Novel Feed Ingredients for Nile Tilapia (*Oreochromis niloticus* L.)

A thesis submitted for the degree of Doctor of Philosophy

Nazael Madalla
B.Sc Animal Science (Sokoine University of Agriculture)
M.Sc Aquaculture (Ghent University)

Institute of Aquaculture
University of Stirling
Scotland
United Kingdom

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to men and women who work tirelessly to improve livelihoods of the world's poor

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 acknowledged.

Nazael Madalla

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Abstract

Lack of affordable feeds is one of the major constraints facing small-scale fish farmers in Tanzania. This study evaluated the suitability of moringa leaf meal (MLM), cassava leaf meal (CLM) and cassava root meal (CRM) as novel ingredients in Nile tilapia, *Oreochromis niloticus* diets. Each of the ingredients was processed in an attempt to remove the most significant antinutritional factor. A series of five experiments was conducted in a recirculation system using juvenile *O. niloticus*. The fish were fed isonitrogenous (30g $100g^{-1}$), isolipidic (10g $100g^{-1}$) and isoenergetic (18 kJ g^{-1}) diets containing graded levels of the processed ingredients to their apparent appetite but not exceeding 10% of their body weight for a period of 8 weeks.

Processing led to the removal of 0.3% of saponin from MLM and 60% and 90% of hydrogen cyanide from CLM and CRM respectively. The contents of other inherent antinutritional factors such as phenols, tannins and phytic acid were little affected. Processed MLM, CLM and CRM had 31.1/29.0/1.5g $100g^{-1}$ of crude protein, 5.9/10.2/2.4g $100g^{-1}$ of crude fibre and 20.1/19.7/15.8k Jg^{-1} of gross energy. The content of sulphur amino acids was higher in CLM (0.47%) than in MLM (0.23%). Digestibe protein and digestible energy was higher in MLM (25.71g $100g^{-1}$/15.44kJ g^{-1}) than in CLM (12.71g 100^{-1}/9.16kJ g^{-1}). CRM had a digestible energy content of 13.5kJ g^{-1}.

Inclusion of either of the leaf meals, even at the lowest level of 15g $100g^{-1}$ of total dietary protein, led to a significant reduction in feed intake, growth and feed utilisation. Liver and small intestine did not show any histopathological changes which could be linked to dietary treatment. Conversely, cassava root meal could replace up to 75% of wheat meal in the diet without significantly affecting performance. The performance of leaf meals was marginally improved by a combination of blending and feeding stimulants, whereby a blend containing 1 part MLM and 2 parts CLM could provide up to 20g $100g^{-1}$ of dietary protein without significantly reducing performance. Biological and economic performance of practical diets containing 30-50g $100g^{-1}$ of dietary protein from moringa and cassava blends (LMB) with feeding stimulants was significantly lower than a fishmeal-meal based diet (FM) but comparable to a soybean meal-based diet (SBM).

The suitability of MLM and CLM as novel protein sources in *O. niloticus* diets will depend on 1) improving reduction/removal of inherent antinutritional factors in MLM and CLM as well as improving digestibility of CLM. On the other hand, the suitability of CRM as a carbohydrate energy source will depend on the availability of cost effective protein sources due to its low protein content.

Table of Contents

List of Tables

List of Figures

CHAPTER 1 General Introduction

1.1 Global Overview of Aquaculture

The Food and Agriculture Organisation of United Nations (FAO, 1990a) defines aquaculture 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".

Fish have been used for food, religious functions as well as a medium of exchange since time immemorial. Several engravings on Egyptian tombs dating as early as 2500 BC stand as testimony to the importance of fish in early civilization (Jhingram, 1987). The engravings apparently show tilapia being fished out of artificial drainable ponds. In Asia, and particularly China, freshwater carp culture has been practiced since approximately 1100 BC (Song, 1996). Around 460 BC Fan Li published what is believed to be the first monograph in aquaculture titled "On Pisciculture". The monograph outlined design and layout of fishponds as well as breeding and rearing of carp. In Europe, the Romans cultivated fish and shellfish over 2200 years ago; this is believed to be first practice of mariculture (Landau, 1992).

From a humble beginning, aquaculture has spread all over the world gradually transforming from a traditional practice into science. It is now the fastest growing animal producing sector with an average growth rate of 8.8% since 1970 outpacing capture fisheries (1.2%) and terrestrial farmed meat production (2.8%) (FAO, 2007b). Its contribution to global aquatic produce supply has increased continuously from 3.9% in 1970 to over 33.7% in 2005 amounting to almost 67 million metric tonnes with a value slightly over USD 86 million (FAO, 2007b). The bulk of global fish production, amounting to about 73 million metric tonnes, comes from developing countries with Asia contributing about 61 million metric tonnes (Figure 1.1). China is the largest producer and accounted for about 63% of production and 50% of value in 2006 (FAO, 2007b). In terms of continents, Africa has the lowest aquaculture production amounting to only about 1% of global production in 2006.

Global finfish production in 2006 amounted to about 33 million metric tonnes and was dominated by freshwater fish which amounted to about 28 million metric tonnes (FAO, 2007a). These freshwater fish are mostly composed of omnivorous/herbivorous or filter feeders cultured in low input-low cost systems varying from extensive to semi–intensive production systems in developing countries (FAO, 2002; Halwart *et al.,* 2003). A combination of fertilization and/or supplementary feeding is used to bolster production in medium stocked earthen ponds where fish thrive

in the absence of formulated feeds due to their ability to feed low on the food chain (Hassan, 2000; Tacon, 2003).

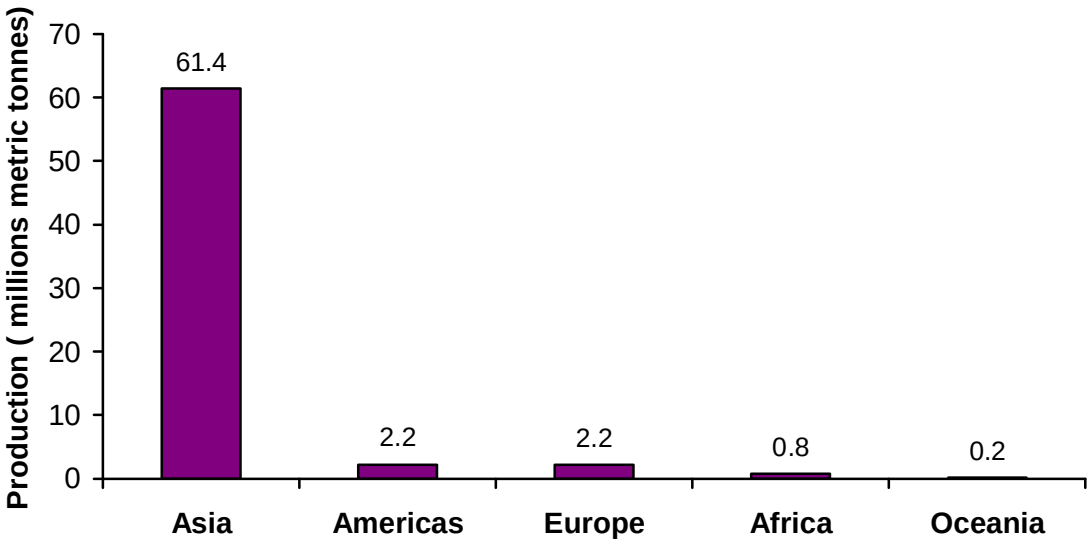


Figure 1.1: Global aquaculture production distribution in 2006
Source: FAO (2007a)

Aquaculture produce plays a vital role in providing affordable high quality protein all around the world. Fish protein has a high nutritional value due to a well balanced amino acid profile, ample amounts of polyunsaturated fatty acids (PUFA) as well as a number of vitamins and minerals (Edwards, 1997). Moreover, fish meat is generally tender because it contains less connective tissue than other meats (beef, mutton, pork, poultry etc) and hence it is easy to chew, particularly for children and the elderly (Tibbets, 2001).

Demand for aquatic products in developing countries has been increasing steadily at 10.4% per annum compared to 4.0% per annum in developed

countries (FAO, 2004b). This is due to the significant contribution of fish to total animal protein intake amounting to nearly 20%. Demand is predicted to increase even further due to population growth, expected economic development and changes in eating habits (Halwart *et al.,* 2003). Similarly, global *per capita* consumption has increased progressively from 9.0 kg in 1961 to an estimated 16.5 kg in 2003 with the exception of sub-Saharan Africa where it declined to 6.6 kg (FAO, 2007b). This has contributed to under nourishment of an estimated 16% of the population in this region. Despite the low *per capita* consumption, fish still plays an important nutritional role providing an average of 45% of total protein intake and in some countries, such as Sierra Leone, it is in excess of 80% (Béné and Heck, 2005; Hecht, 2006).

Aquaculture also plays an important role in providing a livelihood to millions of people around the world. In 2004, FAO (2007b) estimated that 4.5 million people were engaged directly in aquaculture with most of these located in the developing world. Many more are employed in a wide range of activities linked to aquaculture such as provision of inputs as well as processing of output.

1.2 Aquaculture in Sub-Saharan Africa

Aquaculture production in sub-Saharan Africa has increased gradually from 11,372 metric tons with a value of USD 19,884 in 1987 to 160,302 metric tonnes with a value of USD 421,066 in 2006 (FAO, 2007a).

According to Hishamunda (2007), the compounded annual growth rate of aquaculture production in the past two decades has been higher (11.7%) than that of the world average (9.1%). Despite this growth, the contribution of sub-Saharan African aquaculture production has remained low amounting to about 1% compared to 38% from the rest of the world. The top five aquaculture producers are Nigeria 53%, Uganda 20%, Madagascar 7%, South Africa 4% and Zambia 3% (Figure 1.2).

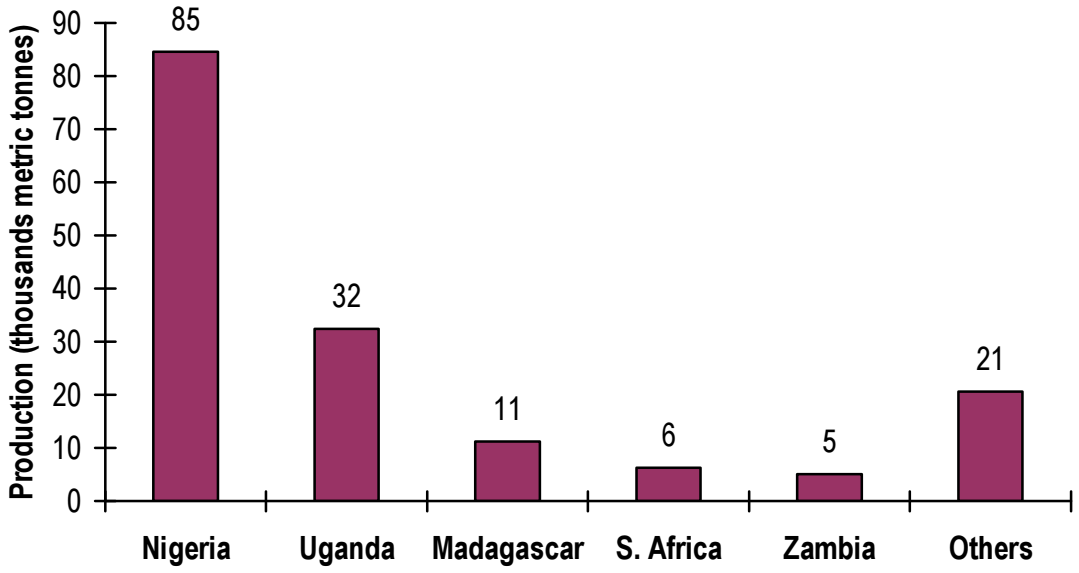


Figure 1.2: Top five aquaculture producers in sub-Saharan Africa in 2006
Source: FAO (2007a)

The main species cultured include finfish (96.2%), aquatic plants (1.8%), crustaceans and molluscs (0.6%). Freshwater finfish makes up about 80% of total aquaculture production.

Aquaculture is a relatively new phenomenon in most parts of sub-Saharan Africa and this has partly contributed to slow growth (Satia and Bartley,

1997). In contrast to terrestrial agriculture, little or no traditional aquaculture knowledge exists among farmers (Machena and Moehl, 2000). Aquaculture in Africa first emerged in the late 1920s in the form of fish culture for sport fishing whereby trout were introduced into high altitude areas in several countries such as Tanzania, Kenya and Madagascar (Coche *et al.*, 1994).

According to Maar *et al.*, (1966) the first African attempts to culture fish were made in Kenya in 1924 using tilapia. This was followed by the Congo in 1937, Zambia in 1942 and Zimbabwe in 1952. Fish culture then spread to other countries and by the late 1950s a total of 300,000 ponds had been constructed (Brummett and Williams, 2000; Satia, 1989). However, fish farming regressed sharply during the early 1960s as ponds were abandoned for a number of reasons including poor yields and lack of government support (Machena and Moehl, 2000). During the 1970s and 80s there was a resurgence of activity in the form of development projects due to their popular appeal among bilateral and multilateral co-operations with remarkable but short lived success (Stomal and Weigel, 1997).

A new wave of optimism occurred during the 1990s as failures of past efforts were better understood and previously unnoticed resources surfaced (Machena and Moehl, 2000). A new series of approaches inspired by new trends in social science were adopted. These included the farming systems approach, participatory approach, advocacy of low cost–low input production systems and market–oriented production (Stomal and Weigel,

1997). For instance, the farming systems approach attempted to reverse the research-development process by emphasizing the need to start with a careful analysis of the real situation of small-scale farmers (Halwart *et al.,* 2003). With this approach farms were viewed as complex integrated plant-animal-fish systems with multiple goals and multiple livelihoods. It also entailed a clear understanding of links between external services and internal functions of the farm system.

Despite being poorly developed, aquaculture in Africa has enormous potential. About 43% of continental Africa has been identified as being suitable for both small-scale and commercial farming of tilapia, African catfish and carp (Aguilar-Manjarrez and Nath, 1998). About 15% of this is considered "most suitable" with a possibility of getting almost two crops per year of both Nile tilapia and African catfish. The suitability consideration was based on a multi-criteria evaluation which took into account factors like water requirements, soil and terrain suitability, inputs availability, farm-gate sales and infrastructure. In the case of commercial aquaculture, almost 23% of southern Africa was ranked as suitable for commercial aquaculture but less than 5% is being utilised (Kapetsky, 1994; Aguilar-Manjarrez and Nath, 1998).

Nevertheless, African aquaculture has remained mostly rural, secondary and a part-time activity taking place in small freshwater ponds within small farm holdings. According to Hecht (2007), over 80% of fish farmers in sub-Saharan Africa are small scale, practicing extensive aquaculture on

a non-commercial basis for improving household food security. According to Machena and Moehl (2000), the ponds are integrated into a mosaic of agricultural activities. A typical fish farmer owns between 1–3 ponds with an average size of 210 m^2 (range 50–1,000 m^2) (Hecht, 2006). The most commonly cultured species is tilapia though it is not uncommon to find polyculture with catfish and carp. Sources of fingerlings vary from government hatcheries, wild stock or even self-owned ponds after harvest and the stocking density varies from 2–5 m^{-2}. Fish farming relies almost entirely on family labour and on–farm inputs (Coche *et al.,* 1994). Pond productivity is enhanced by "green compost" cribs as well as irregular application of inadequate quantities of manure and feed inputs which include cereal bran, kitchen waste and vegetable matter (Hecht, 2007). Fish production ranges from 280–3,200 kg ha^{-1} yr^{-1} with an average of 1,030 kg ha^{-1} yr^{-1} which translates into 20 kg yr^{-1} for a 210 m^2 pond (Hecht, 2006). The harvest is mostly consumed directly and surplus can be bartered or sold locally.

The main reasons for poor aquaculture development have been discussed in detail by Machena and Moehl (2000) and Hishamunda (2007). They include poor aquaculture development policies, few fish farming traditions, lack of access to quality feed, lack of quantity and quality of fingerlings, inadequate research and extension services, limited coordination between research and development sectors and inaccessibility of capital.

1.3 Aquaculture in Tanzania

Aquaculture in Tanzania also started in the form of sport fishing with the introduction of trout in 1927 from Scotland which were released into streams around Mount Kilimanjaro and the Mbeya region (Balarin, 1985). Pond fish culture started in the early 1950s with the establishment of experimental ponds in Korogwe and Malya, located in Tanga and Mwanza regions respectively, for production of tilapia and other non–indigenous fish (Bailey, 1966). However, emphasis shifted from pond culture to reservoir stocking with Korogwe and Malya becoming important fingerling distribution centres. Over 50% of reservoirs constructed to provide water for domestic use, livestock, irrigation and flood control were stocked with tilapia by 1955. After independence in 1961 there was a keen interest in aquaculture in which over 10,000 ponds with a total surface area of 1,000 ha were constructed by 1963 (Ibrahim, 1975). However, this progress was similarly short–lived as more emphasis was given to efficient exploitation of major lakes and marine waters where fish stocks were still abundant (Balarin, 1985).

A more deliberate attempt to improve aquaculture took place in 1972 after its inclusion in the national fishery policy though it remained a low priority sector (Singh, 1975). In the 1970s and 1980s a number of small donor-funded projects were undertaken through the auspices of several institutions including non-governmental, religious and parastatal organisations. These included the US Peace Corps, Church of The Province

of Tanzania, Overseas Development Agency (ODA), Evangelical Lutheran Church of Tanzania, FAO through the Aquaculture for Local Communities (ALCOM) project, Sokoine University of Agriculture and Heifer Project International (Bjoneseth, 1992). Perhaps the most notable project was construction of a national fish farming centre in Morogoro under UNDP/FAO funding in the early 1980s (Bjoneseth, 1992). The objective of the centre was to conduct research and training on various aspects of fish farming as well as fingerling production for distribution to fish farmers. Unfortunately, due to financial constraints it was never completed (Heck, 2003). In general all efforts during this period did not yield the anticipated success.

The new millennium started with optimism to revamp aquaculture development activities using local initiatives. The government, through its ministry of natural resources and tourism, increased budget allocation for aquaculture activities (Osewe Kajitanus, personal communication). One of the important undertakings was completion of the national fish farming centre in Morogoro to realize its intended objectives. Furthermore, a series of training programmes have been conducted for fish farmers all over the country on different aspects of fish farming. This has gone hand in hand with re-establishing fingerling distribution centres to cater for the chronic shortage of fingerlings. In efforts to alleviate personnel shortages two public universities namely Sokoine University of Agriculture and

University of Dar es Salaam launched undergraduate degree programmes in aquaculture from 2004/05 academic year.

Nonetheless, Tanzania is naturally endowed with abundant water resources with a huge potential to develop both marine and freshwater aquaculture. It shares three of the largest inland lakes in Africa (Victoria, Tanganyika & Nyasa) in addition to diverse river systems, numerous wetlands and a stretch of ocean coastline. The freshwaters cover an area of 58,000 km^2 while marine waters cover an area of 64,300 km^2 (MNRT, 1997). Fish production trends from both capture fisheries and aquaculture are shown in Figure 1.3.

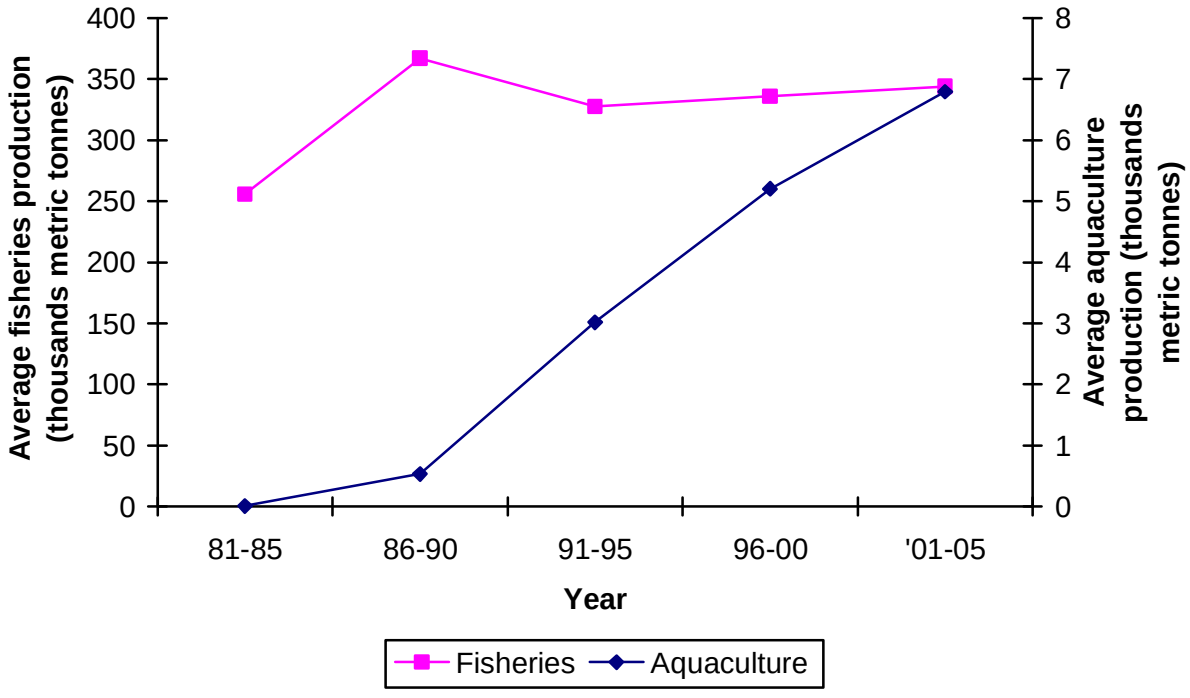


Figure 1.3: Fisheries and aquaculture production trends in Tanzania from 1981 to 2005

Source: FAO (2007a)

The figure reflects a typical global trend whereby fish supply from fisheries has almost stagnated while that from aquaculture is on a steady rise. This generates a need for concerted efforts from government and other stakeholders to address issues hindering aquaculture development as it is likely to be the main source of fish supplies for the future. Closer examination of aquaculture production reveals that the bulk comes from marine waters (Figure 1.4).

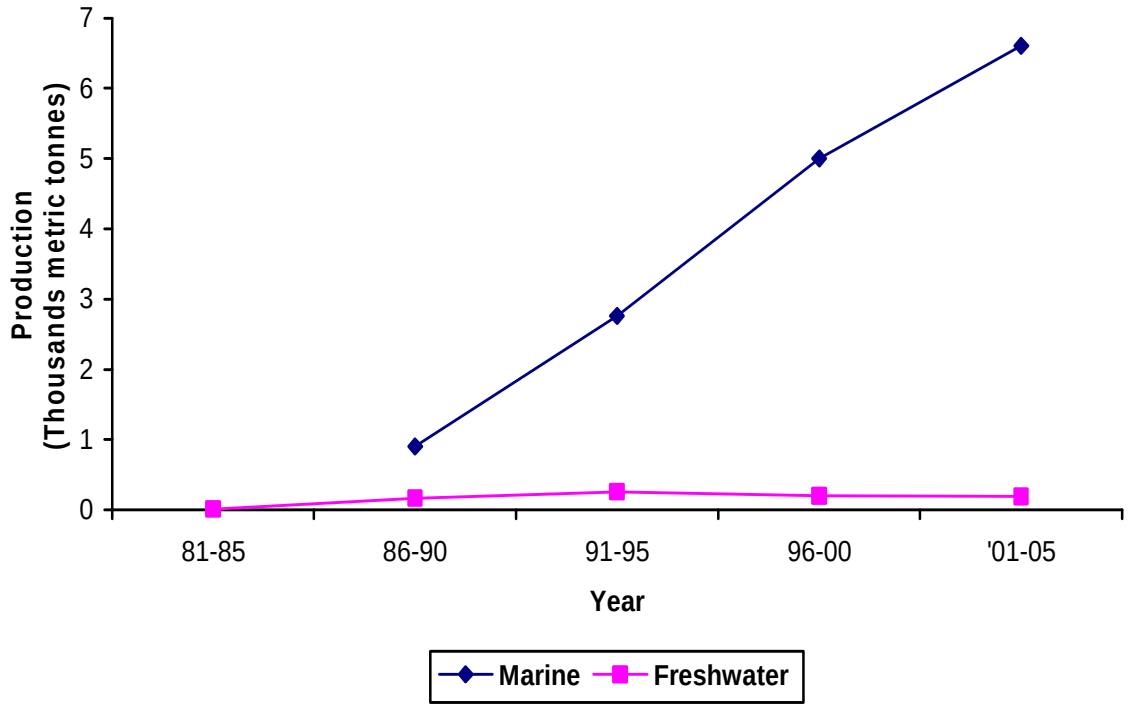


Figure 1.4: Marine and freshwater aquaculture production in Tanzania from 1982 to 2002

Source: FAO (2007a)

The main mariculture produce is seaweeds of the red algal species *Eucheuma denticulatum* and *Kappaphycus alvarezii* cultivated for their carrageenan content. Cultivation was initiated in 1989 and has since thrived, increasing steadily from 600 metric tonnes to 6,000 metric tonnes by 2005 (FAO, 2007a). Seaweed culture is perhaps the most established aquaculture industry in Tanzania. It is an important source of foreign exchange for the country as well as creating job opportunities and income, particularly for women in coastal communities (Bryceson, 2002). Aquaculture of other marine species is still in its infancy. This includes prawns, *Penaeus monodon* (Bryceson, 2002), rabbitfish, *Siganus cannaliculatus* (Bwathondi, 1982) and milkfish, *Chanos chanos* (Mwangamilo and Jiddawi, 2003); clams, ark clam, *Anadara antiquate* and giant clam, *Tridacna maxima*; oysters, pearl oysters *Pinctada margaritifera* and mangrove oysters, *Saccostrea cucullata* and mud crab, *Scylla serrata* (Rice *et al.,* 2006). Milkfish farming is the most promising so far. In 2006, a farmer in Bagamoyo district in the Coast region was dubbed the first "milkfish millionaire" after harvesting a tonne of fish which earned him a total of Tshs 2.4 million (about USD 2,000) (WIOMSA, 2006).

Though it started earlier than mariculture, freshwater aquaculture has been growing very slowly with an average annual production of 206 metric tonnes compared to 3,815 metric tonnes of mariculture between 1982 and 2005 (FAO, 2007a). The main species are tilapia (Nile tilapia, *Oreochromis niloticus,* Mozambique tilapia, *O. mossambicus* and Zanzibar tilapia,

Tilapia hornorum) which are cultured by small scale fish farmers, and rainbow trout, *Oncorhynchus mykiss* which is cultured commercially. Nile tilapia, *O. niloticus* is the most widely cultured species and is employed by over 95% of small scale fish farmers. Tilapia are cultured in semi–intensive earthen ponds with sizes varying from 150 – 500 m^2 (Bjoneseth, 1992). The ponds are normally fertilised with animal droppings or tender leaves as compost manure. The most common feeds used are rice and maize bran, kitchen leftovers and garden remains. Fish are partially or totally harvested after 6 - 12 months with an average yield of 2089 kg ha^{-1} yr^{-1} (Wetengere *et al.,* 1998; Kaliba *et al.,* 2006). The yield is low compared to the 10,000 kg ha^{-1} yr^{-1} which can be achieved if simple supplementary feeds are provided (Jauncey, 1998).

Dwindling fish catch from natural waters and low aquaculture production has contributed to decreased *per capita* consumption of fish and fish products as shown in Figure 1.5. Production of fish from capture fisheries is anticipated to remain constant or even decline as stocks have reached, or are close to reaching, their maximum sustainable yield. This brings aquaculture into perspective to fill the ever widening gap between demand and supply while stabilising prices, making fish and fish products available to a wider range of consumers at affordable prices.

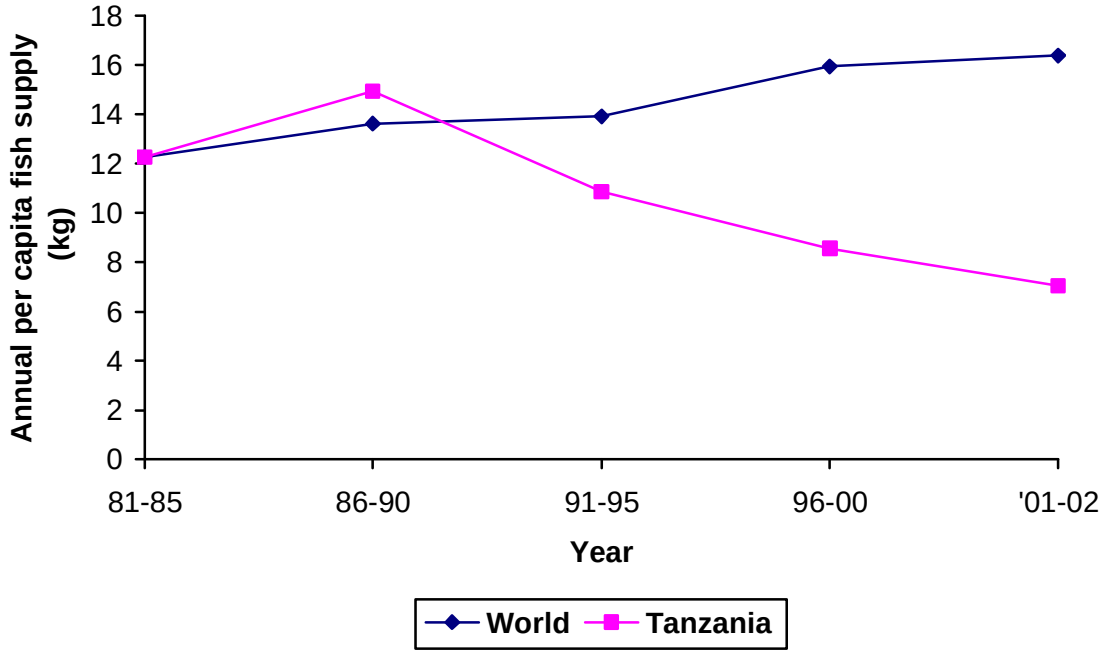


Figure 1.5: Annual food supply per capita from fish and fishery products in Tanzania from 1981 to 2002

Source: FAO (2006)

1.4 The Need for Low Cost Fish Feeds

The failure of aquaculture to meet the challenge of closing the widening gap between fish supply and demand in Tanzania, results from a number of factors including lack of quality feeds. Compost cribs and occasionally animal droppings are usually the main feed input in ponds. These, however, can only promote limited growth and further growth is restricted by insufficiency of nutrients from primary production (Edwards *et al.*, 2000). Further growth is only possible through provision of supplementary feed to sustain the increased demand for nutrients. Currently fish farmers use cereal bran, kitchen leftovers and green leaves as fish feed. This is because commercially formulated feeds are not available in Tanzania and

even if they were to be available they would be expensive and beyond the reach of most fish farmers. According to Hecht (2007) poor financial circumstances of the farmers within sub-Saharan Africa are one of the main constraints impeding aquaculture development. Often feed is the most expensive operating cost item accounting for over 50% of costs in semi–intensive aquaculture (De Silva, 1993) and up to 70% in intensive aquaculture (Thompson *et al.,* 2005). Lack of quality feed is such an issue that nutrition research was given highest overall priority in the synthesis of national reviews and indicative action plans for sub-Saharan African aquaculture (Coche *et al.,* 1994).

Both protein and energy are important components in fish feeds for maintenance, growth and reproduction. Their adequate supply in both quality and quantity enables fish to realise their potential. Conventionally, fishmeal and cereals have been used as protein and energy sources respectively (El-Sayed, 1999; Gatlin *et al.,* 2007). However, they are frequently either not affordable or conflict with food security interests.

1.4.1 Fishmeal as Protein Source

Protein is a critical component in complete fish feeds and generally the most expensive component accounting for more than 50% total feed cost in intensive aquaculture (Thomson *et al.,* 2005). Protein intake by fish is important to provide the amino acids required for synthesis of new tissues (growth & reproduction) or replacing worn out protein (maintenance).

Protein is also the major organic material in fish tissue making up 65 – 75% of total weight on a dry matter basis (Wilson, 2002). Therefore, dietary protein is always given priority in formulation of complete feeds to avoid inadequacy which may lead to poor growth and loss of weight. Depending on the abundance of protein-rich natural food in semi-intensive systems, the importance of dietary protein in supplementary feeds is lessened in favour of carbohydrate energy sources (Hepher, 1988).

Animal proteins are preferred due to their superior nutritional qualities (such as protein content, amino acid profile) but they tend to be expensive. Fish meal in particular is very palatable, highly digestible and rich in essential amino acids, fatty acids, energy and minerals (Ogunji, 2004). However, its supply is unstable (Figure 1.6) because of dwindling fisheries landings (due to over fishing, pollution, climate change), bad weather (El Niño) and increased demand from the fast expanding feed industry (Naylor *et al.,* 2001). For instance, the proportion of global fishmeal used for aquaculture has increased from 10% in 1989 to 35% in 2000 (Hardy, 2000).

