureau *et al*., 1998; Hostettmann and Marston, 1995) and inhibit reproduction (Francis *et al*., 2001). Levels of saponins below 1 $g.kg^{-1}$ of diet are not likely to affect fish growth (Francis *et al*., 2001).

In spite of the adverse effects of saponins, some studies have shown their beneficial effects. According to Makkar *et al.* (1995) complex formation between saponins and other antinutrients like tannin could lead to the inactivation of the toxic effects of both substances. Soy saponins have been reported to have stimulated feeding in oriental clouded yellow larva, *Coliaserate poliographus* (Matsuda *et al.*, 1998). Aqueous extraction is normally used to remove most saponins from feed ingredients since they are highly soluble in water (Tacon, 1997).

1.8 The Aim and Objectives of this Research

From the foregoing discussion it is clear that one of the major problems faced by rapidly growing aquaculture in many developing countries, including Ghana,

is the non-availability of quality and affordable feeds, especially for fry and fingerlings. The aim of the present research was therefore to develop cost-effective diets for Nile tilapia (*O. niloticus)* fingerlings using a mixture of selected oilseed meals/cakes available in Ghana as alternative protein source to fish meal.

The null hypothesis of this study is stated as follows; “there is no significant difference between growth performance, nutrient utilization, digestibility and whole body composition of Nile tilapia fed oilseed meal based and fish meal based diets”.

The main objective of this study was to investigate the nutritional suitability and cost effectiveness of soybean meal (SBM), cottonseed meal (CSM), groundnut cake (GNC) and groundnut husk (GNH) as alternative protein source to fish meal in the diet of Nile tilapia. The specific objectives were to:

- Study the apparent nutrient digestibility and gross energy for soybean meal, cottonseed meal, groundnut cake and groundnut husk in the diet of Nile tilapia;

- Evaluate the nutritive value of these oilseed meals/cake as alternative protein sources in the diet of Nile tilapia;

- Study the nutritive value of using various mixtures/combinations of the oilseed meals/cake in the quest to improve plant protein source use in diets of Nile tilapia;

- Investigate the effect of dietary amino acid supplementation of diets containing mixtures of the oilseed meal based diets on growth performance and feed utilization of Nile tilapia;

- Evaluate the effect of processing/detoxification of these oilseed meals on growth performance and feed utilization of Nile tilapia and

- Assess the cost effectiveness of the formulated diets for Nile tilapia.

1.9 Thesis Structure

This thesis is structured into eight chapters as shown in Figure 1.8 below. Chapters 1 and 2 are the General Introduction and Methodology, Chapters 3 (short-term feed trial, 2-3 weeks), Chapters 4 to 7 (medium-term feed trials, 8 weeks) and Chapter 8 the General Conclusions and Recommendations.

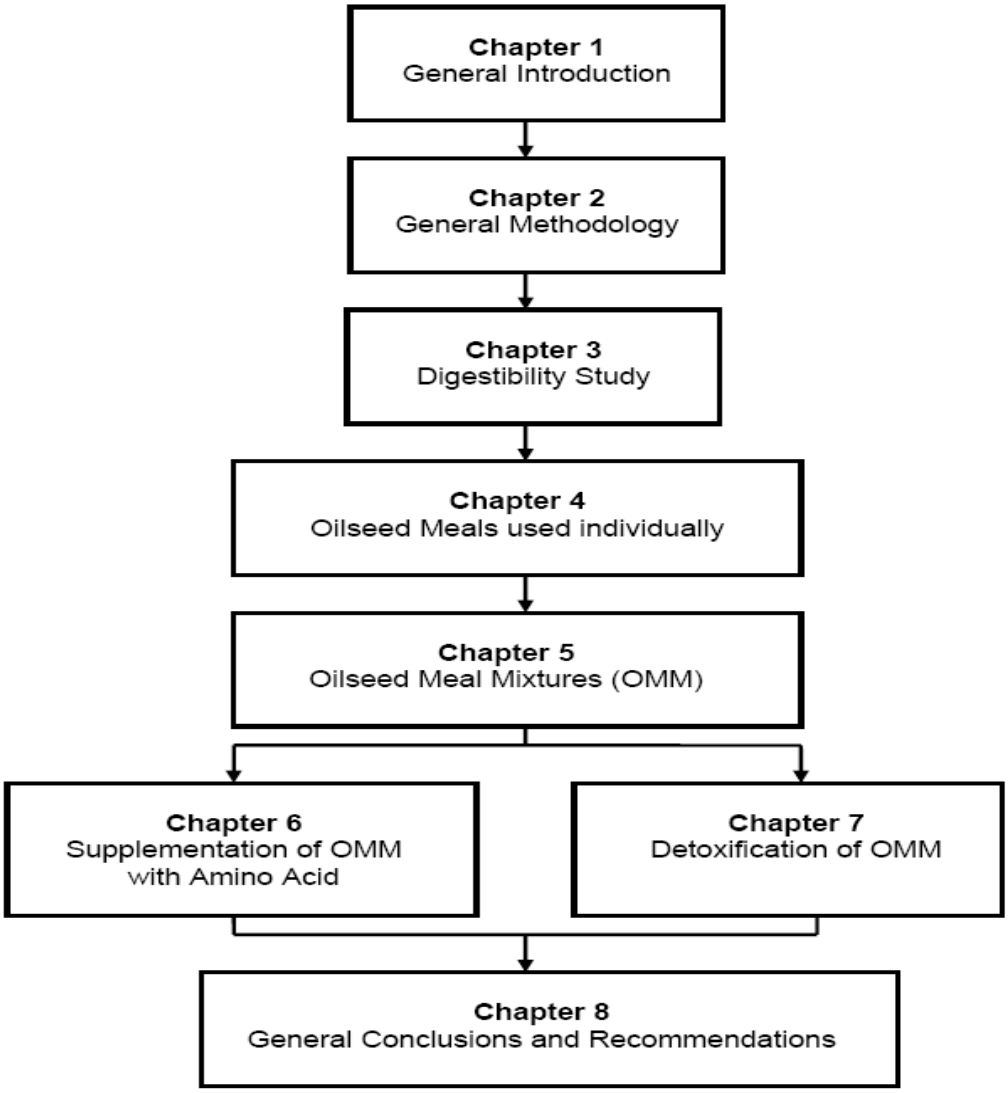

Figure 1.8 Structure of the Thesis

Chapter 2 - General Materials and Methods

2.1 Experimental Facilities

All experiments were conducted in the Tropical Aquarium of the Institute of Aquaculture which is an indoor facility with a re-circulatory water system.

2.1.1 Experimental System for Growth Trial and Fish Husbandry

The re-circulatory water system used in this study consists of 32 tank units of 30 litres capacity each (Figure 2.1). These are connected to a plumbing system that supplies water continuously. Water supply to the tanks was from a header tank through a common inflow pipe. Tanks were each fitted with inlets such that water flow (1 $L.min^{-1}$) 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 2.2). Fitted internally to each tank unit is an overflow and stand drain pipe, onto which a screen is fixed. This maintains water level without letting out fish. Over the drain pipe could be placed a jacket (sleeve) 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 bio-rings, which filtered waste water. Tanks were mounted on a metal framework over the settling tanks that received waste water. These tanks were then in turn connected to a clean water collecting sump from which used water was pumped to a header tank where it was further treated and recirculated.

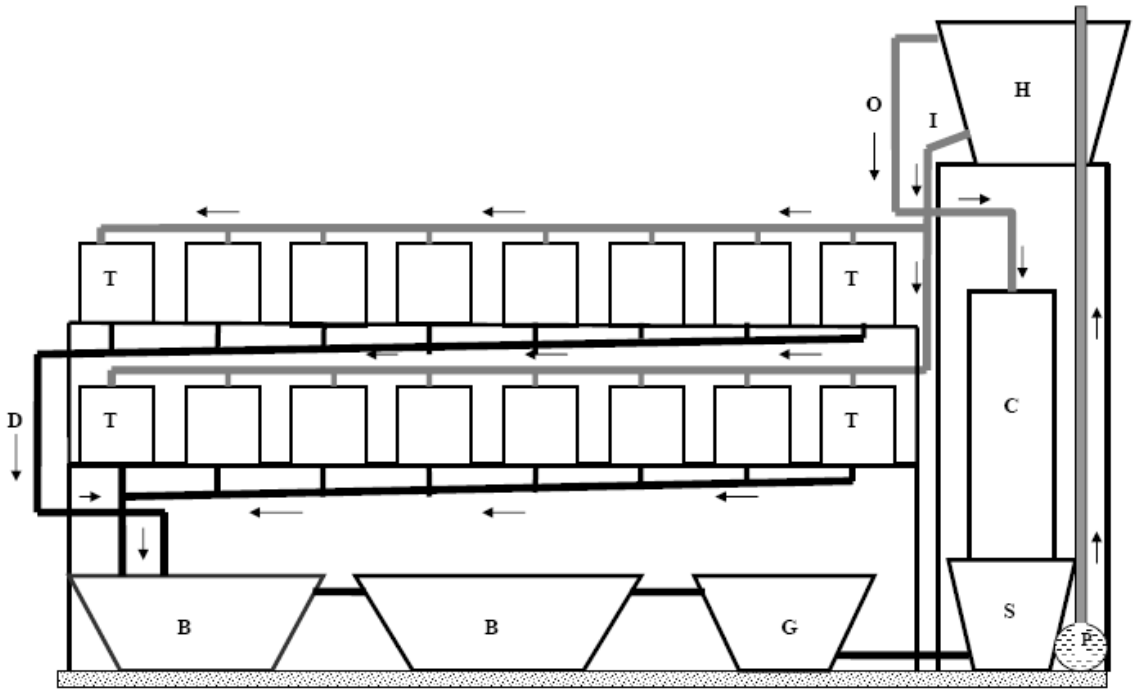

Figure 2.1 The recirculatory water system used for growth and digestibility studies
Key: H = Header tank, C = Vertical filter, S = Sump tank, G = Gravel filter, B = Biological filter, P = Pump, T = Experimental/Rearing tanks, I = Inlet pipes, D = Drainage pipes, O = Overflow pipe, ⟶ Direction of water flow

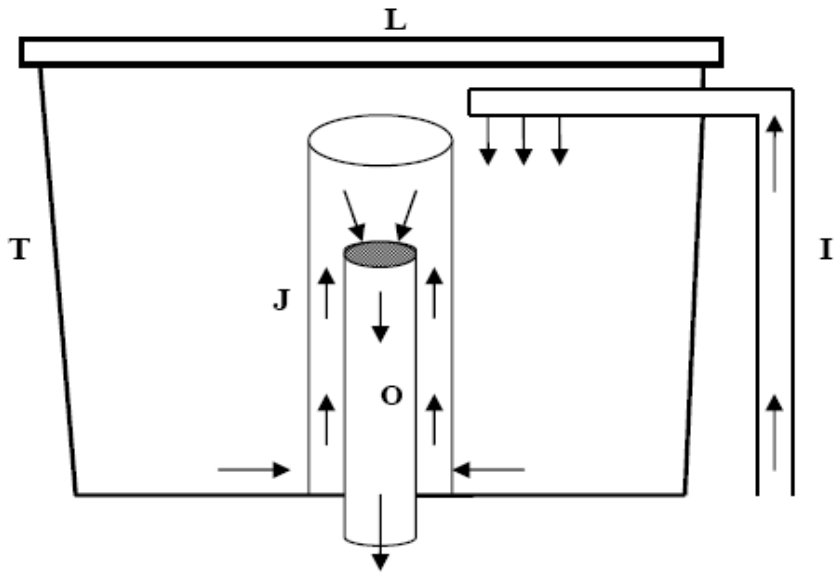

Figure 2.2 Experimental/Rearing tank
Key: L = Tank lid, T = Tank, J = Outer jacket of stand drainage pipe, O = Stand drainage pipe, I = Water inlet pipe, ⟶ Direction of water flow

Temperature was maintained at 27 ± 1 ^{o}C with the aid of submerged heating elements in the header tank. Air was supplied by an external compressor to maintain a dissolved oxygen concentration of, approximately 7 mg.L^{-1}. Water quality parameters including dissolved oxygen, pH, nitrite (NO_2), nitrate (NO_3) and ammonia (NH_3), were monitored weekly. A light:dark regime of 12 h:12 h was maintained using artificial light from fluorescent tube (58 watts, 240 volt, General Electric Hungary) and timer (Sangano, UK).

2.1.2 Faecal Collection System

In this study a settling column system similar to the Guelph system (Cho et al. 1985) was employed for faeces collection, but it was adapted to the 30 L cylindrical tanks used (Figure 2.3). 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 (Centaur 2 Sanyo Centrifuge) 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), phosphorus and gross energy (GE).

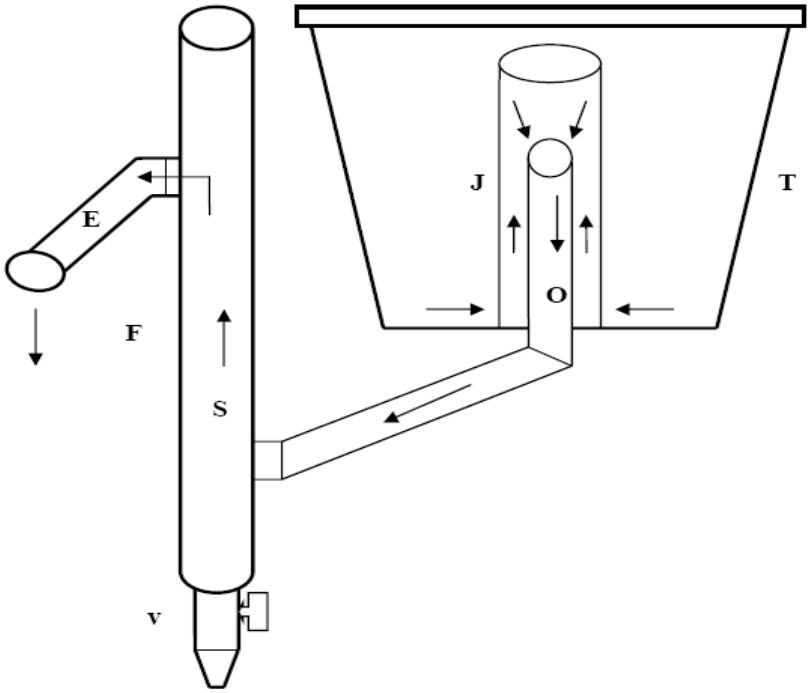

Figure 2.3 Faeces collection system used for the studies
Key: T = Rearing tank, J = Outer jacket of stand drainage pipe, O = Stand drainage pipe, F = Faeces collector, E = Overflow, S = Settling column, V = Valve to collect faeces, ⟶ Direction of water flow

2.1.3 Experimental Fish

Mixed-sex *Oreochromis niloticus* known as the "Red-stirling strain" (Ranson, personal communication) were used in this research. They were bred and reared in the tropical aquarium and hatchery complex of the institute where this research was carried out as described below. They were originally from Lake Manzallah in Egypt and introduced to the University of Stirling in 1979 and underwent natural selection over the years (Majumdar and McAndrew, 1986; McAndrew et al. 1988).

Breeding of *Oreochromis niloticus* was done artificially by stripping the eggs from a gravid broodstock female and fertilising them with milt from a broodstock male. Fertilisation was by the wet method whereby prior to mixing the eggs with

milt, a bit of water was added to ensure good contact of sperm with eggs. The fertilised eggs were incubated for about 2-3 days to hatch into fry. The fry absorbed their yolk in 2-3 days after which they were transferred to rearing/nursing tanks where they were fed with ground commercial trout diet (Nutra Trout Fry 02 from Skretting, U.K.) until ready for the growth trial.

Figure 2.4 Nile tilapia (*Oreochromis niloticus*) fingerlings used for the study

2.1.4 Acclimation and Weighing Procedures

One week before the start of each experiment fish were transferred to the experimental tanks from nursery tanks 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 30-litre tank in triplicates per treatment. Fish were fed with trout pellets during this period. For initial and final samples, all fish were individually weighed and measured under anaesthesia with Benzocaine (50 $mg.L^{-1}$) solution (Ross and Geddes, 1979). 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 a Mettler PC 400

electronic top pan balance and their lengths measured to the nearest 0.1cm using a fish measuring board.

For all intermediate weight measurements fish were bulk weighed, without anaesthesia, weekly. All fish in each tank were netted, using a fine mesh handnet. Excess water was then removed from the fish by blotting the net on a soft paper towel. Fish were then transferred to a tared, 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. Fish were monitored and handled according to procedures under the Animals (Scientific Procedures) Act 1986 enforced by the Home Office UK.

2.2 Diet Formulation and Preparation

The oilseed meals used as protein sources in this study were imported from Ghana from commercial sources. They are locally available and commonly used in fish culture in Ghana. These are; soybean (*Glycine spp)* meal (solvent extracted, dehulled), cottonseed (*Gossypium* spp.) meal (solvent extracted), groundnut (*Arachis hypogaea L*.) cake (mechanically extracted) and groundnut husk. All the ingredients (which came as one batch) were packaged in two layers of plastic bags and stored in a well ventilated room under ambient temperatures (of about 15^{o}C - 20^{o}C) but groundnut cake was stored in a freezer due to fear of aflatoxin contamination. Ingredients were used within 18 months and proximate composition was analized 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}. These levels were based on requirements for Nile

tilapia fry/fingerlings (Anderson *et al*., 1991; Jauncey and Ross, 1982; NRC, 1993). The diets were formulated on as fed basis. Brown fish meal (aquaculture grade) and wheat grain (milled) supplied by Ewos Ltd (Bathgate) were used as the main dietary protein and carbohydrate sources respectively. A mineral premix (Table 2.1) at 40 $g.kg^{-1}$, vitamin premix (Table 2.2) at 20 $g.kg^{-1}$ and a binder (carboxymethyl cellulose, high viscosity, Sigma C5013) at 20 $g.kg^{-1}$ were added. Sunflower oil was used as the source of lipid in the diets. Chromic oxide was added as an indigestible marker for digestibility study (Cho *et al*., 1974; De Silva and Anderson, 1995).

Diets were prepared by wet extrusion. All ingredients were finely ground and sieved through a 790µm sieve to obtain a homogenous mixture. The dry ingredients were then weighed out according to the formulation, placed in the bowl of a Hobart A200 Industrial Food Mixer (Hobart Co Ltd, London, England) and mixed until uniformly blended. Water was added (20%-30%) slowly to the mixture with continuous stirring until dough was formed. A steam conditioned California Pellet Mill (CL2 model, San Francisco, California) was used to pellet the diets using a die size of 1mm. The pellets were air dried using an electric fan convector heater at 35-40^{o}C overnight in a drying cabinet. They were then packaged in polythene bags and stored in a deep freeze at -20^{o}C. Prepared diet samples were analysed for proximate composition, amino acids, energy and phosphorus.

Table 2.1 Composition of mineral premixes used in experimental diets

Minerals	Chemical Formula	$g.kg^{-1}$
Magnesium sulphate	$MgSO_4,7H_2O$	510.00
Sodium chloride	NaCl	200.00
Potassium chloride	KCl	151.11
Iron sulphate	$Fe\ SO_4,7H_2O$	100.00
Zinc sulphate	$ZnSO_4,4H_2O$	22.00
Manganese sulphate	$MnSO_4,4H_2O$	10.15
Copper sulphate	$CuSO_4,5H_2O$	3.14
Cobalt sulphate	$CoSO_4,7H_2O$	1.91
Calcium iodate	$CaIo_3,6H_2O$	1.18
Chromic chloride	$CrCl_3,6H_2O$	0.51

According to Jauncey and Ross (1982)

Table 2.2 Composition of the vitamin premix used in experimental diets

Vitamin	$mg.kg^{-1}$ of Premix*
Thiamine (B_1)	4250
Riboflavin (B_2)	3000
Pyridoxine (B_6)	1250
Pantothenic acid	5250
Inositol	25000
Biotin	90
Folic acid	1000
Ethoxyquin	200
Choline	74050
Nicotinic acid (Niacin)	12500
Cyanocobalamin (B_{12})	1.25
Retinol palmitate(A)	1000
Tocopherol acetate (E)	7000
Ascorbic acid (C)	37500
Menadione (K)	1500
Cholecalciferol (D_3)	4

*The mixture was made up to 1kg with α-cellulose.
According to Jauncey and Ross (1982)

2.2.1 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 (1990) protocols:

Moisture: - Moisture content was determined by air-drying the samples in an oven at 105^oC for 24 hours. 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 (AOAC, 1990). Approximately 1g of sample was weighed into a pre-weighed crucible and incinerated overnight at 600^{o}C using a Gallankamp muffle furnace size 2. 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 according to AOAC (1990) was used for the determination in triplicate as follows; 200mg sample was digested in concentrated sulphuric acid and ammonia from the digest (Digestor 2040) was released when reacted with 40% sodium hydroxide and distilled, trapped in 2% boric acid and quantified by titration against 0.2M hydrochloric acid – both distillation and titration were automated using a Kjeltec 2300 + Analyser (FOSS) and run according to the operation manual from the manufacturer. Percentage protein in the sample was calculated automatically by the analyser.

Crude Lipid: – The method was that of solvent extraction using Soxhlet extraction (Soxtec 2050, FOSS). Approximately 1g sample was weighed into a thimble and corked with cotton. 80ml petroleum-ether (40-60^{o}C) was added to a pre-weighed cup with ten glass balls. The thimbles were placed into the unit by fixing the metal adapters to the magnetic rings at the bottom of each condenser and the corresponding cups then fitted into the unit underneath the thimbles. Extraction which involved boiling, rinsing and evaporation was conducted following the instructions in the manufacturer's manual. Extracted lipid in the

cup was dried in an oven (105^{o}C) for at least an hour before weighing and expressed as a percentage of the original sample.

Crude Fibre: – 1g of defatted sample in a pre-weighed Scintaglass ™ crucible was used for crude fibre determination using acid-base hydrolysis. The crucible was fitted to the Fibertec 2021 Fibercap and run according to the manufacturer's operating instructions. Hydrolysed and oven-dried sample was later ashed in the muffle furnace at 550^{o}C and crude fibre in the defatted sample expressed as a percentage of the original undefatted sample.

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

2.2.2 Amino Acid Analysis

The amino acid contents of the ingredients used for feed formulation were analysed according to the procedure described below.

Sample Preparation – Samples for amino acid analysis were hydrolysed with 5.7 N HCL for 24 hours at 110^{o}C *in vacuo*, following the procedures given in the LKB 4151 Alpha-Plus Instruction manual (1983).

Analysis – All the analyses were carried out using an LKB 4151 Alpha-Plus Amino Acid Analyser (LKB Biochrom Ltd, Cambridge). These analyses were based on the following principles. The prepared sample was loaded onto a column of cation-exchange resin. The amino acids were sequentially eluted by buffers of varying pH and ionic strength. In a high temperature reaction coil ninhydrin was reacted with the column eluent to form coloured compounds, which the colour intensity being directly proportional to the quantity of an amino

acid present in the sample. The amount of each coloured compound is determined by a photometer measuring the amount of light absorbed at 570nm. Amino acids were identified by comparison of peak retention times to a known standard and were quantified by comparison of areas to the same standard mixture.

Chemical Score (CS, %) – This is a parameter used to numerically compare essential amino acid (EAA) profiles of ingredients or diets to the requirements of the target species. It is based on the concept that utilisation of a dietary protein depends upon the level of the EAA in greatest deficit. EAA with CS of 100% or more meet or exceed the requirement for EAA and those with less than 100% are in deficit. The EAA producing the lowest CS is termed the 'first limiting EAA' the next lowest CS 'second limiting EAA' etc (Jauncey, 1998). CS is calculated using % dietary protein as follows:

CS (%) = (% EAA in ingredient or diet / % EAA requirement for fish) x 100

2.2.3 Chromic Oxide Analysis

Chromic oxide was determined according to the method of Furukawa and Tsukahara (1966). The procedure depends upon the digestion of the sample by concentrated nitric acid and oxidising chromic oxide with 70% perchloric acid. The orange colour formed by the oxidation of chromium III to chromium IV is read on a spectrophotometer (Uvikon 860 Kontron instruments) at 350nm against distilled water.

100 mg of sample was weighed into a Kjeldahl flask. 5 ml of concentrated nitric acid were added to the flask and the mixture was boiled (using an electric

mantle) gently for about 20 minutes (taking care not to boil dry). After cooling the sample, 3ml of 70% perchloric acid was added to the flask. The mixture was then gently heated again until the solution turned from a green to an orange colour after which it was left to boil for a further 10 minutes to ensure oxidation was complete. The solution was transferred to a 100 ml volumetric flask and diluted to volume. The absorbance of the solution was determined by spectrophotometer (Uvikon 860 Kontron instruments) at 350nm against distilled water. Chromic III oxide was calculated according to the formula:

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

2.2.4 Energy Determination

Energy contents of the ingredients, diets and faeces were determined using an Adiabatic Autobomb Calorimeter (Parr 6100, USA) and benzoic acid as standard. Approximately 1g of dried sample moulded into a tablet using a briquette press was combusted in a chamber pressurised with oxygen and the resulting heat measured by increase in temperature of water surrounding the bomb. Following the instructions in the operation manual from the manufacturer, the energy content of the sample is automatically determined by the bomb calorimeter.

2.2.5 Phosphorus Analysis

Phosphorous was measured by the method outlined by Stirling (1985). The principle of the method is that phosphorous in the sample is converted to soluble inorganic phosphorous by digestion with nitric acid and perchloric acid. This phosphorous reacts with molybdate to form molybdophosphoric acid which

was then reduced to the intensely coloured molybdenum blue complex and determined using a spectrophotometer (Cecil Elegant Technology – Aquarius-P).

2.2.6 Analysis of Antinutritional factors

Phytic Acid: - An assay kit (Megazyme, K-Phyt 05/07) was used to determine phytic acid. Approximately 1.0 g of a sample was weighed into a 75 mL beaker and 20 mL of 0.66M hydrochloric acid was added. The beaker was covered with foil to avoid spillage and placed on a shaker overnight for extraction. After extraction, 1 mL of the extract was transferred into a 1.5 mL microfuge tube and centrifuged at 13,000 rpm for 10 min. and immediately, 0.5 mL of the supernatant was transferred to a fresh 1.5 mL microfuge tube and neutralised using 0.5 mL of 0.75 M sodium hydroxide. The neutralised sample extract was subjected to enzymatic dephosphorylation and phytic acid was determined colorimetrically following procedures described in the assay manual (Megazyme International, 2007; Fiske and Subbarow, 1925; Lowry and Lopez, 1946).

Trypsin Inhibitor: - Trypsin inhibitor activity was determined in solvent extracted SBM using a modification of the American Association of Cereal Chemists Method 71-10 (AACC, 2000). A 1.0 g sample of SBM was extracted with 50 mL 0.01 N NaOH for 3 h. Samples were transferred to 50 mL scintillation tubes and centrifuged at 1750 g for 10 min. Aliquots of 0, 0.6, 1.0, 1.4, and 1.8 mL extract were brought to 2 mL, mixed with 2 mL trypsin solution (4 mg Type-I from bovine pancreas, (Sigma Chemical Co.) in 200 mL 0.001 M HCl), and warmed to 37^{o}C. Following addition of 5.0 mL *N*α-benzoyl-DL DL-arginine-*p*-nitroanilide hydrochloride (BAPNA) solution [40 mg BAPNA dissolved in 1.0 mL DMSO and diluted to 100 mL with 0.05 M Tris buffer (pH 8.2)], the

solution was incubated at 37^{o}C for exactly 10 min before stopping the reaction with 1.0 mL 300 g.kg^{-1} acetic acid. The solution was twice filtered through Whatman no. 2 filter paper and absorbance read at λ = 410 nm. Activity was expressed as trypsin inhibitor units (TIU), which was defined as an increase of 0.01 absorbance units at 410 nm per 10 mL reaction volume. The TIU were converted to trypsin inhibitor activity (TIA) expressed as mg.g^{-1} sample (Hamerstrand *et al*. 1981; Riche and Garling, 2004).

Gossypol: - Gossypol was determined (using a modification of AOCS (1987) methods) by weighing 10 g of sample and adding Solution A (0.2 mL Glacial Acetic acid per litre Ethanol) and 20 mL Ethyl ether then homogenising for 2 minutes, and cooling in an ice-bath. 3 g Hyflo Supercel was suspended in 15 mL of Solution A then filtered in a Buchner funnel under vacuum. 3 g Hyflo Supercel was added to the homogenate and shaken and poured through the funnel. Solution B (715 mL 95% ethanol diluted to litre with distilled water + 200 mL Ethyl ether and 0.2 ml Glacial Acetic Acid) was used to wash through repeatedly until the combined washings and filtrate were 120-130 mL. 10 mL aliquots were transferred in triplicate to 25 mL volumetric flasks. One replicate (the reference) was made up to 25 mL with Solution B and to the other two was added 0.5 mL aniline. The solutions at this stage were heated in a boiling water bath for 40 minutes cooled and 2 mL Ethyl ether added and made up to 25 mL with Solution B then mixed. These were read at 445µm against the reference.

Gossypol content was calculated from a standard curve prepared thus: 0.025 g gossypol was dissolved in 10 mL ethyl ether then diluted to 100 mL with Solution B, 10 mL was transferred to a 100 mL volumetric flask then diluted to

volume with Solution B. In triplicate at 1mL intervals 1-8 mLs was pipetted into 25 mL volumetric flasks making one flask a reference at each aliquot level. All flasks were diluted to 10 ml with Solution B then 0.5 mL aniline added to all except the references. Flasks were heated in a boiling water bath for 40 minutes cooled and diluted to 25 mL, mixed and read against the appropriate reference at 445µm. A standard curve of µg gossypol/25 mL against absorbance was plotted and then gossypol calculated as follows; µg Gossypol/g Absorbance/g sample in 10 mL aliquot x Absorbance/µg Gossypol in 25 mL Standard.

Saponins: - Saponin content was determined using a spectrophotometric method described by Baccou *et al.* (1977). To 0.5 g defatted ground samples, in a capped 25 mL centrifuge tube, was added 10 mL of 80% aqueous methanol. The tubes were tightly capped and the contents were stirred overnight using a shaker for saponin extraction. The tubes were then centrifuged at 3500 rpm for 10 min. and the supernatants collected in volumetric flasks. The residues were rinsed (re-extracted) three times with 5 mL of 80% methanol, centrifuged and the supernatants again added to the previous collection in the 25 mL flasks. The final volume was made to 25 mL with 80% methanol. Aliquot samples from the flasks were used for saponin determination. The results were expressed as diosgenin equivalents from a standard curve of different concentrations of diosgenin in 80% aqueous methanol.

2.3 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.

2.3.1 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.

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

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 $\%.day^{-1}$ (Ricker, 1979).

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

where D is the number of days between weighings.

2.3.2 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:

$$\mathrm{FCR} = \frac{\text{feed fed}_{(g)}}{\text{live weight gain}_{(g)}}$$

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).

2.3.3 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):

$$\mathrm{PER} = \frac{\text{weight gain}_{(g)}}{\text{crude protein fed}_{(g)}}$$

2.3.4 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 analyses of samples of fish taken before and after feeding with the evaluated protein, and generally expressed as a percentage of the protein fed.

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

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.

2.3.5 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).

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; Forster, 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; D_i=% Cr_2O_3 of diet; F_i=% Cr_2O_3 of faeces

and ADC of ingredients as;

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

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

Digestible protein and energy were calculated as follows:

Digestible protein (DP, gkg^{-1}) = dietary crude protein (gkg^{-1}, dwb) x $ADC_{protein}$

Digestible energy (DE, kJg^{-1}) = gross energy (kJg^{-1}, dwb) x ADC_{energy}

2.3.6 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 2.2.1 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 benzocaine, dissected and livers removed, weighed and used to estimate the hepatosomatic index (HSI)

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

2.4 Histopathological Analysis

Histological analysis was conducted to investigate differences or abnormalities in fish as a result of feeding on oilseed meals as an alternative source of protein. Oilseed meals i.e. soybean meal, cottonseed meal and groundnut meal contain endogenous antinutritional factors or antinutrients which restricts their use (NRC, 1993). The method of Drury and Wallington (1980) was adapted to perform the histological techniques which are also approved by the Institute of Aquaculture, University of Stirling. At the end of each experiment, 6 fish from each treatment, including the control, were euthanized by overdose of benzocaine, dissected and livers and intestines removed and fixed in 10% buffered formalin for histopathological study. Samples were cassetted and

processed using autoembedder (Shandon Excelsior, Thermo) for dehydration, clearing and wax impregnation. Processed fish tissues were embedded in paraffin wax and sectioned by microtome (Leica 2035 Biocut) set at 5µm thickness. Tissue sections were stained with haematoxylin and eosin and examined under a light microscope (Olympus BX51) to assess any histopathological changes in the liver and intestine.

2.5 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 3.2) of ingredients in Ghana in 2007. Vincke (1969) 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. Miller (1976) 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 ¢ 2.00.kg^{-1} fish (Asmah, personal communication).

2.6 Statistical Analysis

The experimental design used in this study was mainly completely randomised 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 SPSS Statistical Package (Version 15.0, SPSS Inc., Chicago, IL). 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 $p < 0.05$. All data were tested for normality using the Kolmogorov-Smirnov test and homogeneity using the Levene's test, also all percentages and ratios were arcsine transformed before analysis (Zar, 1984).

Chapter 3 - Protein and Energy Digestibility of Soybean meal, Cottonseed meal, Groundnut meal and Groundnut husk in Juvenile Nile tilapia, *Oreochromis Niloticus* L.

3.1 Introduction

With the increase in intensive aquaculture, demand for more efficient dry diets for fish is rising. Feed is the principal operating cost in the production of fish and the main protein source has traditionally been fish meal (Glencross *et al*., 2007). Fish meal, the conventional protein source in aquaculture feeds, supports good fish growth because of its protein quality and palatability (Watanabe *et al*., 1997). However, fish meal is often scarce and expensive, especially good quality brands, due to relatively stable to low production and high demand, which often lead to high cost of fish production (El-Sayed, 2004; Hardy, 2006). According to Rumsey (1993) and Tacon (1993), 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, research has concentrated on replacing fish meal with cheaper ingredients of either animal origin or protein-rich plant sources (Higgs *et al*., 1995; Kaushik, 1990; Rumsey, 1993). In this respect, oilseed meals have considerable economic potential (Lim and Dominy, 1991). 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 omnivorous/herbivorous fish species such as those commonly used in African aquaculture, including tilapias (*Oreochromis spp.*) and catfishes (*Clarias spp.*).

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 accepted, 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. Digestibility is a relative measure of the extent to which ingested food and its nutrient components have been digested and absorbed by the animal. It is therefore, necessary to know the digestibility of a feedstuff in order to evaluate its value as a source of nutrients (Anderson and De Silva, 2003). Also important points to note when determining digestibilities are that: digestibility of an ingredient should not be estimated by feeding the ingredient alone to the animal; the ingredient digestibility is best determined by preparing a test diet, including 15 - 30% of the ingredient, with a reference diet of known digestibility and lastly attempts should be made to use endogenous markers (Anderson and De Silva, 2003). The nutritive value of mixed rations depends on the nutrient composition of the individual feed components and the ability of the animal to digest and absorb nutrients (Degani *et al*., 1997; Falaye and Jauncey, 1999; Riche *et al*., 2001; Sklan *et al*., 2004; Watanabe *et al*., 1996). Sklan *et al*. (2004) conducted tests using a compound diet which indicated that ingredient digestibility was additive for protein, lipids, carbohydrates and energy. So they concluded that diets for tilapia may be formulated on the basis of digestibility of individual ingredients.

Digestibility is determined by comparing the quantity of a nutrient consumed with that left in faeces at the end of the digestive process. In practical terms the digestibility of a feed ingredient depends primarily on its chemical composition and the digestive capabilities of the species to which it is fed (McGoogan and

Reigh, 1996). The true digestibility of any nutrient must be corrected for the level of that nutrient that would appear in the faeces even if the nutrient in question were absent from the diet. In practice this is difficult to measure and most data is based on apparent digestibility without this correction (Jauncey, 1998). Digestibility is a measure of the quantity of ingested nutrients retained and is most commonly measured in aquatic animals by indirect methods using inert marker materials. By adding an inert material (external marker) to the feed or measuring an inert natural component of the food (internal marker), apparent digestibility can be calculated by comparing the ratio of the marker in the food and faeces to a specific nutrient. For a marker to be effective, it must be indigestible, non-toxic, inert and should move through the gut at the same rate as the digesta (De Silva and Anderson, 1995). Most digestibility studies conducted have used external markers and chromic oxide (Cr_2O_3) is the most commonly used marker and has been used extensively in studies with tilapia (Fagbenro and Jauncey, 1995; Falaye and Jauncey, 1999; Fontainhas-Fernandes *et al*. 1999; Koprucu and Ozdemir, 2005; Sklan *et al*., 2004; Guimaraes *et al.*, 2007). Incorporation in diets at 0.5-1.0% levels, Cr_2O_3 has been demonstrated to be a reliable indicator for digestibility studies in fish (Cho et al. 1974; De Silva and Anderson, 1995; Inaba *et al*., 1962; Nose, 1960). Feedstuff substitution procedures described by Cho *et al*. (1982) as refined by Forster (1999) and Bureau *et al.* (1999) enable the apparent digestibility of a single ingredient in a multi-ingredient diet to be determined.

According to Lovell (1998) feed ingredients containing 20% or more crude protein are considered protein sources. Soybean meal (SBM), cottonseed meal (CSM), groundnut cake (GNC) and groundnut husk (GNH) were selected as

dietary protein sources for this study on the basis of their high protein content, availability and use in fish feeds in Ghana. Work conducted on SBM, CSM and GNC showed they have good protein contents (26-54%, depending on processing methods) and good amino acid profile (Lovell, 1981). Nutrient digestibility has been conducted more extensively on SBM for many fish species than on CSM and GNC. GNH has not been researched into at all, probably because it is restricted to Ghana and has little value, however, it is a common by-product from processed groundnut in Ghana and usually recommended by Fisheries Directorate as a supplementary feed for tilapia (Hasan, 2007). GNH is actually the testa or skin of the kernels which is removed after roasting and groundnut kernels contain 4.1% testa or skin (De Boer and Bickel, 1988).

This study was conducted to evaluate the apparent digestibility coefficients (ADC) of dry matter (DM), crude protein (CP), gross energy (GE) and phosphorous for SBM, CSM, GNC and GNH for *O. niloticus* before their subsequent use in growth study diets.

3.2 Materials and Methods

3.2.1 Experimental System and Animals

The source of experimental fish and their breeding are described in Section 2.1.1. Fingerlings of Nile tilapia of an average weight of 8.67 ± 1.78 g were stocked at 15 per tank (30L-tank) in a water recirculation system (described in Section 2.1.1, Figure 2.1). 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 an overhead tank thermoregulated at 27 ± 1 ^{o}C and a constant photoperiod of 12 hours Light/12 hours Darkness was maintained (Section 2.1.1). Water quality parameters measured during the experiment averaged (± SD): temperature, 26.91 ± 0.33 ^{o}C; pH, 6.9 ± 0.1; ammonia, 0.22 ± 0.08 mg.L^{-1}; nitrite, 0.20 ± 0.0 mg.L^{-1}; Nitrate, 60 ± 28.28 mg.L^{-1} and dissolved oxygen, 6.89 ± 0.38 mg.L^{-1} and they were within acceptable ranges for tilapia (Table 1.3).

3.2.2 Diet Formulation

A reference diet (Table 3.1) was formulated to satisfy the nutrient requirements of Nile tilapia (De Silva *et al*., 1989; Jauncey and Ross, 1982; 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 meal (SBM), cottonseed meal (CSM), groundnut cake (GNC) and groundnut husk (GNH). All test feed ingredients were obtained from commercial sources in Ghana with the exception of soybean meal which was acquired in the UK (BioMar UK Ltd).

Table 3.1 Composition of reference and test diets (g.kg^{-1}) for the digestibility study

Ingredients	Reference diet	Test diets
Test ingredient	0.0	298.5
Fish meal	300.0	210.0
Soybean meal	81.0	56.7
Wheat grain	472.0	330.4
Sunflower oil	62.0	43.4
Vitamin premix [1]	20.0	14.0
Mineral premix [2]	40.0	28.0
Carboxymethyl cellulose[3]	20.0	14.0
Chromic oxide[4]	5.0	5.0

[1]As listed in Table 2.1, [2]As listed in Table 2.2, according to Jauncey and Ross (1982);
[3]Carboxymethyl cellulose (Sigma, C5013); Chromic oxide (BDH 277574Q)

Four test diets were formulated using 70% reference diet and 30% of each of the test ingredients as described by Cho *et al*. (1985). Chromic oxide (Cr_2O_3) was used as an inert marker at a concentration of 0.5% in the diets. Other

supplements used in the diets are outlined in Table 3.1. Diet preparation is described in Section 2.2. Faeces collection from tanks was conducted using collectors as described in Section 2.1.2.

3.2.3 Analytical Techniques

Proximate analyses of ingredients, diets and faeces samples were conducted using the methods described in Section 2.2.1. The amino acid contents, gross energy and phosphorus of the ingredients, diets and faeces samples as well as some antinutritional factors of the ingredients were also analysed according to the methods described in Sections 2.2.2, 2.2.4, 2.2.5 and 2.2.6. Chromic oxide in diets and faecal samples was determined by acid digestion with concentrated sulphuric acid and perchloric acid following the procedure described in Section 2.2.3. Apparent digestibility coefficients of nutrients, energy and phosphorus of diets and ingredients were determined as described in Section 2.3.5.

3.3 Results

3.3.1 Chemical Composition and Prices of Ingredients

Proximate compositions, energy, phosphorus and some antinutritional factors (ANFs) of the ingredients used in the study are shown in Table 3.2 and Table 3.3. Crude protein for the oilseed meals ranged from 205.6 - 500.3 $g.kg^{-1}$ with SBM the highest and GNH the lowest. In contrast, crude lipid was highest for GNH (256.0 $g.kg^{-1}$) and lowest for SBM (10.1 $g.kg^{-1}$). CSM and GNH had the highest crude fibre levels with 89.5 $g.kg^{-1}$ and 89.2 $g.kg^{-1}$ respectively, about seven times higher than GNC which had the lowest (12.8 $g.kg^{-1}$). Gross energy values for ingredients ranged from 18.85 - 23.17 $kJ.g^{-1}$. Phytic acid content was highest (31.64 $g.kg^{-1}$) for CSM and lowest (3.99 $g.kg^{-1}$) for GNH. With regards to

trypsin inhibitors SBM contained the highest (14.09 $g.kg^{-1}$) and CSM the lowest (1.24$g.kg^{-1}$).

Table 3.2 Proximate composition (gkg^{-1} as-fed), energy ($kJ.g^{-1}$), phosphorous ($g.kg^{-1}$) and prices ($¢.kg^{-1}$) of individual feed ingredients used in this study

Ingredients	DM	CP	CL	CF	Ash	NFE	GE	P	Price
Fish meal	947.3	716.3	95.4	05.6	130.0	T	21.16	11.26	1.17
Soybean meal	894.2	500.3	10.1	38.2	58.9	286.7	20.19	5.35	0.48
Cottonseed meal	902.9	441.4	32.3	89.5	77.1	258.6	19.61	10.71	0.18
Groundnut cake	924.0	430.5	219.0	12.8	43.8	217.9	23.17	6.72	0.40
Groundnut husk	932.5	205.6	256.0	89.2	34.1	347.6	22.18	2.34	0.04
Wheat grain	890.9	95.2	14.9	28.2	18.9	733.7	18.85	3.78	0.10

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, NFE = nitrogen free extract, GE = gross energy, P = phosphorous, T = trace; ¢ = Ghanaian cedis (Exchange rate ¢0.90 = USD 1.00 in 2007)

Saponin content of ingredients ranged from 10.08 – 5.80 $g.kg^{-1}$. The prices of ingredients used in the study are shown in Table 3.2. Fish meal was the most expensive (1.17 $¢.kg^{-1}$) and was more than double the price (0.48 $¢.kg^{-1}$) of SBM which was the most expensive among the oilseed meals.

Table 3.3 Antinutritional factors analyzed in the oilseed meals used in this study ($g.kg^{-1}$)

Ingredients	Phytic acid	Trypsin inhibitors	Saponin	Gossypol
Soybean meal	17.54	14.09	5.80	-
Cottonseed meal	31.64	1.24	6.50	5.60
Groundnut cake	14.86	2.34	8.01	-
Groundnut husk	3.99	-	10.08	-

Fish meal had a good amino acid profile and that of the test ingredients was generally good with the exception of GNH, which had very poor amino acid profile (Table 3.4) compared to the requirements for tilapia. The essential amino acid content of SBM was very close to that of fish meal but methionine and threonine had considerably low values of 0.73 and 1.5 % of protein respectively. CSM and GNC had even lower values of methionine, threonine and lysine (Table 3.4).

Table 3.4 Analysed essential amino acid composition (% of protein) of ingredients used in the study

Ingredient	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine[1]	Phenylalanine[2]	Threonine	Valine
Fish meal	3.68	1.42	2.88	4.64	5.98	2.03	3.82	2.37	3.59
Soybean meal	3.12	1.25	2.30	3.54	5.11	0.73	3.60	1.50	2.55
Cottonseed meal	3.74	1.13	1.36	2.36	4.11	0.77	2.90	1.30	1.80
Groundnut cake	3.31	0.90	1.20	2.11	3.63	0.51	2.70	0.88	1.55
Groundnut husk	0.93	0.37	0.45	0.78	3.08	ND	0.98	0.35	0.61
Wheat grain	0.31	0.21	0.29	0.52	2.62	0.09	0.41	0.21	0.38

[1]Value includes cystine of ingredient, [2]Value includes tyrosine of ingredient. ND = Not detected

3.3.2 Chemical Composition of Test Diets

Proximate, energy and phosphorus compositions of the reference and test diets used in the digestibility study are presented in Table 3.5. Analysed crude protein, NFE, dry matter, ash and energy contents of test diets showed little variation. However, crude lipid and 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 18.09 and 19.22 $kJ.g^{-1}$.

3.3.3 Nutrient and Energy Digestibility

Apparent digestibility coefficients (ADCs) of protein, lipid, dry matter, phosphorus and energy in selected test ingredients for Nile tilapia are shown in Table 3.6. Generally ADCs of the nutrients studied were highest for SBM followed by GNC, CSM then GNH. The only exception was ADC of crude lipid which had its highest value (98.95%) for CSM. The apparent DM digestibility coefficient of ingredients decreased as fibre contents of ingredients increased.

ADC of gross energy and phosphorus followed the same trend with SBM with the highest at 85.99% and 64.31% respectively and GNH the lowest 34.67% and 30.38% respectively. Digestible protein and energy followed a similar trend, however, GNC had the highest digestible energy.

Table 3.5 Proximate composition ($g.kg^{-1}$), energy and phosphorus of reference and test diets

Components	Reference diet	Test diets			
		SBM	CSM	GNC	GNH
Dry matter	950.3	952.1	942.7	945.1	946.4
Crude protein	323.1	380.4	352.3	348.7	290.3
Crude lipid	105.7	90.5	84.1	144.7	156.9
Crude fibre	27.1	29.6	44.8	27.8	49.6
Ash	87.2	81.5	85.9	75.8	73.0
NFE	407.2	370.1	375.6	348.1	376.6
Chromic oxide	4.7	4.7	4.9	4.8	4.7
Gross energy ($kJ.g^{-1}$)	18.09	18.18	18.20	19.22	19.07
Phosphorus ($g.kg^{-1}$)	7.54	7.32	8.06	7.21	6.40

SBM = soybean meal, CSM = cottonseed meal, GNC = groundnut cake, GNH = groundnut husk

Table 3.6 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy, phosphorus 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 meal	Cottonseed meal	Groundnut cake	Groundnut husk
DM	77.47 ± 0.39^{a}	68.55 ± 1.79^{b}	74.33 ± 1.46^{a}	60.62 ± 3.01^{c}
CP	94.50 ± 0.70^{a}	84.93 ± 2.67^{b}	90.01 ± 2.90^{ab}	27.67 ± 2.65^{c}
CL	96.84 ± 1.18^{a}	98.95 ± 1.17^{a}	95.43 ± 0.34^{a}	79.64 ± 1.99^{b}
GE	85.99 ± 0.14^{a}	58.43 ± 4.45^{b}	81.45 ± 3.45^{a}	34.67 ± 5.32^{c}
P	64.31 ± 0.80^{a}	53.54 ± 2.44^{a}	55.36 ± 2.81^{a}	30.38 ± 9.77^{b}
DP	528.7	415.2	419.4	61.0
DE	17.36	11.46	18.87	7.60

DM = dry matter, CP = crude protein, CL = crude lipid, GE = gross energy, P = Phosphorus, DP = Digestile protein, DE = Digestible energy. Values are means ± SD of three replicates, and values within the same row with different letters are significantly different ($P < 0.05$).

3.4 Discussion

Proximate composition of the test ingredients (i.e. SBM, CSM and GNC) used in this study was in agreement with reported values of various oilseed meals as reported by other authors (Table 3.7). The only obvious difference was the high crude lipid value (219.0 $g.kg^{-1}$) for GNC. This might be as a result of poor mechanical extraction of oil from groundnut (kernel), therefore leaving a lot more oil in the cake. As was stated by Lovell (1998) nutrient composition of feedstuffs depends on the origin, state and processing methods used. Crude fibre was high in CSM and it is considered a limiting factor in its use as feed. It

is around 10% in decorticated meals and expellers while its content in corticated meal can exceed 20% (Hertrampf and Piedad-Pascual, 2000).

Table 3.7 Proximate composition (% as fed) of some oilseed meals used as feed ingredient for fish

Ingredients	DM	CP	CL	CF	NFE	Ash
SBM	88.4-90.0	49.9-56.7	0.7-1.4	5.0-6.9	32.1-46.7	6.2-7.6
CSM	87.9-94.5	26.2-47.7	5.4-7.6	7.9-25.6	27.8-47.0	5.0-7.0
GNC	90.4-92.6	49.5-52.0	6.3-9.2	5.3-8.3	27.4-31.5	4.5-5.8
RSM	87.2-94.0	32.1-37.4	7.4-12.8	10.4-16.4	31.4-32.3	7.2-12.9

Values adapted from Jauncey (1998) and NRC (1993). DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, NFE = nitrogen free extract, RSM = Rapeseed meal

The EAA values of ingredients were close to those reported by NRC (1993). Generally the oilseed meals had good essential amino acid profile with the exception of GNH. The EAA profile of SBM compared very well with fishmeal but methionine and threonine were quite low. The same was true for CSM and GNC which had even lower values including lysine (Table 3.4). According to Jauncey (1998) most oilseed meals tend to be rich in protein but their EAA profiles are often unbalanced with lysine, methionine and threonine generally deficient.

The ANFs of the oilseed meals particularly, SBM, CSM and GNC (Table 3.3) were within values reported by various researchers (Cheryan, 1980; Francis *et al*., 2001; Graf, 1983; Hossain, 1988; Snyder and Kwon, 1987; Wolf, 1983).

The present study showed that ADCs of dry matter, protein, lipid, phosphorus, and energy in the test ingredients for Nile tilapia were affected by test ingredients ($p < 0.05$). The differences in ADCs of nutrients and energy may be explained by differences in chemical composition, origin and processing of these feed ingredients (Table 3.2). Results in Table 3.6 indicate that soybean meal was the most digestible with an apparent protein digestibility (APD) of

94.50% and GNH was the least with 27.67%. Previous studies (Anderson *et al*., 1991) have indicated that in tilapia as in other species (Dabrowski and Dabrowska, 1981; Lupatsch *et al*., 1997) CP digestion is relatively high, and this is found even in feeds containing high fibre. In this study although CSM had a relatively high CF the APD was still high.

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. Deficiency of an essential amino acid leads to poor utilization of the dietary protein and consequently reduces growth and decreases feed efficiency (Halver and Hardy, 2002). Proteins in most feedstuffs that have been properly processed are highly digestible to fish. The digestion coefficients for protein-rich feedstuffs are usually in the range of 75 to 95% (NRC, 1993). APD tends to be depressed as the concentration of dietary carbohydrate increases (NRC, 1993), however this was not observed in this study. The APDs (84.93%–94.50%) in test ingredients for Nile tilapia in this study are generally in agreement with the reported APDs in various oilseed ingredients in this species. For example, APDs of soybean meal for tilapia reported by different authors were 94.0% (NRC, 1993); 91.6% (Jauncey, 1998); 96.2% (Sklan *et al*., 2004), 87.4% (Koprucu and Ozdemir, 2005) and 92.4% (Guimaraes *et al.*, 2007), defatted soybean meal 94.4% and fullfat soybean 90.0% (Fontainhas-Fernandes *et al*., 1999); cottonseed meal 31.0% and groundnut meal 79.0% (Luquet, 1989) and cottonseed meal 78.5% (Guimaraes *et al.*, 2007). Part of the variability in APDs may be explained by differences in chemical composition, origin and processing

of these various feed ingredients, methods of faeces collection and calculation of ADCs (Bureau *et al*., 1999; Bureau and Hua, 2006; Forster, 1999). The significantly low APD of GNH may be mainly attributed to its low protein content and very poor amino acid profile shown in Table 3.2 and Table 3.4 respectively. Feed ingredients of plant origin often contain less protein than those of animal origin, but protein in many plant products appears to be digested by carnivorous and omnivorous fish as efficiently as that in animal products (Sullivan and Reigh, 1995; Wilson, 1991; Wu *et al*., 2006). In this study, Nile tilapia generally showed a high capacity to digest protein. APDs of SBM, CSM and GNC compared well and were even higher than that of fish meal reported for tilapia, which range from 85.0% to 90.5% (NRC, 1993; Sklan *et al*., 2004; Koprucu and Ozdemir, 2005).

Lipid, when administered either alone or in a mixed diet, routinely gives digestibility values ranging from 85% to 95% for fish (Aksnes and Opstvedt, 1998; Cho and Slinger, 1979). Sklan *et al*. (2004) reported that for tilapia the ADCs of lipid range between 72–90% for corn gluten, soybean meal, rapeseed meal, sunflower seed meal, wheat, corn, sorghum and wheat bran. The ADCs of lipid (79.64–98.95%) in test ingredients for Nile tilapia in this study are generally in agreement with those reported by Sklan *et al*. (2004) and Köprücü and Özdemir (2005). Lipid digestibility in other species ranged from 70% to 90% (Lupatsch *et al*., 1997) and 92.38–96.93% for plant products (Zhou *et al*., 2004) similar values were observed in this study but were slightly higher.

Apparent digestibility coefficient (ADC) of dry matter (DM) was highest for SBM and GNC. Results indicated that ADC of DM for juvenile Nile tilapia ranged from

60.62-77.47% for SBM, CSM, GNC, and GNH. Apparent DM digestibility coefficients of ingredients decreased as ash or fibre contents of ingredients increased. Fibre in the diet is not digested by fish (NRC, 1993); thus, the ADC of DM may be reduced by high fibre content. Some previous reports have demonstrated that DM ADCs of feed ingredients were negatively correlated to fibre content of feed ingredients (De Silva *et al*., 1990; Falge *et al*., 1978; Hilton *et al*., 1983; McGoogan and Reigh, 1996; Spannhof and Plantikow, 1983; Sullivan and Reigh, 1995). Additionally, high ash content of feed ingredients (especially CSM) in the diet could also decrease DM ADCs (Bureau *et al*., 1999). Generally the present results are lower than DM ADCs as Köprücü and Özdemir (2005) reported for Nile tilapia, which were 90.9% and 93.2% for SBM and corn gluten meal respectively. This might have been caused by the different formula used by these authors to calculate their ADCs as commented by Bureau and Hua (2006). Other studies using the same test ingredients but fed to other fish compared well with the present study. These studies were by Fagbenro (1996) on *Clarias isheriensis* with DM ADC of 67.0% (SBM), 50.0% (CSM) and 58.0% (PM), Zhou *et al.* (2004) on juvenile cobia, which showed a range from 58.52-70.51% for soybean meal, peanut meal and rapeseed meal and Wu *et al*. (2006) on yellowfin seabream with 58.70% (SBM), 65.1% (extruded SBM), 70.6% (Peanut meal) and 33.5% (Rapeseed meal).