Instability in the fishmeal supply has led to sharp increases in price beyond the reach of many resource-poor fish farmers. Moreover, there are ethical concerns about feeding fish to non-piscivorous fish (Mullaney *et al.,* 2000). Also, there are social concerns over feeding farmed fish with wild fish which could be used directly for human consumption (Naylor *et al.,* 2001; Francis *et al.,* 2002; Subasinghe, 2003). This somewhat contradicts

the anticipated role of aquaculture in food security and augmenting dwindling supplies from natural stocks particularly in nutritionally deficient areas of the world.

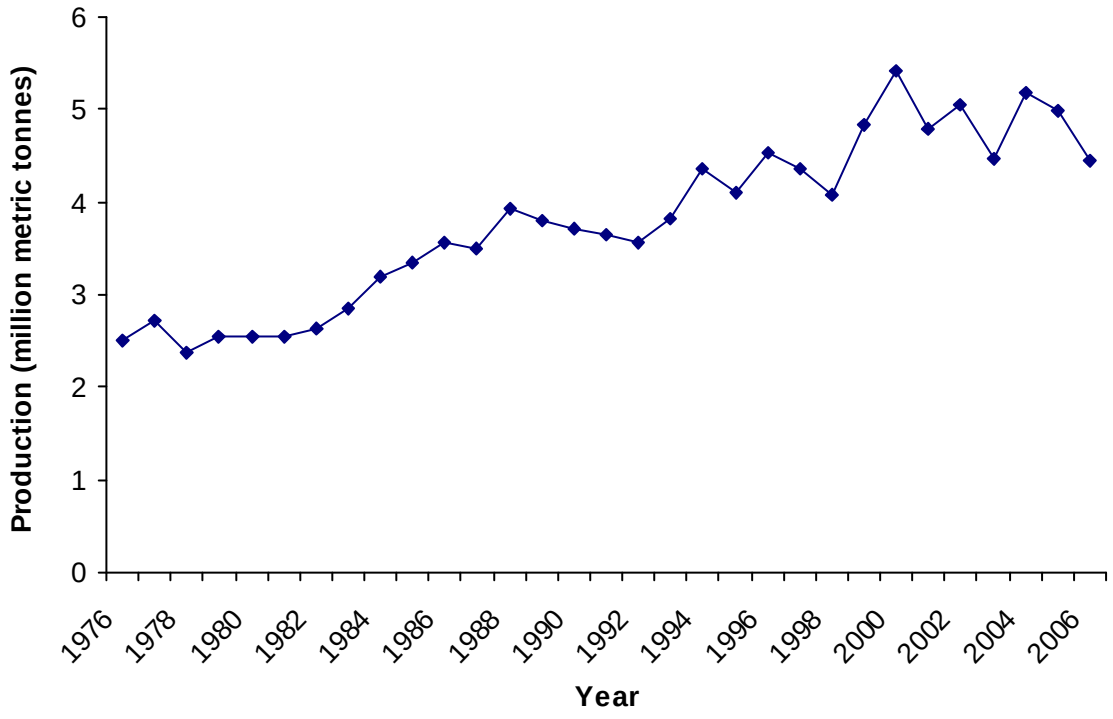


Figure 1.6: World fishmeal production trends (1976–2006)
Source: FAO (2004a)

1.4.2 Cereals as Energy Source

Although carbohydrates are not essential in fish feeds, they constitute an inexpensive source of energy (Kaushik, 1999). In the absence of adequate non-protein energy sources (lipid and carbohydrate), protein is used as an energy source instead of growth. Among preferred sources of carbohydrates are cereals and their by-products. Cereals make up the bulk of energy sources in diets particularly for herbivorous and omnivorous fish, due to their high carbohydrate content (De Silva and

Anderson, 1998). Apart from energy, cereals can contribute to the protein and lipid contents of feeds. A large proportion of cereal carbohydrate is starch (68–72%) which gelatinises during pelleting making pellets more stable in water (Jauncey, 1998; Hardy and Barrows, 2002). Generally cereal by–products such as maize bran, rice bran and hominy meal are very cheap except perhaps in the tropics where cereals are a staple food (Jauncey, 1998). This is very true for countries such as Tanzania where cereals form half of the daily calorie intake, a situation that makes cereals and their by–products scarce and expensive particularly during the dry season (Figure 1.7). Even without food shortages most resource poor farmers tend to reserve maize bran as emergency food for anticipated food shortages during the dry season, thus making their use as fish feed impractical (Brummett, 2002). Availability of cereals is also affected by low production in relation to a fast growing population (Figure 1.8). For instance maize production among Malawian farmers was reported to provide only 37% of the feed requirement of a fish pond (Brummett, 1999). This situation has transformed developing countries from net exporters of grains of about 5 million metric tonnes in the early 1970s to being net importers by 2004 of about 99 million metric tonnes (Machin, 1992; FAO, 2006). The situation is further complicated by insufficient foreign exchange vital for importing cereals hence grossly reducing cereal availability for both human and animal use.

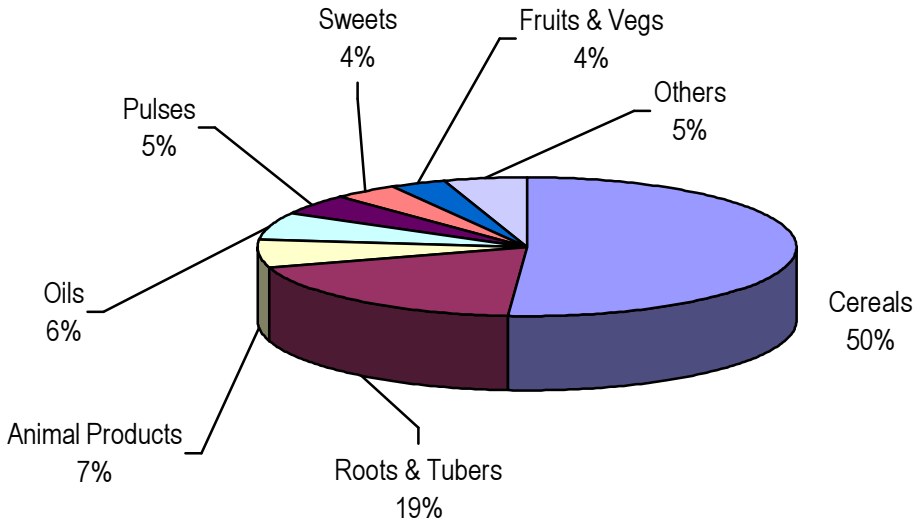


Figure 1.7: Percentage share of dietary components of total energy consumption in Tanzania (2001-2003).

Source: FAO (2001).

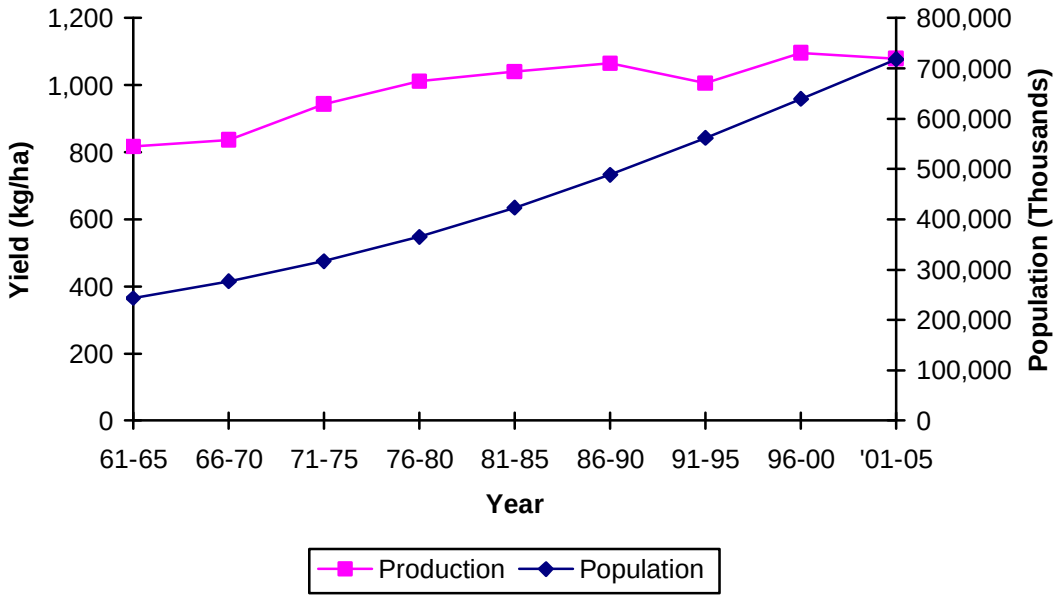


Figure 1.8: Cereal yield and population growth in sub-Saharan Africa (1961–2005)

Source: FAO (2006)

The trend is most likely to persist as projections indicate that developing countries will not be able to meet their demands for cereals resulting in sharp price increases (Rosegrant *et al.,* 2001). Prices of maize, wheat and rice are projected to rise by 40%, 20% and 14% respectively between 2005 and 2017 (ODI, 2008). The critical global food shortages in 2008 led to riots in some developing countries due to high food prices. The situation has been worsened by persistent droughts thought to be linked to climate change (Lobell *et al.,* 2008). The emergence of the bio-fuel industry has also impacted demand for cereals for production of ethanol and/or decreased cereal acreage in favour of biofuel crops (Africa Research Bulletin, 2007; Dickie, 2007).

1.5 Novel Ingredients in Fish Diets

The constraints associated with conventional ingredients have virtually excluded resource poor fish farmers from semi–intensive aquaculture. The search for alternatives to fishmeal has largely been focused on conventional sources such as oil seed cakes and meals due to their higher protein content (Table 1.1).

Table 1.1: Gross nutritional composition of some ingredients used in fish feeds (g 100g^{-1})

	Nutritional Composition				
Feed ingredient	Protein	Lipid	Carbohydrates	Fibre	Ash
Fish meal	60–75	5–20	1–4	1–3	10–25
Oil seed meal & cakes	20–60	1-15	20–55	4–20	6–15
Leaf meals	11–30	2–7	38–65	10–20	4–19
Cereals	5–14	1–9	75–80	2–10	2–5
Cereals by products	6–17	4–15	34–65	10–30	4–18
Root crops	2–10	1–3	70–90	1–4	2–6

Source: Hassan (2000)

These include soybean (Sadiku and Jauncey, 1995), groundnut, sunflower, rapeseed (Jackson *et al.,* 1982), and cottonseed cake (Mbahinzireki *et al.,* 2001). Despite their usefulness, these ingredients are scarce and expensive due to high demand for livestock production. Moreover, their cultivation generally requires high use of inputs and energy subsidies (Francis *et al.,* 2002). This makes them unaffordable, unsustainable and sometimes even conflicts with food security interests, particularly among resource-poor farmers.

Some of the ingredients that may have potential include products from moringa, *Moringa oleifera* and cassava, *Manihot esculenta.* These plants have positive agronomic attributes such as the ability to resist adverse soil and climatic conditions and yet sustain a reasonably high yield (Pérez, 1997; Fuglie, 1999). More than 11,300 hectares of moringa trees had been planted in various parts of Tanzania by 2001, primarily for its oil seeds (Creighton, 2001). Tanzania is among the top five producers of cassava in Africa, which is primarily grown for its starchy roots and it is estimated that about 7 million metric tonnes are produced annually (Lekule and Sarwatt, 1992).

Particular products of interest are moringa and cassava leaf meals as potential dietary protein sources as well as cassava root meal as an energy source. The potential of these products as ingredients in fish feed formulations lies in their local availability, affordability and relatively good

nutritional profile as well as favourable agronomic characteristics such as drought resistance and minimum requirements for inputs. Another advantage is their multiple uses which potentially could serve as a source of additional income to the farmers. For instance, oil from moringa seeds is used in perfumes and lubrication of fine machinery while powder from the seed kernel has coagulant properties which can be used to clarify turbid water (Fuglie, 1999). Cassava starch has a wide range of food and non-food uses (O'Hair, 1995). This has a potential of spreading risks among resource-poor farmers, who are always vulnerable to natural calamities, by offering diversification of farm activities.

1.5.1 Utilization of Leaf Meals in Fish Diets

Several leaf meals have been studied with respect to their suitability as protein sources in fish diets. These include leucaena, *Leucaena leucocephala* (Pantastico and Baldia, 1980; Ferraris *et al.,* 1986; Santiago *et al.,* 1988), sesbania, *Sesbania sesban* (Hafez *et al.,* 2001), sweet potato, *Ipomoea batata* (Borlongan and Coloso, 1994), mulberry, *Morus spp* (Vijayakumar Swamy and Devaraj, 1995), alfalfa, *Medicago sativa* (Yousif *et al.,* 1994), acacia, *Acacia auriculiformis* (Mondal and Ray, 1999), papaya, *Carica papaya* (Eusebio and Coloso, 2000; Palavesam *et al.,* 2001), water spinach, *Ipomoea reptans* (Borlongan and Coloso, 1994), water hyacinth, *Eichornia crassipes* (Hasan and Roy, 1994), duckweed, *Lemna polyrhiza* (Bairagi *et al.,* 2002), duck lettuce, *Ottelia alismoides*, water snowflake, *Nymphoides indicum* (Patnaik *et al.,* 1991) and peanut,

Arachis hypogaea (Garduno-Lugo and Olvera-Novoa, 2008). In most of these studies the leaf meals could only replace ≤ 25% of fishmeal protein. Higher replacement levels led to poor palatability (Hassan *et al.,* 1997), poor nutrient utilisation (Eusebio *et al.,* 2001), poor growth (Eusebio and Coloso, 2000), pathological lesions (Hafez *et al.,* 2001) and/or poor reproductive performance (Santiago *et al.,* 1988).

The causes of these adverse effects are discussed in detail in section 1.6 but endogenous antinutritional factors play a major role. Treatment of leaf meals to remove antinutritional factors has been shown to allow much higher fishmeal replacement without these adverse effects. For instance, soaking of leucaena leaf meal in water eliminated all (Wee and Wang, 1987) or most (Hassan *et al.,* 1994; Mondal and Ray, 1998) of the mimosine, a toxic non–protein amino acid which is largely responsible for the poor performance of this material (El-Sayed, 1999). Inoculation with enzyme-producing bacteria from fish guts allowed duckweed, *Lemna polyrhiza* (Bairagi *et al.,* 2002) and leuceana leaf meal (Bairagi *et al.,* 2004) to replace up to 30% of fishmeal in rohu, *Labeo rohu* diets. The bacteria apparently degraded antinutritional factors as well crude fibre hence increasing nutrient availability.

1.5.2 Utilization of Root Meals in Fish Diets

Studies on alternative sources of cereals have been patchy, probably because carbohydrates are poorly utilised by fish as an energy source

compared to protein and lipid (Shiau, 1997). However, the cost of the diet may be reduced by the protein-sparing action of carbohydrates thus reducing the amount of protein in the diet (Shiau and Peng, 1993). Similarly, a sparing effect of carbohydrates has been suggested in eutrophic ponds rich in bacteria, phytoplankton and zooplankton stocked with herbivorous and omnivorous fish species (Wee and Ng, 1986). Tal and Hepher (1966) suggested that when natural food is utilised by fish, part of the high quality protein is used for maintenance rather than growth. The application of energy-rich supplements in such ponds may therefore spare protein-rich natural food organisms for growth. Feeding of energy-rich feeds in manured ponds significantly increases fish production (Hepher, 1979).

All of this underscores the need to identify alternative energy sources to cereals which are cheaper and available to resource poor farmers. Root crops are primarily grown for their edible starchy roots or underground stems (tubers). According to Jauncey (1998), potential root meals include cassava, sweet potato and yams. Cassava root meal is more abundant than other root meals in Tanzania and is often regarded as a famine crop with lowered demand compared to cereals. Its ease of growth, with minimum requirements for costly inputs, makes cassava cheaper than cereals. Its main limitation as animal feed is the presence of antinutritional factors, mostly in form of cyanogens (Oke, 1978).

1.6 Limitations in Utilizing Plants in Fish Diets

Generally, inclusion of plant materials in fish feeds leads to reduced performance in many cases. This is attributed to a number of factors including poor palatability, poor digestibility, antinutritional factors, high fibre content and poor protein profile in terms of low protein content and poor amino acid profile (Ogunji, 2004; Francis *et al.,* 2001; Francis *et al.,* 2002).

1.6.1 Poor Palatability

Glencross *et al,* (2007) define palatability as acceptable to the taste or sufficiently agreeable in flavour to be eaten. Palatability is determined by both gustatory qualities (taste) and aesthetic qualities (appearance and texture) (Jauncey, 1998; Kasumyan and Doving, 2003). The gustatory system however, plays a predominant role in determining whether feed will be accepted or rejected (Adron and Mackie, 1978). The structural units of the gustatory system are taste buds which are composed of groups of specialised epithelial cells containing receptors or gustatory cells (Bone *et al.,* 1995). The taste buds are generally concentrated in the mouth, pharyngeal region and gill arches of bony fishes and are innervated by different cranial nerves which mediate food search behaviour and palatability (Bond, 1996). The gustatory response triggers reflexive feeding behaviours such as arousal, search, uptake, ingestion or rejection (Kanwal and Finger, 1992). The behavioural response to food is mediated by two distinct but interrelated sub-systems i.e. the extra-oral subsystem and the

oral subsystem (Kasumyan and Doving, 2003). The extra-oral subsystem deals with food search while the oral system deals with selective ingestion. The response also invokes cephalic reflexes which modulate enzyme activities in the gastrointestinal tract for optimal digestion and nutrient absorption of the anticipated incoming feed (Giduck *et al.,* 1987). Takeda and Takii (1992) reported that artificial feed with good chemosensory qualities is digested more effectively.

It has been suggested that incorporation of plant materials in fish diets negatively affects texture and taste thus reducing palatability and consequently feed intake (De Silva and Gunasekera, 1989; El-Sayed, 1999; Francis *et al.,* 2001). Plant ingredients often contain antinutritional factors, some of which act as deterrents to keep away herbivores (Becker and Makkar, 1999). Kasumyan and Doving (2003) define deterrents as substances that make fish abandon feed intake and evoke feed rejection. This was demonstrated by low feed intake in *O. niloticus* fed diets containing extracts from moringa leaves (Afuang *et al.,* 2003; Dongmeza *et al.,* 2006). These extracts contained saponins and tannins which are known to have astringent/bitter taste. Bernays *et al.* (1989) suggest that the deterrence might be due to the astringent taste binding to saliva mucopolysaccharides, epidermis or chemosensory receptors.

Positive gustatory responses can be elicited by inclusion of feeding stimulants which include L–amino acids, nucleotides and nucleosides, extracts from marine animals, betaine and quaternary ammonium bases

either alone or in combination (Takeda and Takii, 1992; Xue and Cui, 2001). Their common properties include low molecular weight, being non–volatile, nitrogenous, amphoteric, water–soluble, stable to heat treatment and having broad biological distribution (Carr *et al.,* 1996; Polat and Beklevik, 1998; Higuera, 2002). Kasumyan and Doving (2003) group these substances into 3 categories i.e. incitants, stimulants and enhancers or potentiators based on their effects on the oral and extra-oral taste systems. Incitants act on the extra-oral taste system while stimulants and enhancers act on the oral taste system. Incitants induce food capture by evoking actions like suction, grasping, snapping, biting, tearing or pinching. Stimulants act by initiating or continuing feeding activity only when fish come in direct contact with the feed and they are characterised by high ingestion rate. Enhancers act by accentuating flavour of food causing an increase in their consumption.

Some studies in fish have shown that inclusion of attractants led to improved feed palatability and intake. This has been demonstrated in seabass, *Dicentrarchus labrax* (Dias *et al.,* 1997), striped bass, *Morone saxatilis* (Papatryphon and Soares, 2000), olive flounder, *Paralichthys olivaceus* (Choi *et al.,* 2004), Nile tilapia, *O. niloticus* (Gaber, 2005), and Indian major carp, *Labeo rohita* (Shankar *et al.,* 2008).

1.6.2 Poor Digestibility

De Silva and Anderson (1998) define digestibility as quantification of digestive processes which gives a relative measure of the extent to which ingested feed and its nutrients have been digested and absorbed by the animal. Increasing levels of plant ingredients in fish diets are often accompanied by decreased diet digestibility (El-Sayed, 1999). One reason for this is high crude fibre content, this fibre serving as a support structure in plants. According to Ahmad *et al.,* (2004), the chemical and physical structure of fibre creates a barrier between nutrients and digestive enzymes hence lowering digestion. Gaber (2006) also suggests that fibre may disrupt enzyme activities through adsorption or immobilization. Antinutritional factors inherent in plants may also lower digestibility. Saponins (Ikedo *et al.,* 1996), tannins (Guillaume and Métailler, 1999) and phytates (Richie and Garling, 2004) are known to form poorly digestible complexes with nutrients. Saponins are also known to inflict damage on the intestinal mucosa thus interfering with the digestion process (Bureau *et al.,* 1998). In addition, plant ingredients tend to have a higher carbohydrate content which is generally poorly utilised by fish (Shiau, 1997). This is partly due to adsorption of amylase to raw starch thus inhibiting its hydrolysis (Spannhof and Plantikow, 1983). However, omnivorous fish such as *O. niloticus* can utilise much higher levels of dietary carbohydrates (Krogdahl *et al.,* 2005).

1.6.3 Antinutritional Factors

Antinutritional factors are defined as substances which themselves or through their metabolic products arising in living systems, interfere with food utilisation thus affecting the health and production of animals (Makkar, 1993). Antinutritional factors are perhaps the main factor limiting inclusion of plant ingredients in fish diets. Their inclusion results in poor palatability, poor feed intake, interference with feed utilisation and alteration of gut morphology (Francis *et al.,* 2001). According to Tacon (1985) antinutritional factors can be grouped into four major groups according to their chemical composition as shown in Table 1.2. Moringa and cassava products are known to contain saponins, cyanogenic glycosides, tannins and phytates. Among these, saponins are more significant in moringa (Makkar and Becker, 1996) while cyanogenic glycosides are more significant in cassava (Ravindran, 1993).

Table 1.2: Major groupings of antinutritional factors

Group	Antinutritional Factors
Proteins	Protease inhibitors, Haemagglutinins
Glycosides	Goitregens, Cyanogens, Saponins, Oestrogens
Phenols	Gossypol, Tannins
Miscellaneous	Anti-minerals (eg Phytic acid), Anti-vitamins, Anti-enzymes, Food allergens, Microbial/plant carcinogens, Toxic amino acids

Source: Tacon (1985)

1.6.3.1 Saponins

Saponins are glycosides found in a wide range of plants. According to Birk and Peri (1980), saponins are characterised by bitter taste, foaming in aqueous solutions and haemolysis of red blood cells. Their chemical structure is made up of two portions i.e. sugar (glycone) and non–sugar

(aglycone). The non–sugar portion, also known as genin or sapogenin, is divided into two major classes i.e. triterpenoids and steroids (Figure 1.9:) (Applebaum and Birk, 1979).

Dietary saponins are known to have several adverse effects on fish performance. Due to their astringent taste, saponins reduce feed intake (Guillaume and Métailler, 1999). However, some saponins may have a liquorice like taste if the sugar on triterpenoid aglycone is replaced by glucuronic acid (Celik *et al.,* 2007).

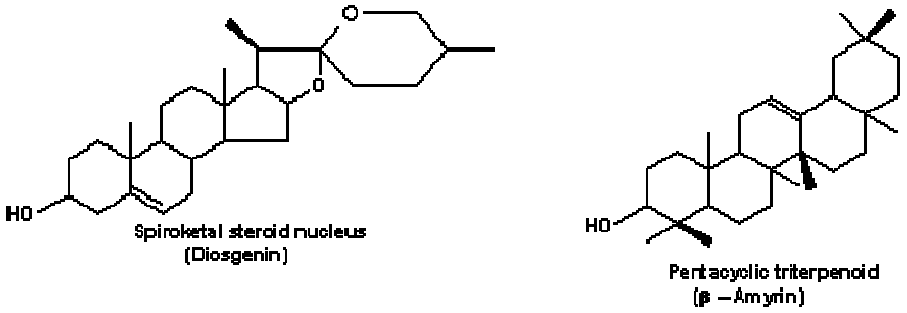


Figure 1.9: Chemical structures of major classes of saponins
Source: Friedli (2006)

Saponins are also known to interfere with digestibility and absorption of nutrients due to formation of sparingly digestible saponin–nutrient complexes (Potter *et al.,* 1993; Ikedo *et al.,* 1996). They may also damage intestinal epithelium mucosa (Bureau *et al.,* 1998), induce stress (Roy and Munshi, 1989), inhibit reproduction (Francis *et al.,* 2001) and damage respiratory epithelium (Hostettmann and Marston, 1995).

Despite deleterious effects, saponins are known to have beneficial effects. They can form complexes with other antinutritional factors like tannins

resulting in inactivation of the toxic effects of both substances (Makkar *et al.,* 1995; Francis *et al.,* 2001). They can also increase digestibility of carbohydrate rich feeds due to their detergent like activity which reduces viscosity thus preventing the normal obstruction action of such foods against digesta movement in the gut (Francis *et al.,* 2001).

Fish diets containing saponins levels below 1g kg^{-1} are unlikely to affect fish growth (Francis *et al.,* 2001). Saponins can be readily removed from plant tissues by aqueous or solvent extraction (Tacon, 1997).

1.6.3.2 Cyanogenic Glycosides

Cyanogenic glycosides are compounds found in a number of pulses, roots and oil seeds (Francis *et al.,* 2001). The cyanogenic glycosides are not toxic *per se*, but hydrogen cyanide (HCN), which is an end product of their hydrolysis, is. This occurs after cyanogenic glycosides (linamarin) are converted enzymaticaly by linamarase to sugar and cyanohydrin (α–hydroxynitrile) which further dissociates into ketone and HCN (Figure 1.10) (Tewe, 1992; Tacon, 1997). HCN toxicity is mostly due to its ability to combine reversibly with enzymes associated with cellular respiration thus suppressing natural respiration and causing cardiac arrest (Conn, 1979; Francis *et al.,* 2001). Reduced growth and feed efficiency was reported when fish were fed diets containing HCN such as in *C. carpio* fed linseed, *Linum usitatissimum*, meal (Hossain, 1988).

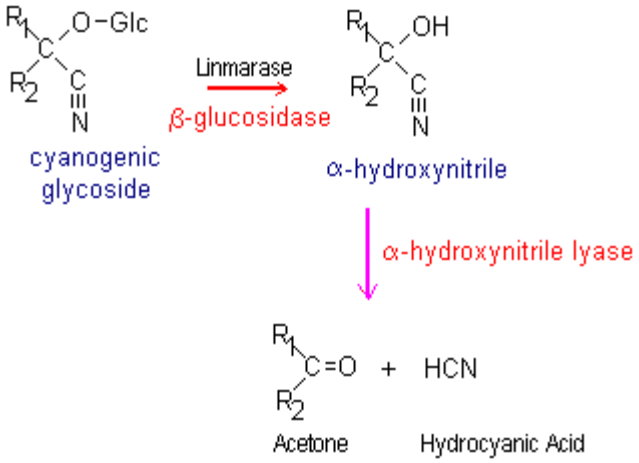


Figure 1.10: Chemical reactions leading to formation of hydrocyanic acid (HCN)

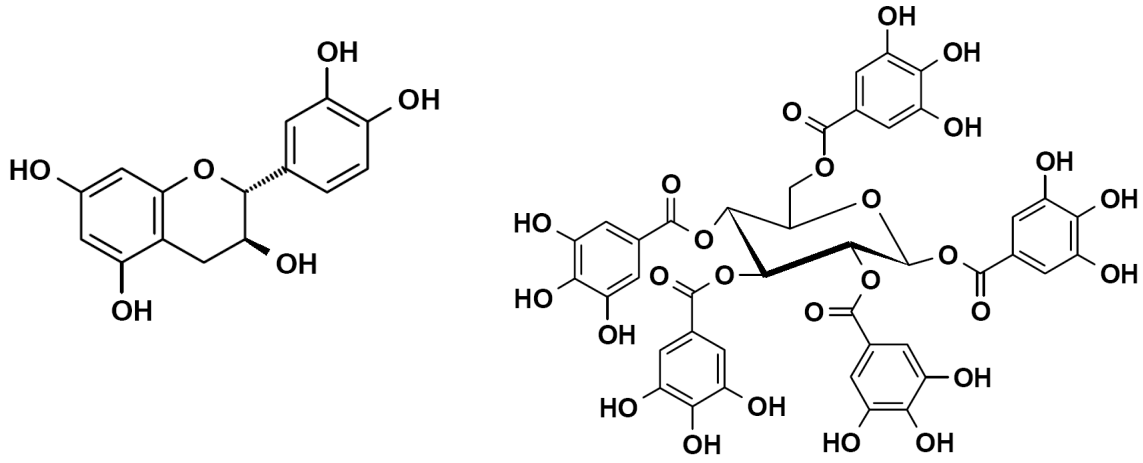
Source: Modified from Rhodes (2006)

Levels of HCN can be reduced significantly by soaking and/or thermal treatment (boiling, roasting and sun drying) and microbial fermentation (Ravindran, 1993). Physical damage/crushing of tissues facilitates the hydrolysis process by bringing together linamarin and linamarase. Padua *et al* (1998) reported complete removal of cyanide in cassava leaf meal after grinding followed by sun drying. Dietary HCN levels of less than 10 mg 100^{-1} g DM are recommended to ensure safety of livestock (Tewe and Egbunike, 1992).

1.6.3.3 Tannins

Tannins are polyphenolic compounds with various molecular weights and variable complexity (Hagerman, 2002). Other polyphenolic compounds include lignins and flavonoids. Tannins are categorised into two major groups (Figure 1.11) i.e. hydrolysable and condensed (Francis *et al.,* 2001). Tannins are known to impart an astringent/bitter taste which

affects palatability and depresses appetite (Makkar, 2003). They are also known to affect digestibility due to the formation of complexes with certain proteins, particularly digestive enzymes (Guillaume and Métailler, 1999) as well as vitamin B12 (Tacon, 1997). Significant negative effects on digestibility were observed in *O. niloticus* fed diets containing 0.68% tannin from barbatimao, *Stryophnodendrum obovatum* (Pinto *et al.,* 2004). In *Tilapia hornorum,* catarrhal enteritis, acute hepatitis and tubular nephrosis were observed after dietary intake of tannin (Romero and Ocampo, 1984).



Condensed tannins–*Catechin* Hydrolysable tannins–β-1,2,3,4,6-pentagalloyl-O-D-glucose

Figure 1.11: Chemical structure of tannins (condensed and hydrolysable)

Source: Hagerman (2002)

Tannins can be inactivated/removed using a number of treatments including soaking in alkaline solution (Ben Salem *et al.,* 2005), fermentation (Mukhopadhyay and Ray, 1999) and supplementation with a tannin complexing agent like polyethylene glycol (PEG) (Makkar, 2003).

1.6.3.4 Phytic Acid

Phytic acid is composed of hexaphosphates of myo – inositol (Figure 1.12) (Tacon, 1997) that, in salt form, are referred to as phytate. Phytic acid is a principal storage form of phosphorus and other minerals in many plant tissues due to its chelating ability (Guillaume and Métailler, 1999; Francis *et al.,* 2001). Hence, phytic acid can make up to 6% by weight in cereals and oil seeds and up to 80% of total phosphorus in plants can be in phytate form (Hossain, 1988).

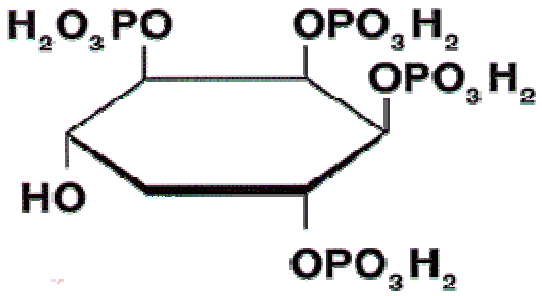


Figure 1.12: Chemical structure of phytic acid

Source: BiogenicStimulants.com (2006)

The main negative effect of phytate is reduced bioavailability of nutrients due to formation of complexes such as a phytate–mineral/protein complex. The complexes are formed due to the presence of six negatively charged phosphate groups in the cyclic structure. Dietary phytic acid has been reported to affect performance in various fish species such as rainbow trout (Spinelli *et al.,* 1983) and common carp (Hossain and Jauncey, 1993). The inability of fish to make use of phytate-nutrient complexes is due to lack of the enzyme phytase (Riche *et al.,* 2001) though there are suggestions that tilapia posses microbially-derived intestinal phytase

activity which could make available phosphorus bound by phytic acid (Ellestad *et al,.* 2002). Moreover, Riche and Garling (2004) suggest that the chelating ability of phytic acid could be beneficial in preventing leaching of nutrients which occurs due to the fine grinding of feed by tilapia using pharyngeal teeth. However, this remains inconclusive and therefore minimising levels of phytic acid in diets is recommended. Phytic acid may cause eutrophication and consequently algal blooms in aquatic environment when phosphorus is released from phytate–phosphorus complexes through microbial processes (Dickie, 2007). However, in the case of pond culture this may be advantageous as the released phosphorus could enhance primary production.