Overall digestion of energy increased with increasing protein and decreased with increasing CF content of the ingredients. Variation in apparent GE digestibility coefficients of ingredients followed the same trend as that of DM digestibility. Ash (Bureau *et al*., 1999) and fibre (Lee, 2002) in feed ingredients and carbohydrate content of plant protein sources (Hepher, 1985; Lupatsch *et*

al., 1997; Storebakken *et al*., 1998) could have an effect on GE ADCs. The ADC of GE of CSM and GNH was significantly lower than that of SBM and GNC due to their high crude fibre content (Hertrampf and Piedad-Pascual, 2000). Sklan *et al*. (2004) and Köprücü and Özdemir (2005) reported ADCs of energy of (39–89%) and (54.8–92.1%) respectively for tilapia. Similar values of (34.67-85.99%) were obtained in this study. The apparent phosphorus digestibility coefficients (30.38-64.31%) in this study are higher than those reported by Köprücü and Özdemir (2005) for Nile tilapia, Ogino *et al*. (1979) for common carp and Wilson *et al*. (1982) for channel catfish.

In conclusion, the results of this study showed that the selected oilseed meals were cheaper than fish meal and they appeared to be good feed ingredients for Nile tilapia diets in terms of overall nutrient composition and digestibility. SBM, GNC and CSM were far better digested than GNH and might have greater potential to be used as a dietary replacement for fish meal in Nile tilapia diets. GNH generally performed poorly, although it contained 205.6 $g.kg^{-1}$ of CP, protein digestibility was very low therefore it seems unsuitable as a protein source. However it contains high levels of lipid and carbohydrate and has good lipid digestibility so could be used as source of lipid and to some extent energy in a compound as well as supplementary feed in a semi-intensive system. The results of this study will serve to aid in the formulation of cost-effective diets for Nile tilapia using SBM, CSM and GNC as a complete/partial replacement of fish meal. Growth trials are needed to further examine the nutritive value of these oilseed meals and their cost-effectiveness. Digestibility information could promote the use of ingredient substitutions in least-cost formulated diets for Nile tilapia.

Chapter 4 - Evaluation of Oilseed By-Products as Alternative Protein Sources in the Diet of Juvenile Nile tilapia

4.1 Introduction

Feed is the single largest expenditure in semi-intensive and intensive fish culture operations. Fish meal features prominently in complete diet formulation globally on account of the good growth response it induces in cultivated species. The dependence of intensive and artificially fed semi-intensive aquaculture on fish meal represents a severe constraint to development and intensification of this industry in many regions. Worldwide, aquaculture production has grown about seven and three fold compared to capture fisheries and terrestrial farmed meat production systems respectively since 1970 (FAO, 2007b). However, world fish meal production is not expected to increase further because it has remained relatively stable (Tacon *et al*., 2006). Fish meal in world markets is not always readily available and the price is ever increasing (Hardy, 2006), therefore in order to develop economically viable aquaculture systems alternate sources of high quality proteins will have to be identified to replace high-cost fish meal. For both economic and practical reasons fish feeds should use locally available protein sources, especially those unsuitable for direct human consumption (Glencross *et al*., 2007).

A number of published reports are available regarding the suitability of plant proteins as alternative protein sources in fish feeds (Tacon, 1993; Gomes *et al*., 1995; Hossain and Jauncey, 1989; Kaushik *et al*., 1995; Thiessen *et al*., 2003; Fournier *et al*., 2004; Kaushik *et al*., 2004). More recent work has been

reviewed in Section 1.5.1. 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 (Balogun and Ologhobo, 1989; Fagbenro, 1999; Francis *et al*., 2001; Mambrini *et al*., 1999; Tacon, 1993).

Considerable research effort has been directed at the use of oilseed meals as fishmeal substitutes. Oilseed meals are important in animal nutrition as they are used in compound feed. Oilseed meals are high in protein with protein content ranging from 20 to 50% (Hertrampf and Piedad-Pascual, 2000). Commonly used oilseed meals in fish nutrition are soybean meal, cottonseed meal, sunflower meal, groundnut meal, sesame meal and palm kernel meal to mention a few (Ogunji, 2004). Soybeans are the leading oilseed crop produced globally and projected production for 2006-2007 is expected to be about 233 mmt (Food Outlook, 2007). More tonnes of soybeans are produced globally than all other major oilseeds combined. Soybean meal is considered to be one of the most suitable and stable supplies of an alternative ingredient for replacing fish meal in commercial fish diets. Because of high protein content, high digestibility, relatively well-balanced amino acid profile and reasonable price it is used in feeds for many aquaculture animals (Kikuchi, 1999; NRC, 1993; Storebakken *et al*., 2000). However, soy protein products contain several compounds that may interfere with the digestive process in fish (NRC, 1993; Rackis, 1974). The response of tilapia and other fish to diets containing soybean meal has been investigated by various authors. Soybean products have been successfully used as partial substitutes for fish meal in diets for

Mozambique tilapia, *O. mossambicus* (Davies *et al*., 1989; Jauncey *et al*., 1983), hybrid tilapia, *O. niloticus* x *O. aureus* (Shiau *et al*., 1987), Nile tilapia, *O. niloticus* (Fagbenro and Davies, 2000; Furuya *et al*., 2004) and African catfish, *Clarias gariepinus* (Balogun and Ologhobo, 1989; Sadiku and Jauncey, 1995). Similar study on other fish species has been reported in Section 1.5.1.

Cottonseed meal on the other hand ranks third in the world in tonnage and production for 2006-2007 is estimated at 47 mmt among vegetable protein concentrates (Gatlin *et al*., 2007) and is available at relatively lower cost than animal proteins (Lovell, 1989). Cotton is widely cultivated in the tropics not only for cotton fibre for fabrics but also for oil production. The by-product, cottonseed cake, is also a major component in domestic animal feeds. The nutritional value of cottonseed has been evaluated for several species of fish such as Chinook salmon, *Oncorhynchus tsawytscha* and coho salmon, *Oncorhynchus kisutch* (Fowler, 1980), tilapia (Jackson *et al*., 1982; Viola and Zohar, 1984; El-Sayed, 1990; Mbahinzireki *et al*., 2001;), and channel catfish, *Ictalurus punctatus* (Robinson, 1991; Robinson and Li, 1994; Robinson and Tiersch, 1995). The results of numerous studies evaluating CSM in catfish, salmonid and tilapia diets indicate that between 10% and 30% of solvent extracted, 40% crude protein CSM can be used in aquaculture diets without growth depression (Gatlin *et al*., 2007). Literature on the effects of feeding CSM to tilapia is contradictory. While the majority of investigators have recommended inclusion of CSM at levels not exceeding 50% (Mbahinzireki *et al*., 2001; Ofojekwu and Ejike, 1984; Robinson *et al*., 1984), a few have even indicated that CSM could totally substitute animal protein in tilapia diets (El-Sayed, 1990; Jackson *et al*., 1982). The extent to which CSM may substitute animal or fish meal protein is limited by

the level of gossypol, a toxic component known to have adverse physiological effects on monogastric animals (Martin, 1990; Mbahinzireki *et al*., 2001; Robinson *et al*., 1984). Furthermore, Rinchard *et al.* (2002) reported that inclusion of CSM in diets for *Oreochromis sp*. was limited by a deficiency in sulphur amino acids and high levels of gossypol. In addition, Jauncey and Ross (1982) reported that the presence of phytic acid in CSM could render lysine and minerals unavailable to fish.

Another oilseed meal of interest is groundnut meal, which is available either mechanically or solvent extracted. Groundnuts production ranks fourth amongst oilseeds and has been estimated at 34 mmt for 2006-2007 (Food Outlook, 2007). Its protein content is high but it has sub-optimal levels of cystine, methionine and lysine and is deficient in calcium and vitamin B_{12} (Jauncey, 1998). Groundnut proved to be an acceptable protein source at a low inclusion level but growth decreased rapidly as the level was increased in the diet of tilapia, *O. mossambicus* (Jackson *et al.*, 1982). The principal contaminant of groundnut is aflatoxin, which is a hepatotoxin (produced by moulds *Aspergillus flavus* and *Aspergillus parasiticus)* and mortality among afflicted animals and fish invariably results from severe liver damage (FAO, 1983; Nwokolo, 1996). The feeding value of groundnut by-products has been poorly investigated in fish. Groundnut meal has been used at varying levels, in combination with other protein sources, in diets e.g. for Indian major carp, *Catla catla*, tilapia, *Tilapia discolor* and mudfish, *Clarias anguillaris* (Oduro-Boateng, 1986; Madu and Ajibola, 1988; Madu and Tsumba, 1988; Nandeesha *et al*., 1989; Garduno-Lugo and Olvera-Novoa, 2008). From the results of these experiments the feeding value of groundnut meal cannot be interpreted due to lack of comparable

parameters (Hertrampf and Piedad-Pascual, 2000). In diets for tilapia, *Sarotherodon mossambicus,* groundnut meal can replace 25% of the fish meal (Jackson *et al*., 1982). The inclusion rates of groundnut meal/cake in experimental and practical diets for aquatic animals are in the range of 5.0 to 61.0% (Abu-Hassan *et al*., 1984; Jauncey and Ross, 1982). Groundnut husk, as it is known in Ghana, is the testa or skin of the kernels, which is usually removed after roasting. It used to be thrown away but for sometime now has been recommended (Ministry of Fisheries, 2005) as a supplementary feed for fish, although little researched. According to De Boer and Bickel (1988) groundnut kernels contain 4.1% testa or skin.

In recent years intensification of tilapia production in Ghana and expansion of aquaculture has generated the need for development of suitable feeds. In contrast to quality commercial poultry feeds that are readily available in Ghana, there is an acute paucity of nutritionally sound, cost-effective feeds for finfish, in general, and for Nile tilapia, in particular. The traditional feed mixture employed in the culture of tilapia is mostly supplementary and unbalanced. There is, therefore, an urgent need to develop low-cost, nutritionally balanced diets that can support increased production levels both intensive and especially semi-intensive systems commonly used in Ghana. The use of fish meal at high levels in fish feeds is not feasible in Ghana because of its high price and limited supply, moreover, most of the fish meal is imported and therefore very expensive. In contrast to this, a large number of oilseed and cereal by-products are available (Table 1.9 and Table 1.10). Very few locally available plant products have been evaluated in fish feed in Ghana, these include groundnut cake (Oduro-Boateng, 1986) and Pito brewery waste (Oduro-Boateng and

Bart-Plange, 1988). This study was conducted to evaluate the possibility of replacing a significant proportion of FM protein in the diet of Nile tilapia, *O. niloticus,* using locally available oilseed meals/cake namely; soybean meal (SBM), cottonseed meal (CSM), groundnut cake (GNC) and groundnut husk (GNH) and to assess possible effects on growth, feed utilization, body composition and their cost effectiveness.

4.2 Materials and Methods

4.2.1 Experimental System and Animals

The experimental systems described in Sections 2.1.1 and Section 2.1.2 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 1 L min^{-1} and a constant photoperiod of 12 hours Light/12 hours Darkness (Section 2.1.1). 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, 7.29 ± 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 4.24 ± 0.20 g were stocked in triplicate 30-L tanks. Fish were hand-fed twice a day (10:00, 16:00) at a rate of 6% of their body weight per day for the first four weeks and reduced to 4% for subsequent weeks as an adjustment to the increase in fish weight in accordance to their feeding rate (Table 1.7). In order to set the feeding rate fish were initially fed to satiety. Feeding rates were adjusted every week and the experiment lasted eight weeks (Figure 4.1). 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.

4.2.2 Faeces Collection

At the end of the growth trial faecal collectors were fitted to rearing tanks and faeces collected for two weeks (see Sections 2.1.2 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 2.1.2. Apparent digestibility coefficients of nutrients, energy and phosphorus of diets were determined as described in Section 2.3.5.

4.2.3 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by the methods described in Section 2.2.1. Energy and phosphorous contents of diets, faeces and carcass were analysed by methods described in Sections 2.2.4 and 2.2.5. Chromic oxide content of the diets and faeces were determined by the method in Section 2.2.3.

4.2.4 Diet Formulation and preparation

Ten isonitrogenous (320 $g.kg^{-1}$ protein), isolipidic (100 $g.kg^{-1}$ lipid) and isoenergetic (18 $KJ.g^{-1}$) diets were formulated for the experiment. The control diet was formulated with fish meal as the sole source of protein and this was replaced at different levels with selected oilseed meal proteins. SBM protein replaced fish meal protein at inclusion levels of 50% (SBM50) and 75% (SBM75), CSM protein at inclusion levels of 25% (CSM25), 50% (CSM50) and 75% (CSM75), GNC protein at levels of 25% (GNC25) and 50% (GNC50) and

Table 4.1 Composition of diets fed to juvenile *O. niloticus* using selected oilseed meals ($g.kg^{-1}$ as-fed) in experiment 2

	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
Ingredients	1	2	3	4	5	6	7	8	9	10
SBM	-	301.0	451.0	-	-	-	-	-	-	-
CSM	-	-	-	170.5	341.0	511.0	-	-	-	-
GNM	-	-	-	-	-	-	175.0	349.5	-	-
GNH	-	-	-	-	-	-	-	-	146.0	293.0
FM	420.0	210.0	105.0	315.0	210.0	105.0	315.0	210.0	378.0	336.0
WG	203.0	204.0	206.0	204.0	204.0	208.0	203.0	205.0	206.0	206.0
SF oil	57.0	74.0	82.5	61.5	66.0	70.5	29.0	1.0	240.0	-
α-cellulose	30.0	20.0	14.0	15.0	-	-	28.5	27.5	17.0	4.0
Corn St.	205.0	106.0	56.5	149.0	94.0	20.5	164.5	122.0	144.0	76.0
CMC[1]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
M premix[2]	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
V premix[3]	20.0	20.0	20.0	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	5.0	5.0	5.0

FM = Fish meal, SBM = Soybean meal, CSM = Cottonseed meal, GNC = Groundnut cake, WG = Wheat grain, SF oil = Sunflower oil, α- Cel. = α-Cellulose, Corn St. = Corn starch, M premix = mineral premix, V premix = Vitamin premix, Cr_2O_3 = Chromic oxide, [1]Carboxymethyl cellulose (Sigma, C5013), [2]As listed in Table 2.1, [3]As listed in Table 2.2, according to Jauncey and Ross (1982)

GNH protein at levels of 10% (GNH10) and 20% (GNH20). Diet preparation and other ingredients used were similar to those described in Section 2.2.

4.2.5 Analysis of Experimental Data

Growth performance and feed utilization were calculated as described in Section 2.3.

4.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish in a completely randomized design. Data was analysed as described in Section 2.6.

4.3 Results

4.3.1 Chemical Composition of Diets

Proximate composition, energy, phosphorous contents and antinutritional factors of experimental diets are presented in Table 4.2. Crude protein contents varied little between the diets (318.2 - 343.1 $g.kg^{-1}$) as did crude lipid (95.10 – 113.3 $g.kg^{-1}$). Nitrogen free extract and energy levels in all experimental diets were very similar. Crude fibre content of the control diet was 33.0 $g.kg^{-1}$ and that of Diet 6 (CSM75) was 73.3 $g.kg^{-1}$, which is more than double that of the control. GNH diets and CSM diets in particular had the highest levels of crude fibre. Phytic acid ranged from 0.5 $g.kg^{-1}$– 16.7 $g.kg^{-1}$, trypsin inhibitors from 0.0 $g.kg^{-1}$ – 6.4 $g.kg^{-1}$, saponin from 1.1 $g.kg^{-1}$ – 4.5 $g.kg^{-1}$ and gossypol from 0.0 $g.kg^{-1}$ – 5.8 $g.kg^{-1}$. The essential amino acid (EAA) contents of all the diets, except for methionine and threonine, were sufficient to satisfy the EAA requirements (Table 4.3). Diets 6 and 8 were, however, deficient in lysine as well.

Table 4.2 Proximate composition ($g.kg^{-1}$ as-fed), energy ($kJ.g^{-1}$), phosphorous and antinutritional factors ($g.kg^{-1}$) of diets used in the study

Components	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
	1	2	3	4	5	6	7	8	9	10
Dry matter	936.1	930.8	939.7	938.9	941.2	944.0	920.2	916.0	923.8	936.0
Crude protein	331.9	336.3	343.1	323.9	318.2	321.1	325.0	326.2	329.0	331.7
Crude lipid	102.2	107.2	113.3	102.7	95.6	95.1	104.9	105.6	101.7	102.6
Crude fibre	33.0	37.1	38.2	45.6	58.5	73.3	37.1	37.9	38.1	46.8
Ash	95.4	85.9	81.7	94.1	93.3	93.4	86.9	79.3	92.3	93.1
Nitrogen free extract	373.6	364.3	363.4	372.6	375.6	361.1	366.3	367.0	362.7	361.8
Chromic oxide	4.7	4.7	4.8	4.7	4.6	4.9	5.0	4.7	4.8	4.8
Gross energy	18.66	18.73	18.83	18.32	18.17	18.49	18.93	18.75	18.29	18.81
Phosphorus	9.08	8.07	7.19	9.62	8.57	9.67	8.13	8.01	8.01	9.18
Phytic acid	0.5	5.8	8.4	5.9	11.3	16.7	3.1	5.7	1.1	1.7
Trypsin inhibitors	0.0	4.2	6.4	0.2	0.4	0.6	0.4	0.8	0.1	0.1
Gossypol	-	-	-	1.0	3.9	5.8	-	-	-	-
Saponin	1.1	2.9	3.8	2.3	3.4	4.5	2.5	3.9	2.6	4.1

Table 4.3 Estimated essential amino acid (EAA) composition (% of dietary protein) of diets used and their chemical score (CS, %)

EAA	Requirement for tilapia[1]	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
		1	2	3	4	5	6	7	8	9	10
Arginine	4.20	5.01	5.53	5.79	5.80	6.58	7.37	5.62	6.21	4.96)	4.90
Histidine	1.70	1.99	2.24	2.36	2.13	2.27	2.40	2.02	2.05	1.98	1.96
Isoleucine	3.10	3.96	4.23	4.37	3.74	3.52	3.30	3.67	3.38	3.79	3.62
Leucine	3.40	6.41	6.69	6.84	6.15	5.88	5.62	6.04	5.67	6.16	5.91
Lysine	5.10	6.23	5.89	5.72	5.79	5.34	4.90 (96)	5.65	5.07 (99)	5.94	5.64
Met + Cys[2]	2.70	2.72	2.05 (76)	1.72 (64)	2.46 (91)	2.21 (82)	1.95 (72)	2.33 (86)	1.94 (72)	2.45 (91)	2.18 (81)
Phe + Tyr[2]	3.80	5.26	6.14	6.58	5.56	5.85	6.14	5.49	5.71	5.21	5.16
Threonine	3.80	3.23 (85)	3.09 (81)	3.02 (79)	3.15 (83)	3.07 (81)	2.98 (79)	2.94 (77)	2.64 (70)	3.08 (81)	2.93 (77)
Valine	2.80	4.94	4.99	5.01	4.73	4.51	4.29	4.61	4.28	4.75	4.56

[1]Values from NRC (1993), [2]The values for methionine and phenylalanine are the requirements in the presence of cystine and tyrosine of the diet, respectively, Values in parenthesis indicate chemical scores (CS, %) of limiting EAAs in the diets

4.3.2 Acceptability of experimental Diets

Diet palatability was assessed subjectively by direct observation of fish behaviour and feeding responses (Section 1.3.2). Fish from all treatments adapted to the experimental diets within 2-3 days of feeding. Acceptability, however, varied for the different diets. The control diet and the diets containing 25% oilseed meals were generally more acceptable since fish were observed to be actively feeding and and the activity ceased in less than 5 minutes and no left over feed was observed. Diets with higher oilseed meals inclusion (50%-75% of total protein), especially 75% inclusion were less readily accepted by fish taking longer periods (about 5-15 minutes). A summary of these observations is shown in Table 4.4. Generally, all experimental diets were well accepted and no pathological signs were observed during the trial.

Table 4.4 Observation on the acceptability of different diets containing oilseed meal proteins fed to Nile tilapia fingerlings

Diet No.	Inclusion of oilseed protein (% of total protein)	Observation on acceptability
1	Control (FM as sole protein source)	Fish fed actively by swallowing directly through out the trial; no leftover feed was observed within 5 minutes of administration; less faeces were observed
4	25% CSM	
7	25% GNC	
9	10% GNH	
10	20% GNH	
2	50% SBM	Fish initially fed actively and no leftover feed was observed within 5-8 minutes of administration; more faeces were observed
5	50% CSM	
8	50% GNC	
3	75% SBM	Fish fed less actively taking longer (up to 15 minutes) to feed; sometimes uneaten food observed in tanks; large amount of faeces were often observed
6	75% CSM	

FM = Fish meal, SBM = Soybean meal, CSM = Cottonseed meal, GNC = Groundnut cake, GNH = Groundnut husk

4.3.3 Growth performance

Growth responses of Nile tilapia fingerlings are presented as initial and final mean weights, percentage weight gain and specific growth rate in Table 4.5 and shown graphically in Figure 4.1. The first three weeks saw a rapid growth rate of fish of all treatments but this reduced in the fourth week as shown in Figure 4.1 since the fish could not feed well because of a water quality problem. It was not clear what actually caused it but it was observed that water appeared cloudy. When water parameters were tested they were all within acceptable ranges for the fish (see Section 4.2.1). This problem was solved by flushing water through the system to refresh it. From the study it was observed that growth responses were significantly affected by both the type and inclusion level of plant protein. In general, growth rate decreased with increase in inclusion level of plant protein. Diet 7 (GNC25) had the highest weight gain (15.44g) followed by the control (15.31 g) and the least (6.19 g) was Diet 6 (CSM75). However, in the case of specific growth rate the control was significantly higher (2.73 $\%.day^{-1}$) than Diet 6 (1.56 $\%.day^{-1}$).

Weight gain of fish fed diets 4, 5, 7, 9, 10, which had lower inclusion levels of plant protein was not significantly different from that of the control. With respect to SGR, diets 3 and 6 resulted in the only values significantly lower than that of the control. This meant that all diets with 50% and below inclusion of oilseed protein had SGRs which were not significantly different from the control.

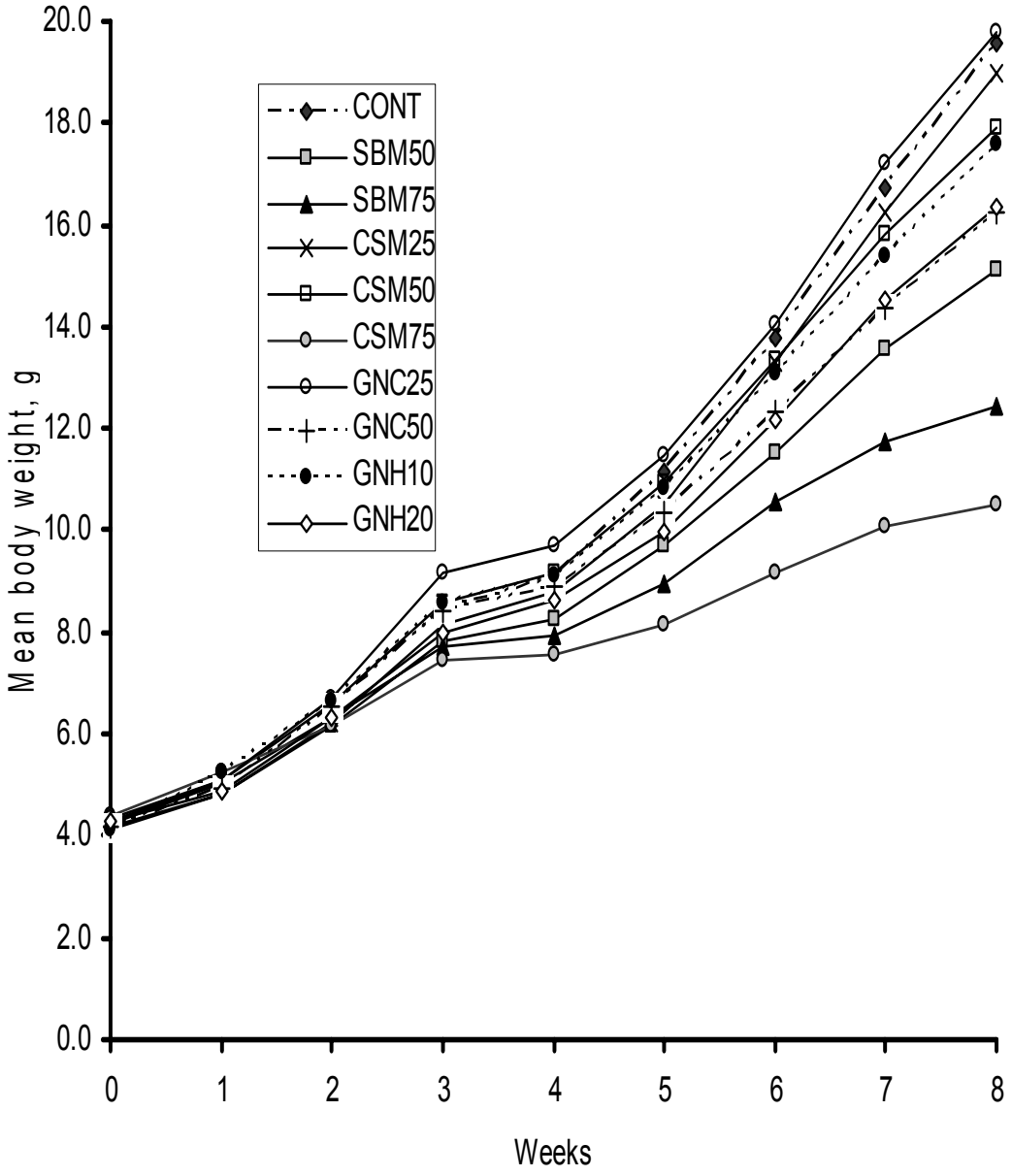

Figure 4.1 Growth response of fish fed oilseed meal based diets for eight weeks

Table 4.5 Growth and feed utilization of juvenile Nile tilapia fed oilseed meal based diets

Parameters	Diets									
	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
	1	2	3	4	5	6	7	8	9	10
IW	4.22 ± 0.13	4.20 ± 0.26	4.23 ± 0.17	4.13 ± 0.19	4.29 ± 0.19	4.42 ± 0.06	4.35 ± 0.43	4.20 ± 0.02	4.14 ± 0.21	4.27 ± 0.20
FW	19.53 ± 1.97^{a}	15.12 ± 1.57^{cd}	12.54 ± 0.50^{de}	18.79 ± 0.90^{ab}	17.92 ± 0.96^{abc}	10.61 ± 0.81^{e}	19.79 ± 0.19^{a}	16.21 ± 1.53^{bc}	17.75 ± 0.56^{abc}	16.29 ± 0.80^{bc}
WG	363.79 ± 59.10^{a}	261.78 ± 54.67^{ab}	196.52 ± 11.19^{bc}	356.02 ± 31.14^{a}	319.06 ± 39.48^{a}	140.21 ± 16.97^{c}	357.47 ± 39.28^{a}	285.85 ± 34.63^{ab}	330.24 ± 32.23^{a}	281.67 ± 23.81^{ab}
SGR	2.73 ± 0.23^{a}	2.28 ± 0.26^{ab}	1.94 ± 0.07^{bc}	2.70 ± 0.13^{a}	2.55 ± 0.17^{a}	1.56 ± 0.13^{c}	2.71 ± 0.16^{a}	2.41 ± 0.16^{a}	2.60 ± 0.14^{a}	2.39 ± 0.11^{a}
S	88.33 ± 5.77	100.00 ± 0.00	88.33 ± 10.41	91.67 ± 10.41	83.33 ± 7.64	86.67 ± 7.64	86.67 ± 7.64	90.00 ± 5.00	98.33 ± 2.89	90.00 ± 8.66
FCR	2.31 ± 0.25^{a}	2.54 ± 0.33^{ab}	3.37 ± 0.24^{bc}	2.05 ± 0.18^{a}	2.39 ± 0.34^{a}	3.91 ± 0.84^{c}	2.27 ± 0.09^{a}	2.58 ± 0.29^{ab}	2.22 ± 0.13^{a}	2.31 ± 0.23^{a}
FI	35.03 ± 2.39^{a}	27.33 ± 0.75^{bc}	27.96 ± 2.15^{bc}	30.02 ± 1.87^{abc}	32.35 ± 3.72^{ab}	23.76 ± 2.19^{c}	35.02 ± 2.08^{a}	30.67 ± 0.82^{ab}	30.33 ± 3.39^{ab}	27.67 ± 0.94^{bc}
PER	1.31 ± 0.13^{a}	1.19 ± 0.15^{ab}	0.87 ± 0.06^{b}	1.51 ± 0.13^{a}	1.34 ± 0.20^{a}	0.82 ± 0.18^{b}	1.36 ± 0.06^{a}	1.20 ± 0.14^{ab}	1.37 ± 0.08^{a}	1.31 ± 0.13^{a}
PPV	20.27 ± 2.07^{a}	17.76 ± 2.27^{ab}	12.38 ± 0.86^{bc}	22.01 ± 1.98^{a}	17.55 ± 2.38^{ab}	11.62 ± 2.95^{c}	20.46 ± 1.05^{a}	18.08 ± 2.17^{a}	20.91 ± 1.17^{a}	18.57 ± 1.78^{a}
ER	14.09 ± 1.48^{ab}	13.67 ± 1.75^{abc}	9.80 ± 0.68^{cd}	17.35 ± 1.44^{a}	13.48 ± 2.00^{abc}	6.72 ± 1.59^{d}	14.47 ± 0.59^{ab}	12.88 ± 1.53^{bc}	16.10 ± 0.93^{ab}	14.16 ± 1.37^{ab}

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 replicates, and values within the same row with different letters are significantly different ($P < 0.05$).

4.3.4 Feed utilization

Food conversion ratio (FCR) followed the same trend as SGR except that Diet 4 (CSM25) had the lowest FCR (2.05) and Diet 6 (CSM75) the highest (3.91). Diet 7 and the control were the next most efficient with FCRs of 2.27 and 2.31 respectively. FCRs of Diets 2, 4, 5, 7, 8, 9 and 10 were not significantly different from that of the control (Table 4.5). Feed intake of the different diets ranged between 23.76 g and 35.03 g at the end of the experiment. Feed intake correlated with diet acceptability and reduced with the increase in inclusion level of plant protein.

Protein utilization efficiency followed the same trend as FCR with Diet 4 having the highest PER (1.51) and PPV (22.01) and Diet 6 the lowest PER (0.82) and PPV (11.62). PER and PPV for Diet 3 and Diet 6 were significantly lower than the control and other diets (2, 4, 5, 7, 8, 9 and 10). Energy utilization followed exactly the same trend as PER and PPV.

4.3.5 Apparent Nutrient Digestibility

Apparent nutrient digestibilities are shown in Table 4.6. Apparent dry matter digestibility (ADMD) of diets ranged from 82.13% to 67.76%. ADMD generally decreased with increase in plant protein, with the exception of Diet 8 which had the highest ADMD (82.13%) followed by the control. Apparent protein digestibility (APD) for all diets was fairly high ranging from 81.06% to 90.97%. Again, Diet 8 (GNC50) had the highest (90.97%) followed by Diet 2 (SBM50) and Diet 10 the lowest protein digestibility and digestible protein followed the same tend. In general APD decreased with increase in plant protein with a few exceptions. Apparent lipid digestibility (ALD) was higher than all the other nutrients studied and did not follow any particular trend. ALD ranged from

90.34% to 97.75%. Apparent energy digestibility ranged from 70.80% to 83.15%. The control diet had the highest (83.15%) and Diet 6 (CSM75) had the lowest energy digestibility. Digestible energy also followed a similar trend. Apparent phosphorous digestibility was between 58.96% and 79.53%.

4.3.6 Body Composition

Whole fish body proximate composition at the start and end of the study is presented in Table 4.7. There was no particular change in whole body composition compared with that at the start of the experiment, however, the MC of carcasses increased with increase in individual oilseed meal inclusion and the opposite was true for CP, CL and ash.

Table 4.6 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy, phosphorous and digestible protein and energy ($g.kg^{-1}$ and $kJ.g^{-1}$ respectively, dry weight basis) in the test diets for Nile tilapia

Components	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
	1	2	3	4	5	6	7	8	9	10
Dry matter	80.58	79.30	78.57	74.18	70.32	67.76	79.76	82.13	74.19	70.00
Crude protein	86.04	90.25	89.29	85.88	82.32	82.52	86.40	90.97	83.97	81.06
Crude lipid	96.00	96.30	97.26	94.95	97.75	96.48	94.64	92.18	90.36	90.34
Gross energy	83.15	81.87	81.12	76.15	72.97	70.80	82.48	84.12	75.82	72.09
Phosphorus	74.89	79.53	67.19	72.03	58.96	62.13	71.42	77.82	78.38	78.63
Digestible protein	305.1	326.1	326.0	296.3	278.3	280.7	305.2	324.0	299.1	287.3
Digestible energy	15.52	15.33	15.28	13.95	13.26	13.09	15.61	15.77	13.87	13.56

Table 4.7 Whole body proximate composition (% wet weight) and energy of Nile tilapia

Components	Initial carcass	Control	SBM50	SBM75	CSM25	CSM50	CSM75	GNC25	GNC50	GNH10	GNH20
		1	2	3	4	5	6	7	8	9	10
MC	74.43	73.69 ± 0.41	73.76 ± 2.00	74.44 ± 2.07	73.26 ± 2.04	75.56 ± 2.90	75.66 ± 1.06	74.11 ± 1.08	74.37 ± 2.16	72.76 ± 1.26	74.42 ± 1.48
CP	14.34	15.18 ± 0.21	14.80 ± 1.15	14.29 ± 1.13	14.84 ± 1.18	14.07 ± 1.70	14.57 ± 0.59	14.79 ± 0.65	14.64 ± 1.14	14.81 ± 0.67	14.00 ± 0.83
CL	7.16	6.43 ± 0.14^{a}	7.33 ± 0.55^{a}	7.12 ± 0.56^{a}	7.60 ± 0.63^{a}	6.60 ± 0.72^{a}	4.81 ± 0.22^{b}	6.91 ± 0.26^{a}	6.94 ± 0.46^{a}	7.58 ± 0.37^{a}	7.25 ± 0.58^{a}
Ash	3.39	4.16 ± 0.05^{ab}	3.39 ± 0.24^{cd}	3.31 ± 0.26^{d}	3.68 ± 0.28^{abcd}	3.19 ± 0.38^{d}	4.03 ± 0.18^{abc}	3.44 ± 0.14^{bcd}	3.40 ± 0.28^{cd}	4.23 ± 0.19^{a}	3.81 ± 0.20^{abcd}
GE	6.21	6.11 ± 0.73	6.35 ± 0.68	6.01 ± 0.53	6.21 ± 0.44	5.72 ± 0.93	5.45 ± 0.14	6.06 ± 0.02	6.10 ± 0.71	6.61 ± 0.20	6.34 ± 0.15

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

4.3.7 Cost Effectiveness Analysis of Diets

Results of cost analysis of diets used in this experiment are presented in Table 4.8. Cost of the feeds was calculated using 2007 market prices of ingredients in Ghana (Section 2.5, Table 3.2). The economics of feed production indicated that the cost of the diets reduced with increase in inclusion levels of individual oilseed meals. After 56 days of feed trial generally, Incidence Cost (IC) of oilseed based diets was lower than the control with the exception of SBM75. In the case of Profit Index (PI) the trend was the direct opposite, i.e. PI of oilseed based diets were higher than the control with the exception of Diet SBM75.

Table 4.8 Cost analysis of diets fed to *O. niloticus* in experiment 2

Diet	Diet cost[1]	Incidence cost[1]	Profit index
1. Control	0.56	1.29	1.55
2. SBM50	0.46	1.15	1.74
3. SBM75	0.41	1.37	1.46
4. CSM25	0.47	1.00	2.10
5. CSM50	0.37	0.88	2.28
6. CSM75	0.27	1.05	1.91
7. GNC25	0.49	1.11	1.80
8. GNC50	0.42	1.07	1.86
9. GNH10	0.50	1.11	1.81
10. GNH20	0.43	1.00	2.01

[1]¢.kg^{-1}, Exchange rate ¢0.90 = USD 1.00, sale price of fish = ¢ 2.00.kg^{-1} fish

4.4 Discussion

In general, growth performance and feed utilization decreased with increase in oilseed meal protein. In this study growth responses were significantly affected by both the type and inclusion level of plant protein. This is in agreement with various authors (Jauncey, 1998; Mbahinzireki *et al*., 2001; McKevith, 2005; NRC, 1993; Ogunji and Wirth, 2001; Pham *et al*., 2007). Results from this study indicate that WG, SGR, FCR, PER, PPV for all diets with oilseed meal inclusion levels up to 50% were not significantly different from the control (FM as sole source of protein). Above 50%, replacement of fish meal protein by oilseed

meal (SBM and CSM) protein, however, resulted in reduced growth rate, poorer feed conversion and poorer protein utilization. Reduced growth response and feed utilization in various warm-water aquaculture species fed diets in which fish meal was replaced with oilseed meals have been explained by sub-optimal amino acid balance, inadequate levels of phosphorus, inadequate levels of energy, low feed intake caused by poor palatability, presence of endogenous antinutrients or dietary level of fish oil (Lim and Dominy, 1991). Lower growth performance above 50% fish meal replacement with SBM and CSM in this study may have been caused by one or more of these factors.

Growth performance and feed utilization of fish fed Diet 2 (SBM50) was higher than for Diet 3 (SBM75), however, there were no significant differences ($p > 0.05$) between them. Diet 3 performed significantly ($p < 0.05$) poorer compared to the control. Earlier studies reported that growth tends to be low in fish fed diets with SBM replacing all the fishmeal (Jackson *et al*., 1982; Webster *et al*., 1992). According to Shiau *et al*. (1989), male tilapia (*O. niloticus* x *O. aureus*) fed diets in which 100% of the fish meal was replaced with SBM either with or without methionine supplementation had significantly lower weight gain, FCR and protein digestibility than that of the groups fed diets containing fish meal as the sole source of protein. However, Davis and Stickney (1978) and El-Saidy and Gaber (2002) reported feeding blue tilapia and Nile tilapia respectively with 100% SBM (with methionine supplementation in the first case and methionine and lysine in the second) with no significant effect on growth and feed utilization. From the present results the methionine content of Diet 3 (SBM75) was lower than Diet 2 (SBM50), which agreed with Dabrowski *et al*. (1989) who

stated that amino acid level, especially methionine, was reduced if soybean meal protein was used in excess of 50% of the diet.

Feed intakes of Diets 2 and 3 were significantly lower than that of the control which suggests that adding high percentages of soy products to fish diets could cause poor palatability and unacceptability, leading to diminished growth (Watanabe *et al.*, 1997). High saponin levels of these diets (Table 4.2) may account for the poor palatability since according to Guillaume and Metailler (1999) the astringent taste of saponin could reduce feed intake. In this study, when the soybean meal constituted more than 50% of the fish meal protein, WG, FCR and ER were negatively affected as was also observed by Ogunji and Wirth (2001). The results also compared well with a review from Gatlin III *et al*. (2007) who stated that soybean meal often constituted 50 to 60% of the total dietary protein for fish. Fagbenro and Davies (2000) and Martinez-Llorens *et al*. (2007) reported successful dietary replacement of FM with 67% SBM for tilapia and 50% SBM for sea bream respectively. Poor fish growth and feed utilization at higher SBM inclusion in the tilapia diet may also be attributed to high levels of antinutritional factors (Table 4.2) namely; trypsin inhibitors with tolerant levels reported to be 1.6 $g.kg^{-1}$ for tilapia (Wee and Shu, 1989) and phytic acid below 5 $g.kg^{-1}$ (Francis *et al*., 2001).

From the present results it was observed that growth performance and feed utilization decreased as CSM inclusion level increased from 25% to 75% (the decrease was pronounced between 50% and 75%). Growth and feed utilization were not significantly different ($p > 0.05$) between Diet 4 (CSM25), Diet 5 (CSM50) and the control. However, growth of fish fed Diet 6 (CSM75) was

significantly ($p < 0.05$) lower than for the control and the other CSM-based diets. This study demonstrates that up to 50% CSM protein could be used to replace fish meal protein in the diet of tilapia without affecting overall growth and feed utilization of fish. Beyond that level, however, growth was depressed drastically. These results are consistent with those reported by other authors such as Mbahinzireki *et al*. (2001) who conducted a similar study on tilapia and reported depressed growth and even mortality in fish when they were fed up to 100% CSM of the dietary protein and recommended an inclusion level of up to 50%. According to Fagbenro and Davies (2000) there was growth retardation and poor feed utilization for tilapia when CSM protein replaced 67% of fish meal protein. Ofojekwu and Ejike (1984) reported that CSM could not be used as a sole protein source for *O. niloticus* because they exhibited poor growth, food conversion and specific growth rate. Similar results, but at lower inclusion levels, were reported by Robinson *et al.* (1984) for channel catfish, Cheng and Hardy (2002) for rainbow trout fingerlings and Pham *et al.* (2007) for Japanese flounder. These findings contradicted the earlier report by Jackson *et al*. (1982) that tilapia grew well on CSM-based protein, even at 100% level of inclusion. The results (Table 4.6) indicated a general decrease in digestibility as inclusion level of CSM increased. This reinforced the earlier observations that Diet 6 (CSM75) was less utilized for growth because feed intake was lowest. According to De Silva *et al*. (1989) acceptability of feed by fish could be affected by increasing levels of plant material since the texture and taste of test diets are bound to differ. Low feed intake and digestibility could have been due to the high fibre content (due to higher inclusion level of CSM), low level of lysine, methionine and threonine and higher levels of antinutritional factors of Diet 6

(Table 4.2 and Table 4.3). The SGR and FCR of fish in the present experiment may be directly affected by dietary protein source, low digestible protein and energy in fish fed with CSM-based diets. Results of the present study also indicate that tilapia cannot be raised successfully by feeding diets formulated on CSM alone as the sole source of protein as also indicated by Mbahinzireki *et al*. (2001).

In the case of GNC-based diets in this study, growth performance and feed utilization were higher for Diet 7 (GNC25) than Diet 8 (GNC50) but there was no significant difference ($p > 0.05$) between them and the control. Fish growth and feed utilization in the present study was not significantly affected when GNC replaced FM at 50%, although this inclusion level of GNC resulted in deficiency of three EAAs (lysine, methionine and threonine) in Diet 8 (Table 4.3). These results agree favourably with Nyina-wamwiza *et al*. (2007) who reported that groundnut oil cake can replace at least 50% of fish meal in the diet of Clarias fingerlings without amino acid supplementation. However, depressed growth responses have been reported for *Oreochromis mossambicus* (Jackson *et al*., 1982), *O. niloticus* (Fagbenro and Davies, 2000; Kamara, 1982) and *Cyprinus carpio* (Hasan *et al*., 1997) when diets with high levels (50% or more) of groundnut meal were fed. Ogunji and Wirth (2001) recommended an inclusion of groundnut cake at about 10% in tilapia diets. Poor growth performances were attributed to deficiency of EAAs (especially, lysine, methionine and threonine) in diets with high inclusion levels of GNC in relation to the requirements for tilapia. Depressed growth of fish in the above mentioned studies could also have been caused by aflatoxin contamination, since GNC tend to have incidences of

aflatoxins (FAO, 1983), however, none of the researchers reported this as the problem.

Diets 9 and 10 with 10% and 20% GNH respectively performed quite well compared to the control. Apart from Diet 10, which had a significantly lower FI, all other growth and feed utilization parameters were not significantly different from the control. Diets 9 and 10 generally had lower digestibilities than the control and were similar to that of Diets 3 and 6 with inclusion levels of 75% SBM and 75% CSM respectively (Table 4.5). This could be due to the poor nutrient digestibility of GNH observed in experiment 1 (Table 3.7) as well as low digestible energy in the present experiment (Table 4.6).

AD of nutrients for all the diets was very high. SBM-based diets performed better than all other diets including the control. AD of nutrients for all the oilseed-based diets in this study was comparable to, and even higher than, the control as was also observed by Sullivan and Reigh (1995) for Stripped bass and Wu *et al*. (2006) for Sea bream. The digestible protein and energy values followed the same trend as the ADCs. The final whole body composition of experimental fish was broadly similar and relatively unaffected by different dietary treatments with the exception of ash content which was significantly lower for diets with 50% or more oilseed meal inclusion. Although there was depressed fish growth and poor feed utilization at higher inclusion of plant protein, fish did not show any poor health or physical deformities and histopathological examination of the intestines and liver revealed no significant changes. Although, mortalities occurred in this study, they were few and did not seem to be treatment related since there were mortalities even in fish fed the

control diet, moreover survival was not significantly different between treatments.

Cost-effectiveness analysis of the present study generally indicated that the oilseed meal diets are more profitable than the control diet and on the average 50% replacement was more cost effective than at 75%. The CSM diets and Diet 10 (GNH20) were the most profitable. These results are similar to reports by El-Sayed (1990) on economic evaluation of cottonseed meal, Wu *et al*. (1995) on corn gluten feed, and El-Saidy and Gaber (2002) on sunflower meal as single protein sources for Nile tilapia. Another study by Oduro-Boateng and Bart-Plange (1988) on brewery wastes for *Tilapia busumana* also indicated that these sources were more cost-effective than FM, even at the total replacement levels. According to Olvera-Novoa *et al.* (2002b) using sunflower seed meal as a partial protein source in *T. rendalli* diets was more profitable than FM protein. The present feed trial, which lasted for 56 days corresponding to more or less the advanced fingerling production phase of tilapia, i.e.10-100g (Green, 2006), showed that oilseed meal base diets were more profitable than the fish meal based diet. However, if fish is to be cultured to market size of 200g by Ghanaian standards (Apawudwa, personal communication) the culture period could be longer for the oilseed meal base diets because of their lower growth rate and poorer feed efficiency and could have other cost implications (such as labour, power etc., which was not considered in the present study) at the long run. In relation to culture period Ogunji (2004) observed that when alternative protein sources are used in tilapia feeds, the rate of fish growth may be reduced leading to increased rearing time. However, the low cost of the protein sources would reduce the entire cost of raising the fish, compensating for the delayed

growth and time lost, consequently, increasing profitability. Studies in Thailand by Middendorp and Verreth (1991) have indicated that poor farmers are more concerned with lowering feed cost even if that would lengthen the rearing period. Although, this might not be entirely good for intensive commercial farmers who are looking for high profits at the shortest possible time, it could immensely benefit small to medium scale semi-intensive farmers who form the majority in Ghana.

The results from this study indicated that the main oilseed protein sources (SBM, CSM and GNC) used could replace at least 50% of fish meal protein in the diet of *O. niloticus* fingerlings without adversely affecting growth and feed efficiency. GNH performed quite well up to 20% inclusion and requires further research into higher inclusion levels because of its availability and low cost in Ghana and could be used in supplementary feed. Generally the oilseed meal diets were more cost-effective than the fish meal based diet particularly, CSM replacing 50% fish meal protein.

Chapter 5 - Study of Different Mixtures of Oilseed Meals as Dietary Protein Sources in Practical Diets of Juvenile Nile tilapia

5.1 Introduction

Attempts to partially or completely replace the fish meal component of practical fish feeds with alternative protein sources have resulted in variable success notably reduced feed efficiency and growth at higher dietary inclusion levels (Jackson *et al*., 1982; Tacon and Jackson, 1985; Viola *et al*., 1982). In most cases single plant protein sources were evaluated at various inclusion levels to substitute fish meal in the diet (El-Saidy and Gaber, 1997; El-Saidy and Gaber, 2002; El-Sayed, 1999; Mbahinzireki *et al*., 2001; Nyina-wamwiza *et al*., 2007; Sklan *et al*., 2004). Such studies have also been conducted for Nile tilapia using various plant proteins (El-Saidy and Gaber, 2003; El-Sayed, 1999; Hossain *et al.,* 1992; Maina *et al*., 2002; Shiau *et al*., 1989; Soltan *et al*., 2008; Webster *et al*., 1992). The majority of plant protein sources tested was oilseed meals.

Although, oilseed meals have high protein levels and favourable essential amino acid (EAA) profiles they are known to contain a variety of growth inhibiting antinutritional factors (Francis *et al*., 2001; NRC, 1993). When a higher level of plant protein is included, the antinutrients in the diets exceed the tolerance limit of the test animal and this often leads to reduced growth, feed utilization and mortalities in some cases. The use of different plant protein sources in combination could prevent high inclusion levels of any single antinutrient in the diet (Francis *et al*., 2001).

The essential amino acid compositions of alternative protein sources for fish are generally not comparable with that of fish meal. Chemical score data show that there is no single foodstuff that can serve as an alternative to fish meal (De Silva and Anderson, 1995). Therefore, combining different alternative protein sources which possess different limiting amino acids which could complement each other has been strongly recommended (Jackson *et al*., 1982; Tacon and Jackson, 1985).

Several researchers have reported comparatively better growth performance of fish fed diets containing different combinations of plant protein sources. Earlier studies by Olukunle (1982) observed that a mixture of groundnut, sunflower seed and sesame meals resulted in better growth of *O. mossambicus* than single meals. Borgeson *et al.* (2006) found improved performance of *O. niloticus* fed a diet containing mixtures of soybean and maize gluten meals as partial substitutes for fish meal protein. Hossain (1988) also conducted similar work with carp and concluded that plant protein sources in various combinations are more effective than single sources. Attempts to reach substitution levels of more than 50% of the fish meal protein, by mixing two or more alternative protein sources, have been scarce although some of the results look promising (Borgeson *et al*., 2006; Fontainhas-Fernandes *et al*., 1999; Jackson *et al*., 1982). However, a report by El-Saidy and Gaber (2003) stated that a plant protein mixture of soybean, cottonseed, sunflower and linseed meals in equal proportions (25% each with lysine and methionine supplementation) completely replaced fish meal in diets of juvenile Nile tilapia.

Information on using plant protein mixtures in Nile tilapia feeds is generally limited. El-Saidy and Gaber (2003) recommended, after their study, further research to determine the feasibility of using plant protein mixtures composed of different combinations and inclusion levels of ingredients. This type of study has been conducted on common carp using different oilseed by-products (Hossain and Jauncey, 1989). It is, therefore, important to evaluate the quality and suitability of different combinations or mixtures of soybean meal (SBM), cottonseed meal (CSM) and groundnut cake (GNC) currently under study as ingredients to replace fish meal in tilapia diets without compromising growth and feed efficiency.

In chapter 4 of this thesis SBM, CSM and GNC were used as single plant protein sources. At higher inclusion levels they resulted in lower growth performance and feed utilization compared to the control. This result was generally attributed to higher levels of antinutritional factors contained in the oilseed proteins as well as their poor EAA profile for tilapia. In view of this, in the present study an attempt was made to partially replace FM with various combinations/mixtures of the above mentioned dietary oilseed proteins in a quest to improve plant protein source utilisation in the diet of Nile tilapia.

5.2 Materials and Methods

5.2.1 Experimental System and Animals

Fingerlings of Nile tilapia of an average weight of 2.46 ± 0.12 g were stocked in triplicate in 30-L tanks. Each tank was randomly stocked with 20 fingerlings under conditions similar to those described in Section 3.2.1. Water quality parameters were measured every week during the experiment and the mean

values (± SD) were as follows: temperature, 26.10 ± 0.44^{o}C; pH, 7.20 ± 0.16; ammonia, 0.06 ± 0.03 mg.L^{-1}; nitrite, 0.25 ± 0.0 mg.L^{-1}; nitrate, 20 ± 0.0 mg.L^{-1} and dissolved oxygen, 7.20 ± 0.67 mg.L^{-1}. The experiment lasted 8 weeks during which fish were hand-fed three times a day (09:30, 13:00 and 16:00) at a rate of 10% of their body weight per day for the first three weeks and 6% for subsequent weeks.

5.2.2 Diet Formulation and Preparation

Nine diets were formulated to contain 320 g.kg^{-1} protein, 100 g.kg^{-1} lipid and about 18 KJ.g^{-1} energy using mixtures of SBM, CSM, and GNC as protein sources. FM was substituted with different mixtures and combinations of SBM, CSM and GNC at 50% and 75% of total protein. Composition of the different oilseed meal mixtures used for diet formulation is presented in Table 5.1 and designation of diets shown correspondingly. Diet preparation and other ingredients used were similar to those described in Sections 2.2 and 4.2.4. Diet formulation is presented in Table 5.2.

Table 5.1 Specification of dietary protein levels (%) in experimental diets used in Experiment 3

Diet No.	Designation	Percentage of total protein contributed by various sources in the diets			
		Fish meal	Soybean meal	Cottonseed meal	Groundnut cake
1	Control	100.00	-	-	-
2	EQ50	50.00	16.67	16.67	16.67
3	SBM50	50.00	25.00	12.50	12.50
4	CSM50	50.00	12.50	25.00	12.50
5	GNC50	50.00	12.50	12.50	25.00
6	EQ75	25.00	25.00	25.00	25.00
7	SBM75	25.00	37.50	18.75	18.75
8	CSM75	25.00	18.75	37.50	18.75
9	GNC75	25.00	18.75	18.75	37.50

EQ = equal contribution of protein from test ingredients to mixture, SBM = 50% contribution from soybean meal to mixture, CSM = 50% contribution from cottonseed meal to mixture, GNC = 50% contribution from groundnut cake to mixture

Table 5.2 Composition of diets fed to juvenile *O. niloticus* using oilseed meal mixtures ($g.kg^{-1}$ of diet) in experiment 3

Ingredient	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
	1	2	3	4	5	6	7	8	9
SBM	-	100.2	150.4	75.2	75.2	150.4	225.4	112.7	112.7
CSM	-	113.5	85.2	170.4	85.2	170.4	127.7	255.5	127.7
GNC	-	116.4	87.4	87.4	175.0	174.6	131.0	131.1	262.0
FM	420.0	210.0	210.0	210.0	210.0	105.0	105.0	105.0	105.0
WG	203.0	206.0	205.5	206.0	205.2	209.0	209.4	208.0	208.8
SF oil	57.0	47.0	53.8	51.7	35.4	42.0	52.1	49.1	24.6
α- Cel.	30.0	15.9	17.5	12.0	19.5	10.0	10.9	02.6	14.0
Corn St.	205.0	106.0	105.2	102.3	109.5	53.6	53.5	51.0	60.2
CMC[1]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
M premix[1]	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
V premix[1]	20.0	20.0	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	5.0	5.0

FM = Fish meal, SBM = Soybean meal, CSM = Cottonseed meal, GNC = Groundnut cake, WG = Wheat grain, SF oil = Sunflower oil, α- Cel. = α- Cellulose, Corn St. = Corn starch, M premix = mineral premix, V premix = Vitamin premix, Cr_2O_3 = Chromic oxide, [1]Carboxymethyl cellulose (Sigma, C5013), [2]As listed in Table 2.1, [3]As listed in Table 2.2, according to Jauncey and Ross (1982)

5.2.3 Faeces Collection

Faeces collection was undertaken as described in Section 4.2.2 (also see Sections 2.1.2 for details). Apparent digestibility coefficients of nutrients, energy and phosphorus of diets were determined as described in Section 2.3.5.

5.2.4 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by methods described in Section 2.2.1. Energy and phosphorous of diets, faeces and carcass were analysed by methods described in Sections 2.2.4 and 2.2.5. Chromic oxide content of diets and faeces was determined by the method in Section 2.2.3.