The negative effect of dietary phytic acid can be minimised through treatment such as heating, aqueous extraction, milling of cereals to remove the outer coat, use of the enzyme phytase directly or indirectly through fermentation with yeast or lactic acid bacteria (Francis *et al.,* 2001; Francis *et al.,* 2001). It is recommended to maintain levels of phytates below 5g kg^{-1} and also to supplement with minerals such as zinc to partially counteract the adverse effects (Francis *et al.,* 2001).

1.6.4 High Fibre Content

Fibre is intrinsic to plants playing an important role as a supporting structure (Lim, 1996). Fibre refers to indigestible plant material such as cellulose, hemicellulose, lignin, pentosans and other complex

carbohydrates found in feed which are generally indigestible to fish (NRC, 1993; Jauncey, 1998). The physical and chemical structure of fibrous polysaccharides limits access by digestive enzymes and hence nutrients become indigestible (Ahmad *et al.,* 2004). This is partly because the cell wall creates a physical barrier between digestive enzymes and nutrients which prevents or delays digestion of nutrients. The inability of fish to digest fibre is due to low microbially-derived celullase activity in the gut (De Silva and Anderson, 1998). It is also suggested by Gaber (2006) that dietary fibre may change enzyme activities through absorption or immobilization of enzymes. A high proportion of plant ingredients in a diet generally results in high crude fibre content. This may result in reduction of feed intake, digestibility, gut transit time as well as dilution of nutrients and increase in faecal output (Yakupitiyage, 1993; NRC, 1993; Edwards, 2004). Moreover, indigestible carbohydrates and proteins not only dilute nutrient density but also increase outputs of both faecal material and faecal nitrogen (Shah *et al.,* 1982; Cho and Bureau, 1995; Phuc *et al.,* 2000). Dioundick and Stom (1990) seem to suggest that a moderate level of dietary fibre is necessary for effective passage of digesta through the gut.

1.6.5 Poor Amino Acid Profile

To date there is no single animal or plant protein available with an essential amino acid profile which can fulfil the dietary requirements of fish apart from fishmeal (Tacon and Jackson, 1985). As a result, efforts

directed towards total or partial replacement of fishmeal in diets have yielded mixed results. This is mainly due to poor amino acid profiles which result in sub-optimal amino acid balance. Fish require 10 essential amino acids namely; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (De Silva and Anderson, 1998). These must be present in the correct relative proportions for *in vivo* protein synthesis to take place, but generally plant proteins tend to be limiting in one or more essential amino acids. Examples of plant proteins and their limiting amino acids in brackets are soybean (lysine, methionine, threonine), cottonseed cake (lysine), peas and lupin (lysine, methionine) (Gatlin *et al.,* 2007).

The amino acid profile may be improved through supplementation with the purified amino acids in deficit. However, this is not practical for resource poor fish farmers in rural areas due to the high cost and/or availability of such supplements (Fagbenro, 1999). Another approach is to formulate a diet with a protein content higher than the requirement in order to reduce the possibility of having sub–optimal amino acid levels in a diet (Jackson *et al.,* 1982). However, this is not practically possible if the protein source has low protein content (11-30%) as higher amounts will be required thus limiting inclusion of other ingredients as well as resulting in higher amounts of antinutritional factors. One practical approach to improve protein quality is to formulate feeds that contain combinations of different protein sources with different amino acid profiles (Jackson *et al.,* 1982;

Tacon and Jackson, 1985; Jauncey, 1998). A combination of soybean, cottonseed, sunflower and linseed meals at 25% each could replace up to 100% of fishmeal without adverse effects on performance of *O. oreochromis* (El-Saidy and Gaber, 2003). Similar results were also obtained when *C. chanos* were fed a diet containing a combination of *I. reptans*, *I. patata, L. leucocephala* & *M. esculanta* leaf meals (Borlongan and Coloso, 1994).

1.7 Tilapia

Tilapia is one of the earliest fish species to be cultured. According to Maar *et al.,* (1966) illustrations on a bas – relief found in Egyptian tombs show tilapia being fished out of a pond suggesting they were already farmed in Egypt about 2500 BC. Also, with reference to biblical passages it is said that tilapia was the fish which was fed to the multitudes (in the miracle of the loaves and fishes) and it is thus referred to as "Saint Peter's fish" (Suresh, 2003). Tilapia belong to the Cichlidae which is a large family of tropical freshwater fish with bilaterally compressed bodies that exhibit parental care. They are mainly indigenous to Africa but they also occur naturally in the Middle East (Wohlfarth and Hulata, 1981). Tilapia is a common name referring to pure species as well as hybrids belonging to the genera *Tilapia, Sarotherodon* and *Oreochromis* which contain most cultured species. *Orechromis niloticus*, Nile tilapia, is most favoured in aquaculture due to its performance under typical culture conditions and from 1993 onwards its production surpassed that of milkfish to become

second to carps. Annual global production of *O. niloticus* has increased from 1,590 metric tonnes in 1950 to 1,988,726 metric tonnes in 2006 accounting for over 84% of all tilapia production. China is the largest producer followed by Egypt, Indonesia, Philippines and Thailand (Figure 1.13)(FAO, 2007a).

The success of Nile tilapia farming is mainly attributed to its ease of culture and desirable qualities as a food fish (Suresh, 2003). These include ease of breeding in captivity, tolerance to both crowding and relatively poor water quality and low susceptibility to diseases. According to Hussain (2004) Nile tilapia can survive well in water temperatures of 12 – 35^{0}C, pH 6.5–8.5, dissolved oxygen 2.0–8.0 mg l^{-1} and salinity levels between 3 and 25 ppt.

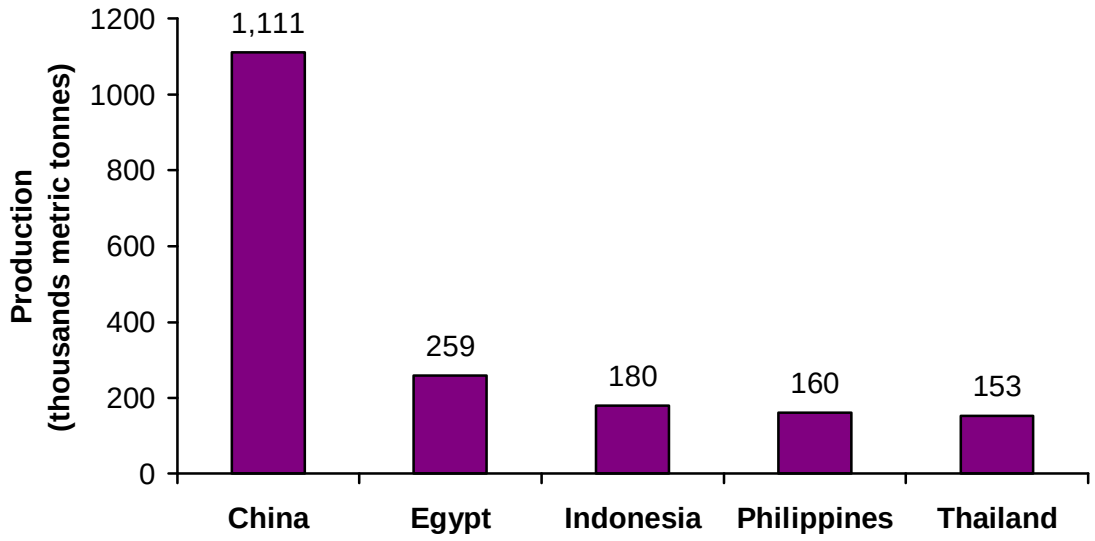


Figure 1.13: Top five Nile tilapia producers in the world 2005

Source: FAO (2007a)

Its quality as a food fish includes white flesh, neutral taste and firm texture making it acceptable to a wide range of tastes and preferences. These qualities have earned Nile tilapia the title of "aquatic chicken" (Pullin, 1984) which could explain why more than 95% of fish farmers in Tanzania culture tilapia (Kaliba *et al.,* 2006).

Adult *O. niloticus* have a high degree of plasticity and opportunism in their feeding behaviour and are hence classified as omnivorous (Jauncey, 1998). They are capable of consuming a wide variety of feed items including phytoplankton, periphyton and detritus (Beveridge and Baird, 2000). Feed intake in adult *O. niloticus* is through filter feeding of entrapped feed particles as well as surface–grazing on periphyton mats. Tilapias have teeth on their jaws as well as on the pharyngeal bones. The teeth are used to crush feed increasing the surface area which in turn facilitates enzyme – substrate interaction in the stomach. Digestion is a two step process involving gastric and intestinal components (Jauncey, 1998). The stomach has an extremely acidic environment, often with very low pH which enables lysis of plant cells exposing them to digestive enzymes in the intestine. The intestine is very long (about 7–13 times total body length of an adult fish), coiled and has a high pH due to secretion of bile salts (Beveridge and Baird, 2000). This facilitates action of enzymes like trypsin, chymotrypsin and amylase. Nutrient requirements for tilapia have been reviewed extensively by Jauncey (1998; 2000).

1.8 Objectives of the Study

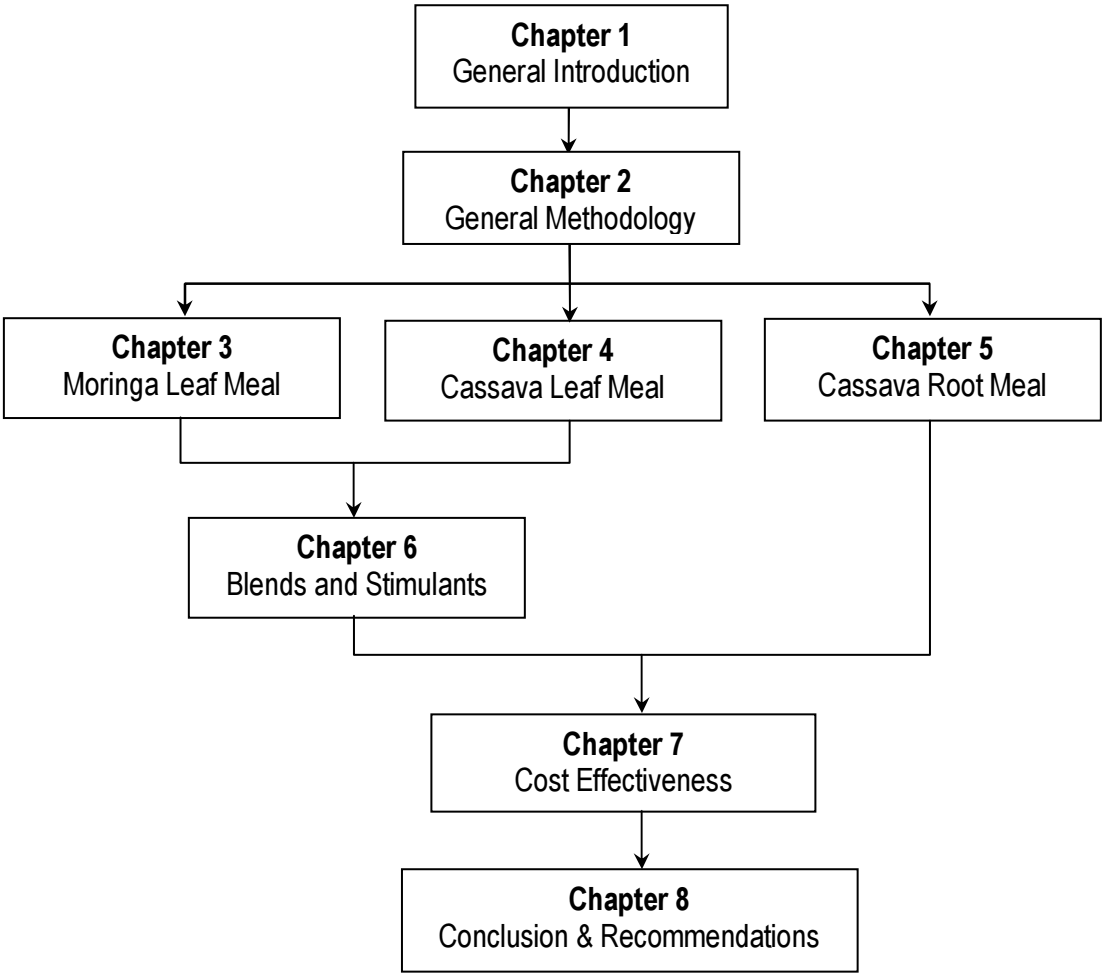
It is clear from the preceding sections that one of the main constraints in Tanzania is lack of affordable fish feeds due to cost and/or unavailability of both commercial feeds and conventional ingredients for on–farm feeds. Novel ingredients such as moringa leaves, cassava leaves and cassava root crop may offer alternatives. However, these ingredients contain inherent antinutritional factors which are known to depress performance. Thus, simple and cost effective processing methods deemed feasible to small-scale fish farmers should be employed to reduce the levels of antinutritional factors.

The main objective of this study was therefore to evaluate the suitability of processed moringa leaf meal (MLM), cassava leaf meal (CLM) and cassava root meal (CRM) as novel ingredients in diets of *O. niloticus*. The specific objectives were to evaluate the:

- nutritional value of processed MLM as protein source
- nutritional value of processed CLM as protein source
- nutritional value of processed CRM as energy source
- effect of blending and feeding stimulants on nutritional value of MLM and CLM as protein sources
- cost effectiveness of practical diets containing MLM, CLM and CRM

1.9 Structure of Thesis

This thesis is organised into eight chapters as follows



CHAPTER 2 General Materials and Methods

2.1 Fish Holding Facility

All experiments were conducted in a recirculation system within the tropical aquarium facility of the Institute of Aquaculture, University of Stirling, Scotland. The system comprised of a header tank which supplied clean water to culture tanks through a series of pipes as shown in Figure 2.1. The culture tanks were made of 30 L circular self-cleaning tanks (Mail box E8830) as shown in Figure 2.2 and were aerated using an air stone.

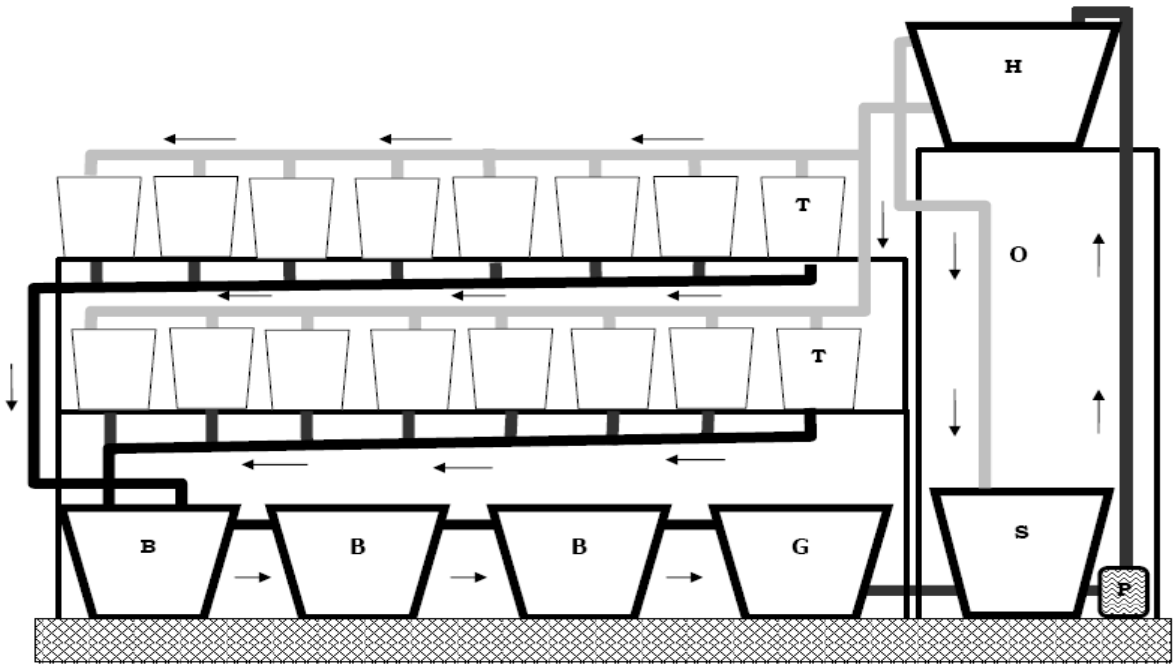


Figure 2.1: Setup of the recirculation system used for growth and digestibility trials

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

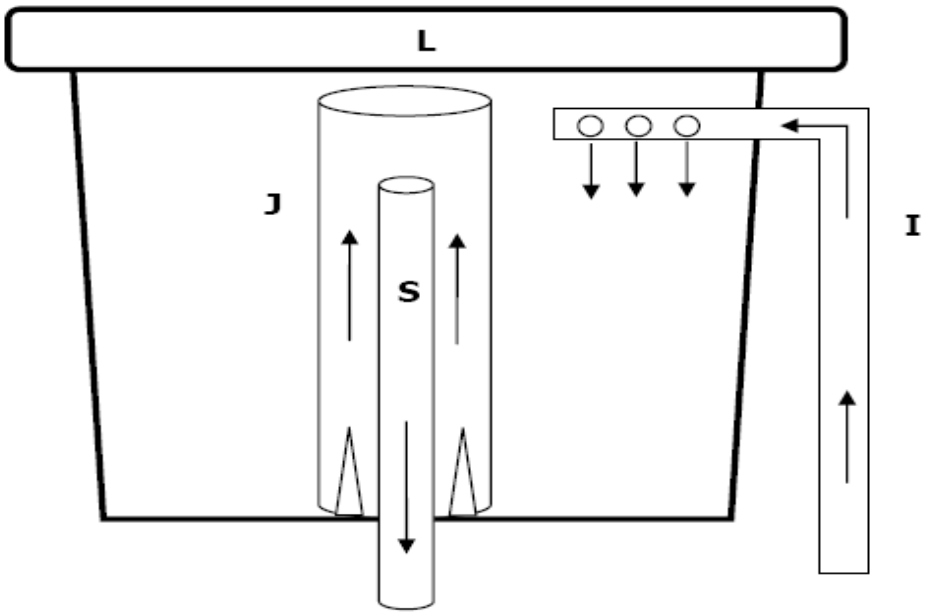


Figure 2.2: Fish tank used during growth trial

Key: L=lid, I=Inlet pipe, J=Outer jacket for the standpipe, S=standpipe/outlet, ⟶=direction of water flow

Clean water was supplied to each culture tank through inlet pipes and the effluent water was drained through stand pipes. Water from inlet pipes was jetted in such a way that it induced a circular flow to facilitate self-cleaning. This was achieved by an outer jacket on each standpipe which had four openings in the shape of an inverted V at the bottom. This enabled removal of solid wastes from the tank which included faeces and uneaten feed together with dissolved wastes such as carbon dioxide and nitrogenous compounds into the filters. The filters comprised of a series of tanks containing bio–rings which served both as mechanical and biological filters.

The water from the filters was then passed through a gravel filter comprising of a tank filled with 12 mm Rossmar limestone chips to buffer the water pH and further filter out remaining solids. The filtered water was then drained into a tank which served as a sump from which the water was pumped (Beresford PV121 220 Lm^{-1}centrifugal pump) back to the header tank for aeration with air stones and heating (Howden 2kW heater). A small amount of water from the system was continuously replaced at a rate of 10 L hr^{-1} to avoid accumulation of excretory products, principally nitrate. The filters were cleaned once every three weeks to get rid of accumulated sludge resulting from faeces and uneaten feed. Water quality was monitored once every week to ensure that it was within acceptable limits for tilapia (Hussain, 2004). Parameters measured included dissolved oxygen and temperature (WTW Multi 340i SET), pH and ammonia/ammonium (Tropic Marin®). Their average values during the study period are as follows; dissolved oxygen 7.26mgL^{-1}, temperature 26.52^{o}C, pH 7.03 and NH_3/NH_4^+ 0.09mgL^{-1}. A constant photoperiod of 12 hr of light and 12 hr of darkness was maintained using artificial light from fluorescent tubes (58W, 240V, General Electric, Hungary) and timer (Sangano Western, UK).

2.2 Experimental Fish and Handling Procedures

The Nile tilapia fingerlings used in this study were of the red Stirling strain (Figure 2.3). The broodstock was originally obtained from lake Manzallah

in Egypt and has been cultured at the Institute of Aquaculture of the University of Stirling since 1979 (Majumdar and McAndrew, 1986; McAndrew *et al.,* 1988).

Figure 2.3: Fingerlings of red Stirling strain of tilapia used for the experiments

Source: Madalla (2005)

Fish were handled according to Home Office regulations as stipulated by The Animals (Scientific Procedures) Act 1986. Fish were individually weighed prior to the start of each experiment in order to obtain fish of uniform size. Fish were anaesthetised using benzocaine (1 mL L^{-1}) to reduce stress. The benzocaine solution was made by dissolving 100g of Benzocaine in 1L of ethanol. Excess water was gently blotted from anaesthetised fish using a paper towel and weighing performed using a top pan balance (Combics 1, Sartorius) to the nearest 0.02g. A total of 20

fingerlings were put into each of the culture tanks and acclimated to experimental conditions for 1 week. Trout diet (Nutra Trout Fry 02, Skretting UK) was fed during the acclimation period. After the acclimation period fish were netted from each tank using a fine mesh hand net and bulk weighed without anaesthesia. Any excess water was removed by gently shaking the net before weighing the fish collectively in a tared water filled container. The dietary treatments were randomly assigned to each of the tanks. Bulk weighing was repeated once every week during the course of growth trials each of which lasted for a period of 8 weeks. The final weights were recorded and 5 fish were randomly sampled from each tank for whole body proximate (3) and histopathology analyses (2). The remaining fish from each replicate were pooled together according to their dietary treatments for faecal collection to determine digestibility of diets as described in section 2.4.

2.3 Biochemical Evaluation of Feedstuffs

2.3.1 Proximate Analysis

Proximate analysis (moisture, crude protein, crude lipid, crude fibre, ash and nitrogen-free extracts) was performed according to standard methods (AOAC, 1990). Moisture content was determined by drying samples to constant weight in an oven (Gallenkamp, UK) at 105°C (12hrs for ingredients & diets and 24hrs for fish carcass). Crude protein was determined using the Kjeldahl method. Samples were digested in

concentrated sulphuric acid using a Digestor 2040 (FOSS, Denmark) followed by distillation using a Kjeltec 2300 auto–analyser (FOSS, Denmark) to determine nitrogen content which was converted to crude protein using a conversion factor of 6.25. Crude lipid was determined using a Soxtec 2050 (FOSS, Denmark) and was extracted using petroleum ether (40-60°C boiling range). Crude fibre was determined using a moisture free defatted sample which was digested by a weak acid followed by a weak base using the Fibertec System 2021 (FOSS, Denmark). Ash was determined by overnight incineration of sample in a muffle furnace at 600^{o}C (Size 2 Gallenkamp, UK). Nitrogen-free extract was determined by subtracting the sum of moisture, crude protein, crude lipid, crude fibre and ash from 100.

2.3.2 Gross Energy Determination

Gross energy was determined using a Parr 6100 Adiabatic Bomb Calorimeter (Parr, USA). The sample was combusted in a chamber pressurised with pure oxygen and resulting heat measured by increase in the temperature of the water surrounding the bomb. Benzoic acid was used as a standard.

2.3.3 Amino Acid Analysis

Samples were hydrolysed with 6N hydrochloric acid in vacuum at 110^{o}C for 24 hrs. Hydrolysed amino acids were quantified using LKB Biochrom 4151 Alpha plus amino acid analyser (LKB Biochrom Ltd, UK). The amino

acid analyser was calibrated using a standard solution (AA-S-18, Sigma). The prepared sample was loaded into ion exchange column. The amino acids were sequentially eluted by buffers of varying pH and ionic strength. The acidic amino acids were removed first, followed by neutral amino acids and finally basic amino acids. Once separated, individual amino acids were quantified by reaction with nihydrin and resultant colour intensity was measured by spectrophotometer at 570nm. The colour intensity was directly proportional to the quantity of an amino acid present in the sample. Amino acids were identified by comparing peak retention times to the standard and quantification was done by comparing area under peak to the area under the standard

2.3.4 Mineral Analysis

Phosphorus was quantified using the method outlined by Allen (1989). Approximately 10 mg of sample was extracted overnight in 25 mL of concentrated nitric acid. This was followed by digestion in concentrated nitric acid and then perchloric acid on a hot plate. Distilled water (20 mL) was added to the sample and boiled until white fumes appeared. This was followed by addition of 5 mL of ammonia solution and further boiling until crystals were formed. The crystals were then dissolved using 20ml of acidified water and 80ml of distilled water followed by 20 mL of a mixed reagent for colour development. The mixed reagent consisted of 250 mL sulphuric acid–antimony, 250 mL sodium molybdate, 500 mL distilled water and 2 g L^{-1} ascorbic acid. The sample was left for 15 minutes for

colour to develop. The quantity of phosphorus was then determined using a spectrophotometer (Cecil Elegant Technology, UK).

Other minerals (sodium, potassium, calcium, iron, zinc, manganese, magnesium and copper) were quantified using a Thermo Xseries 2 inductively coupled plasma mass spectrophotometer (ICP MS) (Thermo Scientific, USA). Samples weighing approximately 80mg were digested in nitric acid for 1 hr in a MarsXpress microwave (CEM Corporation, USA) and then diluted to 10 mL with distilled water ready for quantification.

2.3.5 Antinutritional Factors Analysis

2.3.5.1 Saponins

Total saponins were determined according to the method described by Baccou *et al.,* (1977). About 0.5 g of defatted ground sample was weighed into a capped 25 ml tube. Ten millilitres of 80% methanol (Sigma 320390) were added to the sample which was then placed on a shaker overnight for extraction of saponins. The mixture was then centrifuged at 3500 rpm for 10min after which the supernatant was collected in a 25 mL volumetric flask. The resultant residues were re-extracted three times with 5 ml of 80% methanol, centrifuged and the supernatants pooled in the 25 mL volumetric flask. The final volume was made up to 25 mL with 80% aqueous methanol. 0.5 mL of the methanol extract was transferred into a 15 mL test tube. 2 mL of ethyl acetate (Sigma 319902), 1 mL of 99.5% ethyl acetate reagent (0.5 mL of anisaldehyde [Sigma A0519] and 99.5 mL ethyl acetate reagent) and 1 mL of concentrated sulphuric acid were added

to the tube which was then vortex mixed. The mixture was incubated at room temperature for 30 minutes to allow colour to develop. Absorbance was read on an Uvikon 860 spectrophotometer (Kontron Instruments, France) at 430 nm against a blank. The blank was prepared following the same procedure with the exception that 0.5 mL of distilled water was used in place of the methanol extract. The amount of saponin was determined by means of a standard curve drawn using a series of diosgenin (Sigma D1634) standards (0, 5, 10, 20, 30, 40, 60, 80 and 100 μgmL^{-1}). The standards were treated in the same way as the samples. The diosgenin was dissolved in ethyl acetate to obtain a concentration of 1g mL^{-1} and used to prepare dilutions for the standards.

2.3.5.2 Hydrogen Cyanide

Hydrogen cyanide (HCN) in cassava products was determined as described by Bradbury *et al.,* (1999). About 100 mg of the dried and finely ground sample was weighed into a flat bottomed plastic bottle containing a round paper disc with a buffer at pH 6 and linamarase followed by 0.5 mL of distilled water. A picrate paper attached to a plastic strip was immediately placed in the bottle. The bottle was immediately closed with a screw capped lid and left to stand for 16 hr at room temperature. The picrate paper was carefully removed and transferred to a test tube. Five millilitres of distilled water was added to the tube which was then left for 30 minutes at room temperature. Absorbance of the picrate solution was measured against a blank at 510 nm using an Uvikon

860spectrophotometer (Kontron Instruments, France). The blank was prepared following the same procedure but without the sample. Total HCN content (ppm) was calculated by multiplying the absorbance by 396 and 1ppm = 1 mg HCN kg^{-1} (Bradbury *et al.,* 1999).

2.3.5.3 Tannins

Tannins were quantified using a procedure described by Allen (1989). The procedure involved extraction of total phenolics from a sample. The tannins were quantified by subtracting non-tannin phenols from total phenols. About 0.2 g of a dried and finely ground sample was weighed into a 25ml beaker followed by 10 mL of 70% (v/v) acetone (Sigma 179124). The beaker was placed into an ultrasonic water-bath for 20 minutes at room temperature. The sample was transferred to a centrifuge tube and centrifuged for 10 minutes at 3,000 rpm. The resulting supernatant was collected and kept on ice while the pellet left in the tube was transferred back to the 25 mL beaker. The pellet was re-extracted again twice using 5 mL of 70% (v/v) acetone and centrifuged each time with resultant supernatants pooled. Extract aliquots of 0.02, 0.05, and 0.10 mL were transferred into test–tubes and then made up to 0.50 mL with distilled water. To the tubes, 0.25 ml of 1N Folin and Ciocalteau's reagent (Sigma F9252) and 1.25 mL of 20% sodium carbonate solution (sodium carbonate decahydrate, BDH 102394W) were added. The tubes were vortex mixed and left to stand for 40 minutes for colour to develop. A blank was prepared using 0.5 mL of distilled water, 0.25mL of Folin & Ciocalateau's

reagent and 1.25mL of 20% sodium carbonate solution. The absorbance was read at 725 nm against a blank using a spectrophotometer (Uvikon 860, Kontron Instruments).

Total phenols were determined using a standard curve drawn using blank and a series of tannic acid (Sigma, T8406) standards (2, 4, 6, 8 and 10µgml^{-1}). The standards were treated in the same way as the samples. The tannic acid was dissolved in distilled water to obtain a concentration of 1g mL^{-1}. Tannins were removed from the extracts using insoluble polyvinylpyrrolidone (PVPP) (Sigma, P6755). One hundred milligrams of PVPP were weighed into a test tube followed by 1 mL of distilled water and 1 mL of sample extract. The tubes were vortex mixed and then kept at 4^{o}C for 15 minutes. The tubes were vortex mixed again and then centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected and its non-tannin phenol content was determined by means of the Folin and Ciocalteau method as described earlier but using double the volume for the sample aliquots. Tannin (%) was calculated by the difference between the total phenols (%) and non-tannin phenols (%).

2.3.5.4 Phytic Acid

Phytic acid was determined using an assay kit (Megazyme, K-Phyt 05/07). Approximately 1g of a sample was weighed into a 75mL glass beaker followed by 20mL of 0.66M hydrochloric acid. The beaker was covered with foil and placed on a shaker overnight for extraction. After extraction, 1mL was transferred into a 1.5mL microfuge tube and centrifuged at 13,000

rpm for 10 min. Immediately, 0.5mL of the supernatant was transferred to a fresh 1.5mL microfuge tube and neutralised using 0.5mL of 0.75M 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, 2007).

2.4 Digestibility

Digestibility gives a relative measure of the degree to which feed consumed has been digested and absorbed by the animal (De Silva and Anderson, 1998). Digestibility of ingredients and diets was determined using the indirect method which relies on use of an inert marker. The marker concentrates in faeces relative to the digestible material. Digestibility is therefore determined by the relative quantities of the marker in feed and faeces. In the current study chromium (III) oxide (BDH 277574Q) was used as a marker at an inclusion level of 0.5%. The diets used to determine digestibility of ingredients were formulated according to recommendations by Cho *et al.,* (1982) based on the fact that test ingredients i.e. moringa leaf meal, cassava leaf meal and cassava root meal could not serve as the sole component of a fish diet. The ingredients were therefore combined with a well balanced reference diet. The test diets were formulated using a 7:3 ratio of reference diet to ingredient as shown in Table 2.1. This was done to avoid any influence in terms of poor palatability or nutrient

imbalances of the test ingredients on voluntarily feed intake and/or normal physiological function.

Table 2.1: Formulation of diets used to determine apparent digestibility coefficients of ingredients (g 100g^{-1})

Ingredient	Reference Diet	Test diet
Test Ingredient[1]	0.00	29.85
Fishmeal[2]	30.00	21.00
Soybean meal[3]	8.10	5.67
Wheat meal[4]	47.20	33.04
Sunflower oil	6.20	4.34
Vitamin premix[5]	2.00	1.40
Mineral premix[6]	4.00	2.80
CMC[7]	2.00	1.40
Chromium (III) oxide	0.50	0.50

[1]moringa leaf meal, cassava leaf meal or cassava root meal, [2]brown fishmeal (aquaculture grade), [3]dehulled, solvent extracted, [4]whole grain, [5]As listed in Table 2.2, [6]As listed in Table 2.3, [7]Carboxymethylcellulose (sodium salt, high viscosity)

The composition of mineral and vitamin premixes added to the diets is shown in Table 2.2 and Table 2.3 respectively.