5.2.5 Analysis of Experimental Data

Growth performance and feed utilization were calculated as described in Section 2.3 and 3.2.5.

5.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish by a completely randomized design. Data were analysed as described in Section 2.6.

5.3 Results

5.3.1 Chemical Composition of Diets

Proximate composition, energy, phosphorous contents and antinutritional factors of experimental diets are presented in Table 5.3. Crude protein contents were similar and only varied slightly between the diets (323.8 – 334.9 $g.kg^{-1}$). Energy, crude lipid and NFE levels in all experimental diets were very similar. However, crude fibre content varied ranging between 25.4 $g.kg^{-1}$ and 44.6 $g.kg^{-1}$ where the control diet had the lowest and Diet 6 the highest. Diets 4, 6 and 8,

which contained more CSM, had the highest levels of crude fibre. Phosphorous content of the control diet was highest (9.03 $g.kg^{-1}$). Phytic acid ranged from 0.5 $g.kg^{-1}$– 12.5 $g.kg^{-1}$, TIs from 0.0 $g.kg^{-1}$ – 3.6 $g.kg^{-1}$, saponin from 1.1 $g.kg^{-1}$ – 4.7 $g.kg^{-1}$ and gossypol from 0.0 $g.kg^{-1}$ – 1.4 $g.kg^{-1}$.

Table 5.4 shows the essential amino acid (EAA) composition of all diets vis-à-vis their requirements in Nile tilapia. Methionine + cystine were observed to be the first limiting AA followed by threonine and lysine, in that order. Increasing oilseed meal incorporation reduced the limiting AAs and increased phenylalanine + tyrosine in the diets.

5.3.2 Growth performance

Growth performance of Nile tilapia fingerlings fed the experimental diets is presented as initial weight and final mean weights, weight gain (WG) and specific growth rate (SGR) in Table 5.5 and shown graphically in Figure 5.1. It was observed that growth responses were significantly affected by both mixture composition and the inclusion level of oilseed meal protein right from the end of the second week of the feed trial. In general, growth rate decreased with increase in inclusion of oilseed meal protein mixtures. Best overall growth response was obtained in tilapia fed the control diet. Percentage weight gain and SGR were highest for the control diet (704.24 % and 3.72 $\%.day^{-1}$ respectively) and lowest for Diet 6 containing 75% plant protein (equally contributed by SBM, CSM and GNC) 322.35 % and 2.56 $\%.day^{-1}$ respectively. Weight gains for all oilseed based diets were significantly lower ($p < 0.05$) than the control. SGR followed the same trend with the exception of Diet 2 (EQ50) containing 50% plant protein (equally contributed by SBM, CSM and GNC) which was not significantly different ($p > 0.05$) from the control.

Table 5.3 Proximate composition ($g.kg^{-1}$ as-fed), energy ($kJ.g^{-1}$), phosphorous and antinutritional factors ($g.kg^{-1}$) of diets used in experiment 3

Components	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
	1	2	3	4	5	6	7	8	9
DM	941.6	934.7	936.0	947.7	941.0	938.0	937.1	940.8	936.5
CP	323.8	324.0	330.6	328.6	330.4	330.4	331.7	329.0	334.9
CL	108.0	105.6	105.7	105.9	104.4	104.5	108.5	107.3	104.6
CF	25.4	37.1	34.1	40.2	34.6	44.6	35.2	42.1	33.9
Ash	96.9	87.4	85.9	87.8	84.1	81.1	80.3	85.3	81.5
NFE	387.6	380.6	379.7	385.2	387.4	377.4	381.4	377.1	381.7
Cr_2O_3	5.0	5.1	5.1	5.0	5.0	4.9	5.0	4.9	4.9
GE	18.60	18.79	18.85	18.78	18.86	18.89	18.92	18.79	19.02
P	9.03	7.84	7.70	7.86	7.76	7.42	7.18	7.76	7.48
PA	0.5	7.6	7.1	8.5	7.1	11.1	10.5	12.5	10.4
TI	0.0	1.8	2.4	1.5	1.6	2.7	3.6	2.2	2.3
G	0.0	0.6	0.5	1.0	0.5	1.0	0.7	1.4	0.7
S	1.1	3.4	3.3	3.4	3.5	4.5	4.4	4.5	4.7

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, NFE = nitrogen free extract, Cr_2O_3 = Chromic oxide, GE = gross energy, P = phosphorous, PA = Phytic acic, TI = Trypsin inhibitor, G = Gossypol, S = Saponin

Table 5.4 Estimated essential amino acid (EAA) composition (% of dietary protein) of diets used and their chemical score (CS, %) in the study

EAA	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
Arginine	5.01	6.11	5.97	6.23	6.14	6.66	6.44	6.83	6.69
Histidine	1.99	2.18	2.20	2.20	2.15	2.28	2.30	2.31	2.23
Isoleucine	3.96	3.71	3.84	3.66	3.63	3.59	3.78	3.51	3.46
Leucine	6.41	6.08	6.24	6.03	5.98	5.92	6.15	5.84	5.77
Lysine	6.23	5.43	5.55	5.41	5.34	5.03 (99)	5.20	5.00 (98)	4.90 (96)
Methionine + Cystine	2.72	2.07 (77)	2.06 (76)	2.10 (78)	2.04 (75)	1.74 (65)	1.74 (64)	1.79 (66)	1.70 (63)
Phenylalanine + Tyrosine	5.26	5.90	5.96	5.89	5.85	6.21	6.30	6.20	6.14
Threonine	3.23 (85)	2.93 (77)	2.97 (78)	2.97 (78)	2.86 (75)	2.78 (73)	2.84 (75)	2.83 (75)	2.67 (70)
Valine	4.94	4.59	4.69	4.57	4.52	4.42	4.56	4.39	4.30

Values in parenthesis indicate chemical scores (CS, %) of EAAs in the diets

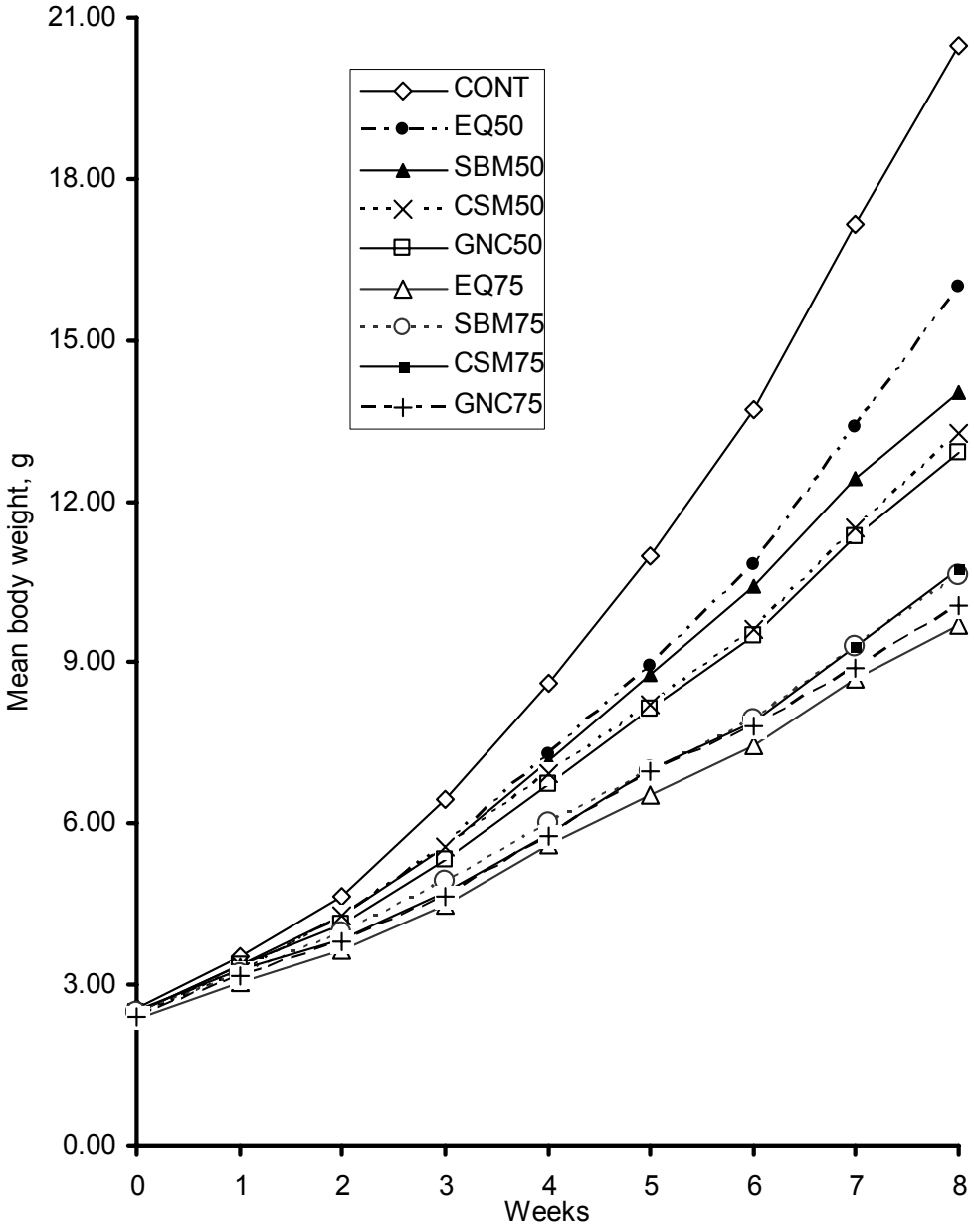

Figure 5.1 Growth response of fish fed diets with oilseed meal mixtures for eight weeks

Table 5.5 Growth performance of Nile tilapia fingerlings fed diets with oilseed meal mixtures for eight weeks

Parameters	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
	1	2	3	4	5	6	7	8	9
IW	2.55 ± 0.14	2.44 ± 0.13	2.47 ± 0.07	2.42 ± 0.05	2.50 ± 0.17	2.35 ± 0.05	2.47 ± 0.07	2.54 ± 0.19	2.39 ± 0.10
FW	20.49 ± 1.91^{a}	15.96 ± 1.03^{b}	13.84 ± 0.69^{bcd}	14.30 ± 2.07^{bc}	12.87 ± 1.02^{bcd}	9.95 ± 1.32^{d}	10.48 ± 1.16^{cd}	11.26 ± 1.64^{cd}	11.18 ± 1.89^{cd}
WG	704.24 ± 55.69^{a}	556.41 ± 57.82^{b}	459.57 ± 25.54^{bcd}	464.46 ± 48.62^{bc}	414.78 ± 23.92^{cde}	322.35 ± 53.37^{e}	324.19 ± 35.92^{e}	334.51 ± 31.64^{de}	352.37 ± 57.55^{cde}
SGR	3.72 ± 0.12^{a}	3.36 ± 0.16^{ab}	3.07 ± 0.09^{bcd}	3.08 ± 0.16^{bc}	2.92 ± 0.08^{bcde}	2.56 ± 0.24^{e}	2.58 ± 0.15^{e}	2.62 ± 0.13^{de}	2.69 ± 0.23^{cde}
S	100.00 ± 0.00	93.33 ± 11.54	90.00 ± 10.00	91.67 ± 2.89	91.67 ± 5.77	95.00 ± 5.00	88.33 ± 5.77	90.00 ± 8.66	90.00 ± 5.00

IW (g) = Initial weight, FW (g) = Final weight, WG (%) = Weight gain, SGR (%.day^{-1}) = Specific growth rate, S (%) = Survival rate, Values are means ± SD of three replicates, and values within the same row with different letters are significantly different ($P< 0.05$)

Table 5.6 Feed utilization of Nile tilapia fingerlings fed diets with oilseed meal mixtures for eight weeks

Parameters	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
FCR	2.07 ± 0.26^{a}	2.52 ± 0.23^{ab}	2.89 ± 0.27^{abc}	2.80 ± 0.20^{abc}	3.17 ± 0.13^{bc}	3.90 ± 0.55^{c}	3.64 ± 0.68^{c}	3.18 ± 0.47^{bc}	3.55 ± 0.44^{bc}
FI	36.83 ± 0.90^{a}	33.98 ± 2.04^{ab}	32.71 ± 1.20^{ab}	31.80± 2.39^{abc}	32.83 ± 2.14^{ab}	29.20 ± 2.60^{bc}	28.67 ± 1.41^{bc}	26.75 ± 1.80^{c}	29.45 ± 2.05^{bc}
PER	1.51 ± 0.19^{a}	1.23 ± 0.11^{ab}	1.05 ± 0.10^{bc}	1.07 ± 0.10^{bc}	0.95 ± 0.04^{bc}	0.79 ± 0.11^{c}	0.85 ± 0.16^{c}	0.97 ± 0.14^{bc}	0.85 ± 0.10^{c}
PPV	22.63 ± 2.83^{a}	17.62 ± 1.63^{ab}	16.30 ± 1.46^{bc}	17.39 ± 1.50^{ab}	15.03 ± 0.65^{bc}	12.08 ± 1.63^{c}	13.36 ± 2.39^{bc}	14.25 ± 2.07^{bc}	13.49 ± 1.52^{bc}
ER	17.10 ± 2.14^{a}	13.37 ± 1.24^{abc}	12.48 ± 1.12^{bc}	14.17 ± 1.03^{ab}	11.62 ± 0.50^{bc}	6.61 ± 0.97^{d}	10.25 ± 1.83^{c}	11.01 ± 1.60^{bc}	10.72 ± 1.20^{bc}

FRC = Feed conversion ratio, FI (g) = Feed intake, PER = Protein efficiency ratio, PPV (%) = Productive protein value, ER (%) = Energy retention. Values are means ± SD of three replicates, and values within the same row with different letters are significantly different ($P< 0.05$)

5.3.3 Feed utilization

Feed efficiency and utilization data are presented in Table 5.6. The control diet was most efficient (FCR, 2.07) and Diet 6 the least (3.90). With the exception of Diets 2, 3 and 4, FCRs of all the plant protein diets were significantly different ($p < 0.05$) from that of the control. Feed intake ranged between 26.75g and 36.83g at the end of the experiment. Oilseed meal inclusion at 75% led to a significantly lower feed intake. Protein utilization efficiency decreased as oilseed meal inclusion increased with the control diet having the highest PER (1.51) and PPV (22.63) and Diet 6 the lowest PER (0.79) and PPV (12.08). PER was significantly ($p < 0.05$) lower for Diets 3, 4, 5, 6, 7, 8 than the control and Diet 2. PPV and energy utilization followed exactly the same trend as PER with the exception of Diet 4 which was not different from the control.

5.3.4 Apparent Nutrient Digestibility

Apparent nutrient digestibility values are presented in Table 5.7. The control diet had the highest apparent protein digestibility (APD) (89.88%) and Diet 8 the lowest (87.41%). APD decreased slightly as plant protein inclusion increased. Apparent energy, dry matter and phosphorous digestibilities followed similar trends to APD. Generally, nutrient digestibility for all diets was high. Digestible protein and energy varied only slightly among the diets.

Table 5.7 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy, phosphorus and digestible protein and energy ($g.kg^{-1}$ and $kJ.g^{-1}$ respectively, dry weight basis) in test diets for Nile tilapia

	1	2	3	4	5	6	7	8	9
DM	81.79	80.60	80.92	78.13	81.17	79.43	78.56	75.31	76.90
CP	89.88	89.08	89.66	87.62	89.75	88.84	88.46	87.41	89.51
CL	96.75	98.85	98.02	97.02	96.91	96.91	96.58	96.07	93.36
GE	83.88	82.74	83.27	80.78	83.34	82.01	81.17	78.10	79.27
P	75.89	73.11	74.48	67.08	72.94	70.53	72.36	64.91	70.53
DP	309.1	308.8	316.7	303.8	315.1	312.9	313.1	305.7	320.1
DE	15.60	15.55	15.70	15.17	15.72	15.49	15.36	14.68	15.08

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, GE = gross energy, P = phosphorous, DP = Digestible protein, DE = Digestible energy

5.3.5 Whole Body Composition

The chemical composition of whole fish body is given in Table 5.8. All fish displayed a change in whole body composition (compared with that at the start of the experiment), which consisted mainly in a decrease in percentage moisture and a corresponding increase in total lipid content. The protein content of fish increased in all dietary treatments compared with the initial sample. Lipid content was significantly higher, especially among diets with higher inclusion of oilseed meal mixtures, however, ash was the direct opposite. HSI values did not show any particular trend relating to diet-treatment, however, the control had the highest value.

5.3.6 Cost-benefit Analysis of Diets

Results of cost analysis of diets used in this experiment are presented in Table 5.9. The cost of the diets reduced with increase in inclusion levels of oilseed meal mixtures. Incidence Cost (IC) of Diets EQ50, CSM50 and CSM75 were lower than that of the control diet and their Profit Index (PI) higher. The PI of the remaining diets was lower than that of the control. In the present experiment it was also observed that the culture periods using the oilseed meal based diets would be longer than the control due to their lower SGRs.

Table 5.8 Whole body proximate composition (% wet weight) and energy of Nile tilapia fed diets with oilseed meal mixtures after experiment 3

	Initial	Control	EQ50	SBM50	CSM50	GNC50	EQ75	SBM75	CSM75	GNC75
		1	2	3	4	5	6	7	8	9
MC	74.06	72.55 ± 0.71	73.97 ± 0.92	72.64 ± 0.47	70.63 ± 1.41	72.27 ± 1.46	73.04 ± 0.91	72.26 ± 1.46	73.64 ± 1.61	72.03 ± 1.29
CP	13.88	14.87 ± 0.37	14.25 ± 0.58	15.19 ± 0.26	15.75 ± 0.73	15.37 ± 0.81	15.02 ± 0.44	15.32 ± 0.81	14.52 ± 0.79	15.06 ± 0.62
CL	7.76	7.81 ± 0.22^{c}	7.62 ± 0.26^{c}	7.99 ± 0.13^{abc}	9.03 ± 0.42^{a}	8.25 ± 0.41^{abc}	7.91 ± 0.25^{bc}	8.22 ± 0.40^{abc}	7.81 ± 0.49^{c}	8.86 ± 0.49^{ab}
Ash	3.27	3.93 ± 0.14^{a}	3.35 ± 0.08^{bc}	3.42 ± 0.04^{bc}	3.67 ± 0.18^{ab}	3.28 ± 0.20^{bc}	3.05 ± 0.11^{c}	3.23 ± 0.17^{bc}	3.08 ± 0.20^{c}	3.36 ± 0.17^{bc}
GE	6.16	6.37 ± 0.04	6.36 ± 0.25^{a}	6.68 ± 0.14^{a}	7.30 ± 0.40^{a}	6.79 ± 0.50^{a}	5.11 ± 0.25^{b}	6.92 ± 0.17^{a}	6.45 ± $0.0.53^{a}$	6.80 ± 0.26^{a}
HSI	-	3.11 ± 0.40^{a}	2.49 ± 0.50^{b}	2.51 ± 0.32^{b}	2.74 ± 0.50^{ab}	2.54 ± 0.48^{b}	2.65 ± 0.43^{ab}	2.92 ± 0.47^{ab}	2.02 ± 0.47^{c}	1.89 ± 0.40^{c}

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

Table 5.9 Cost analysis of diets fed to *O. niloticus* in experiment 3

Diet	Diet cost[1]	Incidence cost[1]	Profit index
1. Control	0.56	1.15	1.73
2. EQ50	0.42	1.05	1.91
3. SBM50	0.43	1.23	1.63
4. CSM50	0.40	1.14	1.75
5. GNC50	0.42	1.32	1.51
6. EQ75	0.34	1.32	1.51
7 SBM75	0.36	1.29	1.56
8. CSM75	0.33	1.02	1.95
9. GNC75	0.34	1.21	1.66

[1]¢.kg^{-1}, Exchange rate ¢0.90 = USD 1.00, Sale price of fish = ¢ 2.00.kg^{-1} fish

5.4 Discussion

Results of the present investigation showed that substitution of fish meal by various plant protein sources in different combinations resulted in improved growth performance compared to that of single plant proteins used at the same level in experiment 2. The weight gain of feed containing protein mixtures at 50% and 75% increased by an average of 184.98% and 164.99% respectively and specific growth rate by 0.70% and 0.89% respectively compared to that of single plant protein source in experiment 2 (Table 4.5 and Table 5.5).

Results obtained here are in agreement with those of Olukunle (1982) and Richards (1983) who observed better growth performance of *O. mossambicus* fed diets containing combinations of groundnut, sunflower seed and sesame meals compared with diets containing the single ingredients. The effectiveness of using various combinations of ingredients in fish feed has been reported by Tacon *et al.* (1984) who successfully reduced the fish meal level from 50% to 10% by using a mixture of soybean, meat and bone meal, brewers yeast, puffed maize and blood meal in the diet of tilapia without reducing the growth performance. Fontainhas-Fernandes *et al.* (1999) incorporated a mixture of extruded pea and defatted soybean meals and Borgeson *et al.* (2006) soybean and maize gluten meals into tilapia diets and reported improved growth

parameters. Hasan (1986) also reported better growth performance of carp fry fed diets containing different mixtures/combinations of linseed, groundnut, mustard and sesame meals.

In the present study growth performance (WG and SGR) of fish fed the control diet were significantly higher than all the test diets with the exception of Diet 2 (50% fish meal protein replaced by equal proportions of SBM, CSM and GNC protein). Among the oilseed-based diets tested, WG and SGR of Diet 2 was not significantly different from Diets 3 and 4 but significantly higher than diets 5, 6, 7, 8, 9 which were mostly 75% plant protein mixtures. Indeed, results from this study indicate that poor growth could be attributed to high levels of phytic acid, trypsin inhibitors and gossypol in the diets (Table 5.3).

Results in this study also indicated that, Diets 2, 3, 4 and 5 at 50% substitution of oilseed meal mixture generally had slightly improved levels of methionine and threonine compared to those in experiment 2 at the same level (Table 4.3). Diets at 75% substitution also followed a similar trend. This seems to support the view of Jackson *et al.* (1982) and Tacon and Jackson (1985) who advocated the use of different plant protein sources in combination as a means of compensating EAA deficiency in tilapia diets.

In the present study feed intake was significantly different among treatments (Table 5.6). Feed intakes were similar to those reported by El-Saidy and Gaber (2003). Feed intakes were significantly higher in fish fed Diets 1, 2, 3, 4 and 5 compared to diets with 75% plant protein mixture and this may be due to relatively high plant protein inclusion which usually results in poor palatability.

FCRs of all 75% plant protein diets and Diet 5 in this study, were significantly different from the control but Diets 2, 3 and 4 were not. These values compare to those of El-Saidy and Gaber (2003) and Borgeson *et al.* (2006).

The present study revealed that protein utilization indices (PER, PPV) and ER in fish fed the oilseed meal mixtures were significantly different (Table 5.6) from the control with the exception of Diet 2 in case of PER, Diets 2 and 4 in case of PPV and ER, which were not different from the control. Within the oilseed meal based diets these parameters were not significantly different with the exception of Diet 6 and 7. The best WG, SGR, PER, PPV and ER values amongst test diets were recorded for fish fed Diet 2 suggesting the superiority of 50% equal mixture of the oilseed meals over the other mixtures for *O. niloticus.* The improved performance of Diet 2 was probably due to improved EAA balance. Mixing the oilseed meals slightly increased the level of methionine + cystine and reduced that of phenylalanine + tyrosine and leucine (but they were still above the requirements for tilapia, Table 5.4) as was also observed by Hossain (1988) and Sadiku and Jauncey (1995). Methionine was identified as the first limiting amino acid in diets with single oilseed meals in chapter 4 but its level was slightly improved by the incorporation of SBM which had a higher level of this amino acid than the other oilseed meals. Although the different oilseed meals complemented each other in terms of AA balance, SBM contributed more because of its superior AA profile. There was also a reduction in levels of individual antinutritional factors (especially TIs and gossypol contents of Diets at 75% inclusion levels (Table 5.3) particularly for SBM and CSM diets which contained higher levels of TIs and gossypol respectively when they were used individually as protein sources in experiment 2 (Table 4.2). However, PA

increased in the mixtures especially at 50% inclusion as compared to that in experiment 2.

Apparent protein digestibility (APD) of the diets fed to fish in this experiment was slightly higher than that of single protein source used in experiment 2 indicating slight improvement through mixing of plant protein source. APD in the present study was similar for all diets up to 75% replacement of the FM protein compared with that of the control diet, even though percentage weight gain was significantly lower for the 50% and 75% replacement groups. APD obtained in this study is higher (87.41% – 89.88%) than the values reported by El-Saidy and Gaber (2003) (80.30% - 85.40%) and Hossain *et al.* (1992) (81.44%) for tilapia.

Whole body composition was little affected by dietary treatments. Total crude protein, moisture content and energy contents of whole body of Nile tilapia were not influenced by dietary treatments since there was no significant difference among them. Similarly, El-Saidy and Gaber (2003) in Nile tilapia, Regost *et al.* (1999) in turbot, Moyano *et al.* (1992) in rainbow trout, Pongmaneerat *et al.* (1993) in carp and Shimeno *et al.* (1993) in yellowtail did not find any effects of dietary mixtures of plant protein on whole body protein content. Ash content was significantly higher for the control diet and Diet 4 (CSM50) compared to the other diets particularly with higher levels of plant protein. However, diets with higher levels of plant proteins produced higher lipid and lower moisture contents as also observed by Nyina-wamwiza *et al*. (2007) for *Clarias gariepinus.* Despite poor fish growth and feed utilization at high inclusion of plant protein (particularly at 75% plant protein) in this study, fish did not show any poor health

or physical deformities and histopathological examination of the intestines and liver revealed no significant changes in morphology.

The economics of feed production indicated that the costs of the diets were minimised by replacing fish meal with the oilseed meal mixtures (Table 5.9). From the results it was observed that the diet with equal contributions of oilseed meal in the mixture (Diet 2) and diets with higher proportions of CSM in the mixture (Diet 4 and 8) were the diets which were more profitable than the control diet. This compares with the results in experiment 2 where CSM based diets were most profitable possibly because CSM was the cheapest oilseed meal (Table 3.2) and had a fairly good growth performance. A similar investigation by Olvera-Novoa *et al.* (2002a) suggested that it is possible to replace up to 65% of animal protein in *O. mossambicus* fry diets using a mixture of plant proteins (alfalfa leaf protein concentrate, soybean and torula yeast) without adverse effects on fish growth and profit. However, another study by El-Saidy and Gaber (2003) evaluating a mixture of SBM, CSM and sunflower meal for Nile tilapia reported that plant protein mixtures were more profitable than the fish meal based diet even at 100% replacement. Moreover, Coyle *et al.* (2004) indicated that efficient and economical tilapia growth can be obtained by feeding diets without fish meal using a combination of distillery by-products, meat and bone meal and SBM.

Results from the present study demonstrate that use of different plant protein sources in various combinations could be more effective than a single source in the substitution of fish meal in tilapia diets. Careful selection and use of different plant protein sources in various combinations could be a means of

compensating for essential amino acid deficiency in any single protein source and also prevent a high inclusion level of any single antinutritional factor in the diet. From the results though Diets EQ50, CSM50 and CSM75 had PIs higher than the control, Diet EQ50 has the best prospects based on growth performance, nutrient utilization and economic benefits.

Chapter 6 - Effects of Dietary Essential Amino Acid Supplementation on the Growth Performance and Feed Utilization of Juvenile Nile tilapia

6.1 Introduction

The scarcity of good quality fish meal and escalating prices in recent years has lead to renewed interest in the use of alternative protein sources for fish. A possible solution to this problem is being sought by using plant proteins, mainly oilseed meals. However, apart from fish meal, there are few animal or plant protein products available for formulation of fish feeds with an essential amino acid (EAA) profile approximating the dietary requirements of cultured fish (De Silva and Anderson, 1995). The EAA profiles of diets formulated with individual oilseed meals (soybean meal (SBM), cottonseed meal (CSM) and groundnut cake (GNC) as well as their mixtures in previous experiments of this study showed clearly deficiency of one or more EAAs (Table 4.3 and 5.4) compared to the requirements of Nile tilapia(Table 1.5 and 4.3).

Supplementing crystalline amino acids to diets in order to optimize the amino acid profile has been commonplace in the terrestrial livestock industry for decades with good results (Lewis and Bayley, 1995). Inclusion of crystalline amino acids in feeds has also been used in aquaculture, both in experimental diets and commercial feed production. Supplementation with crystalline amino acids is actually an attempt to improve the protein quality of fish feeds by addition of the essential amino acids (EAAs) that are in deficit in plant proteins (Robinson, 1991; Webster *et al*., 1995). Some EAAs, such as methionine and lysine, are generally critical in the formulation of fish diets when incorporating

inexpensive plant protein sources (Tacon, 1990). For most EAAs, deficiency is manifest as a reduction in weight gain. In some species of fish, however, a deficiency of methionine or tryptophan leads to pathologies, because these AAs are not only incorporated into proteins but also used for the synthesis of other compounds (Lovell, 1998). For example, cataracts occur in salmonids and rainbow trout as a consequence of methionine (sulphur amino acids) and tryptophan deficiency respectively in their diets (Lovell, 1998).

Tilapias have a requirement for sulphur-containing amino acids (i.e. methionine, cystine and cysteine) which can be met by either methionine alone or a proper mixture of methionine and cystine (Shiau, 2002). Dietary cystine can replace up to 50% of the total sulphur-containing amino acid requirement for *Oreochromis mossambicus* (Jauncey and Ross, 1982). A relationship exists between methionine and cystine, two important sulphur-containing amino acids. Cystine is considered non-essential because it can be synthesized by fish from methionine, an EAA. Therefore, if methionine is fed without cystine, a portion of the methionine is used for protein synthesis and another portion is converted to cysteine for incorporation into protein as cystine. When cystine is included in the diet it reduces the amount of methionine required. Because of this relationship, the fish has a total sulphur amino acid requirement rather than a specific methionine requirement (Wilson, 1989). However, due to the relationship between methionine and cystine mentioned above, methionine can meet the total sulphur amino acid requirements of fish, although some of this requirement may be met by cystine (NRC, 1993). The total sulphur amino acid requirement (methionine + cystine) and lysine for Nile tilapia has been reported to be 2.7% and 5.1% of the total protein respectively (Santiago and Lovell, 1988).

Unlike fish meal, which has a well balanced amino acid profile, the majority of plant protein sources tested were either deficient in one or more EAAs, especially sulphur-containing amino acids (methionine and cystine). Dietary amino acid utilization requires that all amino acids are simultaneously present in adequate concentrations at sites of protein synthesis. Hence, deficiency of an EAA limits protein synthesis to the level of that particular EAA, the remainder being catabolized (Sveier *et al*., 2001). It has been reported that dietary imbalances of AAs can also cause reduced performance in animals through amino acid antagonism or toxicity. Adverse interactions (antagonism) may occur between amino acids that are structurally related when their concentrations in the diet are imbalanced (Lovell, 1998; NRC, 1993). Examples of such antagonisms are lysine-arginine, leucine-valine and leucine-isoleucine (NRC, 1993; Tacon and Jackson, 1985). In some instances, however, dietary excesses of certain amino acids (eg. excesses of leucine for rainbow trout) are directly toxic and their negative effects cannot be ameliorated by additions of other AAs (Lovell, 1998).

Several studies have also indicated that fish utilize crystalline amino acids less efficiently than protein bound forms (Yamada *et al*., 1981; Murai *et al*., 1986; Davies and Morris, 1997; Schuhmacher *et al*., 1997; Sveier *et al*., 2001). Andrews and Page (1974) and Li and Robinson (1998) also reported no beneficial effect of dietary amino acid supplementation in channel catfish. Jauncey *et al.* (1984) reported difficulty in using purified AA test diets with *O. mossambicus*. Different suggestions have been offered to explain the reduced efficacy of crystalline AA as compared to protein-bound AA. A possible reason for the poorer utilization of free compared with protein bound amino acids may

be different rates of absorption in the gut, creating amino acid imbalances in the tissues (Cowey and Sargent, 1979; Cowey and Walton, 1988). Zarate *et al.* (1999) have shown poorer utilization of dietary free lysine compared with protein-bound lysine for growth of channel catfish *(Ictalurus punctatus)*. The authors concluded that crystalline amino acids have a higher rate of stomach evacuation and lower rate of absorption compared with protein-bound amino acids and claim that this led to poorer growth. Another reason for poorer utilization was attributed to leaching of dietary crystalline AA during feeding (Lovell, 1998) because these losses occur to a greater degree than those of protein-bound amino acids (Zarate and Lovell, 1997). According to Tantikitti and March (1995) and Lovell (1998) losses of dietary crystalline AA may be reduced by increasing the feeding frequency to stabilize amino acid plasma concentration and increase protein deposition.

However, crystalline AA have been reported to be successfully used to supplement AA deficient diets, improving fish growth and feed utilization efficiency (Mukhopadhyay and Ray, 1999; Murai *et al*., 1986; Williams *et al*., 2001). Jackson and Capper (1982) assert that free EAA are well utilised by *O. mossambicus.* Odum and Ejike (1991), El-Saidy and Gaber (2002) and Furuya *et al.* (2004) reported increased performance of Nile tilapia when diets were supplemented with amino acids. Similar findings were reported by Robinson (1991) for hybrid tilapia (*Oreochromis niloticus x O. aureus*), Bai and Gatlin (1994) for rainbow trout (*Oncorhynchus mykiss*), and Bai and Gatlin (1994) for channel catfish (*Ictalurus punctatus*). Webster *et al.* (1995) reported that the inclusion of essential amino acids in diets for blue catfish (*I. furcatus*) resulted in

performance comparable to that obtained with diets with high fish meal contents.

In view of contradictory reports on the efficacy of EAA supplementation of tilapia feeds, further study and economic analysis is required to determine whether or not any improvements in growth justify the use of EAAs and the additional feed cost that this would incur. In the previous experiments most of the oilseed meal based diets were deficient in one or more essential amino acids (particularly, methionine, threonine and in some cases lysine) and resulted in lower growth of Nile tilapia compared to the fish meal based diets. The objective of this study, therefore, was to investigate whether supplementing an amino acid (crystalline methionine) to the oilseed meal based diets for Nile tilapia could improve growth and feed utilization.

6.2 Materials and Methods

6.2.1 Experimental System and Animals

Fingerlings of Nile tilapia of average weight 5.48 ± 0.20 g were stocked in triplicate in 30-L tanks. Each tank was randomly stocked with 20 fingerlings under similar conditions to those described in Section 3.2.1. Fish were hand-fed three times a day (09:30, 13:00 and 16:00) at a rate of 6% of their body weight per day for the first three weeks and 4% for subsequent weeks, the experiment lasted 8 weeks. Diets were dispensed in small portions to ensure prompt consumption and avoid amino acid leaching as recommended by Lovell (1998). Water quality parameters measured every week during the experiment included the following: temperature, 26.97 ± 0.26 ^{o}C; pH, 7.29 ± 0.27; ammonia, 0.05 ±

0.03 mg.L^{-1}; nitrite, 0.20 ± 0.0 mg.L^{-1}; Nitrate, 20 ± 0.0 mg.L^{-1} and dissolved oxygen, 7.54 ± 0.52 mg.L^{-1}.

6.2.2 Diet Formulation and preparation

Five isonitrogenous and isoenergetic diets were formulated using a mixture of SBM, CSM, and GNC as protein sources. FM was substituted with different mixtures of SBM, CSM and GNC at 50% of total protein as used in experiment 3. Composition of the different mixtures is presented in Table 5.1. Diets containing different mixtures of oilseed proteins were supplemented with 0.5% DL-methionine (M9500, 99.0% TLC; Sigma) to meet the minimum requirement for Nile tilapia, which is 2.70% of dietary protein (NRC, 1993). In experiment 3 the oilseed meal mixtures at 50% substitution level of FM were deficient in methionine (as the first limiting EAA) and threonine (the second limiting EAA for all the test diets). In this experiment only the first limiting EAA (i.e. methionine) was supplemented because this was deemed more critical for Nile tilapia (Shiau, 2002). Diet preparation and other ingredients used were similar to those described in Sections 2.2 and 4.2.4. Diet formulation is presented in Table 6.1.

6.2.3 Faecal Collection

Faeces collection was undertaken as described in Section 4.2.2 (also see Sections 2.1.2 for details). Apparent digestibility coefficients for nutrients, energy and phosphorus of diets were determined as described in Section 2.3.5.

6.2.4 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by the methods described in Section 2.2.1. Energy and phosphorous of diets, faeces and carcass were analysed by methods described

in Sections 2.2.4 and 2.2.5. Chromic oxide content of the diets and faeces were determined by the method in Section 2.2.3.

Table 6.1 Composition of diets fed to juvenile *O. niloticus* using oilseed meal mixtures ($g.kg^{-1}$ of diet) supplemented with DL-Methionine in experiment 4

Ingredients	Control	EQ50	SBM50	CSM50	GNC50
	1	2	3	4	5
SBM	-	100.2	150.4	75.2	75.2
CSM	-	113.5	85.2	170.4	85.2
GNC	-	116.4	87.4	87.4	175.0
FM	420.0	210.0	210.0	210.0	210.0
Wheat Grain	203.0	206.0	205.5	206.0	205.2
Sunflower Oil	57.0	47.0	53.8	51.7	35.4
α- Cellulose	30.0	10.9	12.5	7.0	14.5
Corn Starch	205.0	106.0	105.2	102.3	109.5
Binder CMC[1]	2.00	2.00	2.00	2.00	2.00
Mineral Premix[2]	40.0	40.0	40.0	40.0	40.0
Vitamin Premix[3]	20.0	20.0	20.0	20.0	20.0
Chromic Oxide	5.0	5.0	5.0	5.0	5.0
DL-Methionine[4]	0.0	5.0	5.0	5.0	5.0

FM = Fish meal, SBM = Soybean meal, CSM = Cottonseed meal, GNC = Groundnut cake, [1]Carboxymethyl cellulose (Sigma, C5013), [2,3]Mineral and vitamin premixes are presented in Table 2.1 and Table 2.2 respectively, [4](Sigma, M9500) Sigma-Aldrich Chemie, Germany

6.2.5 Analysis of Experimental Data

Growth performance and feed utilization of fish were calculated as described in Section 2.3 and 3.2.5. Hepatosomatic index (HSI) was calculated as described in Section 2.3.6.

6.2.6 Statistical analysis

Each experimental diet was fed to three groups of fish by a completely randomized design. Data was analysed as described in Section 2.6.

6.3 Results

6.3.1 Chemical Composition of Diets

The chemical composition of diets used in this study is presented in Table 6.2. CP, CL and energy were similar for all diets as formulated. Ash and phosphorus contents of the control diet were higher than for the plant-based diets. Diet 4 (CSM50) had the highest fibre content reflecting higher inclusion level of cottonseed meal (50% of plant mixture). The ANFs are similar to Diets 1, 2, 3, 4 and 5 in experiment 3 (Table 6.2).

Table 6.3 shows the estimated EAA composition of diets used in the study. The diets met the EAA requirements of Nile tilapia (NRC, 1993) and exceeded this in most cases with the exception of threonine which was slightly deficient for all the diets.

Table 6.2 Proximate composition (gkg^{-1} as-fed), energy ($kJ.g^{-1}$), phosphorous and antinutritional factors ($g.kg^{-1}$) of diets used in experiment 4

Components	Control	EQ50	SBM50	CSM50	GNC50
	1	2	3	4	5
DM	976.7	971.8	973.0	958.9	973.7
CP	333.4	342.7	342.1	337.2	343.6
CL	110.3	111.4	107.8	109.4	112.6
CF	22.8	28.2	26.6	31.9	25.5
Ash	97.8	89.1	88.3	89.7	89.1
NFE	412.4	400.5	408.1	390.7	402.9
Cr_2O_3	5.0	5.2	5.1	5.0	5.1
GE	18.36	18.77	18.75	18.74	18.81
P	8.30	7.01	7.33	7.53	7.77
PA	0.5	7.6	7.1	8.5	7.1
TI	0.0	1.8	2.4	1.5	1.6
G	0.0	0.6	0.5	1.0	0.5
S	1.1	3.4	3.3	3.4	3.5

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, NFE = nitrogen free extract, Cr_2O_3 = Chromic oxide, GE = gross energy, P = phosphorous, PA = Phytic acic, TI = Trypsin inhibitor, G = Gossypol, S = Saponin

Table 6.3 Estimated essential amino acid (EAA) composition (% of dietary protein) of diets fed to Nile tilapia in experiment 4

EAA	Requirement for tilapia[1]	Diets				
		Control	EQ50	SBM50	CSM50	GNC50
		1	2	3	4	5
Arginine	4.20	5.01	6.11	5.97	6.23	6.14
Histidine	1.70	1.99	2.18	2.20	2.20	2.15
Isoleucine	3.10	3.96	3.71	3.84	3.66	3.63
Leucine	3.40	6.41	6.08	6.24	6.03	5.98
Lysine	5.10	6.23	5.43	5.55	5.41	5.34
Met. + Cys[2]	2.70	2.72	3.63	3.62	3.66	3.59
Phe + Tyr[2]	3.80	5.26	5.90	5.96	5.89	5.85
Threonine	3.80	3.23	2.93	2.97	2.97	2.86
Valine	2.80	4.94	4.59	4.69	4.57	4.52

Met. + Cys = Methionine + Cystine; Phe + Tyr = Phenylalanine + Tyrosine, [1]Values from NRC (1993), [2]Values of methionine and phenylalanine are the requirements in the presence of cystine and tyrosine of the diet respectively.

6.3.2 Growth performance and feed utilization

Data on growth performance and feed utilization of Nile tilapia fed the different experimental diets is presented in Table 6.4 and growth responses are also shown in Figure 6.1. All experimental diets were well accepted and no pathological signs were observed during the trial. FW, WG, SGR, FCR, PER and PPV were all significantly higher ($P< 0.05$) for the control diet than for all the oilseed meal based diets (supplemented with methionine). Feed intake of fish fed the control, Diets 4 and 5 was significantly higher than that of fish fed Diets 2 and 3 (Table 6.4). Only Diet 3 (SBM50) had significantly lower energy retention than the control diet.

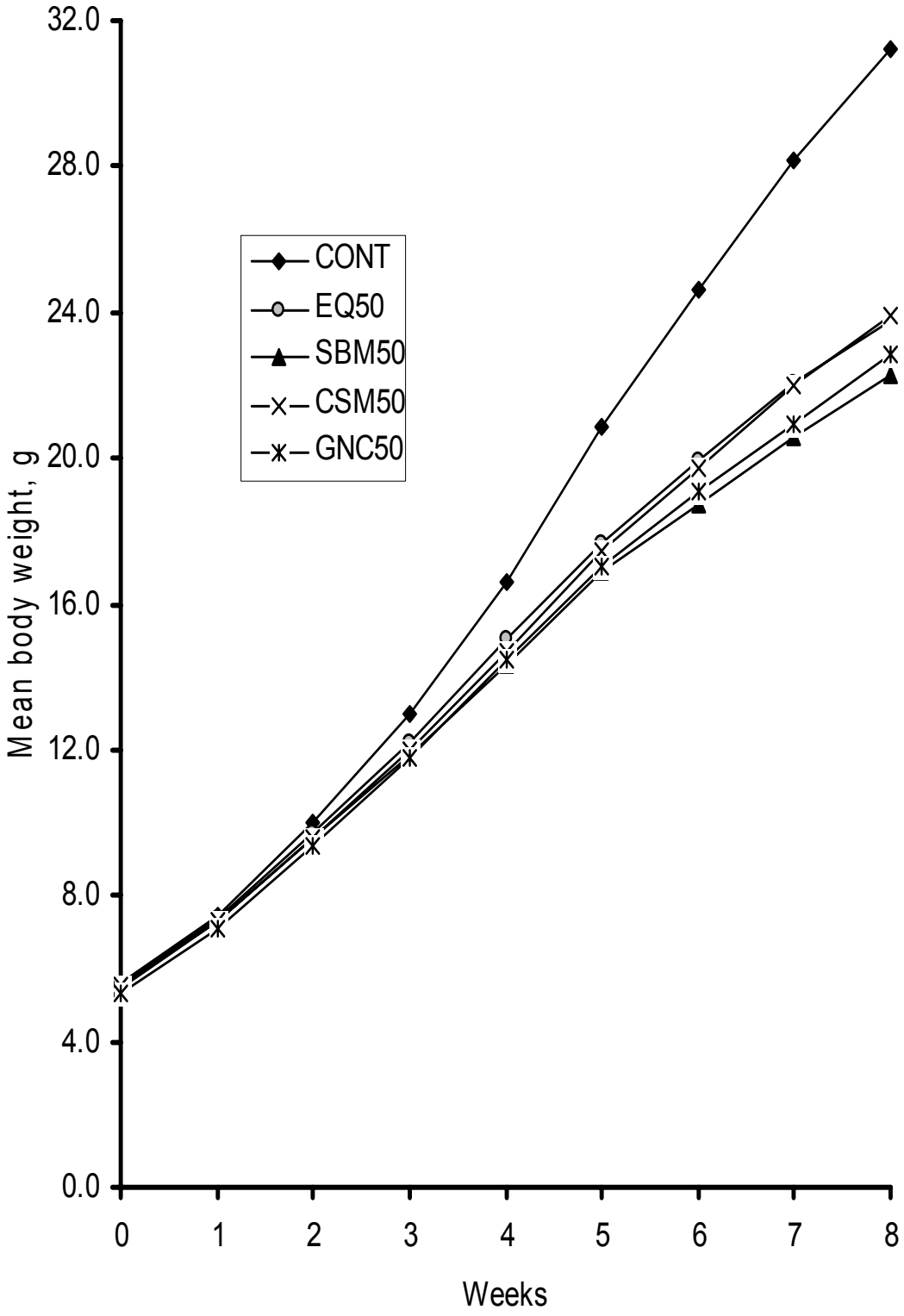

Figure 6.1 Growth response of fish fed oilseed based diets with DL-methionine supplementation for eight weeks

Table 6.4 Growth and food utilization of Nile tilapia fingerlings fed oilseed meal based diets with DL-methionine supplementation for eight weeks

	Control	EQ50	SBM50	CSM50	GNC50
	1	2	3	4	5
IW	5.59 ± 0.27	5.50 ± 0.28	5.47 ± 0.02	5.50 ± 0.19	5.32 ± 0.17
FW	31.20 ± 0.99^{a}	23.80 ± 0.92^{b}	22.29 ± 1.16^{b}	23.88 ± 1.97^{b}	22.90 ± 1.07^{b}
WG	458.63 ± 28.60^{a}	333.12 ± 23.95^{b}	307.65 ± 20.14^{b}	333.78 ± 21.46^{b}	330.34 ± 6.84^{b}
SGR	3.07 ± 0.09^{a}	2.62 ± 0.10^{b}	2.51 ± 0.09^{b}	2.62 ± 0.09^{b}	2.61 ± 0.03^{b}
FCR	1.34 ± 0.05^{a}	1.82 ± 0.09^{b}	1.99 ± 0.13^{b}	1.84 ± 0.16^{b}	1.94 ± 0.10^{b}
FI	34.31 ± 0.07^{a}	33.22 ± 0.11^{b}	33.42 ± 0.03^{b}	34.09 ± 0.28^{a}	34.12 ± 0.04^{a}
PER	2.24 ± 0.08^{a}	1.61 ± 0.08^{b}	1.47 ± 0.10^{b}	1.60 ± 0.16^{b}	1.50 ± 0.08^{b}
PPV	32.68 ± 1.16^{a}	24.00 ± 1.13^{b}	21.75 ± 1.4^{b}	23.10 ± 2.28^{b}	23.26 ± 1.20^{b}
ER	26.70± 1.03^{a}	20.37 ± 2.73^{ab}	19.91 ± 0.90^{b}	22.46 ± 4.36^{ab}	20.48 ± 1.16^{ab}
S	100.00	100.00	100.00	100.00	100.00

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 replicates, and values within the same row with different letters are significantly different (P< 0.05)

Table 6.5 Apparent digestibility coefficient (%) of protein, lipid, dry matter, energy and phosphorus and digestible protein and energy (g.kg^{-1} and kJ.g^{-1} respectively, dry weight basis) in the test diets for Nile tilapia

Components	1	2	3	4	5
DM	84.13	81.02	80.75	78.45	81.11
CP	89.71	89.25	89.04	88.62	89.98
CL	96.72	97.21	96.19	98.92	99.75
GE	85.24	82.87	82.18	80.86	83.31
P	86.17	77.72	82.72	73.58	84.76
DP	306.2	314.7	313.1	311.6	317.5
DE	15.65	15.56	15.41	15.15	15.67

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, GE = gross energy, P = phosphorous, DP = Digestible protein, DE = Digestible energy

6.3.3 Apparent Nutrient Digestibility

Apparent protein digestibility varied only slightly among diets with the exception of Diet 4, where it was lower. The control diet had the highest apparent dry matter, energy and phosphorus digestibilities and Diet 4 again the lowest (Table 6.5). Digestible protein and energy were similar for all diets with only little variations.

6.3.4 Body Composition

At the end of the growth trial there were no significant differences ($P > 0.05$) in whole-body protein, moisture, lipid and energy contents among diets, with the exception of Diet 4 which resulted in a lower protein content (Table 6.6). These values were, however, higher than for initial whole-body composition. Ash contents of fish fed the control diet were significantly higher than those of fish fed the plant-based diets.

Table 6.6 Whole body proximate composition (% wet weight) of Nile tilapia oilseed meal based diets with DL-methionine supplementation after experiment 4

	Initial	Control	EQ50	SBM50	CSM50	GNC50
		1	2	3	4	5
MC	75.48	72.66 ± 0.61	72.44 ± 1.07	71.61 ± 0.20	70.85 ± 1.36	70.98 ± 0.27
CP	13.85	14.45 ± 0.17^{ab}	14.66 ± 0.06^{ab}	14.56 ± 0.24^{ab}	14.27 ± 0.45^{b}	15.13 ± 0.43^{a}
CL	5.73	7.78 ± 0.75	8.43 ± 1.19	9.13 ± 0.42	9.93 ± 1.58	8.89 ± 0.37
Ash	3.90	4.31 ± 0.05^{a}	3.57 ± 0.16^{b}	3.77 ± 0.11^{b}	3.83 ± 0.12^{b}	3.78 ± 0.16^{b}
GE	5.41	6.36 ± 0.27	6.57 ± 0.48	6.93 ± 0.10	7.22 ± 0.63	7.00 ± 0.08
HSI	-	2.95 ± 0.60^{a}	3.55 ± 0.77^{ab}	3.28 ± 0.70^{ab}	3.52 ± 0.70^{ab}	3.73 ± 0.76^{b}

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

6.3.5 Cost-benefit Analysis of Diets

The costs of ingredients used in this analysis are presented in Table 3.2. The cost per kilogram of experimental diets varied little with the control having the highest (¢0.56 kg^{-1}) and Diet 4 (CSM50) the least (¢0.53 kg^{-1})(Table 6.7). The cost analysis however, showed that the highest profit was obtained by the control diet and the lowest by Diet 3 (SBM50). Among the different mixtures Diets 2 and 4 were the most profitable, showing a similar trend in experiment 3.

Table 6.7 Cost analysis of diets fed to *O. niloticus* in experiment 4

Diet	Diet cost[1]	Incidence cost[1]	Profit index
1. Control	0.56	0.75	2.66
2. EQ50	0.54	0.98	2.04
3. SBM50	0.55	1.10	1.82
4. CSM50	0.53	0.98	2.04
5. GNC50	0.54	1.05	1.90

[1]¢.kg^{-1}, Exchange rate ¢0.90 = USD 1.00 (2007), Sale price of fish = ¢ 2.00 kg^{-1} fish, DL-methionine price ¢25.0 kg^{-1} price based on feed grade (MP Biomedicals, Solon, USA).

6.4 Discussion

Results from the present study indicated that there was significantly lower growth performance and feed utilisation (Table 6.4) in Nile tilapia fed oilseed meal based diets supplemented with methionine compared with the control diet (fish meal based diet). However, ER of fish fed Diets 2, 4 and 5 were not significantly different from the control diet (Table 6.4). Amongst test diets, Diets 2 and 4 gave better growth and feed utilisation even though this was not significantly different from the other treatments. This was also the trend in experiment 3 (Table 5.5 and 5.6) where the same oilseed meal mixtures were used at the same inclusion level.

Despite the fact that growth and feed utilization of fish fed test diets were lower than that of the control in this study, they could be considered as showing improvements (particularly, FCR, PER and PPV). Values are significantly higher than in previous experiments (i.e. experiment 2 and 3) at similar plant protein levels and comparable to those of various researchers who reported improved growth of fish with AA incorporation at similar inclusion levels of plant protein (El-Saidy and Gaber, 2002; Furuya *et al*., 2004; Mukhopadhyay and Ray, 2001; Polat, 1999).

Supplementing crystalline AAs in fish diets has had variable success. According to Shiau *et al*. (1989), male tilapia (*O. niloticus* x *O. aureus*) fed diets in which 100% of the fishmeal was replaced with SBM either with or without methionine supplementation had significantly lower weight gain, FCR and protein digestibility than in groups fed diets containing fishmeal as the sole source of protein. Andrews and Page (1974) stated no improvement in growth of channel catfish when L-methionine was supplemented to a soybean meal based diet and also Teshima and Kanazawa (1988) did not observe improved fish growth when they supplemented SBM with the deficient EAA, and therefore concluded that it was unnecessary. Bai and Gatlin (1994) also reported that addition of supplemental L-lysine to a diet with 25% crude protein from soy did not improve growth of channel catfish.

In contrast, Davis and Stickney (1978) and El-Saidy and Gaber (2002) reported feeding blue tilapia and Nile tilapia respectively with 100% SBM (with methionine supplementation in the first instance and methionine and lysine in the second) with no significant effect on growth and feed utilization. Shiau *et al*. (1987) reported improved growth of tilapia with addition of supplemental methionine and Murai *et al.* (1986) also reported that the nutritional value of soy flour was improved by addition of 0.4% crystalline L-methionine. In other studies partial replacement of fish meal was achieved using a combination of plant proteins supplemented with amino acids. A mixture of plant proteins (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin) balanced with EAAs could provide between 50 - 75% of protein in diets fed to gilthead sea bream, *Sparus aurata* without significantly affecting performance (Sitjà-Bobadilla *et al*., 2005). A sizeable proportion of the fish meal in diets for

juvenile turbot, *Psetta maxima* could be replaced by a mixture of plant proteins from lupin, corn gluten and wheat gluten meal supplemented with amino acids without affecting growth and nutrition (Fournier *et al*., 2004).

Even though supplementation of diets with DL-methionine in the present study resulted in improvements in feed utilization compared to the previous experiments, the oilseed meal based diets did not produce growth and feed utilization equivalent to that of fish fed the fish meal based diet. The superiority of fishmeal in this respect may be attributed to the absence of any known antinutritional factors, higher protein levels (Table 3.2) and to the generally higher availability of amino acids in fish meal protein (Hertrampf and Piedad-Pascual, 2000). Reduced efficacy of crystalline-AA compared to protein-bound-AA was also observed in different fish species (Mambrini and Kaushik, 1994; Rodehutscord *et al*., 1995a; Sveier *et al*., 2001; Zarate and Lovell, 1997; Zarate *et al*., 1999). This was attributed to differences in AA absorption rates and the leaching of dietary crystalline-AA during feeding of fish. In the present study in order to avoid leaching of AAs, fish were fed more frequently with smaller quantities of feed as recommended by Tantikitti and March (1995) and Lovell (1998).