Table 2.2: Composition of mineral premix used in the experimental diets

Mineral	Chemical formula	Amount (g kg^{-1})
Magnesium sulphate	$MgSO_4$	510.00
Sodium chloride	NaCl	200.00
Potassium chloride	KCl	151.11
Iron sulphate	$FeSO_4$, $7H_2O$	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

Source: Jauncey and Ross (1982)

Table 2.3: Composition of vitamin premix used in the experimental diets

Vitamin	Amount (mg kg^{-1*})
Vitamin A as Retinol palmitate	1000.00
Vitamin D as Cholecalciferol	4.00
Vitamin E as Tocopherol acetate	7000.00
Vitamin K	1500.00
Vitamin C as Ascorbic acid	37500.00
Vitamin B_{12} as Cyanocobalamin	1.25
Vitamin B_1 as Thiamine hydrochloride	4250.00
Vitamin B_2 as Riboflavin	3000.00
Vitamin B_6 as Pyridoxine hydrochloride	1250.00
Calcium Pantothenate	5250.00
Niacin	12500.00
Vitamin H as Biotin	90.00
Folic acid	1000.00
Choline chloride	74050.00
Inosine	25000.00
Ethoxyquin†	200.00

†Antioxidant to prevent rancidity, *The mixture was made up to 1kg with α–cellulose

Source: Jauncey and Ross (1982)

Faecal collection was conducted using the modified Guelph system (Cho *et al.,* 1985) shown in Figure 2.4. The system involved a sedimentation column made out of a 2 inch diameter pipe (UPVC Durapipe) fitted with an inlet at the bottom and an outlet at the top approximately 2 inches from the PVC pipe end on each side. The bottom end of the PVC pipe was fitted with a stopper which could allow release of collected solids. The inlet of the sedimentation column was attached to the outlet of the fish tank whenever faecal material was to be collected.

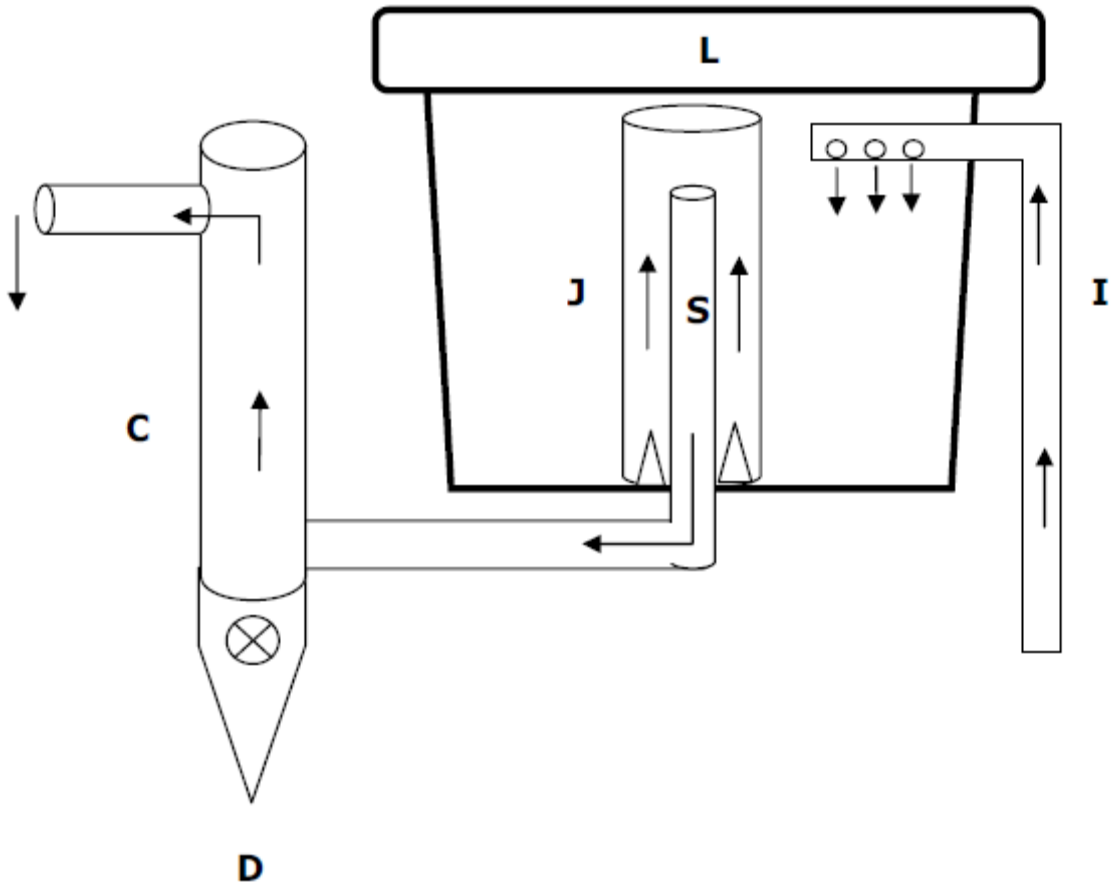


Figure 2.4: Tank used for faeces collection during digestibility trial

Key: L=lid, I=Inlet pipe, J=Outer jacket for the stand pipe, S=standpipe/outlet, C=sedimentation column, D=Faecal collection point, ⟶=direction of water flow

Fish were fed the reference and test diets for a period of 7 days to allow evacuation of all previously ingested feed materials before commencing faecal collection. One hour after the last meal, the tanks were flushed to remove any uneaten feed and the sedimentation column was set to collect faeces overnight. Water flow was reduced to a minimum to facilitate settling of faecal materials in the sedimentation column. The next morning all trapped faeces were removed and centrifuged (MSE Centaur 2, Sanyo-Gallenkamp) at 4,300 rpm for 10 minutes. The supernatant was discarded

and the faecal material stored in a freezer at -20°C. This procedure was repeated until sufficient faecal material was collected.

Before analysis, frozen faecal material was thawed overnight in a refrigerator. Thereafter, the faecal material was dried at 60^0C for 48hrs and then ground to a fine powder using mortar and pestle. Chromic oxide was quantified following the method of Furukawa and Tsukahara (1966). Approximately 50-100mg of sample was digested with 5ml of concentrated nitric acid followed by 3ml of perchloric acid turning the digesta from green to yellow. This was then diluted to 100 ml of distilled water and absorbance read using Uvikon 860 spectrophotometer (Kontron Instruments) at 350nm. The weight and percentage of chromic oxide was calculated using the following formulae:

Weight of Cr_2O_3 *= Absorbance – 0.0032/0.2089*

Digestibility coefficients of both ingredients and diets were estimated to determine bioavailability of nutrients. Apparent digestibility coefficients (ADC) of the feeds were computed using the formula described by Maynard and Loosli (1969).

ADC (%) =100 × (%nutrient in faeces/%nutrient in feed) × (%marker in feed/marker in faeces)

Digestibility coefficients for ingredients were estimated according to Schürch 1969 as cited by Lupatsch (2003) by taking into account the

relative contribution of nutrients from both reference and test diets using the following formula:

$$DC_T=[DC_D -(DC_R\times\{1-t\}]/t$$

where

DC_T = *Digestibility coefficient of the nutrient in test ingredient (%)*

DC_D = *Digestibility coefficient of the nutrient in whole diet (%)*

DC_R = *Digestibility coefficient of the nutrient in reference diet (%)*

t = Contribution of nutrient of test ingredient to total diet; calculated as 100-[(nutrient concentration in R×inclusion of R in D %)/(nutrient concentration in DD)] where R=reference ingredient, T=test ingredient and D=R+T, whole diet.

Digestible protein and digestible energy were calculated as follows;

Digestible protein (g100g^{-1} DM) = Protein digestibility × Crude protein content

Digestible energy (kJ g^{-1}) = Energy digestibility × Gross energy content

Digestible protein to digestible energy ratio (DP/DE ratio) was calculated as mg digestible crude protein/kJ digestible gross energy.

2.5 Diet Formulation and Preparation

Moringa leaf meal (MLM), cassava leaf meal (CLM) and cassava root meal (CRM) were locally acquired in Morogoro, Tanzania. Whole wheat meal, soybean meal (dehulled, solvent extracted) and brown fishmeal (aquaculture grade) were supplied by Ewos Limited, Bathgate. The ingredients were stored in a cool dry environment and used within one year. Their proximate composition and amino acid content is shown in Table 2.4. Diets used for growth trials were formulated to be isonitrogenous (30g 100g^{-1}), isolipidic (10g 100g^{-1}) and isoenergetic (18kJ

g^{-1}). The choice of these nutrient levels, particularly protein, was intended to reflect the practical diets used in Tanzania. The low protein content of leaf meals (30g $100g^{-1}$) was also a constraining factor whereby higher inclusion levels to attain higher dietary protein level would have limited space for inclusion of other ingredients.

During compounding of diets, the relevant proportions of dry ingredients were weighed (Mettler PM6000) and then thoroughly mixed using a food mixer (A200 Hobart Ltd) before adding sunflower oil (Tesco ltd, UK) and water. The mixture was steam-pelleted using a California Pellet Mill (Model CL2) with a 1.2 mm die. The resulting pellets were dried for 48 hrs at 50°C and then packed in polythene bags and frozen.

Table 2.4: Biochemical composition of soybean, fishmeal and wheat meal used in formulation of experimental diets.

	Soybean[1]	Fishmeal[2]	Wheat meal[3]
Proximate composition (g $100g^{-1}$, as fed)			
Dry matter	89.42	92.71	89.09
Crude protein	44.74	66.41	8.48
Crude lipid	0.90	8.84	1.33
Crude fibre	3.42	0.52	2.51
Ash	5.27	12.05	1.68
Nitrogen free extracts	35.10	4.89	75.09
Gross energy (kJ g^{-1})	20.19	21.16	18.85
Amino acid composition (g $100g^{-1}$ feed)			
Arginine	3.12	3.68	0.31
Histidine	1.25	1.42	0.21
Isoleucine	2.30	2.88	0.29
Leucine	3.54	4.64	0.52
Lysine	2.86	4.62	0.28
Methionine + Cystine	0.73	2.03	0.09
Phenylanine + Tyrosine	3.60	3.82	0.41
Threonine	1.50	2.37	0.21
Valine	2.55	3.59	0.38

[1]dehulled, solvent extracted, [2]Brown fishmeal (aquaculture grade), [3]Whole grain.

An inert marker, chromium (III) oxide (BDH 277574Q) was included in all diets at 0.5g $100g^{-1}$ to allow indirect determination of digestibility as described in section 2.4. Carboxymethylcellulose sodium salt (high viscosity) (Sigma C5013) was added as a binder to improve water stability. In some diets α-cellulose (Sigma C8002) was added to balance crude fibre content between experimental diets and also as a bulking agent in the vitamin premix.

2.6 Feeding Regime

Fish were hand fed three times per day to apparent satiation but not more than 10% of body weight. The diets were offered at 09:00, 13:00 and 17.00 hours in two rounds. In the first round, half of the meal was offered followed by the second round where the remaining half of the meal was offered in small amounts until 2-5 feed particles remained on the tank bottom for 20-30 seconds without being consumed. At this point, it was assumed that satiation had been achieved. The ration was adjusted weekly according to fish weight with care being taken to avoid feed wastage. Any remaining feed rations at the end of the week were weighed to estimate actual feed intake.

2.7 Growth and Nutrient Utilisation

Growth and nutrient utilisation were determined in terms of feed intake (FI), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU), energy retention (ER) and hepatosomatic index (HSI) as follows:

FI (g fish^{-1} day^{-1}) = Total feed intake per fish/ number of days

ADG (g fish^{-1} day^{-1}) = final weight – initial weight/ number of days

SGR (% day^{-1}) = 100 × (ln[final body weight]- ln[initial body weight])/ no. of days

FCR = feed intake/ live weight gain

PER = live weight gain/ crude protein intake

ANPU (%) = 100 × (final fish body protein (g) - initial fish body protein (g))/ crude protein intake (g)

ER (%) = 100 × (final fish body energy - initial fish body energy)/ gross energy intake

HSI = 100 × (liver weight/ total body weight)

2.8 Whole Body Composition

Whole body proximate analysis was conducted to determine whole body composition as described in section 2.3.1. Samples were analysed for moisture, crude protein, crude lipid and ash and results expressed as percentage of live weight.

2.9 Liver and Gut Histopathology

Histological analyses of liver and gut were carried out to detect any pathological changes due to dietary treatments. Fish were sampled after

the trial and relevant tissues fixed in 10% neutral buffered formalin. Histological evaluation of the tissues was performed according to procedures described by Drury and Wallington (1980). Samples were cassetted and mounted in an autoembedder (Shandon Excelsior, Thermo) for dehydration, clearing and wax impregnation. Processed samples embedded in paraffin wax were sectioned (5 µm thickness) using a microtome (Leica 2035 Biocut). Samples were stained with haemotoxylin and eosin and examined under a light microscope (Olympus BX51).

2.10 Data Analysis

A completely randomised design (CRD) was used in assigning dietary treatments to culture units. The main statistical hypothesis tested was "there is no significant difference between treatment means". One way analysis of variance (ANOVA) was used to determine differences between treatment means which were deemed significant at $P<0.05$. Post-hoc analysis was done where significant differences existed between treatments means using Tukey's Honest Significant Difference Test (Steele and Torrie, 1980). Analyses were performed using SPSS software version 13 (SPSS Inc.). Before analysis data were tested for normality using the Kolmogorov–Smirnov test and homogeneity of variance using Levene's test and transformed in case of non–conformity. Percentage data was first transformed using arcsine before performing ANOVA (Ott and Longnecker, 2001).

CHAPTER 3 Moringa Leaf Meal as Protein Source

3.1 Introduction

Moringa, *Moringa oleifera* Lamarck is a small tree that grows to a height of about 7–12 m, has a grey bark and fragrant white flowers (Figure 3.1). It belongs to the family Moringaceae and although indigenous to the sub-Himalayan tracts of northern India, it is now found in other parts of the world with tropical and sub-tropical climatic conditions (Makkar and Becker, 1996; Fuglie, 1999). Moringa grows fast bearing long green pods after 6–8 months and production may continue for 30–40 years. Moringa has a high forage yield which may amount to 120 metric tonnes dry matter ha^{-1} yr^{-1} (Makkar and Becker, 1999).

Figure 3.1: Moringa leaves with flowers

Source: FloralPix (2006)

Products from moringa have a wide range of applications in agricultural, industrial and pharmaceutical processes and quite often moringa is regarded as a "wonder tree" (Fuglie, 1999). Moringa leaves have a relatively high crude protein content which varies from 25% (Makkar and Becker, 1996) – 32% (Soliva *et al.,* 2005). A high proportion of this protein is potentially available to digestion due to a high proportion of pepsin soluble nitrogen (82–91%) and low proportion (1–2%) of acid detergent insoluble protein (Makkar and Becker, 1996). The protein contains high levels of sulphur containing amino acids and compares well with soybean, which is usually regarded as a source of high quality plant protein (Francis *et al.,* 2002). In addition the leaves are also rich in vitamins and minerals (Fuglie, 1999). Its crude lipid fraction has a high proportion of n-3 (ω3) fatty acids in the form of linolenic acid accounting for almost 67% of total fatty acids (Soliva *et al.,* 2005).

There have been several studies on utilisation of moringa leaf meal (MLM) as a protein source in terrestrial animals such as cattle (Sarwatt *et al.,* 2004; Reyes Sanchez *et al.,* 2006), goats (Aregheore, 2002), (Sarwatt *et al.,* 2002), pigs (Ly *et al.,* 2001) and poultry (Kaijage, 2003). Only a few studies have been performed in aquatic animals, namely abalone, *Haliotis asinina* and Nile tilapia (Richter *et al.,* 2003; Afuang *et al.,* 2003; Dongmeza *et al.,* 2006). Good growth rate, low feed conversion ratio and high protein utilisation were reported in abalone, *Haliotis asinina* fed a diet containing 13% crude protein from moringa leaf (Reyes and Fermin,

2003). In Nile tilapia, inclusion of 12% raw MLM to provide 10% of total dietary protein did not significantly affect growth (Richter *et al.,* 2003). Higher inclusion levels led to a significant reduction in performance due mainly to high levels of anti-nutritional factors (ANFs) particularly saponins and to a lesser extent tannin, phytic acid and hydrogen cyanide (HCN). Makkar and Becker (1996) and Makkar and Becker (1997) showed that significant amounts of saponins from moringa leaves can be removed through solvent extraction and aqueous extraction respectively. Using solvent extracted moringa leaf meal, the inclusion level could be tripled (33%) to replace up to 30% of fish meal without significantly affecting performance of Nile tilapia (Afuang *et al.,* 2003).

Solvent extraction, however, may not be technically and financially feasible for small-scale fish farmers. Given the relatively high solubility of saponin, the current study explored the suitability of aqueous extracted moringa leaf meal as a protein source for Nile tilapia. Aqueous extraction may be more technically and financially feasible than solvent extraction for small scale fish farmers in rural areas. The objective of the present study therefore, was to evaluate the suitability of aqueous extracted moringa leaf meal as a protein source for Nile tilapia.

3.2 Methodology

3.2.1 Processing of Moringa Leaves

Leaves were harvested during the dry season from a moringa tree plot located within the premises of Sokoine University of Agriculture in Tanzania. The leaves were soaked overnight in a tank containing still tap water at 1:1 w/v in attempt to remove saponins and other soluble antinutritional factors (Figure 3.2).

a) Freshly harvested moringa leaves

b) Overnight soaking of leaves in water

c) Drying under shade

d) Foam from water used for soaking

Figure 3.2: Procedure used for the processing of moringa leaves

Source: Madalla (2005)

Soaked leaves were placed on a wire mesh to drain excess water and then spread on plastic sheets to dry in the shade to avoid loss of vitamins (through photodynamic damage/oxidation) as suggested by Fuglie (1999). The leaves were threshed to strip off dry leaves from stalks to reduce the crude fibre content in the meal. The dried leaves were then ground into a fine powder using a hammer mill (Lab Mill, screen size 0.2 mm) and stored in plastic bags at room temperature.

3.2.2 Biochemical Analysis and Digestibility of MLM

Proximate analysis, gross energy, amino acid profiles, minerals and antinutritional factors in MLM shown in Table 3.1 were determined following procedures described in section 2.3. Apparent digestibility coefficients of processed MLM were determined using a total of 45 Nile tilapia of an average weight of 12.89 ± 1.16g following procedures described in section 2.4.

3.2.3 Experimental Setup and Diet Formulation

The experimental fish were kept in 15 self–cleaning circular plastic tanks of 30L capacity in a recirculation system as described in section 2.2. Each tank contained 20 Nile tilapia fingerlings with an average weight of 4.68 ± 0.34 g. Fish were weighed in bulk once every week to monitor growth and adjust feed ration during the 8 week the trial. Five diets were formulated to contain 30g 100 g^{-1} crude protein, 18kJ g^{-1} and 10g 100 g^{-1} lipid for the growth trial (Table 3.2) as described in section 2.5. Five diets were

formulated in which processed moringa leaf meal provided 0, 15, 30, 45 and 60g $100g^{-1}$ g of total dietary crude protein. The diets were identified as MLM0, MLM15, MLM30, MLM45 and MLM60 respectively. Diet MLM0 contained fishmeal as the main source of protein and served as control.

Table 3.1: Biochemical composition of unprocessed and processed MLM

	Unprocessed MLM	Processed MLM
Proximate composition (g 100^{-1} g, as fed)		
Dry matter	95.77	92.99
Crude protein	34.92	31.07
Crude lipid	5.89	4.45
Crude fibre	7.09	5.89
Ash	8.04	5.52
Nitrogen free extract	39.83	46.09
Gross energy (kJ g^{-1})	19.88	20.11
***Amino acid composition* (g $100g^{-1}$ feed)**		
Arginine	3.23	2.01
Histidine	1.31	0.83
Isoleucine	2.84	1.69
Leucine	4.70	2.90
Lysine	2.25	1.50
Methionine +Cystine	0.76	0.23
Phenylalanine+ Tryptophan	5.01	3.92
Threonine	2.24	1.45
Valine	3.50	2.17
Mineral composition (mg g^{-1}, DM)		
Phosphorus	3.72	3.42
Sodium	0.22	0.36
Magnesium	3.26	3.16
Potassium	15.4	6.21
Calcium	14.98	11.41
Iron	0.13	0.23
Copper	0.02	0.03
Zinc	0.05	0.25
Antinutritional factors (g $100g^{-1}$, DM)		
Saponins[1]	1.19	1.19
Phenols[2]	4.13	3.36
Tannins[2]	1.19	1.62
Phytic acid[3]	0.06	0.15
HCN[4]	0.16	0.16

[1]As diosgenin equivalent, [2]As tannic acid equivalent, [3]As phosphorus equivalent, [4]Hydrocyanic acid (mg $100g^{-1}$)

Diets were randomly assigned to the tanks and were fed to appetite but not exceeding 10% of body weight in three equal rations daily at 09:00, 13:00 and 17:00 as described in section 2.6. At end of the trial, fish weight was recorded and 5 fish from each tank were sacrificed for whole body proximate and histopathology analyses as described in sections 3.2.4 and 3.2.5 respectively. Remaining fish were pooled according to dietary treatment and used for faecal collection to determine digestibility of diets as described in section 2.4.

Table 3.2: Formulation of diets fed to *O. niloticus* during the growth trial for moringa leaf meal (g 100 g^{-1})

Ingredient	MLM0	MLM15	MLM30	MLM45	MLM60
Fish meal[1]	36.0	31.0	26.0	21.0	16.0
Moringa leaf meal	0.0	14.5	29.0	42.0	58.0
Wheat meal[2]	47.5	38.5	29.5	22.5	11.5
Sunflower	6.0	6.0	6.0	6.0	6.0
Vitamin premix[3]	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	4.0	4.0	4.0	4.0	4.0
CMC[5]	2.0	2.0	2.0	2.0	2.0
α-cellulose	2.0	1.5	1.0	0.0	0.0
Chromium oxide.	0.5	0.5	0.5	0.5	0.5

[1]Brown fishmeal (aquaculture grade), [2]Whole grain, [3]As listed in Table 2.2, [4]As listed in Table 2.3, [5]Carboxymethylcellulose

3.2.4 Performance Parameters

Growth performance and nutrient utilisation were evaluated according to procedures described in section 2.7 while whole body composition was evaluated according to the procedures described in section 2.8.

3.2.5 Histopathology of Liver and Gut

The sampled fish were dissected to remove the gastrointestinal tract and liver which were fixed in 10% neutral buffered formalin. Liver and gut histology was evaluated according to procedures described in 2.9.

3.2.6 Data Analysis

The data collected on the performance parameters was analysed as described in section 2.10.

3.3 Results

3.3.1 Biochemical Composition and Digestibility of MLM and MLM Diets

The biochemical composition of unprocessed and processed MLM is shown in Table 3.1. In general, aqueous extraction led to reduction of most nutrients. Crude protein content was reduced by about 10%, though it still remained high above 30%. The amino acid content was also reduced and in particular the sulphur amino acids i.e. methionine+cystine was most affected with nearly 67% reduction. The amounts of sodium, iron and zinc were noticeably higher in processed MLM than in unprocessed MLM. On the contrary, less potassium and calcium were observed in processed MLM compared to raw MLM. Aqueous extraction had no effect on saponin content. The contents of other antinutritional factors with the exception of phytic acid, were little affected. Results from the digestibility trial showed that processed MLM was well digested by Nile tilapia as demonstrated by fairly high values of apparent digestibility coefficients

which were also reflected in the high values of digestible crude protein and gross energy shown in Table 3.3.

Table 3.3: Digestibility of moringa leaf meal (MLM)

	Reference Diet	Test diet	MLM
Proximate composition (g 100g^{-1}, as fed)			
Dry matter	93.08	94.13	
Crude protein	31.56	32.85	
Crude lipid	10.69	9.73	
Crude fibre	2.81	4.39	
Ash	8.66	8.01	
Nitrogen free extract	39.36	39.15	
Gross energy (kJ g^{-1})	17.92	19.19	
Apparent digestibility coefficients (%)			
Dry matter	73.31	71.83	**68.5**
Crude protein	84.34	85.85	**89.0**
Crude lipid	98.39	90.72	**65.2**
Gross energy	70.05	72.38	**76.8**
Digestible protein (g 100g^{-1})	24.78	26.55	**25.71**
Digestible energy (kJ g^{-1})	12.55	13.89	**15.44**

The biochemical composition of the diets used for the growth trial is shown in Table 3.4. A slight variation in proximate composition was observed among the diets. The crude fibre content increased with an increase in MLM with diet MLM60 having almost double the fibre content of diet MLM0. Phosphorus content in diets decreased with increase in MLM inclusion. The essential amino acid contents (calculated from raw ingredient data) in all diets met Nile tilapia requirements with the exception of methionine + cystine in diets MLM30, MLM45 and MLM60. Antinutritional factor levels in MLM diets increased with inclusion level of MLM with higher levels of phenols, tannins and saponins.

Table 3.4: Biochemical composition and apparent digestibility coefficients of MLM diets used for the growth trial

	MLM0	MLM15	MLM30	MLM45	MLM60	Req*
Proximate composition (g 100g^{-1}, as fed)						
Dry matter	95.15	95.26	95.06	96.53	95.94	
Crude protein	32.55	33.66	33.75	34.19	34.21	
Crude lipid	10.63	10.99	11.47	12.18	12.33	
Crude fibre	3.35	3.97	4.82	4.88	5.82	
Ash	9.18	9.17	9.18	9.19	9.13	
Nitrogen free extract	39.44	37.46	35.84	36.08	34.45	
Gross energy (kJ g^{-1})	19.09	19.39	20.11	20.44	20.64	
Phosphorus (mg g^{-1})	7.61	7.37	6.64	6.28	5.66	
Amino acids (% Protein)						
Arginine	6.00	6.14	6.21	6.27	6.35	4.20
Histidine	2.34	2.41	2.45	2.49	2.54	1.72
Isoleucine	4.22	4.44	4.62	4.78	4.97	3.11
Leucine	7.97	8.22	8.37	8.54	8.68	3.39
Lysine	6.93	6.72	6.43	6.13	5.85	5.12
Methionine +Cystine	3.91	3.48	3.01	2.57	2.06	3.21
Phenylalanine+ Tryrosine	6.99	7.49	7.91	8.30	8.75	5.54
Valine	5.73	5.98	6.17	6.33	6.53	2.80
Antinutritional factors (g 100g^{-1}, DM)						
Saponins[1]	-	0.17	0.35	0.50	0.69	
Phenols[2]	-	0.49	0.97	1.41	1.95	
Tannins[2]	-	0.23	0.47	0.68	0.94	
Phytic acid[3]	-	0.02	0.04	0.06	0.09	
HCN[4]	-	0.02	0.05	0.07	0.09	
Apparent digestibility coefficients (%)						
Dry matter (%)	76.6	78.4	77.3	74.1	78.6	
Crude protein (%)	91.0	89.3	85.5	84.6	85.8	
Crude lipid (%)	94.3	92.0	88.5	86.5	90.2	
Gross energy (%)	79.8	80.2	79.1	77.1	80.6	
Phosphorus (%)	75.2	78.0	77.6	74.6	75.1	
Digestible protein (DP) ((g 100g^{-1})	29.6	30.1	28.9	28.9	29.3	
Digestible energy (DE) (kJ g^{-1})	15.2	15.6	15.9	15.8	16.6	
DP/DE ratio (mg kJ^{-1})	20.4	20.3	19.1	19.0	18.4	

*Req=*O. niloticus* amino acid requirements (Santiago and Lovell, 1988) [1]As diosgenin equivalent, [2]As tannic acid equivalent, [3]As phosphorus equivalent, [4]Hydrocyanic acid (mg 100g^{-1}).

The digestibility of MLM diets was high with apparent digestibility coefficients above 70%. Generally, apparent digestibility coefficients decreased with increasing levels of MLM in the diets with few exceptions. Digestible protein (DP), digestible energy (DE) and digestible protein to digestible energy ratios were more or less similar between the MLM diets. However, the intakes of DP and DE significantly declined with increasing inclusion levels of MLM.

3.3.2 Growth Performance and Feed Utilisation

The difference in body weight between the control diet and the different experimental diets containing graded levels of moringa leaf meal was noticeable after 1 week (Figure 3.3). The difference in body weight between diets containing MLM became noticeable after 3 weeks. Inclusion of MLM in experimental diets led to a significant decline in performance as shown in Table 3.5. Fish showed reluctance to consume diets containing MLM and were even observed to spit out pellets a few times before actual ingestion resulting in a significant decline in daily feed intake as well as digestible protein and energy. This feed rejection was more pronounced in fish fed diets MLM45 and MLM60. It was noted that fish fed diets containing MLM fed actively on diet that had adhered to the tank sides since the previous meal and thus been exposed to prolonged immersion.

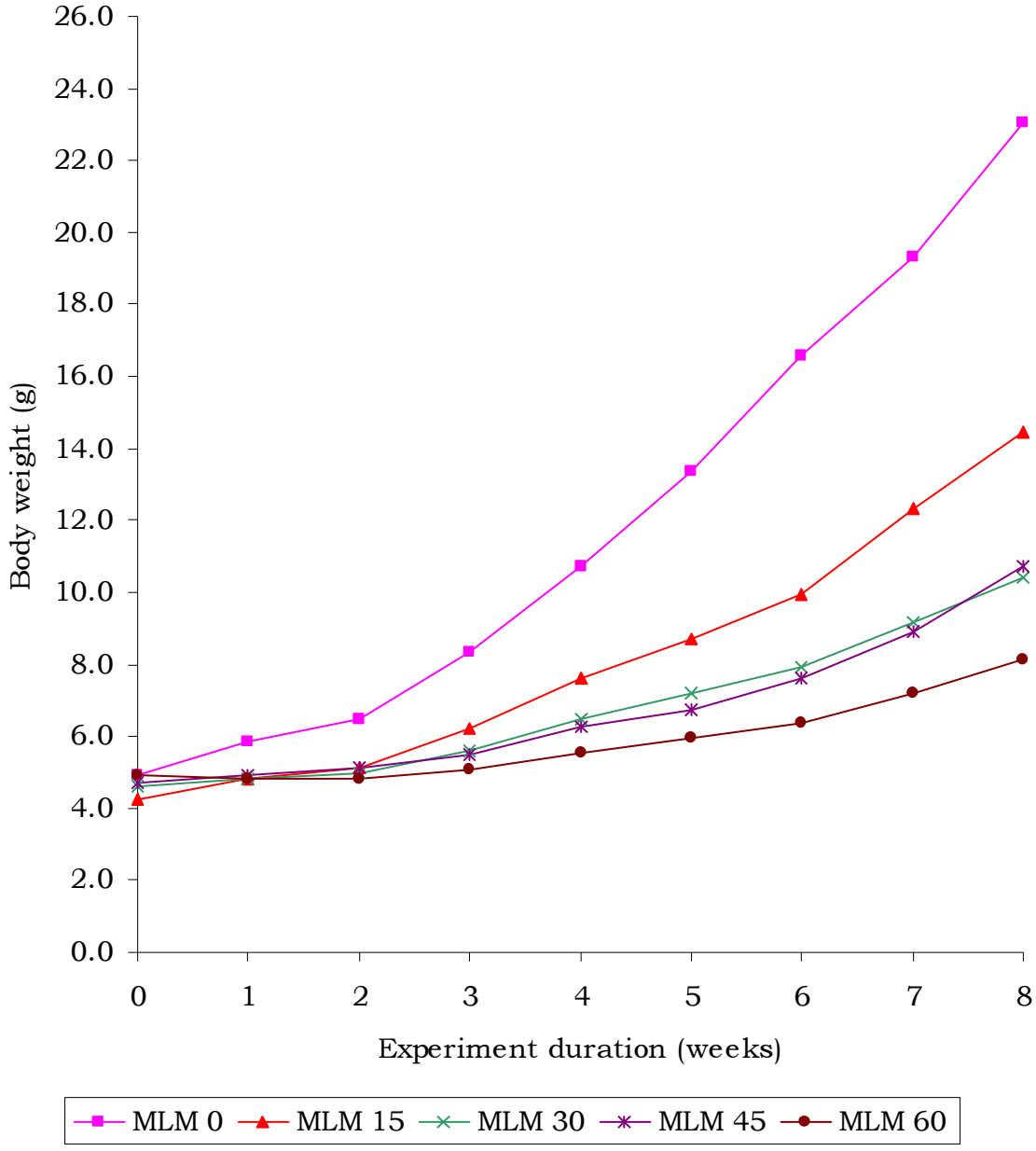


Figure 3.3: Change in body weight of *O. niloticus* fed MLM diets

The decline in intake was accompanied by a significant decline in growth in terms of final weights (FW), average daily gain (ADG), specific growth rate (SGR) and nutrient utilisation in terms of feed conversion ratio (FCR),

protein efficiency ratio (PER), apparent net protein utilisation (ANPU), energy retention (ER) and hepatosomatic index (HSI).

3.3.3 Whole Body Composition

Whole body proximate composition of fish at the beginning and end of the experiment (% fresh weight basis) is shown in Table 3.6. The final whole body moisture content was significantly higher in fish fed diets with high levels of MLM and the converse was true for the crude lipid and gross energy content. As a consequence fish fed on diet MLM60 had less than half the body lipid content of those fish fed on diet MLM0. The crude protein and ash contents varied little between the fish fed the different diets. The ash content, however, significantly declined with increased MLM.