Apparent protein digestibilities (APD) in this study were similar (88.62% - 89.98%) with little variation for all diets including the control diet. Diet 4, which contained more CSM in the oilseed meal mixture, had the lowest APD as was also observed in previous experiments. This was most likely due to the higher fibre level in the diet (Table 6.2). In a similar study El-Saidy and Gaber (2002) reported APD values of 74.5% - 86.5% for Nile tilapia using 100% SBM

supplemented with L- methionine and L-lysine in the diet. The APD in this study also compared well with that reported by Mukhopadhyay and Ray (2001) where rohu fingerlings fed linseed meal (up to 50% replacement of FM) supplemented with AAs had higher APDs of (84.1% - 89.9%) than diets without AAs (82.8% – 84.9%). Apparent dry matter, energy and phosphorus digestibilities of the control diet were higher than the test diets. Generally, apparent nutrient digestibilities of the test diets followed the same trend as that of the individual ingredients (Table 3.6).

Carcass composition was little affected by dietary treatments at the termination of the feed trial. Total crude protein and energy contents of Nile tilapia were not significantly different between dietary treatments and the control with the exception of CP of fish fed Diet 4 which was significantly lower than the control. However, there was significant increase in protein and fat in comparison with the initial carcass values in all the dietary treatments. Similar results were reported by Polat (1999) in *T. zillii*, El-Saidy and Gaber (2002) in Nile tilapia and Mukhopadhyay and Ray (2001) in rohu.

Cost analysis of diets in this study revealed that the diets had higher cost per kilogram as compared to the same diets in experiment 3 due to supplementation of methionine, which is quite expensive (¢25.0 kg^{-1}) compared to all the other ingredients used to formulate the diet (Table 3.2). However, their cost per kilogram was still slightly lower than that of the control. Nonetheless, the incidence costs were higher with corresponding lower PIs, which showed that the control was more profitable than the oilseed meal based diets supplemented with DL-methionine. The result indicated that DL-methionine

supplementation of tilapia diets in this study did not lead to improved cost-effectiveness. This appears to concur with concerns raised by Jauncey (1998) about EAA supplementation in tilapia feeds and whether any improvements in growth justify the additional costs. Moreover, amino acid supplementation may not be feasible in Ghana since supplements are likely to be unavailable and unaffordable to majority of farmers. However, if cheaper sources of methionine are found cost effectiveness could improve substantially, especially for Diets 2 and 4. Methionine price used in cost anaysis in this study was based on feed grade price in the USA (MP Biomedicals, Solon, USA) because most feed supplements are usually imported from Europe or USA.

To conclude, the present study demonstrated that utilization of crystalline methionine (0.5%) by Nile tilapia was not effective in improving the nutritive value and cost effectiveness of the diets containing oilseed meal mixtures (SBM, CSM and GNC replacing 50% FM protein) compared with the fish meal based diet. However, FCR, PER and PPV improved compared to the previous studies where there was no methionine supplementation. The lower performance of fish fed the oilseed based diets compared to the control may be attributed to poor utilization of crystalline methionine and the presence of various antinutritional factors, among others in the selected oilseed meals.

Chapter 7 - The Effects of Oilseed Meals Detoxification on Growth Performance and Feed Utilization in Juvenile Nile Tilapia

7.1 Introduction

Despite the fact that most oilseed meals are readily available at a lower cost than fish meal, their use within compound aquafeeds is usually restricted by relatively low protein content, unbalanced essential amino acid profile (Jauncey, 1998), high levels of fibre and starch, especially non-soluble carbohydrates (Gatlin *et al*., 2007) and the presence of one or more endogenous antinutritional factors (ANFs) (Kaushik, 1989; Krogdahl, 1989; NRC, 1993). To improve the nutritive value of plant products, ingredients have been modified by chemical, mechanical and biological methods to remove antinutrients and/or fractions of low nutritive value that results in fairly good or high-protein plant products (Adelizi *et al*., 1998; Kaushik *et al*., 1995) and also supplementing limiting free amino acids or by mixing complementary protein sources in the case of essential amino acid deficiencies (Tacon and Jackson, 1985). It is generally believed that the presence of naturally occurring ANFs within oilseeds is the most important factor limiting their use at high dietary inclusion levels within compound aquafeeds (Tacon, 1995b).

Oilseed meals, particularly soybean meal (SBM), cottonseed meal (CSM) and groundnut cake (GNC) as used in this study, contain a number of ANFs (Table 3.3). ANFs common to the selected oilseed meals are; trypsin inhibitors (TIs), phytic acid and saponin. TIs, phytic acid and saponin are of great importance in the present study because diets from the oilseed meal mixtures still had

considerably high concentrations (Table 5.3) particularly phytic acid. Gossypol contained in CSM is also of importance in this experiment because even at low concentrations it could be deleterious to fish (Francis *et al*., 2001). Based on common methods of detoxification, ANFs can be classified as heat-labile (TIs, lectins, goitrogens, anti-vitamins) and heat-stable (phytic acid, gossypol, saponins, tannins, oestrogens, non-starch polysaccharides and protein antigens) (Csaky and Fekete, 2004; Drew *et al*., 2007; Francis *et al*., 2001; Refstie *et al*., 2001; Rumsey *et al*., 1993).

Trypsin inhibitors, one of the major problems limiting oilseed products utilization in fish feed, can be reduced or eliminated using a variety of methods such as; fermentation, germination, soaking, boiling, roasting, toasting, extrusion, autoclaving and enzyme treatment (Fagbemi *et al*., 2005; Francis *et al*., 2001; Marsman *et al*., 1997; Raj Bhandari and Kawabata, 2006). According to Fagbemi *et al*. (2005) fermentation reduced TI activity in breadnuts, cashewnuts and fluted pumpkin seed flours better than boiling, germination and roasting. Vinay and Sindhu Kanya (2008) reported reduction in TI activity (84%) when they treated Karanja (*Pongamia pinnata)* seed meal with 2% hydrochloric acid. According to Mumba *et al*. (2004) soaking soybeans in water can effectively remove the TIs. However, heat treatment (i.e. autoclaving) has been recommended as an effective means of reducing the concentration of TIs below the critical levels in ingredients (Norton, 1991). Heat treatment of SBM using an autoclave (172,253 Pa at 121 ^{o}C for 20 min) has been reported to have lowered the level of TIs to between 79% - 99% (Arndt *et al*., 1999; McNaughton *et al*., 1981). It has also been reported by others that TI concentrations were lowered to similar levels when SBM was autoclaved for 10 min at 141,855 Pa compared

to 45 min at 34,451 Pa (Borchers *et al*., 1947). Furthermore, McNaughton and Reece (1980) and McNaughton *et al*. (1981) reported that when water (10–25%) was added to SBM prior to autoclaving, the heating time to inactivate TI was shortened, and the potential for damage to protein and amino acids was thereby reduced.

Although, heat treatment is an effective method of reducing TIs, overheating may result in decreased bioavailability or loss of essential amino acids, particularly lysine, and may denature protein (Francis *et al*., 2001; NRC, 1993; Viola *et al.*, 1983). The degree of destruction or inactivation of TIs depends on the temperature, duration of heating, particle size and moisture conditions (Fagbemi *et al*., 2005; Francis *et al*., 2001; Lim and Dominy, 1991; Rehman and Shah, 2005). In general the destruction or inactivation of TIs, together with any other heat-labile ANFs, is accompanied by a marked improvement in the nutritive value of the protein source (Borgeson *et al*., 2006; Drew *et al*., 2007; Francis *et al*., 2001; Hossain, 1988; Marsman *et al*., 1997; Rumsey *et al*., 1993).

One other major problem limiting the use of oilseed products in fish feed is the presence of phytate, which is the major phosphorus (P) storage compound in plant seeds and can account for up to 70% of total phosphorus (Baruah *et al*., 2004; Lovell, 1998; NRC, 1993). Phytates, particularly in cereals, are normally concentrated in the outer endosperm. Therefore, milling to remove the outer layer of seeds reduces the phytate content of seeds considerably (Francis *et al*., 2001). Fermentation has also been shown to reduce the phytic acid content of grains because of the action of phytases produced by yeast or lactic acid

bacteria (Duffus and Duffus, 1991; Mukhopadhyay and Ray, 1999). The addition of minerals such as Zn has been observed to be only partially capable of counteracting the negative effects of dietary phytate (Francis *et al*., 2001). Exogenous phytase has been used more successfully to hydrolyse phytate and increase nutrient digestibility (Cao *et al*., 2007; Davies *et al*., 1993; Drew *et al*., 2007; NRC, 1993). Specifically for hydrolysis of phytate, phytase is most promising in producing cost-effective fish feed formulae that can reduce the need to supplement inorganic P in feeds and also lower phytate P levels in fish excretions (Liener, 1994; Simons *et al*., 1990). Incorporation of microbial phytase in diets has resulted in an increase in phosphorus bioavailability, protein utilization and growth performance in several fish species including rainbow trout (Cain and Garling, 1995; Cheng *et al*., 2004; Ketola and Harland, 1993; Riche and Brown, 1996; Vielma *et al*., 1998) common carp (Schafer *et al*., 1995), channel catfish (Eya and Lovell, 1997; Jackson *et al*., 1996; Li and Robinson, 1997), African catfish (Van Weerd *et al*., 1999), striped bass (Hughes and Soares, 1998; Papatryphon *et al*., 1999) and Nile tilapia (Furuya *et al*., 2001; Portz and Liebert, 2004). According to Furuya *et al*. (2001) and Portz and Liebert (2004) SBM diets supplemented with between 500 and 1500 $FTU.kg^{-1}$ of phytase showed increased calcium and phosphorus availability, growth performance and protein digestibility in Nile tilapia.

Gossypol is a phenolic compound mostly found in cottonseeds and in large quantities has been shown to be toxic to monogastric animals including fish (Herman, 1970). However, CSM with low levels of gossypol performed excellently when fed to *O. mossambicus* (Jackson *et al*., 1982). Several methods have been reported by different researchers to have been used to

reduce free gossypol in CSMs. Jackson *et al*. (1982) explained that gossypol in cottonseed is concentrated in the pigment glands of the seed and during mechanical processing the gossypol is released and reacts with the amino groups of lysine, rendering it unavailable. According to Liu *et al*. (2007) gossypol was effectively extracted from cottonseed prepressed cake or flakes using 20-30% (by wt) of ethyl alcohol (90% in vol) with commercial hexane as a mixed solvent. It has also been reported that iron, as ferrous sulphate, has been successfully used to counteract the toxicity of free gossypol in diets of monogastric, terrestrial animals (Jones, 1987; Martin, 1990). Hertrampf and Piedad-Pascual (2000) and El-Saidy and Gaber (2004) further confirmed that iron salts (particularly ferrous sulphate) are most effective in blocking the toxic effects of gossypol and it can completely be eliminated by including iron on a 1:1 weight ratio of iron to free gossypol when its level is above 100 ppm. As a preventive measure it is recommended that 0.05% iron (0.25% ferrous sulphate) should be incorporated in all diets containing CSM, to block the toxic effects of gossypol in the organism (Hertrampf and Piedad-Pascual, 2000).

There is a paucity of information on the use of detoxified oilseed meal mixtures by heat processing and phytase and ferrous sulphate supplementation in tilapia diets, although work has been done mainly on including individual processed ingredients. Considering the reduced performance observed in Nile tilapia fed diets containing unprocessed SBM, CSM and GNC mixtures in the previous experiments, the present study attempted to detoxify (i.e. reduce or eliminate the ANFs from) these oilseed meals/cake by using simple methods such as heat processing (i.e. autoclaving), phytase and ferrous sulphate

supplementation and evaluate their nutritive value and cost-effectiveness in the diet of juvenile Nile tilapia.

7.2 Materials and Methods

7.2.1 Experimental System and Animals

Twenty Nile tilapia fingerlings of average weight of 2.46 ± 0.12 g were stocked in triplicate in 30-L tanks. The rearing conditions maintained in this experiment are described in Section 2.1.1. Water quality parameters were measured every week during the experiment and the mean values (± SD) were as follows: temperature, 26.41 ± 0.35 ^{o}C; pH, 7.23 ± 0.13 ammonia, 0.02 ± 0.02 mg.L^{-1}; Nitrate, 20 ± 0.0 mg.L^{-1} and dissolved oxygen, 6.26 ± 0.26 mg.L^{-1}. The experiment lasted 8 weeks during which fish were hand-fed three times a day (09:00, 13:00 and 16:00) at a rate of 6% of their body weight per day for the first three weeks and 4% for the subsequent weeks (feeding rates were adjusted every week).

7.2.2 Detoxification of Oilseed Meals

Proximate composition, trypsin inhibitors, phytic acid and saponins of oilseed meals were analysed before and after processing (methods are described in Section 2.2.6). ANFs were detoxified in the meals using the methods described below.

Heat Processing – SBM, CSM and GNC were heat treated using an autoclave to eliminate completely or reduce trypsin inhibitors which are common to all the oilseed meals. Samples (500 g) were put in autoclave bags and the bags loosely tied were heated in an autoclave at 172,253 Pa, 121^{o}C for 30 minutes

(Norton, 1991; Arndt *et al*., 1999). Samples were then dried at 40^{o}C using an electric fan convector heater and later cooled to room temperature.

Enzyme Supplementation - Microbial phytase (P1259, Sigma) was supplemented to diets at 0.5 g.kg^{-1} (500 FTU.kg^{-1}) as recommended by Cheng *et al*. (2004). One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1 micromol of inorganic phosphorus per minute from 0.0051 mol.L^{-1} sodium phytate at pH 5.5 and 37°C (Engelen *et al*., 1994). The phytase was first dissolved in distilled water (100 ml) then added to the ingredients and mixed together. The diets were cold pelleted at a temperature not exceeding 40^{o}C to avoid denaturation of phytase following the method used by Portz and Liebert (2004). Pellets were dried in a ventilated cabinet at 35 – 40^{o}C for 24 hours followed by cooling to room temperature. The pellets were then stored in sealed plastic bags at -20^{o}C until used.

Iron Supplementation - Ferrous sulphate (BDH 101124V) was incorporated in the diets at 0.25%, to block the toxic effects of gossypol in the fish as recommended by Hertrampf and Piedad-Pascual (2000) and El-Saidy and Gaber (2004).

7.2.3 Diet Formulation and Preparation

Seven isonitrogenous and isoenergetic diets were formulated using SBM, CSM, and GNM as protein sources. The control diet was prepared with FM as the sole source of protein. For the plant-based diets, FM was substituted with equal mixtures of the detoxified meals (by heat and/or addition of supplements) at 50% of total protein as used in experiment 4. The treatments and their designations used in this experiment are presented in Table 7.1. Diet

preparation and other ingredients used were similar to those described in Sections 2.2 and 4.2.4. Formulation of experimental diets is presented in Table 7.2.

Table 7.1 Treatments and designations of experimental diets used in this study

Diet no.	Designation	Treatment	Purpose
1	Control	Fish meal	sole protein source
2	AQ	Autoclaved	reduce trypsin inhibitors only
3	AQP	Autoclaved + Phytase	reduce trypsin inhibitors and phytic acid
4	AQF	Autoclaved + Ferrous sulphate	reduce trypsin inhibitors and gossypol
5	QPF	Phytase + Ferrous sulphate	reduce phytic acid and gossypol
6	AQPF	Autoclaved + Phytase + Ferrous sulphate	reduce all three ANFs
7	AQPFM	Autoclaved + Phytase + Ferrous sulphate + Methionine	reduce all three ANFs and EAA supplemented

ANFs = Antinutritional factors

7.2.4 Faeces Collection

Faeces collection was undertaken as described in Section 4.2.2 (also see section 2.1.2 for details). Apparent digestibility coefficients for nutrients, energy and phosphorus of diets were determined as described in Section 2.3.5.

7.2.5 Analytical Techniques

Ingredients, diets, faeces and carcass samples were analysed for their proximate composition by the methods described in Section 2.2.1. Energy and phosphorous contents of diets, faeces and carcass were analysed by methods described in Sections 2.2.4 and 2.2.5. Chromic oxide contents of diets and faeces were determined by the method in Section 2.2.3.

Table 7.2 Composition of diets fed to juvenile *O. niloticus* ($g.kg^{-1}$) using detoxified oilseed meals in experiment 5

Ingredients	Control 1	AQ 2	AQP 3	AQF 4	QPF 5	AQPF 6	AQPFM 7
ASBM	0.0	94.3	94.3	94.3	0.0	94.3	94.3
ACSM	0.0	117.9	117.9	117.9	0.0	117.9	117.9
AGNM	0.0	115.7	115.7	115.7	0.0	115.7	115.7
SBM	0.0	0.0	0.0	0.0	100.2	0.0	0.0
CSM	0.0	0.0	0.0	0.0	113.5	0.0	0.0
GNM	0.0	0.0	0.0	0.0	116.4	0.0	0.0
FM	420.0	210.0	210.0	210.0	210.0	210.0	210.0
Wheat Grain	203.0	206.0	206.0	206.0	206.0	206.0	206.0
Sunflower Oil	57.0	48.2	48.2	48.2	47.0	48.2	48.2
α- Cellulose	30.0	11.9	11.9	14.4	13.4	14.4	13.9
Corn Starch	205.0	111.0	111.0	106.0	106.0	106.0	101.5
Binder CMC[1]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Mineral Premix[2]	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin Premix[3]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Chromic Oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL-Methionine[4]	0.0	0.0	0.0	0.0	0.0	0.0	5.0
Ferrous Sulphate[5]	0.0	0.0	0.0	2.5	2.5	2.5	2.5

ASBM = autoclaved soybean meal, ACSM = autoclaved cottonseed meal, AGNM = autoclaved groundnut meal, SBM = unprocessed soybean meal, CSM = unprocessed cottonseed meal, GNM = unprocessed groundnut meal, FM = fish meal, [1]Carboxymethyl cellulose (Sigma, C5013), [2]As listed in Table 2.1, [3]As listed in Table 2.2, [4]DL-mehionine (Sigma, M9500) was supplemented at 0.5%, [5](BDH, 101124V)

7.2.6 Analysis of Experimental Data

The growth performance and feed utilization of fish were calculated as described in Section 2.3 and 3.2.5. At the end of the experiment, 20 fish were randomly selected from each treatment, including the control, and euthanized by overdose of benzocaine, dissected and livers and intestines removed, weighed and used to estimate the hepatosomatic index (HSI) (Section 2.3.6).

7.2.7 Statistical analysis

Data was analysed as described in Section 4.2.5.

7.3 Results

7.3.1 Chemical Composition of Processed Oilseed Meals and Experimental Diets

The proximate composition, energy, trypsin inhibitor (TIs), phytic acid and saponin levels in the heat processed and unprocessed oilseed meals are presented in Table 7.3. There were only minor changes in proximate composition between the heat processed and unprocessed ingredients and they were not in any particular order. Phytic acid and saponin contents of ingredients also showed no differences. However, TI levels in oilseed meals were reduced by between 75.20% and 78.57% after heating.

Table 7.3 Proximate composition ($g.kg^{-1}$ as-fed), energy ($kJ.g^{-1}$) and antinutritional factors ($g.kg^{-1}$) of heat processed (autoclaved) and unprocessed test ingredients used in experiment 5

Components	SBM	ASBM	CSM	ACSM	GNC	AGNC
Dry matter	894.2	952.9	902.9	960.7	924.0	969.2
Crude protein	500.3	511.9	441.4	425.2	430.5	433.3
Crude lipid	10.1	8.6	32.3	26.4	219.0	215.3
Ash	58.9	61.4	77.1	77.1	43.8	43.6
Gross energy	20.19	19.57	19.61	19.27	23.17	22.88
Trypsin inhibitors	14.09	3.00	1.24	0.31	2.34	0.51
% reduction of TI	-	78.57	-	75.20	-	77.98
Phytic acid	17.54	17.24	31.64	28.65	14.86	14.87
Saponin	5.80	5.01	6.50	6.33	8.01	8.88

ASBM = autoclaved soybean meal, ACSM = autoclaved cottonseed meal, AGNC = autoclaved groundnut cake, SBM = unprocessed soybean meal, CSM = unprocessed cottonseed meal, GNC = unprocessed groundnut cake

The proximate composition, energy and phosphorous contents of experimental diets are presented in Table 7.4. The crude protein contents were similar with very little variation between the diets (318.4 - 332.2 $g.kg^{-1}$) as for crude lipid (98.3 – 105.9 $g.kg^{-1}$). Energy levels in all experimental diets were very similar. Crude fibre content of the control diet was lower (18.3 $g.kg^{-1}$) than that of the oilseed meal based diets (27.4 – 32.5 $g.kg^{-1}$). The control diet had higher ash and phosphorus levels than the oilseed meal based diets Table 7.4.

Table 7.4 Proximate composition (g.kg^{-1} as-fed), energy (kJ.g^{-1}), phosphorous (g.kg^{-1}) and antinutritional factors (g.kg^{-1}) of diets used in the experiment 5

Components	Control	PQ	PQP	PQF	QPF	PQPF	PQPFM
	1	2	3	4	5	6	7
Dry matter	964.7	964.1	915.8	951.8	923.8	918.6	927.0
Crude protein	324.0	329.5	318.4	332.2	320.9	320.6	326.9
Crude lipid	104.9	105.9	98.3	102.7	101.2	101.7	103.3
Crude fibre	18.3	29.1	32.4	30.8	27.4	32.5	31.1
Ash	99.6	90.4	84.8	88.3	86.3	85.6	86.3
Chromic oxide	4.8	5.0	4.6	4.7	4.7	4.7	4.7
Gross energy	18.86	19.19	19.20	19.10	19.10	19.20	19.32
Phosphorus	8.68	7.45	7.33	7.40	7.56	7.58	7.32
Phytic acid	0.6	7.3	7.3	7.3	7.6	7.3	7.3
Trypsin inhibitors	0.0	0.4	0.4	0.4	1.8	0.4	0.4
Gossypol	0.0	0.7	0.7	0.7	0.6	0.7	0.7
Saponin	1.1	3.4	3.4	3.4	3.4	3.4	3.4

7.3.2 Growth performance

Growth performance and feed utilization of Nile tilapia fingerlings fed the experimental diets are presented in (Table 7.5) and shown in Figure 7.1. The growth performance (SGR and WG) of fish in this study did not significantly differ ($P > 0.05$) for fish fed the control diet and all the oilseed meal based diets, though values for the control diet were higher. FCRs of Diets 2 and 5 were not significantly different from the control diet but that of Diets 3, 4, 6 and 7 were significantly higher. ER of all diets with the exception of Diet 3 were not significantly different from the control diet. Protein utilization (i.e. PER and PPV) of fish fed Diets 2, 5 and 6 was not different from the control (with the exception of Diet 6 in the case of PPV) however, that of Diets 3, 4 and 7 were lower than that of the control.

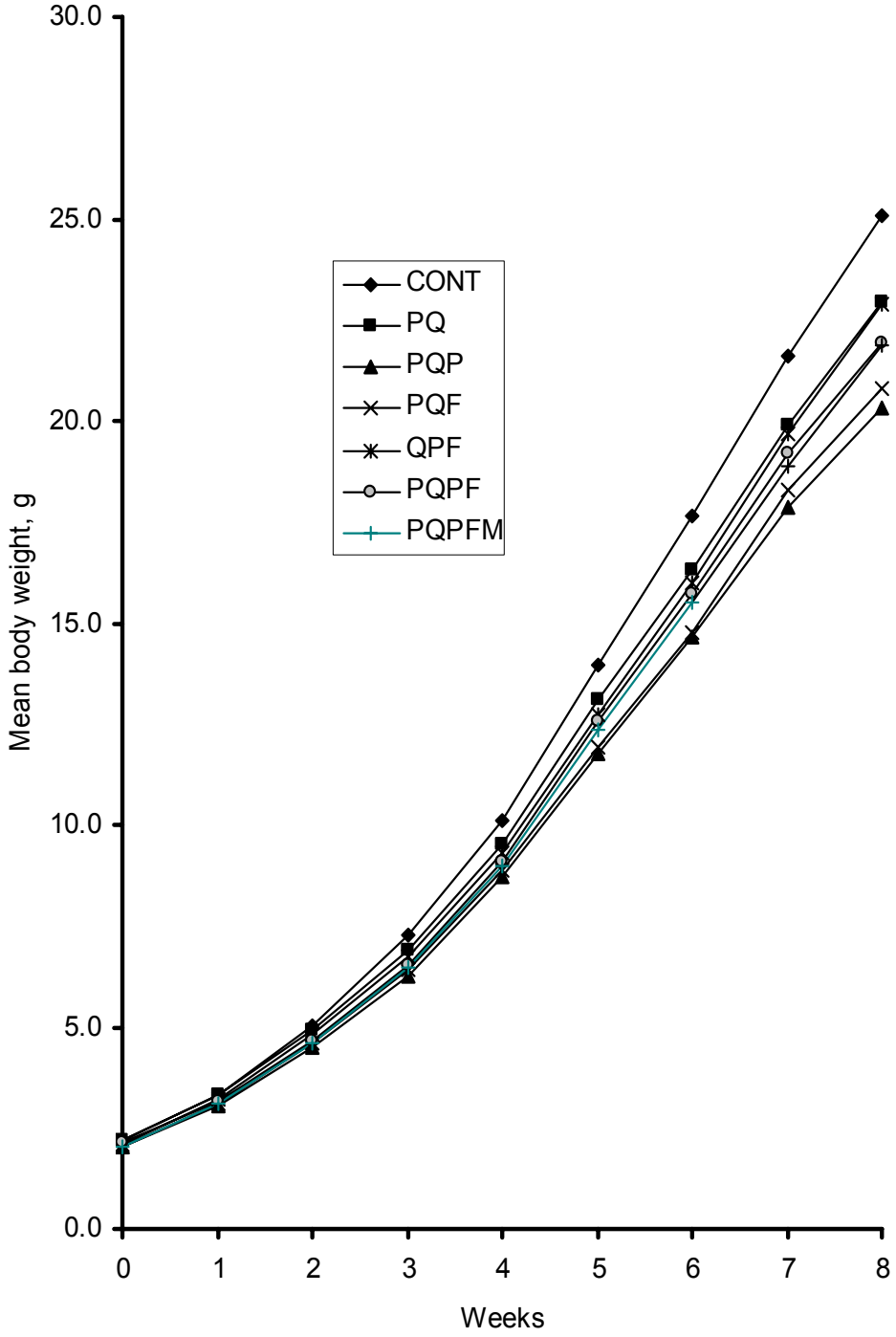

Figure 7.1 Growth response of fish fed detoxified oilseed meal based diets for eight weeks

Table 7.5 Growth performance and feed utilization of Nile tilapia fingerlings fed detoxified oilseed meal based diets in experiment 5

Parameters	Control	PQ	PQP	PQF	QPF	PQPF	PQPFM
	1	2	3	4	5	6	7
IW	2.17 ± 0.20	2.18 ± 0.26	2.01 ± 0.22	2.03 ± 0.20	2.09 ± 0.13	2.12 ± 0.14	2.09 ± 0.33
FW	25.09 ± 0.74^{a}	22.93 ± 0.20^{ab}	20.27 ± 1.52^{c}	20.78 ± 1.30^{bc}	22.89 ± 1.04^{abc}	21.92 ± 0.22^{bc}	21.85 ± 0.82^{bc}
WG	1062.85 ± 139.50	962.21 ± 130.36	915.30 ± 126.20	928.99 ± 122.68	998.13 ± 114.03	937.30 ± 78.60	1001.88 ± 153.06
SGR	4.37 ± 0.21	4.21 ± 0.22	4.13 ± 0.22	4.15 ± 0.21	4.27 ± 0.19	4.17 ± 0.14	4.27 ± 0.25
S	100.00 ± 0.00	100.00 ± 0.00	98.33 ± 2.89	98.33 ± 2.89	100.00 ± 0.00	98.33 ± 2.89	100.00 ± 0.00
FCR	1.13 ± 0.04^{a}	1.23 ± 0.02^{ab}	1.40 ± 0.12^{b}	1.37 ± 0.10^{b}	1.24 ± 0.07^{ab}	1.29 ± 0.03^{b}	1.29 ± 0.04^{b}
FI	25.83 ± 0.01^{a}	25.59 ± 0.06^{b}	25.51 ± 0.08^{b}	25.67 ± 0.09^{ab}	25.79 ± 0.04^{a}	25.51 ± 0.09^{b}	25.52 ± 0.01^{b}
PER	2.74 ± 0.10^{a}	2.46 ± 0.05^{ab}	2.25 ± 0.18^{b}	2.20 ± 0.16^{b}	2.51 ± 0.14^{a}	2.42 ± 0.05^{ab}	2.38 ± 0.07^{b}
PPV	42.71 ± 2.00^{a}	38.30 ± 1.19^{ab}	34.10 ± 2.79^{b}	33.34 ± 2.94^{b}	38.67 ± 2.11^{ab}	36.07 ± 0.22^{b}	36.42 ± 0.51^{b}
ER	31.32 ± 2.44^{a}	28.71 ± 1.05^{ab}	24.82 ± 3.25^{b}	26.21 ± 2.56^{ab}	29.50 ± 2.56^{ab}	27.15 ± 1.81^{ab}	27.88 ± 0.43^{ab}

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 (%) = Apparent energy retention. Values are means ± SD of three replicates, and values within the same row with different letters are significantly different ($P < 0.05$)

7.3.3 Apparent Nutrient Digestibility

Apparent protein and lipid digestibilities varied slightly among diets ranging from 88.58% to 91.55% and 98.27% to 99.3% respectively (Table 7.6). Apparent energy and phosphorus digestibilities were similar for Diets 3, 5, 6, 7 and the control but those of Diets 2 and 4 were slightly lower. Generally, apparent nutrient digestibility of the control diet was higher than that of the oilseed meal based diets with the exception of lipid and phosphorus which were slightly lower. Between the test diets it was observed that those supplemented with microbial phytase (Diets 3, 5, 6 and 7) had higher apparent protein, energy and phosphorus digestibilities than those without phytase (Diets 2 and 4) (Table 7.6).