3.3.4 Histopathology

Examination of histology samples of liver and small intestine did not show any obvious pathological changes which could be linked to dietary treatment. However, lipid deposition in the liver was observed to decrease with increased inclusion levels of dietary moringa leaf meal.

3.4 Discussion

Processing of moringa leaves using aqueous extraction of MLM led to reduction of most nutrients most likely due to leaching of soluble nutrients.

Table 3.5: Growth performance and nutrient utilisation of *O. niloticus* fed MLM diets (mean ± SD, n=3)

	MLM0	MLM15	MLM30	MLM45	MLM60
Initial weight (g)	4.92 ± 0.22^{a}	4.23 ± 0.25^{a}	4.60 ± 0.14^{a}	4.72 ± 0.11^{a}	4.92 ± 0.34^{a}
Final weight (g)	22.86 ± 3.42^{a}	14.29 ± 0.65^{b}	10.31 ± 0.53^{bc}	10.55 ± 0.53^{bc}	8.07 ± 0.46^{c}
Feed intake (g fish^{-1} day^{-1})	0.71 ± 0.40^{a}	0.56 ± 0.40^{b}	0.50 ± 0.40^{bc}	0.45 ± 0.10^{cd}	0.40 ± 0.01^{d}
Average daily gain (g fish^{-1} day^{-1})	0.32 ± 0.56^{a}	0.18 ± 0.2^{b}	0.10 ± 0.01^{c}	0.10 ± 0.01^{c}	0.06 ± 0.01^{c}
DP intake (g fish^{-1} day^{-1})	0.200 ± 0.011^{a}	0.159 ± 0.011^{b}	0.138 ± 0.011^{bc}	0.126 ± 0.003^{cd}	0.114 ± 0.002^{d}
DE intake (kJ fish^{-1} day^{-1})	10.28 ± 0.56^{a}	8.22 ± 0.56^{b}	7.62 ± 0.63^{bc}	6.86 ± 0.18^{c}	6.44 ± 0.08^{c}
Specific growth rate (% day^{-1})	2.73 ± 0.22^{a}	2.18 ± 0.17^{b}	1.44 ± 0.12^{c}	1.44 ± 0.08^{c}	0.97 ± 0.09^{d}
Feed conversion ratio	2.26 ± 0.41^{a}	3.11 ± 0.44^{ab}	4.97 ± 0.64^{c}	4.34 ± 0.26^{bc}	7.27 ± 1.14^{d}
Protein efficiency ratio	1.39 ± 0.23^{a}	0.97 ± 0.14^{b}	0.60 ± 0.07^{cd}	0.68 ± 0.04^{bc}	0.41 ± 0.06^{d}
Apparent net protein utilization (%)	20.85 ± 4.00^{a}	14.82 ± 2.66^{b}	9.10 ± 1.29^{bc}	11.22 ± 1.44^{bc}	6.07 ± 0.89^{c}
Energy retention (%)	12.75 ± 2.06^{a}	8.72 ± 1.27^{b}	4.75 ± 0.59^{c}	5.37 ± 0.32^{c}	2.24 ± 0.43^{d}
Hepatosomatic index	3.01 ± 0.39^{a}	2.32 ± 0.34^{b}	2.15 ± 0.38^{b}	1.62 ± 0.54^{c}	1.54 ± 0.32^{c}
Survival (%).	80.00 ± 22.91^{a}	86.67 ± 15.28^{a}	86.67 ± 12.58^{a}	96.67 ± 5.77^{a}	98.33 ± 2.89^{a}

Table 3.6: Whole body proximate composition of *O. niloticus* fed MLM diets before and after the experiment (% fresh weight basis, mean ± SD, n=3)

	Initial	MLM0	MLM15	MLM30	MLM45	MLM60
Moisture content	77.23	73.77 ± 1.54^{a}	74.18 ± 0.78^{a}	76.18 ± 0.77^{ab}	74.54 ± 1.15^{a}	78.35 ± 0.88^{b}
Crude protein	13.39	14.67 ± 0.88^{a}	14.70 ± 0.49^{a}	14.34 ± 0.49^{a}	15.13 ± 0.68^{a}	13.98 ± 0.59^{a}
Crude lipid	5.60	7.34 ± 0.46^{a}	6.82 ± 0.14^{a}	5.18 ± 0.17^{b}	5.58 ± 0.27^{b}	3.11 ± 0.10^{c}
Ash	3.10	3.07 ± 0.47^{a}	3.24 ± 0.03^{b}	3.65 ± 0.04^{c}	3.77 ± 0.05^{d}	4.09 ± 0.03^{e}
Gross energy (kJ g^{-1})	5.80	5.35 ± 0.01^{a}	5.18 ± 0.01^{a}	4.92 ± 0.02^{b}	4.94 ± 0.01^{b}	4.45 ± 0.11^{c}

Different superscripts in the same row indicate significant difference ($p<0.05$)

Martinez et al (2001) attributed the dry matter loss in *Lupinus campestris* seeds after soaking to leaching of soluble carbohydrates. One of the most noticeable losses in proximate composition was that of crude protein which was reduced by almost 10%. Similar losses in crude protein after aqueous extraction have also been observed in corn, *Zea mays* (Akobundu and Hoskins, 1982), leucaena, *Leucaena leucocephala* (Soedarjo and Borthakur, 1996), cowpeas, *Vigna unguiculata* (Wang *et al.,* 1997) and mung bean, *Phaseolus aureus* (Mubarak, 2005). There is a possibility that the protein loss observed in this study could be attributed to non-protein nitrogen (NPN). A similar protein loss observed in redwood, *Acacia villosa,* following aqueous extraction was attributed to loss of NPN (Wina *et al.,* 2005). Moringa leaves are known to contain appreciable amounts of NPN which ranges between 5% (Makkar and Becker, 1996) and 13% (Makkar and Becker, 1997). The loss of protein in this study is, however, contrary to the increase in protein content reported after solvent extraction using ethanol (Makkar and Becker, 1996) or methanol (Afuang *et al.,* 2003). The loss of amino acids could be explained by the reduction in crude protein.

According to Lyimo *et al.* (1992) loss of protein and other nitrogenous compounds tends to be associated with loss of amino acids. Sulphur amino acids i.e. methionine and cystine were worst affected with a loss of almost 67%. This led to deficiency in diets MLM 30, MLM45 and MLM60 compared to the recommended levels (Santiago and Lovell, 1988). Similarly, the low content of some minerals in the processed MLM could be

explained by the reduction in ash due to leaching although they still remained generally high. All in all, the proximate composition the processed MLM remained within values observed in earlier studies (Makkar and Becker, 1996; Richter *et al.,* 2003; Soliva *et al.,* 2005) and the nutrient content was favourable with high protein and low fibre compared to other leaf meals as also reported by Devendra (1991).

Contents of most antinutritional factors remained more or less the same after processing indicating that aqueous extraction in this study was ineffective. In a study by Makkar and Becker (1997) aqueous extraction of moringa leaves reduced saponins and tannins by 93% and 100% respectively. This is likely to be due to differences on how the aqueous extraction was conducted. In the current study, moringa leaves were soaked overnight in still tap water at 1:1 w/v while in the previous study (Makkar and Becker, 1997) leaves were soaked for 20 minutes in distilled water at 1:50 w/v with stirring. Processing actually led to a slight increase in contents of tannins and phytic acid. A similar increase in tannin content was observed in dolichos lablab bean, *Lablab purpuresusin* after overnight soaking in water at a ratio of 1:10 w/v (Osman, 2007). According to Vijayakumari *et al* (1998) the increase in tannin content could be due to degradation of high molecular weight insoluble polymers into smaller molecular weight polymers that give a stronger colour reaction with the reagents. Makkar and Becker (1997) also observed an increase in phytic acid content after aqueous extraction of moringa leaves but contents in

the current study remained very low in any case. The remaining antinutritional factor was HCN whose level in both raw and processed MLM (1.58 mg kg^{-1}) was below 'safe' level of 100 mg HCN kg^{-1} recommended by the European Union Commission.

Crude protein in moringa leaf was well digested by Nile tilapia (89%) which fell within limits regarded as high i.e. 75%-95% (Cho and Kaushik, 1990). Crude lipid digestibility (65%) was, however, low compared to the routinely reported range of 85–95% (NRC, 1993). This is likely to be because the lipid fraction of MLM contains significant amounts of indigestible waxes (Afuang *et al.,* 2003). The fairly high digestibility of moringa leaf was reflected in the values of digestible protein and energy which were high and comparable to the values reported by Anderson (1991). The good digestibility of moringa leaf meal could explain the high digestibility coefficients for the MLM diets used in the growth trial. Consequently DP and DE values were high with the DP/DE ratio above the optimal value of 18 mg kJ^{-1} suggested for Nile tilapia by Kaushik *et al.,* (1995).

Poor feed intake is perhaps one of the most important factors responsible for poor growth. The presence of antinutritional factors, particularly saponins and tannins, could be responsible for lowering palatability by imparting an astringent/bitter taste responsible for observed feed rejection and poor feed intake (Jansman, 1993; Hostettmann and Marston, 1995; Guillaume and Métailler, 1999; Francis et al., 2001; Makkar, 2003). Poor feed intake may have led to apparent starvation similar to observations in

Chinook salmon, *Oncorhynchus tshawytscha* fed diets containing saponin-rich extracts from soy products whose intestinal morphology resembled that of starved fish (Bureau *et al.,* 1998) although such pathology was not observed in the present experiment. Poor feed intake of diets containing saponin and/or tannins has been observed by Afuang *et al.,* (2003) and Dongmeza *et al.,* (2006) in Nile tilapia (Dongmeza *et al.,* 2006) and also by Becker and Makkar (1999) in common carp. Astringency can be defined as a dry or puckering mouth feel detectable throughout the oral cavity which reduces the lubricating property of saliva thus affecting the ability to swallow food (Drewnowski and Gomez-Carneros, 2000). The astringency causes deterrence by binding to saliva mucopolysaccharides, epidermis or chemosensory receptors (Bernays *et al.,* 1989). However, it is likely that saponins played a bigger role than tannins in reducing palatability. When Al–Owafeir (1999) fed diets containing graded levels of saponins (0.08-0.42 $100g^{-1}$) and tannins (0.05-0.71g $100g^{-1}$) to Nile tilapia, significant reduction in feed intake was only observed in diets containing saponin. This supports the observation of active feeding on feed from the previous meal that had adhered to the tank sides where additional leaching of soluble antinutritional factors, like saponins, could have improved palatability. Drying and/or grinding of moringa leaves before aqueous extraction might improve of removal of soluble antinutritional factors.

Another reason for poor performance could be sulphur amino acid deficiency in some diets. Methionine is essential for normal growth while

cystine is a conditionally indispensable amino acid which has a sparing effect on methionine (Hepher, 1988; Lovell, 1998; Wilson, 2002). Methionine deficiency leads to reduced fish performance in terms of growth and FCR (De Silva and Anderson, 1998). This deficiency could have been further complicated by the fact that availability coefficients for sulphur amino acids in diets containing plant ingredients tend to be lower than for most of the other amino acids (Swick, 1995).

Fish fed diets higher in MLM had higher body moisture content while the opposite was true for lipid and gross energy. Similar results have been reported for carp fed diets containing high amounts of plant proteins such as mustard oilcake, linseed and sesame meal (Hossain, 1988). The decrease in lipid content is probably due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. The presence of saponins may also have contributed to inhibited pancreatic lipase activity and hence delayed intestinal absorption of dietary fat (Han *et al.,* 2000). The increase in ash with increased MLM could also be linked to starvation. A similar increase in ash content was observed in *O. mossambicus* after 60 days of starvation (Pandian and Raghuraman, 1972).

The results of this study show that overnight aqueous extraction of moringa leaves was not effective in removing saponins and other soluble antinutritional factors resulting in poor diet palatability which led to a significant reduction in feed intake. However, high digestibility and lack of

any obvious histopathological signs of abnormality in liver and intestine suggest that MLM has potential to serve as a protein source. This, however, will depend on the efficiency of removing antinutritional factors to improve palatability and thus increase feed intake. This calls for further studies to explore more effective means of removing the antinutritional factors. This study suggests that aqueous extracted MLM be restricted to less than 15g $100g^{-1}$ of dietary protein in Nile tilapia diets which was the lowest level tested.

CHAPTER 4 Cassava Leaf Meal as Protein Source

4.1 Introduction

Cassava, *Manihot esculenta* Crantz is a multipurpose perennial woody shrub with edible leaves and roots. It is a member of the family Euphorbiaceae with origins in Latin America and can grow up to 4m in height (O'Hair, 1995). Cassava was introduced to Africa by Portuguese and Arab traders between the 16th and 18th centuries (FAO, 1990b). It is drought resistant, capable of growing on marginal soils (acidic and/or low fertility) and can give good yields without excessive use of costly inputs (Phuc *et al.,* 2000). Additionally, vegetative propagation using stem cuttings together with flexible harvesting time makes cassava a crop highly appreciated by subsistence farmers. It is primarily grown for its starchy roots which form a major source of low cost carbohydrates in humid tropical regions (Africa, Latin America and Asia) and in some countries leaves are also used as a green vegetable (Calpe, 1991; O'Hair, 1995; Nweke, 2004). Leaf harvesting can start after 4–5 months of growth and yields of up to 10 tonnes dry matter per hectare are possible without adversely affecting root production (Khajarern and Khajaren, 1992).

Proximate composition of cassava leaves (Figure 4.1) tends to vary widely due to differences in cultivars, stage of maturity, sampling procedure, soil fertility and climate (Ravindran, 1993). Crude protein (CP) content varies with variety and age but on average is 25%, of which almost 85% is true protein (Smith, 1992; Ravindran, 1992). Rogers and Milner (1963) studied

20 cultivars and reported a range of 17.8% to 34.8% CP. Ravindran and Ravindran (1988) observed a crude protein content of 38.1% in very young leaves which decreased to 19.7% in mature leaves, amino acids also followed a similar trend.

Figure 4.1: Cassava leaves

Source: Agricultural scenes (2005)

The amino acid profile of cassava leaves compares well with that of soybean meal except that they are both deficient in sulphur containing amino acids (Eggum, 1970). Cassava leaves are also good sources of vitamins such as ascorbic acid, vitamin A and riboflavin as well as minerals like iron, manganese and zinc (Ravindran, 1993).

Despite their nutritional potential, cassava leaves have limitations, particularly for monogastric animals, as they have high levels of anti-nutritional factors (hydrogen cyanide and tannins) and a high fibre content (Ravindran *et al.,* 1987a). Hydrogen cyanide (HCN) is released when cynanogenic glycosides, namely linamarin and lotaustralin are hydrolysed in the presence of the enzyme linamarase after tissue damage (Tewe, 1992; Tacon, 1997). Hydrogen cyanide is the most significant antinutritional factor in cassava leaves. Its acute toxicity results in sudden death while less acute toxicity may cause gastrointestinal disorders and growth depression. The amount of HCN is influenced by the cassava cultivar, environmental conditions, culture practices and plant age (McMahon *et al.,* 1995).

Several methods, such as ensiling and sun drying, can be used to reduce the HCN content in cassava leaves to safer levels. Ensiling is a process which involves fermentation of forages by lactic acid bacteria under anaerobic conditions (Oude Elferink *et al.,* 2000). Ensiling reduces HCN by causing disintegration of intact glycosides through cell disruption, a fall in pH of the ensiled medium and intense heat generation (Tewe, 1992). Ensiling is convenient during the rainy season when sun drying is not feasible but has high labour demands. In addition, during ensiling unfavourable microbial processes may also lower palatability and nutrient content. Sun drying removes HCN by reducing moisture content together with dissolved cyanogenic glycosides. According to Ravindran *et al.,*

(1987a), drying can reduce HCN content by more than 90%. Removal of HCN can be enhanced by grinding the leaves before sun drying to disrupt cell structure and enhance hydrolysis of cyanogenic glycosides to HCN which is volatilized during drying.

Studies on the utilisation of cassava leaf meal as a dietary protein source have been conducted in a number of animals, both terrestrial and aquatic. In terrestrial animals studies have been conducted in cattle (Moore and Cock, 1985; Wanapat *et al.,* 1997), goats (Sokerya and Lylian, 2001; Sokerya and Preston, 2003), pigs (Ravindran *et al.,* 1987b; Phuc *et al.,* 2000) and poultry (Akinfala *et al.,* 2002; Ravindran *et al.,* 1986). In aquatic animals some studies have reported good performance such as in Nile tilapia (Nieves and Barro, 1996) and milkfish (Borlongan and Coloso, 1994) when cassava leaf meal did not exceed 15% of the diet. In other studies higher inclusion levels caused poor growth in Nile tilapia (Ng and Wee, 1989), pacu, *Piaractus mesopotamicus* (Padua *et al.,* 1998), low protein digestibility in Asian sea bass, *Lates calcarifer* (Eusebio and Coloso, 2000) and increased susceptibility to diseases in African catfish, *Clarias gariepinus* (Bureau *et al.,* 1995). The poor growth observed in Nile tilapia by Ng and Wee (1989) was partly due to high dietary crude fibre content (16.7%) in diets with high inclusion levels of CLM.

Despite poor performance, cassava leaves still have a potential to serve as a cheap source of protein in fish feeds in Tanzania due to their abundance. A large proportion of the cassava leaves go to waste as a by–

product of cassava root production. The current study explores the potential of cassava leaves processed using a simple traditional method. The method involves removal of petioles to reduce crude fibre content and grinding using a wooden mortar and pestle followed by sun drying to remove hydrocyanic acid. The main objective of this study was therefore to evaluate the suitability of ground and sun dried cassava leaf meal as a protein source in diets for Nile tilapia.

4.2 Methodology

4.2.1 Processing of Cassava Leaves

Mature leaves from a variety locally known as "betauje" were harvested during the dry season from the slopes of the Uluguru Mountains in Morogoro, Tanzania. The freshly harvested leaves were manually stripped of petioles and then ground using a traditional wooden mortar and pestle (Figure 4.2). The ground leaf meal was spread on plastic sheets to sun dry for 24 hours. The dried leaf meal was ground into a fine powder using a hammer mill (Lab Mill, screen size 0.2mm) and then stored in a plastic bag at room temperature. Grinding facilitates hydrolysis of linamarin to HCN by linamarase while sun drying volatilises the HCN.

1. Grinding of cassava leaves using mortar and pestle

2. Sun drying of ground cassava leaves

Figure 4.2: Processing of cassava leaf meal

Source: Madalla (2005)

4.2.2 Biochemical Analysis and Digestibility of CLM

Proximate analysis, gross energy, amino acid profiles, minerals and antinutritional factors shown in Table 4.1 were quantified using the procedures described in section 2.3. The digestibility of processed CLM was determined using a total of 45 fish with an average weight of 13.1 ± 1.89 g following procedures described in section 2.4.

4.2.3 Experimental Setup and Diet Formulation

A growth trial was conducted in a recirculation system containing fifteen 30L capacity circular self cleaning plastic tanks described in section 2.1. A total of five diets were formulated to contain 30g $100g^{-1}$ crude protein, 10g $100g^{-1}$ lipid and 18kJ g^{-1} as described in section 2.5. The cassava leaf meal provided 0, 15, 30, 45 and 60g $100g^{-1}$ of total dietary crude protein for diets CLM0, CLM15, CLM30, CLM45 and CLM60 respectively.

Table 4.1: Biochemical composition of unprocessed and processed CLM

	Unprocessed CLM	Processed CLM
Proximate composition (g 100^{-1} g, as fed)		
Dry matter	95.94	93.36
Crude protein	28.79	29.00
Crude lipid	5.02	2.37
Crude fibre	13.01	10.21
Ash	5.64	5.95
Nitrogen free extract	43.51	45.84
Gross energy (kJ g^{-1})	20.58	19.66
Amino acid composition (g 100g^{-1} feed)		
Arginine	1.53	1.77
Histidine	0.73	0.77
Isoleucine	1.54	1.62
Leucine	2.44	2.67
Lysine	1.58	1.49
Methionine +Cystine	0.47	0.47
Phenylalanine+ Tryptophan	2.73	2.81
Threonine	1.33	1.27
Valine	1.89	1.99
Mineral composition (mg g^{-1}, DM)		
Phosphorus	4.06	4.11
Sodium	0.21	0.18
Magnesium	2.54	2.45
Potassium	12.44	14.45
Calcium	5.98	6.29
Iron	0.12	0.15
Copper	0.02	0.01
Zinc	0.07	0.06
Antinutritional factors (g 100g^{-1}, DM)		
Hydrocyanic acid[1]	4.34	1.74
Saponins[2]	1.33	1.06
Phenols[2]	5.43	4.94
Tanins[3]	2.70	2.12
Phytic acid[4]	0.19	0.24

[1]mg 100g^{-1}, [2]As diosgenin equivalent, [3]As tannic acid equivalent, [4]As phosphorus equivalent

Diet CLM0 served as the control diet and contained fishmeal as its main source of protein (Table 4.2). Dietary treatments were randomly assigned in triplicate to tanks containing Nile tilapia fingerlings with an average weight of 3.86 ± 0.31g. The fingerlings were handled as described in section 2.2. Fish were fed the formulated diets three times a day at 09:00, 13:00 and 17:00 hrs as described in section 2.6. Each meal was offered to appetite but not more than 10% of body weight per day. Fish were bulk weighed once a week to monitor growth and to adjust feed ration. At the end of the trial, final weights were recorded and 5 fish from each replicate were sacrificed for histopathology and whole body composition analyses as described in sections 4.2.4 and 4.2.5.

Table 4.2: Formulation of diets fed to *O. niloticus* during the growth trial for cassava leaf meal (g 100 g^{-1}, as fed)

Ingredients	CLM0	CLM15	CLM30	CLM45	CLM60
Fishmeal[1]	36.5	31.5	26.0	21.0	16.0
Cassava leaf meal	0.0	15.5	31.5	48.5	62.5
Wheat meal[2]	43.5	34.0	24.5	14.0	6.0
Sunflower oil	6.0	6.5	6.5	7.0	7.0
Vitamin premix[3]	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	4.0	4.0	4.0	4.0	4.0
CMC[5]	2.0	2.0	2.0	2.0	2.0
α-cellulose	5.5	4.0	3.0	1.0	0.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5

[1]Brown fishmeal (aquaculture grade), [2]Whole grain, [3]As listed in Table 2.2, [4]As listed in Table 2.3, [5]Carboxymethylcellulose

Remaining fish were pooled according to their dietary treatments for faecal collection in order to determine digestibility of the CLM diets as described in section 2.4.

4.2.4 Performance Parameters

Feed intake, growth and nutrient utilisation were determined as outlined in section 2.7 and the whole body compositions were evaluated as outlined in section 2.8.

4.2.5 Liver and Gut Histopathology

Histopathology of the liver and gut was evaluated according to the procedures described in section 2.9. Fish were dissected to remove the gastrointestinal tract and liver which were fixed in 10% neutral buffered formalin.

4.2.6 Data Analysis

The performance data collected was analysed as described in section 2.10.

4.3 Results

4.3.1 Biochemical Composition and Digestibility of CLM and CLM Diets

The biochemical composition of processed and unprocessed cassava leaf meal is shown in Table 4.1. Grinding followed by sun drying of cassava leaf meal resulted in a loss of about 53% of crude lipid and about 22% of crude fibre. There was a slight increase in crude protein and ash which was reflected by the increased content of some amino acids and minerals respectively. Processing was fairly effective in removing about 60% of the hydrogen cyanide. Similarly, the contents of other antinutritional factors

i.e. saponins, phenols and tannins were also reduced but that of phytic acid increased.

Results from the digestibility trial showed that CLM had low digestibility. With exception of crude lipid, whose apparent digestibility coefficients (ADC) were above 70%, the ADC for other components was less than 50% (Table 4.3). The poor digestibility subsequently led to poor digestible crude protein and energy values.

Table 4.3: Digestibility of cassava leaf meal (CLM)

	Reference Diet	Test diet	CLM
Proximate composition (g 100g^{-1}, as fed)			
Dry matter	93.08	94.81	
Crude protein	31.56	31.35	
Crude lipid	10.69	8.62	
Crude fibre	2.81	4.85	
Ash	8.66	8.18	
Nitrogen free extract	39.36	41.81	
Gross energy (kJ g^{-1})	17.92	19.22	
Apparent digestibility coefficient (%)			
Dry matter	73.3	62.7	**38.3**
Crude protein	84.3	72.4	**44.5**
Crude lipid	98.4	95.8	**88.3**
Gross energy	70.1	61.9	**44.2**
Digestible protein (g 100g^{-1})	24.8	22.4	**12.7**
Digestible energy (kJ g^{-1})	12.6	11.9	**9.2**

Biochemical analysis of the CLM diets (Table 4.4) showed they had similar contents of crude protein, crude lipid and ash irrespective of CLM inclusion level. However, there was an increase in crude fibre content and gross energy as CLM inclusion increased while the converse was true for phosphorus content.

Table 4.4: Biochemical composition of CLM diets used for the growth trial

	CLM0	CLM15	CLM30	CLM45	CLM60	Req*
Proximate composition (g 100g^{-1}, as fed)						
Dry matter	95.02	95.49	95.3	94.58	95.13	
Crude protein	32.38	31.42	32.02	31.51	31.33	
Crude lipid	11.30	11.05	11.23	11.42	11.44	
Crude fibre	5.98	6.06	6.88	7.51	8.06	
Ash	9.42	9.46	9.64	9.61	9.69	
Nitrogen free extract	35.94	37.5	35.53	34.53	34.61	
Gross energy (kJ g^{-1})	18.39	18.89	19.12	19.95	20.43	
Phosphorus (mg g^{-1})	9.22	7.84	7.73	7.58	6.59	
Amino acids (g 100g^{-1} feed)						
Arginine	6.09	6.11	6.13	6.16	6.17	4.20
Histidine	2.36	2.40	2.45	2.49	2.53	1.72
Isoleucine	4.27	4.48	4.69	4.91	5.09	3.11
Leucine	7.99	8.12	8.26	8.39	8.52	3.39
Lysine	7.07	6.84	6.58	6.34	6.10	5.12
Methionine + Cystine	3.96	3.61	3.25	2.87	2.55	3.21
Phenylalanine + Tryrosine	7.05	7.43	7.83	8.23	8.59	5.54
Valine	4.00	4.06	4.12	4.18	4.23	2.80
Antinutritional factors (g 100g^{-1}, DM)						
Hydrogen cyanide[1]	-	0.27	0.55	0.84	1.09	
Phenols[2]	-	0.77	1.56	2.40	3.09	
Tannins[2]	-	0.33	0.67	1.03	1.33	
Saponins[3]	-	0.16	0.33	0.51	0.66	
Phytic acid[4]	-	0.04	0.08	0.12	0.15	
Apparent digestibility coefficient (%)						
Dry matter (%)	75.8	71.0	65.9	61.4	58.9	
Crude protein (%)	90.7	81.8	75.7	69.1	65.1	
Crude lipid (%)	94.7	90.4	88.9	87.1	88.4	
Gross energy (%)	75.5	70.6	65.8	62.2	60.4	
Phosphorus (%)	80.4	78.1	78.4	75.1	71.9	
Digestible protein (DP) (g 100g^{-1})	29.4	25.7	24.2	21.8	20.4	
Digestible energy (DE) (kJ g^{-1})	13.9	13.3	12.6	12.4	12.3	
DP/DE ratio (mg kJ^{-1})	22.3	20.2	20.2	18.6	17.4	

*Req=*O. niloticus* amino acid requirements (Santiago and Lovell, 1988), [1]Hydrocyanic acid (mg 100g^{-1}), [2]As tannic acid equivalent, [1]As diosgenin equivalent, [4]As phosphorus equivalent

The CLM diets met the essential amino acid requirements of Nile tilapia with the exception of diets CLM45 and CLM60 which were deficient in methionine + cystine. Levels of antinutritional factors in the growth trial diets increased with increasing CLM inclusion with contents of phenols, tannins and saponins being greatest. With the exception of lipid, the digestibility of other nutrients declined sharply with increasing CLM inclusion reflecting its poor digestibility. There was a noticeable decline in digestible protein in comparison to that of digestible energy, resulting in a decline in DP/DE ratio with increased CLM inclusion.

4.3.2 Growth Performance and Nutrient Utilisation

Fish fed CLM diets gained weight during the trial but the gain decreased as fish were fed increasing levels of CLM (Figure 4.3). Growth performance in terms of final weight, average daily gain and specific growth rate declined significantly with increasing CLM inclusion level (Table 3.5). Fish did not show obvious signs of feed rejection but feed intake declined with increasing CLM inclusion although not significantly in some cases. Digestible energy and digestible protein intakes followed a similar trend. Feed conversion ratio, protein efficiency ratio, apparent net protein utilization, energy retention and hepatosomatic index also declined significantly with increasing CLM inclusion level. There was, however, no significant difference in fish survival between different dietary treatments.

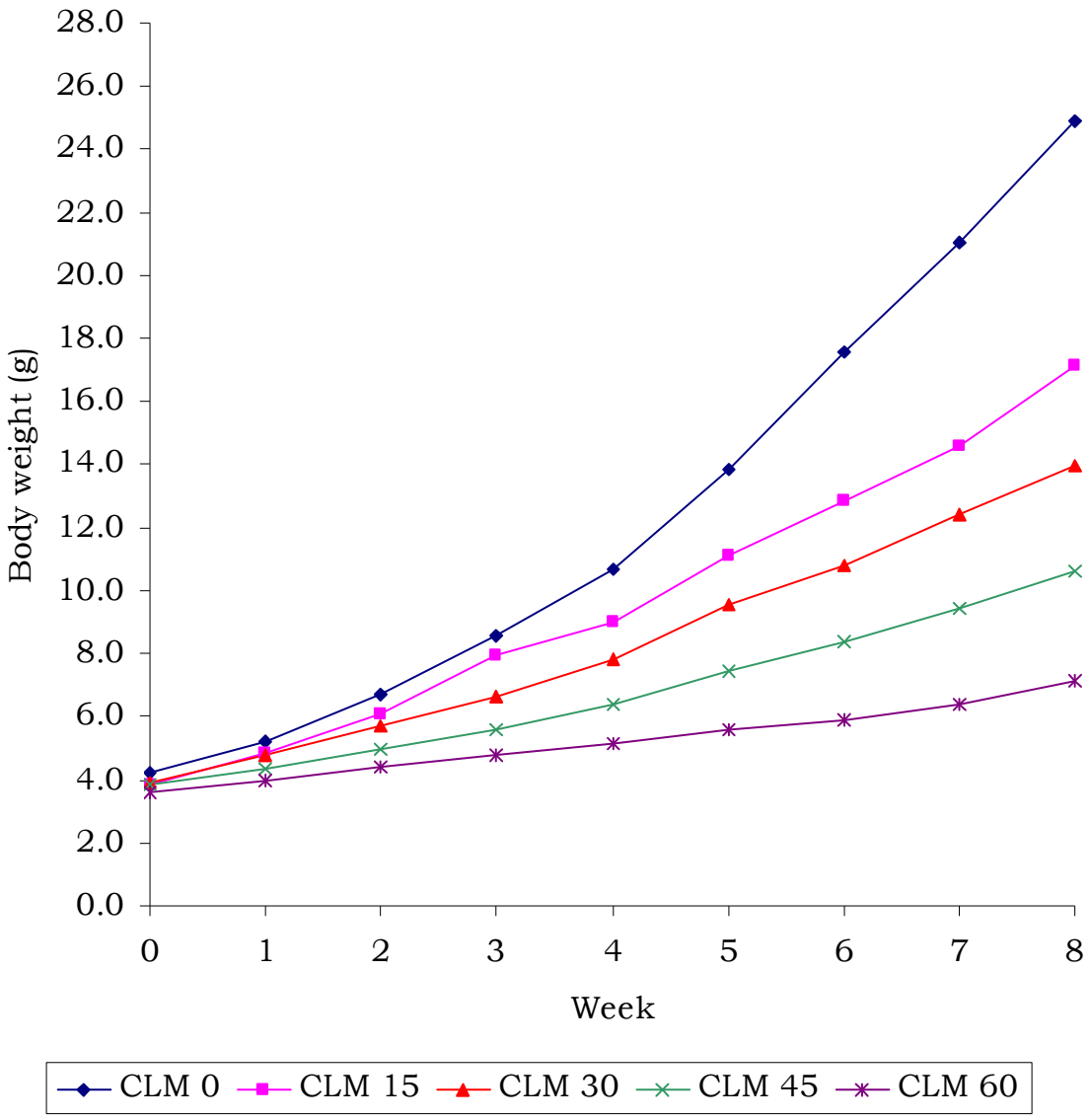


Figure 4.3: Change in body weight of *O. niloticus* fed diets containing graded levels of processed cassava leaf meal

4.3.3 Whole Body Composition

Initial and final whole body proximate composition is shown in Table 4.6. Final body moisture and ash contents were significantly higher in fish fed diet CLM60 while lipid and gross energy content was significantly lower. Crude protein contents were more or less similar except for fish fed diet CLM60 which had the lowest value, this was, however, not significant.

Table 4.5: Growth performance and nutrient utilisation of *O. niloticus* fed CLM diets (mean ± SD, n=3)

	CLM0	CLM15	CLM30	CLM45	CLM60
Initial weight (g)	4.22 ± 0.07^{a}	3.86 ± 0.24^{a}	3.93 ± 0.07^{a}	3.70 ± 0.46^{a}	3.61 ± 0.28^{a}
Final weight (g)	24.96 ± 1.47^{a}	17.45 ± 1.22^{b}	13.97 ± 0.56^{c}	10.57 ± 1.15^{d}	6.90 ± 0.54^{e}
Feed intake (g fish^{-1} day^{-1})	0.53 ± 0.04^{a}	0.45 ± 0.02^{ab}	0.40 ± 0.01^{bc}	0.35 ± 0.04^{c}	0.29 ± 0.01^{d}
DP intake (g fish^{-1} day^{-1})	0.157 ± 0.013^{a}	0.110 ± 0.006^{b}	0.097 ± 0.002^{bc}	0.076 ± 0.007^{cd}	0.060 ± 0.002^{d}
DE intake*(kJ fish^{-1} day^{-1})	7.43 ± 0.61^{a}	5.98 ± 29^{b}	5.08 ± 0.10^{bc}	4.32 ± 0.42^{c}	3.61 ± 0.12^{c}
Average daily gain (g fish^{-1} day^{-1})	0.37 ± 0.03^{a}	0.24 ± 0.02^{b}	0.18 ± 0.01^{c}	0.12 ± 0.02^{d}	0.06 ± 0.01^{e}
Specific growth rate (% day^{-1})	3.17 ± 0.08^{a}	2.69 ± 0.09^{b}	2.26 ± 0.09^{c}	1.88 ± 0.20^{d}	1.15 ± 0.09^{e}
Feed conversion ratio	1.45 ± 0.12^{a}	1.85 ± 0.06^{b}	2.26 ± 0.13^{c}	2.85 ± 0.18^{d}	5.02 ± 0.44^{e}
Protein efficiency ratio	2.14 ± 0.18^{a}	1.72 ± 0.06^{b}	1.39 ± 0.08^{cd}	1.12 ± 0.07^{bc}	0.64 ± 0.05^{d}
Apparent net protein utilization (%)	35.94 ± 3.75^{a}	27.41 ± 1.03^{b}	21.54 ± 2.50^{bc}	18.55 ± 1.02^{c}	10.16 ± 2.05^{d}
Energy retention (%)	19.28 ± 1.12^{a}	15.02 ± 0.96^{b}	12.73 ± 0.35^{b}	9.30 ± 0.68^{c}	4.14 ± 0.23^{d}
Hepatosomatic index	3.41 ± 0.84^{a}	2.87 ± 0.62^{ab}	2.57 ± 0.47^{b}	2.41 ± 0.41^{b}	1.65 ± 0.47^{c}
Survival (%).	95.00 ± 8.66^{a}	96.67 ± 5.77^{a}	100.00 ± 0.00^{a}	98.33 ± 2.89^{a}	98.33 ± 2.89^{a}

Table 4.6: Whole body proximate composition of *O. niloticus* fed CLM diets before and after the experiment (% fresh weight basis, mean ± SD, n=3)

	Initial	CLM0	CLM15	CLM30	CLM45	CLM60
Moisture	76.04	70.99 ± 0.87^{a}	71.60 ± 1.08^{a}	73.01 ± 1.26^{a}	72.90 ± 1.66^{a}	77.62 ± 1.97^{b}
Crude protein	13.64	16.23 ± 0.44^{a}	15.41 ± 0.65^{a}	14.98 ± 0.70^{a}	15.59 ± 1.01^{a}	14.72 ± 1.25^{a}
Crude lipid	6.22	8.29 ± 0.24^{a}	8.35 ± 0.28^{a}	7.37 ± 0.39^{b}	6.67 ± 0.37^{b}	2.61 ± 0.22^{c}
Ash	3.01	3.03 ± 0.02^{a}	3.31 ± 0.13^{b}	3.43 ± 0.02^{b}	3.59 ± 0.03^{c}	4.52 ± 0.02^{d}
Gross energy (kJ g^{-1})	5.26	5.34 ± 0.03^{a}	5.28 ± 0.05^{ab}	5.15 ± 0.03^{bc}	5.03 ± 0.05^{c}	4.26 ± 0.21^{d}

Different superscripts in the same row indicate significant difference ($p<0.05$)

4.3.4 Liver and Gut Histopathology

Liver and gut tissues did not show any pathology that could be linked to dietary treatment. However, liver tissues from fish fed diets containing high levels of CLM had less lipid deposition.

4.4 Discussion

Grinding of cassava leaves followed by sun drying did not cause any significant change in protein or amino acid contents. This corroborates earlier reports that processing has little influence on CLM crude protein content (Ravindran, 1985). Crude protein contents of CLM both raw (28.79%) and processed (29.0%) were higher than the reported average of 25% (Montaldo, 1977) but within the reported range of 17.8% to 34.8% (Rogers and Milner, 1963). The content of some amino acids and minerals increased slightly to reflect the slight increase in crude protein and ash contents respectively. However, in CLM diets there was gradual decline in essential amino acid content as CLM inclusion level increased. Which led to deficiency of sulphur amino acids in diets CLM45 and CLM60 (Santiago and Lovell, 1988). Increasing CLM levels led to a decline in phosphorus content. The reduction in crude fibre content was due to removal of petioles during processing, similar to observations by Ravindran (1985) who reported a 17% reduction after a similar procedure with mature cassava leaves. The crude fibre content, however, still remained high (10g $100g^{-1}$) and consequently fibre content in diet CLM60 (8g $100g^{-1}$) was

higher than recommended (5g $100g^{-1}$) (Anderson *et al.,* 1984; Ali *et al.,* 2003; Dioundick and Stom, 1990).

The removal of almost 60% of the HCN content shows the effectiveness of grinding and sun drying similar to earlier observations (Ravindran et al., 1987a; Ravindran, 1992; Phuc et al., 2000; Fasuyi, 2005). Grinding disrupts cellular structure and facilitates a reaction between cyanogenic glycosides (linamarin and lotaustralin) and the enzyme linamarase which are stored separately in plant cells (Oke, 1978). This facilitates hydrolysis of cyanogenic glycosides into HCN which is then volatilised during sun drying. The contents of other antinutritional factors like saponins, phenols and tannins were also reduced after processing. Reduction of phenols and tannins in cassava leaves processed using similar treatment was also reported by Ravindran et al (1987a) and Fasuyi (2005). Makkar and Singh (1993) suggest that such a reduction is possibly due to oxidation of tannins by polyphenol oxidase. However, the reduction was not as effective as that of HCN and according to Rickard (2006) residual tannins may be a major factor limiting the nutritional value of cassava leaf meal. The content of all antinutritional factors increased with increasing CLM inclusion level in the growth trial hence resulting in reduced feed intake, growth and nutrient utilisation (Mehansho *et al.,* 1987). Sub-lethal doses of HCN remaining after processing are known to trigger detoxification processes which tend to increase the demand for methionine. According to Oke (1978), HCN is converted to thiocyanate within the body in the

presence of the enzyme rhodanase using methionine as a sulphur donor. This process can potentially result in amino acid imbalance as the bioavailability of methionine in cassava leaves is poor (Eggum, 1970).

Results from the digestibility trial showed that CLM was poorly digestible in terms of dry matter, crude protein and gross energy. The apparent protein digestibility coefficient (45%) was very low compared to the range of 75%-95% suggested by NRC (1993) for dietary protein sources thus low digestible protein. Similarly the apparent gross energy digestibility also had a low value (44.17%) also resulting in low digestible energy. Consequently diets with high CLM inclusion could not meet energy requirement of fish. Fish are generally known to compensate for low energy density in feed by eating more as long as the physical capacity of the digestive tract permits and the feed is sufficiently palatable (Cho and Bureau, 1995). Poor digestibility also affected DP/DE ratio, particularly for diet CLM60 whose value was below the 18 mg kJ^{-1} recommended for Nile tilapia (Kaushik *et al.,* 1995).

Poor digestibility of cassava leaf meal is possibly due to a number of reasons. According to Ravindran (1993) only 85% of CLM protein is true protein and the remaining 15% is non-protein nitrogen (NPN) which tilapias are incapable of utilising (Viola and Zohar, 1984). Moreover, Reed *et al.,* (1982) found that a large proportion of the protein in cassava leaves is bound to its crude fibre fraction thus making it unavailable to digestive enzymes. Moreover, Reed *et al.,* (1982) also found that the bound protein

was strongly associated with condensed tannins which are known to form indigestible protein-tannin complexes. Thus, despite attempts to balance crude fibre content in CLM diets, protein digestibility declined drastically exhibiting a 25% difference between diet CLM0 and diet CLM60 while crude fibre only varied by 2%. Moreover, the α-cellulose added to diet CLM0 (5.5g $100g^{-1}$) was approximately within the range of 2.5 to 5.0g $100g^{-1}$ which resulted in better growth and feed conversion ratio in *O. mossambicus* (Dioundick and Stom, 1990). This suggests that it is quality rather than quantity of crude fibre which affected digestibility in CLM diets. However, there is also a possibility that the carbohydrate content of CLM is likely to be fibrous rather than starchy due to its fibre content hence contributing to poor digestibility. According to Bureau *et al.,* (2002), the digestible energy of such ingredients tends to be less than half of their gross energy content. This was the case in the current study where DE (8.04kJ g^{-1}) was less than half of GE (19.66kJ g^{-1}). Similarly, Sklan *et al.,* (2004) fed tilapia with wheat bran with 8.6% crude fibre and reported poor DE (6.95 kJ g^{-1}) which was less than half of the GE (17.94 kJ g^{-1}). Similar observations were also made for common carp fed diets whose carbohydrate fraction was rich in crude fibre that resulted in low energy digestibility (56%) (Kirchgessner *et al.,* 1986). The very low body lipid content of fish fed diet CLM60, which paralleled a low hepatosomatic index, reaffirms poor energy intake. Fish tend to utilise lipid reserves to

sustain metabolism when food energy is not sufficient hence resulting in body lipid loss (Hepher, 1988).

Results from this study have shown that grinding followed by sun drying was fairly effective in removing HCN from cassava leaves but not other antinutritional factors. CLM was poorly digestible and its inclusion in diets led to poor intakes of digestible energy and digestible protein which consequently resulted in poor overall performance. Further studies are recommended to explore means of improving CLM digestibility by reducing crude fibre and antinutritional factors, particularly tannins. The study recommends inclusion of ground and sun dried cassava leaf meal at less than 15g $100g^{-1}$ of dietary protein in Nile tilapia diets, the lowest level tested.

CHAPTER 5 Cassava Root Meal as an Energy Source

5.1 Introduction

Cassava root (*Manihot esculenta*) is an important tropical crop which is grown throughout tropical and subtropical areas approximately 30°N and 30°S of the equator and up to an altitude of 1500m (FAO, 1990b). It is mainly grown for its starchy tuberous roots (Figure 5.1) which may be eaten boiled, baked or fried (Calpe, 1991; O'Hair, 1995; Nweke, 2004).

Figure 5.1: A harvest of cassava tubers

Source: IPM Collaborative Research Support Program (2006)

Its importance as a crop stems from its peculiar comparative advantages over other crops such as cereals. These advantages include high tolerance to adverse conditions such as poor soils, low and erratic rainfall, diseases

and pests (Lekule, 1990). Other positive aspects include low labour requirements in terms of planting and harvesting and low production costs because inputs such as fertilizers are not required. Additionally, cassava is propagated vegetatively and thus planting material does not constitute edible matter. Average cassava root yield within traditional cultivation methods in Africa is 5-12 metric tonnes ha^{-1} but depending on variety, soil fertility and management it is possible to realize yields of up to 68 metric tonnes ha^{-1} (Tewe and Egbunike, 1992; Tewe, 2004). These qualities have made cassava an important crop providing subsistence food for more than half a billion people worldwide. (FAO, 1990b)

A mature cassava plant is composed of approximately 50% root, 44% stem and 6% leaves (Devendra, 1977). The roots tend to have high moisture content which results in low dry matter of only about 30%. Over 85% of the dry matter is composed of starch in the form of amylopectin, amylose and to a lesser extent sugars in the form of sucrose (Johnson and Raymond, 1965; Pérez, 1997; Tewe, 2004). Importantly, cassava starch has excellent binding properties which are very useful when pelleting feed and hence eliminate the need for expensive artificial binders (Adebayo *et al.,* 2003).

The protein content of cassava root meal (CRM) is poor in both quantity and quality. It varies from 0.7–2.5% and contains very low amounts of amino acids such as lysine, methionine and tryptophan and hence compares unfavourably with cereal grains (Khajarern and Khajaren, 1992).

In addition, about 30% of the measured crude protein is in the form of non–protein nitrogen which cannot be utilized by monogastric animals (Muindi and Hanssen, 1981). Similarly, the lipid content in CRM is also very low, ranging from 0.3–1.2% and effectively cassava root is considered to be devoid of lipid. The roots also contain small quantities of minerals and vitamins which tend to vary according to soil types and processing methods. Since cassava roots are rich in carbohydrate and poor in protein and lipid, they are mostly regarded as an energy source (Smith, 1992).

Utilization of cassava root as animal feed has several potential constraints. Firstly, due to the low protein content, CRM requires higher amounts of protein supplementation compared to conventional energy sources like cereals (Gomez, 1977). Thus its use in animal feeds depends greatly on the price and nutritive value of a range of protein sources (Khajarern *et al.,* 1977). Secondly, and perhaps more importantly, is the presence of cyanogenic glycosides in the form of linamarin, (93%) and lotaustralin (7%) (Nartey, 1968). Upon catalytic hydrolysis they yield hydrogen cyanide (HCN) which is responsible for raw CRM toxicity. However, adequate processing through soaking, grating, drying or fermentation can reduce levels of cyanogens to non-toxic levels (McMahon *et al.,* 1995). Another constraint is its powdery nature which leads to decreased feed intake due to irritation of respiratory organs and eye infections in pigs (Khajarern *et al.,* 1977) and poultry (Garcia and Dale, 1999). The powdery nature may also result in ulceration of the gastric mucosa (Oke, 1978). However, the

dustiness of CRM can be addressed by adding molasses or oil in a mix as well as by pelleting (Oke, 1978). Finally, the high moisture content of fresh cassava roots makes them bulky and highly susceptible to deterioration after harvest which may reduce their palatability. Drying the roots until moisture content falls below 14% significantly increases shelf life and reduces bulk (Ospina and Wheatley, 1992).

Cassava root meal has been utilised as the main source of energy in a wide range of farm animals including cattle (Smith, 1992), goats (Mouro *et al.,* 2002), pigs (Phuc *et al.,* 2000), chicken (Akinfala *et al.,* 2002) and rabbits (Omole, 1992). Several studies have also been conducted in fish which recommended various inclusion levels. The recommendations were 45% for mirror carp, *Cyprinus carpio*; 30% for rainbow trout, *Salmo gairdneri* (Ufodike and Matty, 1984) and 60% for Nile tilapia (Wee and Ng, 1986). The main objective of this study was to evaluate the suitability of grated and sun dried cassava root meal as a replacement for wheat meal in Nile tilapia diets.

5.2 Methodology

5.2.1 Processing of Cassava Root Meal

Fresh cassava roots from a variety locally known as "Betauje" were peeled, washed and then grated into small chips using a hand operated grater (Figure 5.2).

1. Freshly harvested cassava roots

2. Peeling of cassava roots

3. Peeled and washed cassava roots

4. Grating of cassava roots

Figure 5.2: Processing of cassava roots for preparation of cassava root meal (CRM).

Source: Madalla (Madalla, 2005)

The chips were then spread on plastic sheets to dry under the sun. Grating and sun drying was done to reduce levels of hydrogen cyanide which is usually present in cassava roots in appreciable quantities.

5.2.2 Biochemical Analysis and Digestibility of CRM

Proximate analysis, gross energy content and antinutritional factors shown in Table 5.1 were analysed following procedures described in section 2.3. Apparent digestibility coefficients for cassava root meal were

determined using 45 fish with an average weight of 12.30 ± 1.60 g following procedures described in section 2.4.

Table 5.1: Biochemical composition of unprocessed and processed CRM

	Unprocessed CRM	Processed CRM
Proximate composition (g 100^{-1} g, as fed)		
Dry matter	97.4	90.91
Crude protein	0.91	1.53
Crude lipid	0.61	0.48
Crude fibre	2.79	2.42
Ash	2.90	2.11
Nitrogen free extract	90.19	84.37
Gross energy (kJ g^{-1})	15.80	15.82
Antinutritional factors (g 100g^{-1}, DM)		
Hydrocyanic acid[1]	4.33	0.39
Saponins[2]	0.26	0.24
Phenols[2]	0.30	0.29
Tanins[3]	ND	ND
Phytic acid[4]	0.001	0.150

[1]mg 100g^{-1}, [2]As diosgenin equivalent, [3]As tannic acid equivalent, [4]As phosphorus equivalent

5.2.3 Experimental Setup and Diet Formulation

A growth trial was conducted in a recirculation system consisting of fifteen 30L circular self-cleaning plastic tanks as described in section 2.1. A total of 300 *O. niloticus* fingerlings with an average weight of 2.86 ± 0.16g were equally distributed in the culture tanks following procedures described in section 2.2. A total of five diets i.e. CRM0, CRM25, CRM50, CRM75 and CRM100 (Table 5.2) were formulated in which cassava root meal replaced wheat meal at 0%, 25%, 50%, 75% and 100% respectively.

Table 5.2: Composition of diets fed to *O. niloticus* during the growth trial for cassava root meal (CRM) (g 100 g^{-1}, on as fed basis)

Ingredient	CRM0	CRM25	CRM50	CRM75	CRM100
Fishmeal[1]	35.5	37.0	38.5	39.5	41.0
CRM	0.0	11.5	22.5	33.5	44.5
Wheat meal[2]	50.0	37.0	24.5	12.5	0.0
Sunflower oil	6.0	6.0	6.0	6.0	6.0
Vitamin premix[3]	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	4.0	4.0	4.0	4.0	4.0
CMC[5]	2.0	2.0	2.0	2.0	2.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5

[1]Brown fishmeal (aquaculture grade), [2]Whole grain, [3]As listed in Table 2.2, [4]As listed in Table 2.3, [5]Carboxymethylcellulose

Diet CRM0 had 100% wheat meal as the main source of carbohydrate energy and served as a control. The diets were formulated to contain 30g 100 g^{-1} crude protein, 18kJ g^{-1} and 10g 100 g^{-1} lipid and compounded as described in section 2.5. The diets were randomly assigned to tanks in triplicate. During the 8 week experiment, fish were bulk weighed once every week to monitor growth and adjust feed ration as described in section 2.2. Fish were hand fed three times a day to appetite but not exceeding 10% of body weight as described in section 2.6. At the end of the trial 3 fish from each tank were sacrificed for whole body proximate analysis as described in section 5.2.4. Remaining fish were pooled according to dietary treatment for faecal collection to determine digestibility of the experimental diets as described in section 2.4.

5.2.4 Performance Parameters

Growth and nutrient utilisation was evaluated as described in section 2.7 while whole body composition was determined following procedures described in section 2.8.

5.2.5 Data Analysis

Performance data on feed intake, growth and feed utilisation was analysed as described in section 2.10.

5.3 Results

5.3.1 Biochemical Composition and Digestibility of CRM and CRM Diets

The biochemical composition of unprocessed and processed CRM is shown in Table 5.1. Proximate composition of cassava root meal remained largely the same even after processing. Grating followed by sun drying removed over 90% of hydrogen cyanide. The contents of other antinutritional factors declined slightly with the exception of phytic acid but they all remained very low. Cassava root meal was highly digestible which subsequently resulted in high digestible energy contents as shown in Table 5.3.

The biochemical composition and apparent digestibility coefficients of diets used for the growth trial are shown in Table 5.4. Diets had low contents of crude fibre and antinutritional factors. Their digestibility was high and was reflected by high values for digestible energy and DP/DE ratio in all diets.

Table 5.3: Digestibility of cassava root meal (CRM)

	Reference Diet	Test diet	CRM
Proximate composition (g 100g^{-1}, as fed)			
Dry matter	93.08	92.99	
Crude protein	31.56	22.89	
Crude lipid	10.69	7.63	
Crude fibre	2.81	2.87	
Ash	8.66	6.98	
Nitrogen free extract	39.36	52.62	
Gross energy (kJ g^{-1})	17.92	17.23	
Apparent digestibility coefficient (%)			
Dry matter	73.3	76.3	**83.4**
Gross energy	70.1	74.2	**83.3**
Digestible energy (kJ g^{-1})	12.6	12.8	**13.2**

Table 5.4: Biochemical composition and apparent digestibility coefficients of CRM diets used for the growth trial

	CRM0	CRM25	CLM50	CLM75	CLM100
Proximate composition (g 100g^{-1}, as fed)					
Dry matter	94.34	94.17	93.58	93.89	93.99
Crude protein	31.85	31.97	31.07	29.8	29.52
Crude lipid	7.70	10.85	11.21	11.43	10.32
Crude fibre	3.37	2.13	2.17	3.32	2.19
Ash	9.32	9.58	9.76	9.96	10.28
Nitrogen free extract	42.1	39.64	39.37	39.38	41.68
Gross energy (kJ g^{-1})	18.23	18.08	18.01	17.92	17.77
Phosphorus (mg g^{-1})	7.62	8.10	7.33	7.86	8.12
Antinutritional factors (g 100g^{-1}, DM)					
HCN[1]	-	0.10	0.20	0.29	0.39
Saponins[2]	-	0.03	0.05	0.08	0.11
Phenols[3]	-	0.03	0.07	0.10	0.13
Tannins[3]	-	ND	ND	ND	ND
Phytic acid[4]	-	0.02	0.03	0.05	0.07
Apparent digestibility coefficient (%)					
Dry matter (%)	79.2	79.9	77.5	77.7	74.7
Crude protein (%)	88.4	90.3	90.0	89.1	85.9
Crude lipid (%)	82.2	94.7	94.0	95.3	91.6
Gross energy (%)	77.9	81.2	79.0	79.6	75.2
Phosphorus (%)	73.8	78.2	73.8	74.1	73.0
Digestible energy (DE) (kJ g^{-1})	14.2	14.7	14.2	14.3	13.4
DP[5]/DE ratio (mg kJ^{-1})	21.0	20.9	21.0	19.8	20.2

[1]Hydrocyanic acid (mg 100g^{-1}), [2]As diosgenin equivalent, [3]As tannic acid equivalent, [4]As phosphorus equivalent, [5]digestible protein, ND=not detected

5.3.2 Growth Performance and Nutrient Utilisation

Weight gain during the growth trial is shown in Figure 5.3. Fish actively consumed all diets except diets CRM0 and CRM100 where they showed reluctance to feed and this contributed to low weight gain.

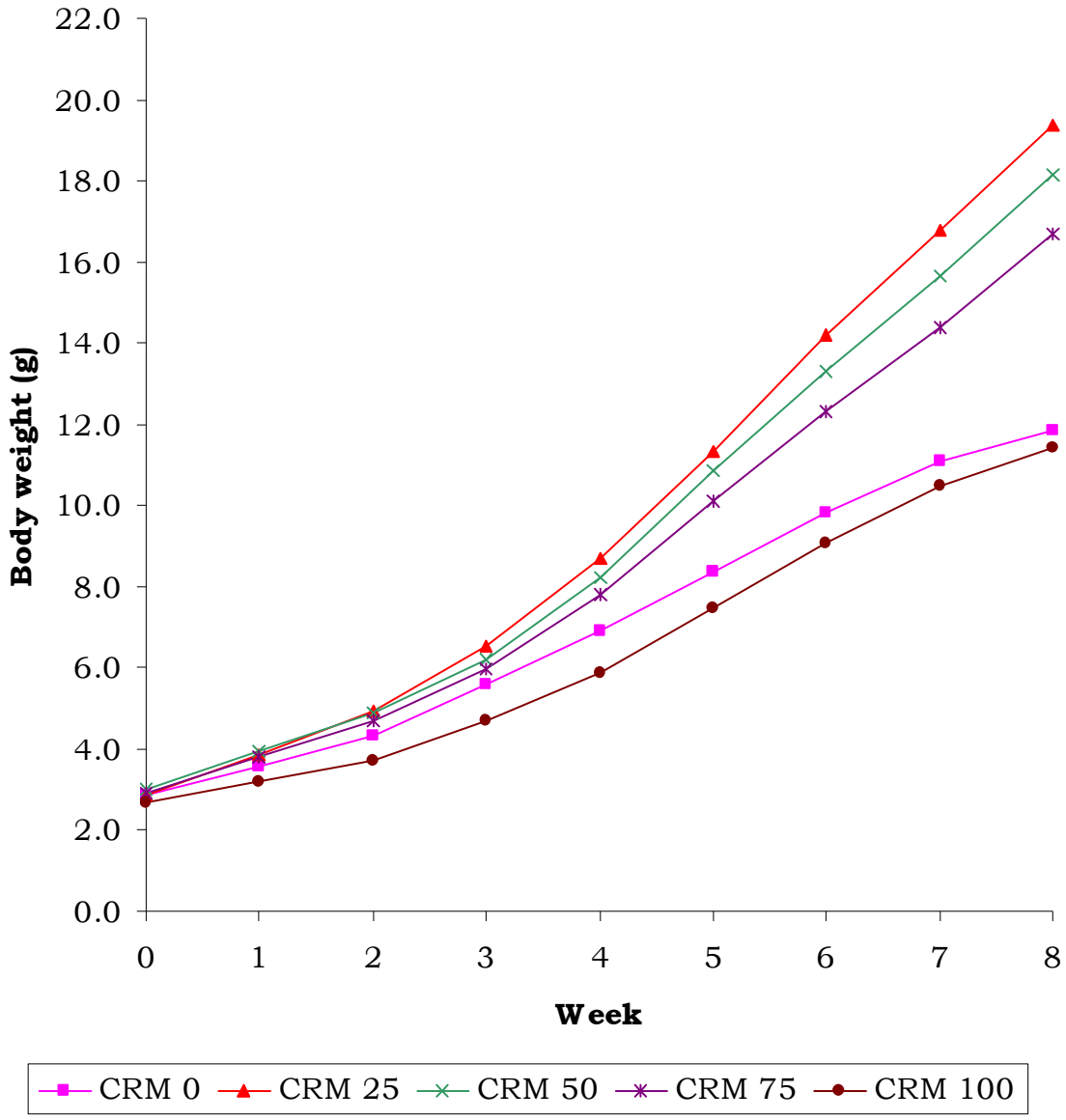


Figure 5.3: Change in body weight of *O. niloticus* fed diets containing graded levels of cassava root meal

Reluctance to consume diet CRM0 was unexpected because it was the control diet. Other performance parameters shown in Table 3.5 followed a similar trend to that of feed intake whereby fish fed diets CRM0 and CRM100 had significantly poorer performance than those fed the remaining diets. In general, substituting wheat meal with cassava root meal at 25–75% did not impair growth and nutrient utilisation. There was no significant difference in fish survival between treatments.

5.3.3 Whole Body Composition

The initial and final whole body proximate composition on a fresh weight basis is shown in Table 5.6. There was no significant difference in moisture and protein contents. Fish fed diet CRM100 had significantly lower lipid and gross energy contents and the converse was true for ash content.

5.4 Discussion

Proximate composition of both raw and processed cassava root meal fell within ranges reported in the literature (Lekule, 1990; Wu, 1991; Smith, 1992). In the current study gross energy content, together with other nutrients, was not significantly affected by processing. Processing, however, was very efficient for HCN content which was reduced by over 90%. Gomez *et al.,* (1984) also reported HCN reduction of over 90% when they subjected cassava root meal to a similar processing procedure.

Table 5.5: Growth performance and nutrient utilisation of *O. niloticus* fed CRM diets (mean ± SD, n=3)

	CRM0	CRM25	CRM50	CRM75	CRM100
Initial Weight (g)	2.88 ± 0.20^{a}	2.85 ± 0.06^{a}	2.99 ± 0.06^{a}	2.90 ± 0.18^{a}	2.60 ± 0.07^{a}
Final Weight (g)	11.74 ± 0.68^{b}	19.37 ± 2.02^{a}	18.84 ± 1.93^{a}	16.70 ± 0.59^{a}	11.88 ± 0.45^{b}
Feed Intake (g fish^{-1} day^{-1})	0.42 ± 0.04^{b}	0.53 ± 0.03^{a}	0.51 ± 0.04^{a}	0.51 ± 0.03^{a}	0.41 ± 0.01^{b}
DP intake (g fish^{-1} day^{-1})	0.125 ± 0.010^{bc}	0.163 ± 0.010^{a}	0.152 ± 0.010^{a}	0.144 ± 0.009^{ab}	0.111 ± 0.004^{c}
DE intake*(kJ fish^{-1} day^{-1})	5.92 ± 0.52^{b}	7.79 ± 0.45^{a}	7.25 ± 0.49^{a}	7.26 ± 0.44^{a}	5.52 ± 0.19^{b}
Average Daily Gain (g fish^{-1} day^{-1})	0.16 ± 0.02^{b}	0.29 ± 0.038^{a}	0.28 ± 0.03^{a}	0.25 ± 0.01^{a}	0.17 ± 0.01^{b}
Specific Growth Rate (% day^{-1})	2.51 ± 0.15^{b}	3.42 ± 0.16^{a}	3.28 ± 0.15a	3.13 ± 0.10^{a}	2.67 ± 0.05^{b}
Feed Conversion Ratio	2.64 ± 0.27^{c}	1.81 ± 0.14^{a}	1.81 ± 0.17^{a}	2.06 ± 0.05^{ab}	2.51 ± 0.03^{bc}
Protein Efficiency Ratio	1.20 ± 0.13^{c}	1.73 ± 0.13^{b}	1.79 ± 0.16^{b}	1.62 ± 0.04^{ab}	1.35 ± 0.02^{bc}
Apparent Net Protein Utilization (%)	18.05 ± 2.03^{c}	26.07 ± 2.07^{a}	26.58 ± 2.92^{a}	23.93 ± 1.59^{ab}	19.40 ± 2.12^{bc}
Energy Retention (%)	11.39 ± 1.47^{b}	16.08 ± 1.48^{a}	15.45 ± 1.46^{ab}	14.39 ± 0.11^{ab}	10.90 ± 0.10^{b}
Hepatosomatic index	2.07 ± 0.44^{c}	2.96 ± 0.52^{a}	2.55 ± 0.49^{b}	2.14 ± 0.30^{c}	1.81 ± 0.28^{c}
Survival (%).	81.67 ± 20.21^{a}	96.67 ± 2.89^{a}	98.33 ± 2.89^{a}	93.33 ± 7.64^{a}	98.33 ± 2.89^{a}

Table 5.6: Whole body proximate composition of *O. niloticus* fed CRM diets before and after the experiment (% fresh weight basis, mean ± SD, n=3)

	Initial	CRM0	CRM25	CRM50	CRM75	CRM100
Moisture	73.44	73.47 ± 0.58^{a}	72.61 ± 0.20^{a}	73.71 ± 1.53^{a}	73.59 ± 0.87^{a}	75.21 ± 1.98^{a}
Crude protein	15.29	15.13 ± 0.15^{a}	15.04 ± 0.06^{a}	14.95 ± 0.71^{a}	14.82 ± 0.60^{a}	14.56 ± 1.13^{a}
Crude lipid	6.06	6.51 ± 0.11^{b}	7.62 ± 0.07^{a}	6.67 ± 0.36^{b}	6.94 ± 0.20^{bc}	5.30 ± 0.45^{c}
Ash	3.14	3.54 ± 0.03^{b}	3.36 ± 0.07^{a}	3.33 ± 0.07^{a}	3.43 ± 0.07^{ab}	3.95 ± 0.06^{c}
Gross energy (kJ g^{-1})	5.39	5.29 ± 0.04^{b}	5.31 ± 0.06^{a}	5.24 ± 0.04^{b}	5.28 ± 0.01^{b}	4.95 ± 0.01^{b}

Different superscripts in the same row indicate significant difference ($p<0.05$)

Grating facilitates the reaction between cyanogenic glycosides and linamarase to form volatile hydrocyanic acid which is vaporized during sun drying (Khajarern *et al.,* 1977). Removal of the peel may also have contributed to lower cyanogenic glycoside levels as it contains a higher cyanide content than the pulp (Johnson and Raymond, 1965). The toxicity of cassava peel limited its inclusion in diets of juvenile Nile tilapia to a maximum of 10% (Mgbenka *et al.,* 2004).