Table 7.6 Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy, phosphorus and digestible protein and energy ($g.kg^{-1}$ and $kJ.g^{-1}$ respectively, dry weight basis) in the test diets for Nile tilapia

Components	Control	PQ	PQP	PQF	QPF	PQPF	PQPFM
	1	2	3	4	5	6	7
DM	82.02	76.42	81.45	78.54	81.85	80.66	81.12
CP	91.55	88.58	90.21	89.77	90.77	89.71	90.37
CL	98.27	99.09	99.30	99.29	98.85	99.11	99.18
GE	84.44	79.22	83.64	81.09	84.05	82.85	83.39
P	81.46	72.88	79.14	70.39	82.10	82.07	83.44
DP	307.5	302.7	313.6	313.3	315.3	313.1	318.7
DE	15.93	15.20	16.06	15.49	16.05	15.91	16.11

DM = dry matter, CP = crude protein, CL = crude lipid, CF = crude fibre, GE = gross energy, P = phosphorous, DP= Digstible protein, DE = Digestible energy

7.3.4 Body Composition

There were no significant differences ($P > 0.05$) in whole body protein, moisture, lipid and energy contents among the diets and the control at the end of the feed trial with the exception of ash content of Diet 2 which was higher than the control (Table 7.7). With the exception of moisture and ash contents of initial whole body composition of fish which were higher, all the other components (i.e. CP, CL and GE) were lower than that of the test diets.

Table 7.7 Whole body proximate composition (% wet weight) of Nile tilapia fed detoxified oilseed meal based diets after experiment 5

	Initial	Control	PQ	PQP	PQF	QPF	PQPF	PQPFM
		1	2	3	4	5	6	7
MC	76.23	72.53 ± 0.55	72.81 ± 0.66	73.01 ± 0.82	72.85 ± 0.50	72.23 ± 0.55	72.95 ± 0.79	72.20 ± 0.79
CP	14.02	15.45 ± 0.17	15.41 ± 0.34	15.06 ± 0.63	15.04 ± 0.42	15.27 ± 0.34	14.82 ± 0.26	15.21 ± 0.44
CL	4.32	7.22 ± 0.72	7.61 ± 0.42	7.44 ± 0.99	8.02 ± 0.50	8.20 ± 0.68	7.75 ± 0.82	8.02 ± 0.52
Ash	4.37	3.99 ± 0.14^{a}	3.32 ± 0.16^{b}	3.67 ± 0.04^{a}	3.40 ± 0.09^{ab}	3.58 ± 0.08^{ab}	3.57 ± 0.19^{ab}	3.66 ± 0.03^{a}
GE	5.01	6.51 ± 0.25	6.63 ± 0.23	6.48 ± 0.34	6.66 ± 0.19	6.80 ± 0.22	6.55 ± 0.35	6.76 ± 0.26
HSI	-	3.67 ± 0.51^{a}	3.12 ± 0.39^{b}	3.43 ± 0.62^{ab}	3.64 ± 0.30^{a}	3.05 ± 0.44^{b}	3.24 ± 0.55^{ab}	3.32 ± 0.44^{ab}

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

7.3.5 Cost-benefit Analysis of Diets

The costs of ingredients used in this analysis are presented in Table 3.2. The cost per kilogram of experimental diets varied drastically due to the different treatments with Diet 7 having the highest (¢10.74 kg^{-1}) and Diet 2 the least (¢0.46 kg^{-1}). Prices of phytase and ferrous sulphate were from Sigma, Germany (2007) but based on the assumption that feed grade would be about half. The cost analysis revealed that the highest profit was obtained by Diet 2 followed by the control diet, Diet 4 and the lowest by Diet 3 (PQP).

Table 7.8 Cost analysis of diets fed to *O. niloticus* in experiment 5

Diet	Diet cost[1]	Incidence cost[1]	Profit index
1. Control	0.56	0.63	3.16
2. PQ	0.46	0.57	3.52
3. PQP	10.46	14.62	0.14
4. PQF	0.61	0.84	2.38
5. QPF	10.57	13.10	0.15
6. PQPF	10.61	13.68	0.15
7. PQPFM	10.74	13.81	0.15

[1]¢.kg^{-1}, Cost of heating = ¢0.05 kg^{-1}, Phytase = ¢20,000.00 kg^{-1}, Ferrous sulphate = ¢30.00 kg^{-1}, DL-methionine = ¢25.00 kg^{-1}, Exchange rate ¢0.90 = USD 1.00)

7.4 Discussion

In the present study, heat processing (autoclaving) did not significantly affect proximate composition, energy, phytic acid or saponin contents of the oilseed meals (Table 7.3). This suggests that there was no major effect of heat processing on these ingredients. Reports by other researchers also indicated no significant effect of heat processing on some plant products (Hossain, 1988; Osman, 2007). Heat processing, however, reduced TIs in oilseed meals by between 75.20% and 78.57% (Table 7.3). There have been similar reports that heat-labile factors, including trypsin inhibitor and lectins, can be eliminated or reduced by heat treatment during processing (McNaughton *et al*., 1981; Arndt *et al*., 1999; Francis *et al*., 2001; Raj Bhandari and Kawabata, 2006). Arndt *et al*. (1999) reported a reduction of 99% TI when SBM was autoclaved at 172,253 Pa, 121 ^{o}C for 20 min and Borchers *et al*. (1947) reported a similar reduction of TI at 141,855 Pa. Since autoclaving in this study reduced TI by almost 80% in the test ingredients, it is likely that other heat-labile ANFs in the oilseed meals might have been reduced as well.

Generally, growth and feed utilization of fish in this study was significantly improved compared to all previous studies although feed intake was the lowest. This experiment indicates that there was no significant difference in growth performance of Nile tilapia fed all the oilseed meal based diets compared with the control diet (Table 7.5). However, feed utilization (FCR, PER and PPV) in fish fed Diets 3, 4, 6 and 7 was significantly lower than for the control diet but not different from the other oilseed meal based diets. The results suggest that heat treatment alone (Diet 2), i.e. reducing heat-labile ANFs, improved growth and feed utilization in Nile tilapia. This compares with reports by Arndt *et al*.

(1999) and Peres *et al*. (2003) who indicated that Coho salmon and channel catfish respectively fed diets containing heat-treated SBM gained more weight than those fed untreated SBM. Borgeson *et al*. (2006) also reported superior growth performance in Nile tilapia when fish meal was replaced with a mixture of heat processed plant ingredients (i.e. SBM, maize gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) compared with unprocessed SBM and corn gluten meal.

Diets 3, 5, 6 and 7 with phytase supplementation also performed well. Furuya *et al.* (2001), Portz *et al.* (2003) and Portz and Liebert (2004) observed that growth and feed efficiency in Nile tilapia were improved by phytase supplementation. Sugiura *et al.* (2001) and Forster *et al.* (1999) also reported similar results in trout, Schäfer *et al*. (1995) in carp and Jackson *et al*. (1996) in channel catfish fed diets supplemented with as little as 500 $FTU.kg^{-1}$ diet phytase. Growth and feed utilization of fish fed Diet 5 (composed of unprocessed oilseed meals with phytase and ferrous sulphate) was not significantly different from the Control diet. This suggests it could not have been heat-labile ANFs (particularly TIs) alone which caused poor growth and feed utilization in Nile tilapia in the previous experiments but phytic acid and possibly gossypol. El Saidy and Gaber (2004) reported better final body weight and SGR of tilapia fed CSM based diets supplemented with ferrous sulphate than those without, indicating that ferrous sulphate was efficient in blocking the effects of gossypol. Early studies have indicated that the amount of CSM that can be used in Nile tilapia feed depends mainly on the level of free gossypol and available lysine content of the meal. Ofojekwu and Ejike (1984) and Robinson *et al.* (1984) found that *O.*

aureus fed CSM-based diets performed poorly. The authors attributed poor performance to the gossypol contained in CSM.

Fish fed Diet 7 (with heat processed and supplementation of phytase, ferrous sulphate and DL-methionine) showed growth that was not different to those fed the control diet, but protein utilization and FCR were significantly lower than for the control diet (Table 7.5). Despite amino acid supplementation in Diet 7, it did not perform better than the other oilseed meal based diets as was expected. This corroborates results from experiment 4 where a similar DL-methionine supplementation was not effective in improving growth and feed utilization to the level of the fish meal based diet. Therefore, improvement of the nutritional value of Diet 7 could mostly be attributed to reduction of ANFs in the diet. In the present study generally all the different treatments (i.e. heat processing and supplementation with phytase and ferrous sulphate) led to improvement in growth and feed utilization although feed utilization of some of them were significantly lower than the control. This suggests that reduction in any one or two of the (i.e. heat-labile or heat-stable) ANFs in the oilseed meals through detoxification by heat processing and use of microbial phytase and ferrous sulphate might have improved their nutritional value as indicated by other researchers (Bureau *et al*., 1998; Drew *et al*., 2007; El-Saidy and Gaber, 2004; Hendriks *et al*., 1990; Portz *et al*., 2003; Refstie *et al*., 1998; Rumsey *et al*., 1993).

Generally all treatments of the oilseed meal based diets in this study showed high apparent nutrient digestibilities, however, diets (3, 5, 6 and 7) supplemented with phytase had higher ADC of crude protein, energy,

phosphorus and dry matter compared to those without (this confirmed the fact that the phytase in the diets was active, since phytase activity was inadvertently not analysed before feeding the diets to the fish). The positive effect of phytase supplementation on ADC of nutrients (phosphorus in particular) has been observed in Nile tilapia (Borgeson *et al*., 2006; Furuya *et al*., 2001; Portz *et al*., 2003; Portz and Liebert, 2004), salmonids (Cain and Garling, 1995), rainbow trout (Lanari *et al*., 1998; Sugiura *et al*., 2001) and Korean rockfish (Yoo *et al*., 2005). This result is also in agreement with a number of studies which have shown that phytase supplementation increased digestibility of crude protein and amino acids (Cheng *et al*., 2004; Ramseyer *et al*., 1999; Riche and Garling, 2004; Sugiura *et al*., 2001; Vielma *et al*., 2000).

Carcass composition was not affected by dietary treatments at the end of the feed trial (Table 7.7). The MC, CP, CL, ash and energy contents of whole body of Nile tilapia were not significantly different between the dietary treatments and the control with the exception of ash of fish fed Diet 2, which was significantly lower than the control. However, there was significant increase in CP, CL and energy in comparison with the initial carcass values in all the dietary treatments. Similarly, Polat (1999) in *T. zillii*, El-Saidy and Gaber (2003) in Nile tilapia, Nyina-wamwiza *et al*. (2007) in *Clarias gariepinus* and Regost *et al.* (1999) in turbot did not find any effects of dietary mixtures of plant protein on whole fish body composition.

Results from cost effective analysis revealed that the more treatments the diets underwent the higher the cost per kilogram, particularly supplementation of phytase drastically increased diet cost because it was very expensive (Table

7.8). It was realized from the result that only the heat processed oilseed meal mixture diet (Diet 2) was more profitable than the control diets. All the other diets had very low PIs (0.14 – 0.15) with the exception of Diet 4, which had a relatively higher value (2.3). Cheaper phytase and ferrous sulphate supplements could improve cost effectiveness of the diets particularly Diet 4. The economic superiority of Diet 2 in this study could be attributed to the fact that it was the cheapest diet since it underwent only heat processing and also because it was one of the diets (apart from Diet 5) which performed very well in terms of growth and nutrient utilization compared with the control diet.

Based on the growth performance and nutrient utilization, it could be concluded that Diet 2 (50% equal mixtures of heat processed oilseed meals/cake) and Diet 5 (50% equal mixtures of unprocessed oilseed meals with phytase and ferrous sulphate supplementation) are the best and could replace the fish meal based control diet. However, in terms of cost-effectiveness it is only Diet 2 which is more superior to the control diet. The study revealed that simple heat processing alone (i.e. autoclaving) of the oilseed meals in the diet of juvenile Nile tilapia may improve their utilization and cost-effectiveness.

Chapter 8 - General Conclusions and Recommendations

One of the major problems faced by aquaculture in Ghana is the non-availability of quality and affordable fish feeds in contrast to quality commercial poultry feeds that are readily available. The traditional feed mixture employed in the culture of tilapia is mostly supplementary and unbalanced. If aquaculture is to thrive in Ghana, nutritionally sound and cost-effective feeds based on local agricultural by-products that can support increased production levels in both intensive and especially semi-intensive systems commonly used need to be developed since fish meal (FM), the traditional protein source, is in short supply and very expensive. Amongst the feed ingredients identified in Ghana based on the their quality, availability, affordability and supply, the most promising alternatives to fish meal in (juvenile) tilapia diets are the oilseed meals/cakes namely; soybean meal (*Glycine spp*), cottonseed meal (*Gossypium spp*.) and groundnut cake (*Arachis hypogaea* L.).

Although, tilapia diets these days contain less fish meal about 5% (little, personal communication) and mostly contain plant ingredients this study compared oilseed protein to fish protein because the author agrees with Drew *et al.* (2007) who stated that fish meal is the “gold standard” to which plant proteins must be compared in terms of protein quality, fish growth performance, health and cost.

The main objective of this study was to investigate the nutritive value of the selected oilseed meals/cakes and their suitability as alternative protein sources

to fish meal in the diet of Nile tilapia (*Oreochromis niloticus* L.). Investigations included:

- quantification of the proximate composition, amino acid composition, gross energy and important antinutritional factors (ANFs) present in these oilseed meals/cakes;

- determination of apparent nutrient digestibility coefficients for these oilseed meals for Nile tilapia;

- study of the nutritive value of individual oilseed meals as partial substitutes for fish meal protein on fish growth and feed utilization;

- evaluation of the effect of various mixtures/combinations of the oilseed meals on fish growth and feed utilization;

- evaluation of the effect of dietary methionine supplementation of diets containing mixtures of the oilseed meals on growth and feed utilization;

- detoxification of these oilseed meals by heat processing and addition of supplements to the diets ;

- assessment of cost effectiveness of the formulated diets for Nile tilapia

Major conclusions from the study are as follows:

1. Protein contents of SBM, CSM and GNC used in this research were fairly high and values ranged from 500.3 $g.kg^{-1}$, 441.4 $g.kg^{-1}$ and 430.5 $g.kg^{-1}$

respectively, which qualifies them as good protein sources, but that of GNH was low 205.6 $g.kg^{-1}$. Generally the oilseed meals had good essential amino acid profiles with the exception of GNH. The EAA profile of SBM compared very well with fishmeal but methionine and threonine were quite low (0.73 and 1.50 % of protein respectively). The same was true for CSM and GNC which had even lower values of methionine and threonine as well as lysine. Crude fibre contents of the oilseed meals varied drastically with levels as high as 89.5 $g.kg^{-1}$ and 89.2 $g.kg^{-1}$ for CSM and GNH respectively, more than double that of SBM (38.2 $g.kg^{-1}$) and about seven times higher than GNC which had the lowest (12.8 $g.kg^{-1}$). Gross energy for the oilseed meals was between 19.61 $kJ.g^{-1}$ and 23.17 $kJ.g^{-1}$. Important ANFs analyzed in the oilseed meals were as follows: phytic acid content of CSM was highest (31.64 $g.kg^{-1}$) about double that of SBM (17.54 $g.kg^{-1}$) and GNC (14.86 $g.kg^{-1}$) and almost ten fold that of GNH (3.99 $g.kg^{-1}$). With regards to trypsin inhibitors SBM contained the highest (14.09 $g.kg^{-1}$) and CSM the lowest (1.24 $g.kg^{-1}$). Saponin content of ingredients ranged from 10.08 – 5.61 $g.kg^{-1}$ and gossypol was 5.6 $g.kg^{-1}$ for CSM.

2. Nutrient digestibility studies revealed that Nile tilapia may be able to utilize SBM, CSM and GNC efficiently as dietary protein sources due to high apparent protein digestibilities of 94.50%, 84.93% and 90.01% respectively. Generally, nutrient digestibility of SBM was clearly the highest followed by GNC and CSM. GNH, however, may not be suitable because of very low apparent protein digestibility of 27.67%. Apparent energy and phosphorus digestibilities followed the same trend with SBM

having the highest (85.99% and 64.31% respectively) and GNH the lowest (34.67% and 30.38% respectively). Lower nutrient digestibility of GNH and particularly CSM was attributed to high crude fibre and phytic acid levels.

3. Evaluation of the oilseed meal proteins (SBM, CSM and GNC) individually as partial substitutes for fish meal protein at various protein substitution levels (i.e. 25%, 50% and 75%) in Nile tilapia diets demonstrated that they can be used at levels up to 50% of the FM protein without any adverse effect on growth and feed efficiency. Higher inclusion levels led to growth depression in fish, which may be attributed to high levels of ANFs, high fibre content and poor essential amino acid profile. However, there were no histopathological changes in liver and intestine related to dietary treatments.

4. Combination of oilseed meals in different proportions was more effective than the single individual sources. Growth and feed utilization of fish fed the oilseed meal mixtures showed that up to 50% replacement could be more effective than a single source in the substitution of fish meal in tilapia diets. This was particularly evident with the diet containing equal proportions of all oilseed meals (EQ50). This could be due to a compensatory effect which led to some reduction of antinutritional factors and improved essential amino acid profile in the diet as a result of mixing.

5. All oilseed meal based diets, even after combining the different meals, were still deficient in one or more EAAs. Although growth depression in fish was believed to be principally due to the higher levels of ANFs, EAA supplementation (particularly methionine the most critical EAA for tilapia) was done to determine whether it would improve their nutritive value. The results showed that Nile tilapia can utilize crystalline methionine leading to improvement in feed utilization but without significantly improving the nutritive value compared with the FM protein.

6. Heat processing of the oilseed meals through autoclaving (intended to reduce heat labile ANFs) was very effective in reducing the trypsin inhibitors in SBM, CSM and GNC by 78.6%, 75.2% and 78.0% respectively but not phytic acid, saponins and or proximate composition which remained virtually unaffected. Detoxification of meals by heat processing and/or addition of supplements (viz. phytase and ferrous sulphate, meant to reduce the effects of phytic acid and gossypol respectively) at 50% inclusion in diets improved growth and feed utilization compared to the unprocessed meals used in previous experiments and was generally not significantly different from the FM diet. The best performing diets were those with heat processed meals only (PQ) and that supplemented with phytase and ferrous sulphate (QPF).

7. In terms of feed ingredient prices, SBM was the highest (¢0.48 kg^{-1}) followed by GNC (¢0.40 kg^{-1}), CSM (¢0.18 kg^{-1}) and GNH the lowest (¢0.04 kg^{-1}). These ingredients were cheaper than fish meal, which was

almost two and a half times more expensive (¢1.17 kg^{-1}) than SBM which was the most expensive among the oilseed meals. This therefore supports the need to use alternative cheaper protein sources as complete/partial substitutes for fish meal in formulation of diets for Nile tilapia. Cost effectiveness of all diets containing individual oilseed meals up to 50% and 75% in the case of CSM replacement were higher than FM diet. A similar trend was observed using the oilseed meal mixtures, where Diets EQ50, CSM50 and CSM75 had higher profit indices than the control. Cost analysis showed that methionine supplementation of diets was not cost effective compared to the control diet. Despite the improvement in feed utilization, methionine supplementation could not justify the additional diet costs since cost effectiveness was lower. With regards to detoxification, only the heat processed diet (PQ) among all the diets was more profitable than the control diet, which suggests that simple heat processing alone of the oilseed meals in the diet of juvenile Nile tilapia could improve their utilization and cost-effectiveness.

From the studies increased growth and feed utilization of fish was achieved through various ingredient improvement methods in the diet of Nile tilapia. Combination of oilseed protein at inclusion levels of 50% and 75% replacing fish meal protein (in experiment 3) increased specific growth rate from an average of 2.4 to 3.1%.day^{-1} and 1.75% to 2.6 %.day^{-1} and weight gain increased by 185.0% and 165.0% respectively compared with that of single plant protein source in experiment 2 (Table 4.5 and Table 5.5). Feed conversion ratio (FCR) did not show any significant difference between the experiments. Supplementing with methionine (in experiment 4) only had slight increase in

growth (0.1%) but FCR improved from 2.5 to 1.9. Finally, an average growth rate of 4.2 %.day^{-1}, weight gain of 957.3% and FCR of 1.3 were obtained from detoxification of ingredients in experiment 5. This obviously indicates that detoxification of ingredients indeed drastically improved growth and feed utilization of Nile tilapia.

In the present study extrapolated yield of the least performed diets (75% inclusion) and the best performed diets (50% inclusion) averaged between 7,265 kg.ha^{-1}.yr^{-1} and 15,133 kg.ha^{-1}.yr^{-1} respectively. Generally, yield of tilapia fingerlings is reported to vary from 7,000 kg.ha^{-1}.yr^{-1} to 14,000 kg.ha^{-1}.yr^{-1} in intensively managed ponds (Green and Engle, 2000), and all-male tilapia from 12,952 kg.ha^{-1}.yr^{-1} to 15,920 kg.ha^{-1}.yr^{-1} in fertlized ponds with formulated feed (Diana *et al.*, 1996). Despite using mixed-sex tilapia in this study yields achieved are comparable to that from commercial farming particularly the detoxified diets. Even though, 75% inclusion of oilseed meal based diets performed poorly as compared to the control and other test diets they yielded an average of 7,265 kg.ha^{-1}.yr^{-1}, which is more than double the current productivity of, 2,500 kg.ha^{-1}.yr^{-1} by Ghanaian farmers (Awity, 2005). This shows that the performance of these diets could more than double the current production of farmers and if used as supplementary feed in fertilised ponds could even be prepared without vitamin and mineral premixes to reduce their cost even further for resource poor farmers since there would be production of natural food to provide these nutrients. According to Shroeder (1980) natural food accounted for 50-70% of total available food for tilapia in pond culture even when complete diet is provided. Moreover, Diana *et al.* (1994) suggested that a

combination of feed and fertilizer is the most efficient in growing Nile tilapia compared to complete feeding or fertilisation alone.

From the performance of the oilseed meals, it was observed that although SBM was the most digestible followed by GNC and the lowest was CSM and GNH (Table 3.6), growth and cost-effectiveness of fish fed SBM based diets was not impressive (as purported by some authors). In some cases GNC and CSM based diets did better than SBM based diet. This gives an indication that Ghana does not need to rely on SBM as a protein source since they are more expensive and mostly imported (Hecht, 2007). Inclusion of GNH up to 20% performed very well although protein digestibility was not high. GNH contains high levels of lipid and carbohydrate (Table 3.2) and has good lipid digestibility (79.64%) so could be used as a source of lipid and to some extent energy in a compound as well as supplementary feed in a semi-intensive system. Since Nile tilapia is an omnivorous fish and can utilize variety of feeds when they are available they are considered as opportunistic and this provides an advantage to farmers because the fish can be reared in extensive situations that depend upon the natural productivity of a water body or in intensive systems that can be operated with lower cost feeds (Fitzsimmons, 1997).

It was realized that simple heat processing to eliminate heat labile antinutritional factors actually yielded the best result. This is important because it seems potentially and practically feasible in Ghana since heat processing is simple (and could be operated under field conditions), cheap and commonly in use in Ghana. Generally, cost analysis from the study revealed that the use of individual, mixtures/combinations and heat processed oilseed meals were more

profitable than using supplements (i.e. DL-methionine, phytase and ferrous sulphate) in the diet of Nile tilapia. The use of supplements in the diets added a lot of cost to feed production, to the extent that some oilseed meal diets were even more expensive than fish meal diets. This suggests that methionine supplementation and detoxification using phytase and ferrous sulphate may not be feasible in Ghana since these supplements are likely to be unavailable and unaffordable to the majority of farmers.

The present study has demonstrated that individual oilseed meals SBM, CSM and GNC, their equal mixtures and heat processed with/without supplements could be used as partial substitutes for fish meal protein in tilapia diets at levels up to 50% without sacrificing growth and feed utilization. Moreover, simple economic analysis indicated that feeding with oilseed based diets even at 75% (in the case of CSM) substitution was more profitable than fish meal based diets with the exception of those using supplements. However, it was observed that fish fed diets with oilseed protein would take longer to attain harvest size compared with FM protein, since growth and feed utilization were lower. This could probably lead to an increase in production costs or a decrease in the number of production cycles which could be achieved within a year. Generally, there is nutritional and economic justification for using SBM, CSM and GNC as partial replacement for FM in diets of Nile tilapia. Based on growth performance, nutrient utilization and economic benefits the diet with heat processed oilseed meal mixtures (containing equal proportions of 16.67% each) at 50% inclusion has the best prospects of replacing FM protein in the diets of *O. niloticus*.

Recommendations for Future Studies

1. The results of the present study indicate that SBM, CSM and GNC can partially substitute fish meal in tilapia diets at levels not more than 50% dietary protein without sacrificing growth and food utilization. However, there is a possibility that the juvenile tilapias used in this investigation are more sensitive to the antinutritional factors than larger fish would be. Therefore, long term studies should be conducted using fish of a larger size range than those used in the present study.

2. This study was carried out under laboratory conditions. It would be interesting to see if the laboratory conditions can be extrapolated to field conditions, especially the semi-intensive system commonly practised in ponds in Ghana. There is a possibility that better and practical results may be achieved due to contribution of natural food. Cottonseed meal and groundnut husk could further be studied as supplementary feed in Ghana since they performed creditably and were more cost-effective.

3. From the results it was observed that the higher the inclusion level of plant protein the longer it could take fish to reach marketable size since growth performance and feed utilization were lower. The additional days for culture could probably lead to increased production cost. It is therefore suggested that a more holistic economic analysis (eg. cost benefit analysis) be conducted taking into account all key factors in fish production (such as labour, power etc.), since the present study considered only feeding cost.

4. Other potential plant protein sources such as palm kernel cake, copra cake, cowpea cake and cocoa cake, which are also readily available and cheap in Ghana, should be similarly studied to develop a variety of plant protein mixtures as suitable alternatives to fish meal in the diets of Nile tilapia and other aquaculture species.

5. This study demonstrates that local or sub-regional agricultural by-products could provide nutritionally sound and cost-effective feeds to support increased fish production levels in both intensive and especially semi-intensive systems. In order to increase and sustain aquaculture production, there is the need to encourage use of the abundant locally available ingredients to develop low cost feeds and discourage import of very expensive formulated/pelletised feed from abroad.

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