Fish showed considerable reluctance to consume diet CRM0 resulting in poor feed intake and consequently poor performance. This was unexpected as it was the control diet and the reasons for this poor palatability remain unclear. Palatability of diets CRM25, CRM50 and CRM75 was good as indicated by active feeding and high feed intake. Nevertheless, in diet CRM100 where CRM served as the main source of carbohydrate energy, palatability was poor and feed intake low. Studies in terrestrial animals show that palatability of diets containing CRM is usually affected by cyanogenic glycosides and the powdery nature of the meal. Presence of cyanogenic glycosides has been linked to poor intake in pigs (Phuc *et al.,* 2000) and poultry (Garcia and Dale, 1999) fed diets containing CRM. Similarly, the powdery nature of CRM has led to reduction in feed intake in pigs and poultry due to irritation of respiratory tract, crop compaction and ulcerogenic effects on the gastric mucosa (Oke, 1978; Ukachukwu, 2005). Even though the processing of CRM and pelleting of CRM diets may have minimised these effects in the current study, their influence in diet CRM100 may not be ruled out.

Growth performance correlated positively with the trend shown in feed intake where Nile tilapia fed diets CRM25, CRM50 and CRM75 displayed significantly higher growth. These diets contained 11.5–33.5% inclusion levels of CRM. The inclusion of 33.5% was higher than earlier recommendations under similar experimental conditions. Boscolo *et al.,* (2002) recommended inclusion of 24% in a trial using Nile tilapia with average body weight of 0.85g. However, higher inclusion was recommended under pond conditions using Nile tilapia with an average weight of 4.0g (Wee and Ng, 1986). This was probably due to nutrient contributions from primary production. The high inclusion levels which were realised in this study were due to the fact that CRM is an excellent source of energy with starch content varying from 60–70% (Garcia and Dale, 1999) and also that the diets were fairly well balanced due to high levels of fish meal which assured an ample supply of amino acids and other essential nutrients. Supply of sulphur amino acids is important for detoxification of the sub-lethal doses of HCN which remained after processing. Detoxification requires methionine as the main source of sulphur to convert HCN to thiocyanate which is less toxic and more readily removed from the body (Oke, 1978).

Though fish are generally poor in utilising carbohydrates Nile tilapia are exceptional because of their high amylolytic activity when fed diets rich in starch (Anderson *et al.,* 1984; Hepher, 1988; Shiau, 1997). This was however not the case in fish fed starch rich diet CRM100. Dietary carbohydrate utilisation depends on both complexity and chemical

composition of the carbohydrate. Simple carbohydrates tend to be absorbed completely as opposed to partially absorption of complex carbohydrates like starch (Spannhof and Plantikow, 1983). A large proportion of cassava starch is composed of amylopectin (70%) which is poorly digested by fish (Khajarern *et al.,* 1977; Oke, 1978; Sveinbjörnsson, 2006). Studies in rainbow trout showed that blood glucose level after intake of amylopectin was lower than after intake of amylose (Spannhof and Plantikow, 1983). Moreover, feed utilization in mirror carp fed diets containing cassava root meal was lower than that in fish fed diets containing rice (Ufodike and Matty, 1983). Moreover, starch also accelerates passage of chyme through the intestine hence reducing the time available for absorption (Spannhof and Plantikow, 1983). Heat treatment may improve digestibility of poorly digestible starches as shown by better utilization of cooked starch compared to raw starch in Nile tilapia (Adikwu, 1987; Offner *et al.,* 2003). The low crude lipid and high moisture content in bodies of fish fed diet CRM100 is similar to that observed by Anderson (1984) when Nile tilapia were fed complex carbohydrates.

This study has demonstrated that grating followed by sun drying of cassava roots can eliminate most of the HCN. The results have also shown that it is possible to replace up to 75% of wheat meal with CRM in diets of Nile tilapia without significantly affecting performance even at a total inclusion level of 33.5%.

CHAPTER 6 Effect of Blending and Feeding Stimulants on Utilisation of Moringa and Cassava Leaf Meals

6.1 Introduction

Inclusion of graded levels of processed moringa leaf meal (chapter 3) and processed cassava leaf meal (chapter 4) as protein sources in *O. niloticus* led to reduced performance even at the lowest inclusion level. This was attributed to a number of factors including poor palatability, poor feed intake, low digestibility, poor nutrient utilization, poor amino acid profiles and inherent antinutritional factors. Several practical ways to improve utilisation of plant proteins have been suggested including blending (Jackson *et al.,* 1982) and feeding stimulants (Takeda and Takii, 1992).

Blending of two or more plant protein sources has been reported to improve performance as opposed to a single protein source (Borgeson *et al.,* 2006). The improved performance is attributed to a number of factors including improved amino acid profiles (Jackson *et al.,* 1982; Gomes *et al.,* 1995), improved feed intake (El-Saidy and Gaber, 2003), improved digestibility (Lanari and D'Agaro, 2005) and reduced exposure to antinutritional factors (Borgeson *et al.,* 2006). Blending is more practical for small scale fish farmers in developing countries where amino acid supplementation may not be viable due to costs and availability (Fagbenro, 1999). Combinations of plant proteins have been shown in some cases to be able replace fish meal totally (Webster *et al.,* 1992; El-Saidy and Gaber, 2002).

Replacing fishmeal with plant proteins decreases the level of an extremely palatable component. Most terrestrial plants contain feeding deterrents i.e. inherent antinutritional factors which naturally function as protection against grazing by herbivores. The undesirable taste associated with these deterrents results in poor palatability and consequently feed intake (Refstie *et al.,* 1997). This was most noticeable with moringa leaf meal in the present study (chapter 3) as well as in other moringa studies (Afuang et al., 2003; Richter et al., 2003; Dongmeza et al., 2006). Palatability can be improved using feeding stimulants which mask such issues. The stimulants provide chemosensory stimuli which facilitate the feeding process including searching, intake and tasting as well as activation of the digestive system, particularly enzyme secretion (Kolkovski, 2000). According to Kasumyan and Doving (2003) feeding stimulants act by either inducing food capture (incitants), initiating or continuing feeding activity (stimulants) or accentuating flavour of food (enhancers or potentiators).

In the present study, betaine and hydrolysed fish protein were used in combination as feeding stimulants. Stimulants given together are often synergistic (Kasumyan and Doving, 2003). This has been demonstrated in pigfish, *Orthopristis chrysopterus* whereby the stimulatory effect of a mixture of 19 amino acids and betaine was higher than either individually (Carr, 1976). Similar results were also reported by Takeda et al (1984) where a combination of betaine and a mixture of the amino acids glycine,

alanine, proline and histidine gave best results in stimulating feed intake in Japanese eel, *Anguilla japonica*.

Betaine (glycine betaine, trimethylglycine) is a highly water soluble compound which is known to stimulate the olfactory bulb of fish (Polat and Beklevik, 1998). It is found in invertebrates, microorganisms and some plants which are consumed by fish suggesting that its dietary inclusion might help to mimic the smell and taste of a natural fish diet (Felix and Sudharsan, 2004). Betaine has been shown to act as a feed attractant in red sea bream, *Chrysophyrys major* (Goh and Tamura, 1980), Dover sole, *Solea solea* (Mackie and Mitchell, 1982), rainbow trout and European eel, *Anguilla anguilla* (Mackie and Mitchell, 1983; Polat and Beklevik, 1998).

Hydrolyzed fish protein is a non-conventional ingredient which is used as a protein supplement as well as feed attractant and palatability enhancer (Hardy, 1991; Oliveira and Cyrino, 2004; Oliveira and Cyrino, 2004). It is produced from fish industry by–products through processes involving hydrolytic enzymes and subsequent heating. The enzymatic treatment degrades the protein making it more digestible (Refstie *et al.,* 2004). The degradation of protein results in shorter peptides and free amino acids which are known to stimulate feeding (Berge and Storebakken, 1996). Improved feed intake of diets supplemented with hydrolysed fish protein has been observed in lake sturgeon, *Acipenser fulvescens* (Moreau and Dabrowski, 1996), Atlantic salmon, *Salmo salar* (Berge and Storebakken,

1996) and largemouth bass, *Micropterus salmoides* (Oliveira and Cyrino, 2004).

Studies in Nile tilapia have shown increased feed intake when betaine was fed in combination with choline (Kasper *et al.,* 2002; El-Husseiny *et al.,* 2008). Also, free amino acids (acidic, neutral and basic) have been reported to enhance feed intake in tilapia (Johnsen *et al.,* 1990; Yacoob *et al.,* 2001). Some of the effective amino acids include the L–amino acids histidine, arginine, serine, methionine, glutamine, tryptophan and alanine (Yacoob *et al.,* 2001). Feed intake in redbelly tilapia, *Tilapia zillii* was reported to be enhanced by inclusion of amino acids (glutamic acid, alanine, aspartic acid, lysine and serine) (Johnsen and Adams, 1986) and organic acids (citric acid and acetic acid) (Adams *et al.,* 1988).

The main objective of the present study was to evaluate the effect of blending and feeding stimulants on the nutritive value of moringa and cassava leaf meals as protein sources in Nile tilapia diets.

6.2 Methodology

6.2.1 Preparation of Leaf Meal Blends

Leaf meal blends (LMB) were prepared from processed moringa and cassava leaf meals in different proportions. Moringa and cassava leaf meals were processed as described in sections 3.2.1 and 4.2.1 respectively. LMB1 was prepared by blending 2 parts of moringa leaf meal to 1 part of cassava leaf meal by weight. LMB2 was prepared by blending

equal parts of moringa and cassava leaf meals while LMB3 was prepared by blending 1 part of moringa leaf meal to 2 parts cassava leaf meal. Ingredients were thoroughly blended using a food mixer (Hobart Ltd, London, UK).

6.2.2 Biochemical Analysis and Digestibility of Blends

Proximate analysis, gross energy, amino acids, minerals and antinutritional factors shown in Table 6.1 were analysed following the procedures described in section 2.3. Digestibility was determined following procedures described in section 2.4 using Nile tilapia with an average weight of 13.07 ± 1.87g.

6.2.3 Experimental Setup and Feed Formulation

The growth trial was conducted in 30L circular self cleaning plastic tanks connected to a recirculation system as described in section 2.1. A total of 600 Nile tilapia fingerlings with an average weight of 4.99 ± 0.17g were were handled as described in section 2.2 and were equally distributed into thirty experimental tanks. A total of ten diets were formulated to contain 30% crude protein, 18kJ g^{-1} and 10% lipid as described in section 2.5. The control diet (LMB0) had fish meal as the main source of protein whilst in the remaining diets, LMBs provided between 10% (LMB1 10, LMB2 10 and LMB3 10) and 20% (LMB1 20, LMB2 20 and LMB3 20) of total dietary crude protein (Table 6.2).

Table 6.1: Biochemical composition of moringa and cassava leaf meal blends in comparison with processed moringa (MLM) and cassava (CLM) leaf meals

	LMB1[1]	LMB2[2]	LMB3[3]	MLM[4]	CLM[5]
Proximate composition (g 100^{-1} g, as fed)					
Dry matter	89.38	89.58	89.94	92.99	93.36
Crude protein	32.16	31.44	30.59	31.07	29.00
Crude lipid	4.80	3.62	3.52	4.45	2.37
Crude fibre	8.42	8.85	9.39	5.89	10.21
Ash	5.49	5.43	5.63	5.52	5.95
Nitrogen free extract	38.49	40.24	40.81	46.09	45.84
Gross energy (kJ g^{-1})	20.00	20.01	20.06	20.11	19.66
Amino acid composition (g 100g^{-1} feed)					
Arginine	1.93	1.89	1.85	2.01	1.77
Histidine	0.81	0.80	0.79	0.83	0.77
Isoleucine	1.67	1.66	1.64	1.69	1.62
Leucine	2.83	2.79	2.74	2.90	2.67
Lysine	1.50	1.50	1.49	1.50	1.49
Methionine +Cystine	0.31	0.36	0.39	0.23	0.47
Phenylalanine+ Tryptophan	3.00	2.95	2.90	3.10	2.81
Threonine	1.39	1.36	1.33	1.45	1.27
Valine	2.11	2.08	2.05	2.17	1.99
Mineral composition (mg g^{-1}, DM)					
Phosphorus	3.72	3.88	4.26	3.42	4.11
Sodium	0.32	0.21	0.19	0.36	0.18
Magnesium	2.98	2.66	2.35	3.16	2.45
Potassium	10.67	10.44	10.94	6.21	14.45
Calcium	11.73	9.37	7.49	11.41	6.29
Iron	0.33	0.24	0.19	0.23	0.15
Copper	0.02	0.02	0.01	0.03	0.01
Zinc	0.30	0.20	0.15	0.25	0.06
Antinutritional factors (g 100g^{-1}, DM)					
Saponins[6]	1.19	1.16	1.25	1.19	1.06
HCN[7]	0.50	0.72	1.09	0.16	1.74
Phenols[8]	3.72	4.23	4.84	3.36	4.94
Tannins[8]	1.85	1.99	2.43	1.62	2.12
Phytic acid[9]	0.92	2.21	1.22	1.49	2.44

[1]2MLM:1CLM, [2]1MLM:1CLM, [3]1MLM:2CLM, [4]processed moringa leaf meal from Table 3:1, [5]processed cassava leaf meal from Table 4:1, [6]As diosgenin equivalent, [7]Hydrocyanic acid (mg 100g^{-1}), [8]As tannic acid equivalent, [9]As phosphorus equivalent

Hydrolysed fish protein (CPSP G, Sopropêche, France) and betaine hydrochloride (Sigma, B3501) were added as feeding stimulants at 5% and 1% respectively to a further set of diets containing LMBs at 20% (LMB1 20 ST, LMB2 ST and LMB3 20 ST). The diets were randomly assigned to the experimental tanks in triplicate and fish were hand fed three times a day to appetite but not exceeding 10% of the body weight as described in section 2.6. Once a week the fish were bulk weighed to monitor growth and adjust feed ration. Final body weights were recorded at the end of the trial and 3 fish from each tank were sacrificed for whole body proximate composition analysis as described in section 6.2.4. The rest were pooled according to dietary treatment for faecal collection to determine digestibility as described in section 2.4

6.2.4 Performance Parameters

Growth and feed utilisation were measured as described in section 2.7 and whole body composition was determined according to sections 2.8.

6.2.5 Data Analysis

Data on performance was analysed as described in section 2.10.

Table 6.2: Composition of the moringa and cassava leaf meal blends (LMB) diets fed to *O. niloticus* during the growth trial (g 100 g^{-1}, on dry weight basis)

Ingredient	LMB0	LMB1 10	LMB2 10	LMB3 10	LMB1 20	LMB2 20	LMB3 20	LMB1 20 ST	LMB2 20 ST	LMB3 20 ST
LMB1[1]	-	10.0	-	-	20.0	-	-	20.0	-	-
LMB2[2]	-	-	10.0	-		20	-	-	20.0	-
LMB3[3]	-	-	-	10.0	-	-	20.0	-	-	20.0
Fishmeal[4]	36.0	31.5	28.0	31.5	28.0	31.5	28.0	24.0	24.0	24.0
Wheat meal[5]	48.5	44.0	37.5	44.0	37.5	44.0	37.5	36.0	36.0	36.0
Sunflower oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	5.5	5.5	5.5
Mineral premix[6]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix[7]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
CMC[8]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromium oxide.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Hydrolysed fish protein[9]	-	-	-	-	-	-	-	5.0	5.0	5.0
Betaine[10].	-	-	-	-	-	-	-	1.0	1.0	1.0

[1]2MLM:1CLM, [2]1MLM:1CLM, [3]1MLM:2CLM, [4]Brown fishmeal (aquaculture grade), [5]Whole grain, [6]As listed in Table 2.2, [7]As listed in Table 2.3, [8]Carboxymethylcellulose-sodium salt, high viscosity, [9]CPSP G, Sopropêche (72% crude protein, 20% crude lipid, 3% ash, 5% nitrogen free extract), France, [10]betaine hydrochloride (Sigma, B3501)

6.3 Results

6.3.1 Biochemical Composition and Digestibility of LMB and LMB Diets

The biochemical composition of the blends in comparison with moringa and cassava leaf meals is shown in Table 6.1. Protein and lipid contents in the leaf meal blends increased as the proportion of MLM increased. The crude fibre content increased as the proportion of CLM increased in blends. Quantitatively, amino acid levels in blends were higher than in CLM but lower than in MLM. The mineral content of blends reflected the mineral contents of individual leaf meals. Saponin contents of blends were more or less similar to that in the individual leaf meals. In contrast, the amount of HCN, phenols, tannins and phytic acid in leaf meal blends was lower than in CLM and higher than in MLM.

Apparent digestibility coefficients of blends are shown in Table 6.3 in comparison with those of moringa and cassava leaf meals. Replacing MLM with CLM in LMBs resulted in decreased ADCs for dry matter, protein, lipid gross and energy reflecting the low digestibility of CLM. Proximate composition and digestibility of LMB diets used for the growth trial are shown in Table 6.4.

Table 6.3: Digestibility of LMB in comparison with MLM and CLM

	RD	TD[1]	TD[2]	TD[3]	LMB1	LMB2	LMB3	MLM[1]	CLM[2]
Proximate composition (g 100g^{-1}, as fed)									
Dry matter	91.01	90.21	90.37	90.43					
Crude protein	33.67	33.70	33.37	33.15					
Crude lipid	9.37	8.78	8.36	8.04					
Crude fibre	2.81	4.38	4.50	4.66					
Ash	8.54	7.95	7.87	7.86					
Nitrogen free extract	45.61	45.19	45.90	46.29					
Gross energy (kJ g^{-1})	18.66	19.79	19.78	19.81					
Apparent digestibility coefficient (%)									
Dry matter	80.8	70.6	69.8	65.8	**46.8**	**44.0**	**30.7**	**68.4**	**38.3**
Crude protein	90.5	84.5	82.5	99.4	**70.6**	**63.5**	**53.2**	**89.3**	**44.5**
Crude lipid	94.9	87.2	86.5	85.5	**68.0**	**64.5**	**59.8**	**71.1**	**88.5**
Gross energy	81.6	72.2	70.9	67.1	**51.3**	**47.3**	**35.2**	**78.5**	**44.2**
Digestible protein (g 100g^{-1})	21.6	21.5	19.2	16.0	**20.3**	**17.9**	**14.6**	**25.9**	**13.0**
Digestible energy (kJ g^{-1})	15.2	14.3	14.0	13.3	**10.3**	**9.5**	**7.3**	**15.8**	**8.0**

RD=Reference diet, TD=Test diet (1=LMB1, 2=LMB2, 3=LMB3), [1]processed moringa leaf meal from Table 3.3, [2]processed cassava leaf meal from Table 4.3

Table 6.4: Proximate composition (g 100g-1), gross energy (kJ g-1) and phosphorus (mg g-1) content of diets containing moringa and cassava leaf meal blends (LMB) used for O. niloticus growth trial (on dry weight basis)

	LMB0	LMB1 10	LMB2 10	LMB3 10	LMB1 20	LMB2 20	LMB3 20	LMB1 20 ST	LMB2 20 ST	LMB3 20 ST
Proximate composition (g 100g^{-1}, as fed)										
Dry matter	92.21	91.67	91.64	91.57	92.00	92.02	92.66	92.05	92.43	92.72
Crude protein	30.87	31.07	30.66	30.98	30.95	30.83	30.76	31.95	32.31	31.51
Crude lipid	8.91	9.80	10.16	9.35	9.66	9.85	10.18	10.45	10.14	10.36
Crude fibre	1.96	2.37	3.40	2.64	3.58	2.88	3.42	3.40	3.16	3.32
Ash	9.55	9.46	9.20	9.39	9.24	9.34	9.36	8.88	9.02	9.16
Nitrogen free extract	40.92	38.97	38.22	39.21	38.57	39.12	38.94	37.37	37.8	38.37
Gross energy (kJ g^{-1})	19.22	18.84	19.12	18.83	19.38	19.18	19.17	19.87	19.71	19.61
Phosphorus (mg g^{-1})	8.73	7.51	7.63	8.21	7.56	8.65	8.44	7.13	6.66	6.66
Apparent digestibility coefficient (%)										
Dry matter (%)	83.7	81.8	78.3	80.9	84.1	83.8	81.3	84.3	79.2	78.8
Crude protein (%)	89.0	87.1	85.7	85.1	87.7	87.0	84.5	88.0	85.0	83.6
Crude lipid (%)	95.8	94.6	93.1	93.5	95.0	94.7	93.8	96.1	92.3	93.4
Gross energy (%)	84.6	82.6	79.2	81.6	84.7	84.6	81.8	85.4	80.2	79.8
Phosphorus (%)	80.8	77.3	82.5	81.1	93.1	82.0	81.2	85.2	80.5	75.0
Digestible protein (DP) (g 100g^{-1})	27.5	27.1	26.6	26.2	26.9	26.9	26.0	28.1	27.5	26.3
Digestible energy (DE) (kJ g^{-1})	16.3	15.6	14.9	15.6	16.2	16.4	15.7	17.0	15.8	15.6
DP/DE ratio (mg kJ^{-1})	16.9	17.4	17.8	16.8	16.6	16.4	16.6	16.6	17.4	16.8

Nutrient contents were largely similar between diets with the exception of crude fibre which was slightly higher in diets containing blends. Diets were highly digestible with coefficients above 75%. In general, increasing the proportion of CLM in blends resulted in a decrease in ADCs. Values for digestible protein and energy were also high and similar between the different diets resulting in a DP/DE ratio of about 17mg kJ^{-1}.

6.3.2 Feed Intake, Growth and Nutrient Utilisation

Differences in body weight gain of fish fed the experimental diets became noticeable after the first two weeks of the experiment as shown in Figure 6.1. Performance in terms of intake, growth and nutrient utilisation is shown in Table 6.5. There was no significant difference in feed intake between the control diet and LMB diets except with diet LMB1 20. Addition of feeding stimulants to diets containing 20% LMB did not significantly improve feed intake, except that of diet LMB1 20 ST which was slightly improved and comparable to the control diet. Digestible protein and energy intakes followed the same trend. Inclusion of the leaf meal blends in diets affected growth and nutrient utilisation. However, there was no significant difference in average daily gain between fish fed the control diet and those fed diet LMB2 10 while fish fed diet LMB1 20 had least average daily gain. Similarly, there was no significant difference in specific growth rate between fish fed control diet and those fed diets LMB1 10%, LMB2 10% and diet LMB3 20 ST. Those fed diet LMB1 20 had lowest specific growth rate.

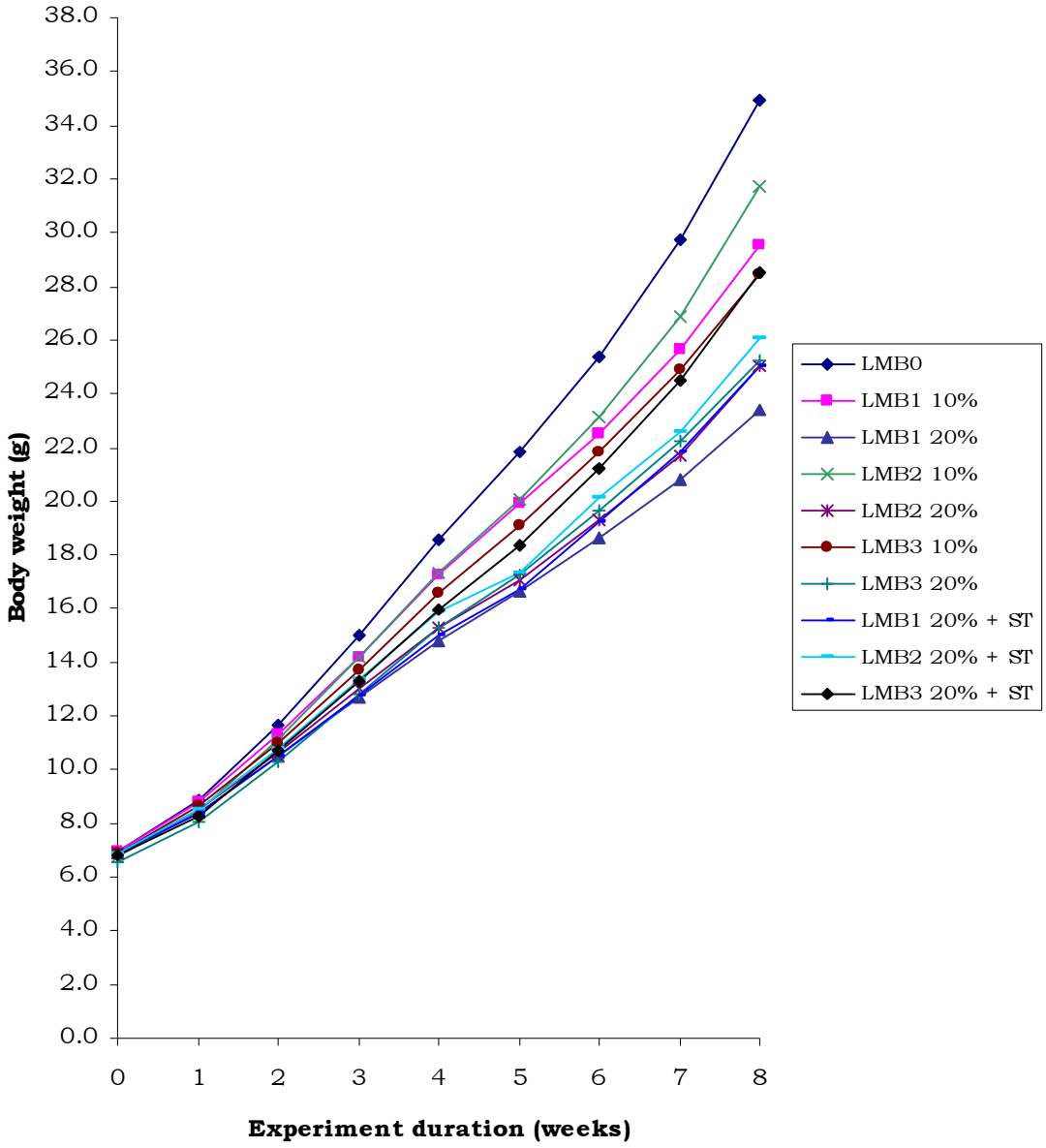


Figure 6.1: Change in body weight of *O. niloticus* fed diets containing moringa and cassava leaf meal blends (LMB).

Feed conversion ratios for diets containing 10% LMBs and diet LMB3 20 + ST did not significantly differ from the control and highest FCR was exhibited by fish fed diet LMB1 20. Fish fed diets LMB1 10, LMB2 10 and LMB3 20 ST exhibited better protein utilisation as indicated by protein

efficiency ratio and apparent net protein utilisation which did not differ significantly from the control. Energy retention was higher in fish fed diets containing 10% LMB and LMB3 ST which was comparable to the control. Fish fed diet LMB3 10 had significantly higher hepatosomatic index while the lowest value was observed in fish fed diet LMB1 20. There was no significant difference in survival between treatments.

6.3.3 Whole Body Composition

Whole body proximate composition is shown in Table 6.6. There were no significant differences in all proximate composition parameters between the fish fed the different LMB diets. Nevertheless, there was a slight decrease in crude lipid as the level of leaf meal blends doubled in the diets. However, this was not the case in diets LMB1 20 ST and LMB3 20 ST which contained feed attractants.

6.4 Discussion

The increase in protein, lipid gross energy and some amino acids in the blends was a simple additive effect since MLM had a higher content of these nutrients than CLM. The same phenomenon accounts for increased crude fibre and sulphur amino acids with increased proportions of CLM. The reduction in HCN, phenols, tannins and phytic acid in blends compared to CLM confirms the suggestion of Borgeson *et al.,* (2006) that blending can result in reduced exposure of fish to antinutritional factors.

Table 6.5: Growth performance and nutrient utilisation of *O. niloticus* fed LMB diets (mean ± SD, n=3)

	LMB0	LMB1 10	LMB2 10	LMB3 10	LMB1 20	LMB2 20	LMB3 20	LMB1 20 ST	LMB2 20 ST	LMB3 20 ST
Initial weight (g)	6.97 ± 0.10^{a}	6.93 ± 0.09^{a}	6.73 ± 0.12^{a}	6.89 ± 0.20^{a}	6.90 ± 0.03^{a}	6.91 ± 0.04^{a}	6.54 ± 0.21^{a}	6.89 ± 0.10^{a}	6.91 ± 0.08^{a}	6.82 ± 0.10^{a}
Final weight (g)	34.74 ± 0.87^{a}	29.36 ± 1.64^{bc}	31.91 ± 2.05^{ab}	28.44 ± 1.77^{bc}	23.35 ± 1.43^{d}	24.97 ± 0.76^{cd}	25.29 ± 1.64^{cd}	25.07 ± 1.26^{bd}	26.10 ± 1.68^{cd}	29.13 ± 2.82^{bc}
Feed intake (g fish^{-1} day^{-1})	0.594 ± 0.031a	0.567 ± 0.029^{ab}	0.562 ± 0.025^{ab}	0.564 ± 0.017^{ab}	0.513 ± 0.008^{b}	0.537 ± 0.008^{ab}	0.525 ± 0.018^{ab}	0.535 ± 0.018^{ab}	0.559 ± 0.035^{ab}	0.560 ± 0.051^{ab}
DP intake (g day^{-1})	0.163 ± 0.009^{a}	0.153 ± 0.008^{ab}	0.147 ± 0.007^{abc}	0.151 ± 0.005^{ab}	0.136 ± 0.003^{c}	0.144 ± 0.003^{abc}	0.136 ± 0.005^{bc}	0.151 ± 0.005^{ab}	0.153 ± 0.009^{ab}	0.147 ± 0.015^{ab}
DE intake (kJ fish^{-1} day^{-1})	9.63 ± 0.49^{a}	8.87 ± 0.46^{ab}	8.77 ± 0.38^{abc}	9.23 ± 0.31^{ab}	7.63 ± 0.15^{c}	8.70 ± 0.10^{abc}	8.23 ± 0.25^{bc}	9.07 ± 0.32^{ab}	8.80 ± 0.53^{abc}	8.73 ± 0.78^{abc}
Average daily gain (g fish^{-1} day^{-1})	0.497 ± 0.012^{a}	0.400 ± 0.026^{bc}	0.450 ± 0.040^{ab}	0.383 ± 0.032^{bc}	0.293 ± 0.023^{d}	0.323 ± 0.012^{cd}	0.333 ± 0.032^{cd}	0.327 ± 0.023^{cd}	0.340 ± 0.030^{cd}	0.400 ± 0.053^{bc}
Specific growth rate (% day^{-1})	2.87 ± 0.04^{a}	2.57 ± 0.13^{abc}	2.77 ± 0.11^{ab}	2.53 ± 0.15^{bc}	2.17 ± 0.10^{d}	2.29 ± 0.06^{cd}	2.41 ± 0.14^{cd}	2.31 ± 0.08^{cd}	2.37 ± 0.10^{cd}	2.59 ± 0.17^{abc}
Feed conversion ratio	1.20 ± 0.88^{a}	1.43 ± 0.19^{ab}	1.25 ± 0.06^{a}	1.47 ± 0.10^{abc}	1.75 ± 0.15^{c}	1.67 ± 0.10^{bc}	1.57 ± 0.10^{bc}	1.65 ± 0.05^{bc}	1.63 ± 010^{bc}	1.41 ± 0.05^{ab}
Protein efficiency ratio	2.71 ± 0.19^{a}	2.32 ± 0.29^{abc}	2.58 ± 0.12^{ab}	2.21 ± 0.16^{bcd}	1.87 ± 0.17^{d}	1.94 ± 0.11^{cd}	2.07 ± 0.13^{cd}	1.90 ± 0.06^{cd}	1.90 ± 0.11^{cd}	2.25 ± 0.31b^{cd}
Apparent net protein utilization (%)	38.73 ± 1.86^{a}	33.34 ± 4.24^{abc}	37.39 ± 1.35^{ab}	31.54 ± 3.49^{bcd}	26.54 ± 2.66^{cd}	28.55 ± 0.94^{cd}	30.06 ± 2.14^{cd}	25.13 ± 1.18^{d}	25.58 ± 2.09^{d}	32.74 ± 2.32^{abc}
Energy retention (%)	21.89 ± 0.10^{a}	19.63 ± 2.62^{ab}	22.14 ± 1.13^{a}	18.65 ± 1.72^{abc}	15.00 ± 2.75^{c}	15.97 ± 1.28^{bc}	16.82 ± 1.31^{bc}	15.85 ± 0.87^{bc}	15.65 ± 0.66^{bc}	18.98 ± 0.60^{abc}
Hepatosomatic index	2.51 ± 0.70^{ab}	2.56 ± 0.50^{ab}	2.49 ± 0.42^{ab}	2.89 ± 0.57^{a}	1.90 ± 0.63^{c}	2.08 ± 0.59^{bc}	2.54 ± 0.43^{ab}	2.09 ± 0.56^{bc}	2.47 ± 0.55^{bc}	2.58 ± 0.63^{ab}
Survival (%).	97.50 ± 03.54^{a}	91.67 ± 14.43^{a}	85.00 ± 10.00^{a}	90.00 ± 10.00^{a}	96.67 ± 05.77^{a}	90.00 ± 08.66^{a}	95.00 ± 05.00^{a}	95.00 ± 05.00^{a}	88.33 ± 07.64^{a}	81.67 ± 27.54^{a}

Figures followed by different letters in superscripts in the same rows are significantly different at $p<0.05$

Table 6.6: Whole body proximate composition of *O. niloticus* fed LMB diets before and after the experiment (% fresh weight basis, mean ± SD, n=3)

	Initial	LMB0	LMB1 10	LMB2 10	LMB3 10	LMB1 20	LMB2 20	LMB3 20	LMB1 20 ST	LMB2 20 ST	LMB3 20 ST
Moisture content	77.36	75.45 ± 0.67[a]	74.47 ± 0.75[a]	74.88 ± 0.38[a]	74.93 ± 1.51[a]	75.03 ± 1.77[a]	74.62 ± 0.14[a]	75.21 ± 0.52[a]	75.59 ± 0.76[a]	75.58 ± 0.31[a]	74.47 ± 0.12[a]
Crude protein	13.57	14.15 ± 0.28[a]	14.20 ± 0.34[a]	14.31 ± 0.61[a]	14.10 ± 1.03[a]	14.02 ± 0.26[a]	14.40 ± 0.32[a]	14.26 ± 0.20[a]	13.33 ± 0.14[a]	13.50 ± 0.23[a]	14.06 ± 0.45[a]
Crude lipid	4.19	5.60 ± 0.23[a]	6.30 ± 0.52[a]	6.10 ± 0.21[a]	6.11 ± 0.92[a]	5.71 ± 1.92[a]	5.79 ± 0.48[a]	5.27 ± 0.47[a]	6.03 ± 0.55[a]	5.77 ± 0.65[a]	6.07 ± 0.13[a]
Ash	4.29	3.95 ± 0.27[a]	3.78 ± 0.06[a]	3.70 ± 0.07[a]	3.57 ± 0.10[a]	3.87 ± 0.44[a]	3.74 ± 0.43[a]	3.83 ± 0.13[a]	3.83 ± 0.22[a]	3.89 ± 0.09[a]	3.58 ± 0.05[a]
Gross energy (kJ g^{-1})	4.83	4.99± 0.11[a]	5.12± 0.08[a]	5.14± 0.03[a]	5.14± 0.14[a]	4.94± 0.33[a]	5.06± 0.10[a]	5.00± 0.10[a]	5.09± 0.11[a]	4.98± 0.21[a]	5.15± 0.04[a]

Different superscripts in the same row indicate significant difference ($p<0.05$)

Comparable feed intakes between the control diet and diets LMB2 20 and LMB3 20 is an improvement compared to either of the leaf meals fed individually (chapters 3 and 4) in which inclusion levels of 15g $100g^{-1}$ led to a significant reduction in feed intake. These findings are similar to those observed in rohu, *Labeo rohita* where feed intake was significantly higher when soybean meal, groundnut meal and canola meal were fed in combination rather than individually (Khan *et al.,* 2003). Similar results were also observed in Atlantic cod, *Gadus morhua* fed diets containing a mixture of soybean meal and corn gluten (Albrektsen *et al.,* 2006). Poor intake of diet LMB1 20 which contained a high proportion of MLM, reaffirmed the poor palatability of moringa leaf meal observed in chapter 3. Hence improved intake of diets LMB2 20 and LMB3 20, which contained less MLM, is probably due to reduced exposure to the antinutritional factors responsible for suppressing feed intake. It has been suggested that combining several plant protein sources with inherent antinutritional factors may reduce exposure of fish to those antinutritional factors due to lower inclusion levels of each of the individual protein sources in the combined mixture (Fontaínhas-Fernandes et al., 1999; Borgeson et al., 2006). Also, blending may have led to interactions between various antinutritional factors contained in MLM and CLM with the potential of decreasing their deleterious effects. Reduction of individual toxicity due to such interactions has been reported between saponin–tannin (Freeland *et*

al., 1985) and tannin–cyanogens (Goldstein and Spencer, 1985) all of which were present in both MLM and CLM.

Hydrolysed fish protein and betaine only had a marginal effect in improving intake, as exhibited by a slight increase in feed intake of diet LMB1 20 ST. It was anticipated that betaine would reinforce the stimulatory effect of hydrolysed fish protein which contains free amino acids (Berge and Storebakken, 1996) as observed in juvenile yellowtail, *Seriola quinqueradiata* (Harada and Matsuda, 1984) and Nile tilapia (Kasper *et al.,* 2002). There is a probability that these attractants do not form a major part of the diets naturally preferred by Nile tilapia as gustatory sensitivity and specificity in fish is developed evolutionarily and ontogenetically in relation to feeding habits (Yacoob *et al.,* 2001; Kasumyan and Doving, 2003). The natural feed of Nile tilapia is mainly composed of plant material and/or detritus of plant origin (Beveridge and Baird, 2000). Betaine is found in high concentrations in crustaceans which are not a natural feed of Nile tilapia (Kubitza *et al.,* 1997). Yacoob *et al.,* (2001) observed low relative stimulatory effects after exposing Nile tilapia to betaine. Possibly citric acid may be a more promising stimulant as it was reported to increase feed intake in redbelly tilapia, *Tilapia zillii,* which has similar feeding habits (Adams *et al.,* 1988).

There is also a possibility that the high percentage of fishmeal in the diets could have masked any stimulatory effect of hydrolysed fish protein in this study. In a similar study, significantly higher feed intake and growth was

observed when krill was used as an attractant in *O. niloticus* diets with soybean meal as the main source of protein (Gaber, 2005). There is also a possibility that the free amino acids found in hydrolysed fish protein are less effective compared to the inosine 5' – monophosphate (IMP-5') that is abundant in fish muscle and consequently in fish meal (Oliveira and Cyrino, 2004). For instance, turbot, *Scophthalmus maximus* juveniles responded positively when fed diets containing inosine and IMP-5' (Mackie and Adron, 1978) but negatively to diets containing hydrolysed fish protein (Oliva-Teles *et al.,* 1999). It is also worthwhile to note that feed attractants gradually lose their potency if fed in diets for prolonged periods, particularly when feeding at high levels, as fish get conditioned to the taste (Kamstra and Heinsbroek, 1991).

Nevertheless, the combination of blending and feeding stimulants had a positive effect on growth and feed utilisation in fish fed a diet containing 20g $100g^{-1}$ of dietary protein from LMB3 (diet LMB3 20 ST). This is an improvement compared to the poor performance shown in chapters 3 and 4 even at the lowest inclusion level of 15g $100g^{-1}$. It is a well established fact that plant protein sources are generally of poorer quality than fish meal. Plant proteins are generally characterised by poor palatability, poor amino acid profiles, poor digestibility and high levels of antinutritional factors and crude fibre. Because of essential amino acid imbalance in plant proteins, chemical scores commonly indicate that they have limited value as fishmeal replacers when used individually (De Silva and

Anderson, 1998). Chemical score is obtained by dividing percentage of each essential amino acid in a test protein by the percentage of corresponding amino acid in egg protein (Hepher, 1988).

Studies have shown that fish tend to perform better when fed diets containing a combination of two or more plant protein sources as opposed to a single plant protein source. This is partly due to protein complementation where two or more plant protein sources with complementary amino acid profiles are blended to obtain the required optimal amounts of essential amino acids (EAA) (Jackson *et al.,* 1982; Tacon and Jackson, 1985). In this study, processed MLM had quantitatively higher amino acid content, except sulphur amino acids, compared to processed CLM which had quantitatively lower amino acid content but higher sulphur amino acid content. Espe *et al.,* (2007) reported that a combination of plant proteins could replace up to 95% of fishmeal in Atlantic salmon, *Salmo salar* diets as they could mimic the amino acid profile of fishmeal. Similar results were also observed in Atlantic cod, *Gadus morhua* fed diets containing a mixture of soybean meal and corn gluten which provided up to 68% of total protein (Albrektsen *et al.,* 2006). In another trial, Nile tilapia fed diets containing a combination of plants proteins (canola and pea or flax and pea) performed better than those fed diets containing the plant proteins individually (Borgeson *et al.,* 2006).

Inclusion of hydrolysed fish protein in this diet may have also contributed towards improved growth, particularly in fish fed diet LMB3 20 ST. This could be due to greater amino acid availability, similar to that observed in Atlantic salmon fed diets containing hydrolysed fish protein which led to more efficient protein utilization and growth (Refstie *et al.,* 2004). Total replacement of fish meal was reported in some studies where plant proteins were combined with animal proteins. A combination of distillers dried grain solubles, soybean meal and meat and bone meal could totally replace fish meal without affecting growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* (Coyle *et al.,* 2004). Similar findings were also observed in juvenile rainbow trout, *Oncorhynchus mykiss* fed a diet containing a mixture of plant proteins (cottonseed meal and soybean mean) and animal by–products (Lee *et al.,* 2002). Remarkably, total replacement of fishmeal was attained in *O. niloticus* using a combination of soybean, sunflower, cottonseed and linseed meals at 25% each supplemented with 0.5% of both methionine and lysine (El-Saidy and Gaber, 2003).

To a large extent the digestibility of blends was reflective of the digestibility of individual ingredients. Increasing the proportion of CLM, which is less digestible, and decreasing the proportion of MLM, which is more digestible, resulted in decreased digestibility. This is in agreement with findings by Eggum and Christensen (1973) who found that digestibility of a mixture of two protein sources is directly related to digestibility of those individual

proteins determined separately. Digestibility of LMB diets also followed the same trend where it decreased with increase in both proportion of CLM in blends and inclusion levels of blends in diets. This observation is in agreement with the suggestion that digestibility of a compound diet will depend on the digestibilities of individual ingredients (Fontaínhas-Fernandes *et al.,* 1999). This is due to the fact that individual digestibilities are additive though a potential for interaction also exists. This was demonstrated in hybrid tilapia, *Oreochromis niloticus x Oreochromis aureus* (Sklan *et al.,* 2004) as well as in gilthead sea bream, *Spaurus aurata* (Lupatsch *et al.,* 1997) in which individual nutrient digestibility values could be used for estimating digestibilities in compounded feeds.

In this study, blending and use of feeding stimulants did not influence whole body proximate composition. Similar results have been reported earlier in channel catfish, *Ictalurus punctatus* (Robinson and Li, 1994) and Nile tilapia (El-Saidy and Gaber, 2003) fed diets containing a combination of plant protein sources. However, LMB diets had an influence on the hepatosomatic index which differed significantly between diets. This is probably due to increased lipid deposition in the liver. Plant proteins tend to increase daily fat gain and reduce nitrogen retention (Fournier *et al.,* 2004).

The results from this study show that the blending of MLM and CLM complemented each other in terms of proximate composition and amino

acids. The digestibility of blends reflected the digestibility of individual leaf meals i.e. higher with high proportion of MLM which is more digestible and lower with higher proportion of CLM which is less digestible. Feeding stimulants only had a marginal effect in improving feed intake of the diet containing LMB1. However, the combination of blending and stimulants had a positive effect on growth and nutrient utilisation in fish fed diet containing LMB3 at 20g $100g^{-1}$. Further studies are recommended to identify other leaf meals with complementary nutritional profiles to MLM and CLM to improve the nutritional value of blends as well as more effective feeding stimulants.

CHAPTER 7 Cost Effectiveness and Growth Performance Using Practical Diets made from Moringa and Cassava

7.1 Introduction

The majority of aquaculture development programmes in sub Saharan Africa have focused on promotion of small-scale fish farming for food security and improved nutrition. This may be is one of the biggest mistakes made by the early proponents of aquaculture as such an approach lacks commercial elements that tend to attract more investment and lead to sustainability (Hecht, 2000). Small–scale aquaculture with intrinsic commercial elements has huge potential for poverty reduction through income generation, diversification of farm enterprises as well as job creation in rural areas in addition to improving household nutrition and food security (Hishamunda, 2007). This benefit was evident from Rwandese farmers who regarded fish as a cash crop rather than a protein source (Engle, 1997). Similarly, Tanzanian farmers ranked fish produce highly in terms of marketability and profitability in comparison to traditional farm produce (Nilsson and Wetengere, 1994).

Transforming subsistence aquaculture into semi–commercial aquaculture depends on the economic viability of fish farming in rural settings which are characteristically poor with very little disposable cash. Success will therefore depend upon technological innovations which can lead to yield improvement and yet remain cost effective (Coche *et al.,* 1994). This is particularly crucial for small scale farmers who naturally tend to minimise

risks by limiting capital inputs; this means that new activities are only attempted if profitability is obvious and capital requirements are low (Middendorp and Verreth, 1992).

Feed is the most expensive cost item in aquaculture and may account for up to 60% of total operational expenditure (Virk and Saxena, 2003). High cost and/or unavailability of feed inputs have contributed to failure of fish farming in rural areas thus excluding many populations from the socioeconomic benefits of aquaculture (Abou *et al.,* 2007). Availability of a cost-effective diet can make a difference between a profitable and unprofitable operation and hence determine the economic viability of a fish farming operation. Therefore, success of aquaculture among rural communities will obviously largely depend on availability of cost-effective feeds compounded from inexpensive locally available ingredients. This approach was recently further emphasised by Kaliba *et al.,* (2006) in an economic analysis of Nile tilapia production in Tanzania.

Most nutrition studies on alternative ingredients have focused on biological performance with less attention given to economic performance. However, Meade (1989) points out that economic evaluation of new inputs in aquaculture has a vital influence on economic viability and hence long term sustainability. Moreover, such evaluation is important in decision making as some novel ingredients may display poor biological performance but prove to be cost effective nonetheless (El Sayed and Tacon, 1997).

The objective of this study was therefore to evaluate the biological and economic performance of Nile tilapia fed practical diets made from moringa and cassava.

7.2 Methodology

7.2.1 Biochemical Analysis

Proximate analysis, gross energy, amino acid profiles, antinutritional factors and mineral contents of ingredients are shown in Table 2.4 and Table 6.1.

7.2.2 Diet Formulation and Experimental Setup

A total of 540 fingerlings with an average weight of 4.91 ± 0.37g were equally distributed between eighteen 30L circular self cleaning tanks in a recirculation system described in section 2.1 following procedures described in section 2.2. A total of six diets shown in Table 7.1 were formulated to contain 30g $100g^{-1}$ crude protein, 18kJ g^{-1} and 10g $100g^{-1}$ lipid as described in section 2.5. The practical LMB diets were formulated to contain leaf meal blends as the main source of protein providing between 30g $100g^{-1}$ (30LMB2 and 30LMB3) and 50g $100g^{-1}$ (50LMB2 and 50LMB3). The two leaf meal blends were prepared as described in section 6.2.1. The choice of the two blends was based on their performance in chapter 6. Hydrolysed fish protein (5g $100g^{-1}$) and betaine (1g $100g^{-1}$) were added as feeding stimulants to diets LMB and soybean meal (SBM) because they contained little or no fish meal. This was based on the

presumption that high levels of fishmeal in diets used in chapter 6 could have masked their feeding stimulating effect. The practical diets were compared with a fishmeal-based diet (FM) containing 10g $100g^{-1}$ of fish meal and a soybean meal-based diet (SBM) containing 42g $100g^{-1}$ of soybean meal. Diet FM simulated current commercial tilapia diets while diet SBM simulated the oilseed cake based diets intended for use in semi–intensive tilapia culture. All diets were formulated to reflect practical *O. niloticus* diets with minimum inclusion levels of fishmeal and hydrolysed fish protein which did not exceed 10g $100g^{-1}$. Diets were randomly assigned to culture tanks in triplicate and fish were hand fed 5% of their body weight per day, to reflect practical feeding practices in Tanzania, and were fed in three portions following the procedure described in 2.6.

Table 7.1: Composition of practical diets fed to *O. niloticus* (g $100g^{-1}$)

Ingredients	FM	30LMB2	50LMB2	30LMB3	50LMB3	SBM
LMB2[1]	-	32.0	47.0	-	-	-
LMB3[2]	-	-	-	32.0	47.0	-
Fishmeal[3]	10.0	5.0	5.0	5.0	5.0	-
Soybean meal[4]	42.0	24.0	15.0	24.0	16.0	50.0
Wheat meal[5]	15.5	8.5	6.0	8.5	5.5	13.0
CRM	14.5	9.0	6.0	9.0	5.5	14.0
α-cellulose	1.0	0.0	0.0	0.0	0.0	1.0
Sunflower oil	8.5	7.0	6.5	7.0	6.5	8.5
Vitamin premix[6]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[7]	4.0	4.0	4.0	4.0	4.0	4.0
CMC[8]	2.0	2.0	2.0	2.0	2.0	2.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Fish protein hydrolysate[9]	-	5.0	5.0	5.0	5.0	5.0
Betaine[10]	-	1.0	1.0	1.0	1.0	-

[1]1MLM:1CLM, [2]1MLM:2CLM, [3]Brown fishmeal (aquaculture grade), [4]dehulled, solvent extracted [5]Whole grain, [6]As listed in Table 2.2, [7]As listed in Table 2.3, [8]Carboxymethylcellulose (Sigma C5013), [9]CPSP G, Sopropêche, France, [10]betaine hydrochloride (Sigma, B3501)

At the end of 8 weeks of growth, final body weights were recorded and 3 fish from each tank were sacrificed to determine whole body proximate composition as described in section 7.2.3. The rest of the fish were pooled according to dietary treatment for faecal collection as described in section 2.4 to determine apparent digestibility coefficients of the diets.

7.2.3 Growth Performance Parameters

Feed intake, growth and feed utilization were measured according to procedures described in section 2.7. Apparent digestibility coefficients and whole body composition were determined according to procedures outlined in sections 2.4 and 2.8 respectively.

7.2.4 Cost Analysis

Simple cost analysis was performed to determine cost effectiveness of diets. It was assumed that all other operating costs remained constant and only the variable cost of ingredients was used in calculations. The cost of the diets was calculated using prevailing prices for the feed ingredients in Tanzania as follows; fishmeal, 1,600 Tshs kg^{-1}; soybean, 600 Tshs kg^{-1}, hydrolysed fishmeal, 3,200Tshs kg^{-1}; Betaine, 41,280Tshs kg^{-1}, wheat meal, 66 Tshs kg^{-1} and sunflower oil, 1,000 Tshs kg^{-1} (1,200 Tshs = 1USD). Moringa and cassava products were assumed to be on–farm produce and hence available at zero real cost. Harvest weight was assumed to be 150g which is a common table size for Nile tilapia (Nilsson

and Wetengere, 1994). The following key economic indicators were computed according to Vincke (1976) and Miller (1976) as follows;

$$\text{Incidence cost} = \frac{\text{cost of feed}}{\text{quantity of fish produced}}$$

$$\text{Profit index} = \frac{\text{value of fish}}{\text{cost of feed}}$$

7.3 Data Analysis

Data analysis was carried out as described in section 2.10.

7.4 Results

7.4.1 Proximate Composition and Digestibility of Practical Diets

Proximate composition and digestibility of the practical diets used in the trial are shown in Table 7.2. Most nutrient contents were similar amongst diets except for crude fibre which was higher in diets containing LMB. Apparent digestibility coefficients for diets FM and SBM were high, above 80%, reflecting the high digestibility of the ingredients used. Digestibility coefficients for LMB diets were affected by both the inclusion level as well as composition of the blend. There was a reduction in digestibility coefficients as blend inclusion levels increased from 30% to 50%. The diets containing LMB3 had lower digestibility than those containing LMB2. Digestible protein and energy values were more or less the same between the different diets with the exception of diet 50LMB3 whose values were lowest.

Table 7.2: Proximate composition and apparent digestibility coefficients of LMB practical diets

	FM	30LMB2	50LMB2	30LMB3	50LMB3	SBM
Proximate composition (g 100g^{-1}, as fed)						
Dry matter	94.41	94.51	94.76	95.25	93.09	93.70
Crude protein	31.53	32.23	32.61	32.41	31.62	31.53
Crude lipid	10.66	11.07	11.22	10.86	10.35	10.21
Crude fibre	2.60	4.29	4.79	4.58	4.97	3.37
Ash	8.02	8.04	8.31	8.10	8.18	7.20
Nitrogen free extract	41.60	38.88	37.83	39.30	37.97	41.39
Gross energy (kJ g^{-1})	18.81	20.20	20.73	20.25	20.53	19.19
Apparent digestibility coefficient (%)						
Dry matter	81.7	78.8	78.0	75.0	65.1	82.3
Crude protein	92.5	87.2	86.9	85.2	78.7	90.6
Crude lipid	98.1	95.4	95.3	95.4	90.5	98.9
Gross energy	84.5	81.1	80.5	77.2	68.1	84.8
Digestible protein (DP) (g 100g^{-1})	29.2	29.8	30.2	30.0	29.2	29.2
Digestible energy (DE) (kJ g^{-1})	15.9	16.4	16.7	15.6	14.0	16.3
DP/DE ratio (mg kJ^{-1})	18.4	18.2	18.1	19.2	20.9	17.9

7.4.2 Feed Intake, Growth and Feed Utilization

Differences in growth rate became clear after 1 week with fish fed diet FM exhibiting fastest growth followed by diets SBM, LMB30 and diet LMB50 which produced least growth (Figure 7.1). Other performance parameters are shown in Table 7.3. Feed intake decreased with increased inclusion of leaf meal blends in diets. Least feed intake was observed for fish fed 50LMB2 while fish fed diet FM had significantly higher feed intake. Digestible protein and digestible energy intake followed a similar trend. Feed intake was reflected in average daily gain (ADG). Fish fed diet FM had significantly higher ADG than those fed either LMB diets or diet SBM which did not differ significantly from each other. There was a significant difference in specific growth rate (SGR) which was higher in fish fed diets FM and SBM and lower in fish fed LMB diets. There was mortality in all treatments which appeared to be linked to aggression rather than dietary treatment.

Feed conversion ratio (FCR) was high for diets LMB and SBM. Diet 50LMB2 had significantly high FCR while diet FM had significantly low FCR. Protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and energy retention (ER) for diet FM was significantly higher than that of the plant based diets. Though not significantly different, the values of PER, ANPU and AER for diet SBM were higher than those for LMB diets in which they declined with increasing inclusion of leaf meal blends.

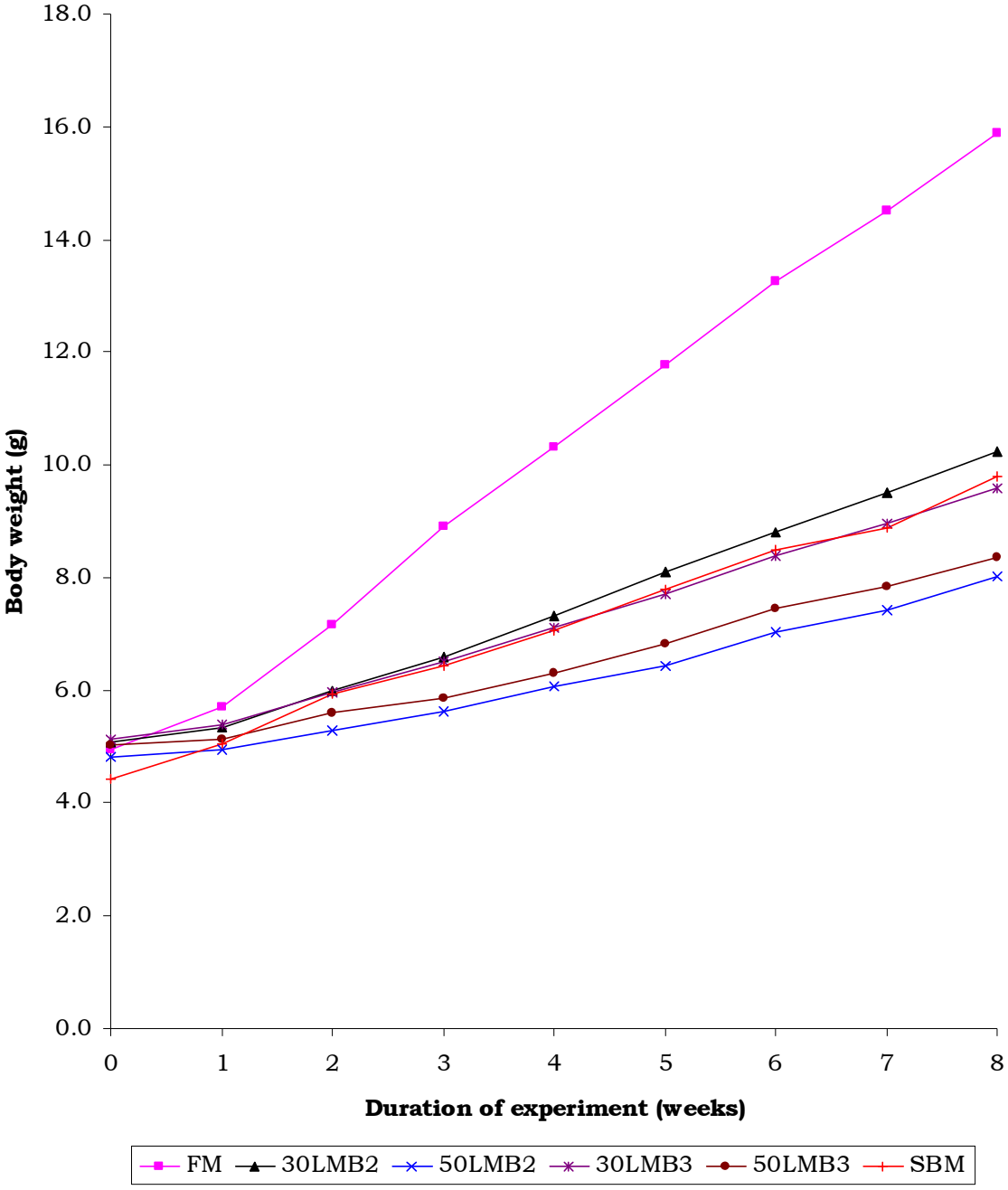


Figure 7.1: Change in body weight of *O. niloticus* fed practical diets containing leaf meal blends (LMB) and cassava root meal (CRM)

Hepatosomatic index (HSI) also differed significantly with fish fed diet FM displaying significantly higher values while lowest values were observed for fish fed diets 50LMB2 and 50LMB3. Price of LMB diets was higher than that of diets FM and SBM. Diet FM demonstrated low incidence cost and high profit index compared to diets LMB and SBM.

7.4.3 Whole Body Composition

Initial and final whole body proximate composition is shown in Table 7.4. Final moisture content was significantly higher in fish fed diet LMB than in fish fed diets FM and SBM. There was no significant difference in crude protein content of fish fed diets FM and SBM. Fish fed diets 50LMB2 and 50LMB3 had lowest protein content but were not significantly different from fish fed diets 30LMB2 and 30LMB3. Final lipid content of fish fed diets 30LMB2, 50LMB2, 30LMB3 and 50LMB3 was significantly lower than that of fish fed diet FM which in turn was not significantly different from that of fish fed diet SBM. The final ash content of fish fed diets FM and SBM was significantly lower than that of fish fed LMB diets.

Table 7.3: Growth performance, nutrient utilisation and cost effectiveness of practical diets containing leaf meal blends (LMB) fed to *O. niloticus* (mean ± SD, n=3)

	FM	30LMB2	50LMB2	30LMB3	50LMB3	SBM
Initial weight (g)	4.95 ± 0.25^{a}	5.08 ± 0.29^{a}	4.81 ± 0.40^{a}	5.12 ± 0.38^{a}	5.03 ± 0.41^{a}	4.44 ± 0.25^{a}
Final weight (g)	15.90 ± 0.85^{a}	10.03 ± 1.07^{b}	7.93 ± 1.58^{b}	9.63 ± 0.46^{b}	8.35 ± 1.26^{b}	9.87 ± 1.46^{b}
Average feed intake (g fish^{-1} day^{-1})	0.39 ± 0.02^{a}	0.34 ± 0.01^{b}	0.30 ± 0.02^{c}	0.33 ± 0.01^{b}	0.30 ± 0.01^{bc}	0.35 ± 0.03^{b}
DP intake (g fish^{-1} day^{-1})	0.114 ± 0.004^{a}	0.102 ± 0.004^{bc}	0.090 ± 0.007^{cd}	0.100 ± 0.001^{bc}	0.088 ± 0.003^{d}	0.100 ± 0.007^{b}
DE intake (kJ fish^{-1} day^{-1})	6.22 ± 0.24^{a}	5.61 ± 0.22^{ab}	4.96 ± 0.35^{b}	5.18 ± 0.05^{b}	4.22 ± 0.13^{c}	5.61 ± 0.41^{ab}
Average daily gain (g fish^{-1} day^{-1})	0.197 ± 0.023^{a}	0.090 ± 0.026^{b}	0.057 ± 0.023^{b}	0.083 ± 0.005^{b}	0.057 ± 0.021^{b}	0.100 ± 0.026^{b}
Specific growth rate (% day^{-1})	2.08 ± 0.17^{a}	1.21 ± 0.27^{b}	0.87 ± 0.25^{b}	1.13 ± 0.09^{b}	0.90 ± 0.26^{b}	1.41 ± 0.29^{b}
Feed conversion ratio	2.01 ± 0.18^{a}	4.08 ± 1.22^{ab}	6.01 ± 2.59^{b}	4.13 ± 0.26^{ab}	5.52 ± 1.80^{ab}	3.71 ± 0.86a^{ab}
Protein efficiency ratio	1.59 ± 0.14a	0.80 ± 0.22b	0.57 ± 0.19b	0.75 ± 0.05b	0.62 ± 0.22b	0.89 ± 0.24b
Apparent net protein utilization (%)	27.75 ± 1.22^{a}	14.57 ± 3.28^{b}	11.02 ± 2.83^{b}	13.45 ± 0.63^{b}	11.90 ± 2.57^{b}	16.74 ± 3.53^{b}
Energy retention (%)	15.16 ± 1.70^{a}	5.74 ± 2.56^{b}	2.82 ± 2.01^{b}	5.43 ± 0.55^{b}	3.37 ± 1.20^{b}	8.13 ± 2.55^{b}
Hepatosomatic index	3.43 ± 0.99^{a}	2.13 ± 0.62^{bc}	1.81 ± 0.75^{c}	2.15 ± 0.47^{bc}	1.84 ± 0.41^{c}	2.49 ± 0.86^{b}
Survival (%).	85.00 ± 08.66	76.67 ± 22.55	95.00 ± 08.66	88.33 ± 12.58	96.66 ± 05.77	60.00 ± 15.00
Feed price[1]	507	870	809	870	815	554
Incidence cost[1]	0.79	1.52	1.73	1.82	1.66	1.63
Profit index	1.91	0.98	0.87	0.82	0.90	0.92

Different superscripts in the same row indicate significant difference ($p<0.05$)

[1]Tshs kg^{-1}, Tshs 1,200= USD 1

Table 7.4: Whole body proximate composition and hepatosomatic index (HSI) of *O. niloticus* fed practical diets containing leaf meal blends (LMB) (% fresh weight basis, mean ± SD, n=3)

	Initial	FM	30LMB2	50LMB2	30LMB3	50LMB3	SBM
Moisture content	80.36	73.04 ± 0.78^{b}	77.61 ± 1.40^{a}	79.13 ± 0.54^{a}	78.19 ± 0.64^{a}	78.39 ± 1.11^{a}	74.61 ± 1.16^{b}
Crude protein	10.29	15.26 ± 0.42^{a}	14.15 ± 0.66^{bc}	13.86 ± 0.15^{c}	13.87 ± 0.41^{bc}	13.86 ± 0.31^{c}	14.95 ± 0.19^{ab}
Crude lipid	6.42	7.81 ± 0.92^{a}	3.97 ± 1.37^{bc}	2.16 ± 0.82^{c}	3.75 ± 0.19^{c}	2.74 ± 0.85^{c}	6.48 ± 1.29^{ab}
Ash	3.48	2.69 ± 0.14^{b}	3.62 ± 0.48^{b}	4.21 ± 0.44^{b}	3.47 ± 0.12^{ab}	4.17 ± 0.42^{b}	2.60 ± 0.13^{b}
Gross energy (kJ g^{-1})	5.39	5.60 ± 0.14^{a}	4.91 ± 0.32b^{c}	4.49 ± 0.30^{c}	4.98 ± 0.06^{bc}	4.64 ± 0.19^{c}	5.47 ± 0.15^{ab}

Different superscripts in the same row indicate significant difference ($p<0.05$)

7.5 Discussion

This study compared biological and economic performance of LMB diets with fishmeal-based and soybean meal-based diets which are options available to small scale fish farmers depending on their financial capability and accessibility factors. LMB diets had highest inclusion levels of plant proteins where between 56–64% of the total diet was composed of blends and soybean meal. This, however, led to poor performance observed in fish fed LMB diets in terms poor feed intake, growth and nutrient utilisation. It is well established that high inclusion levels of plant proteins leads to poor growth (El-Sayed, 1999). Findings in chapters 3 and 4 showed both moringa and cassava leaves used to compound the blends contain saponins and tannins which are known to lower palatability (Francis *et al.,* 2001). Inclusion of feeding stimulants did not have any effect on improving feed intake. These findings do not support the earlier suggestion that the high levels of fish meal in the LMB diets used in chapter 6 could have inhibited the feeding stimulating effect of hydrolysed fish protein and betaine. Thus more effective processing to remove inherent antinutritional factors from moringa and cassava leaf meals would appear more appropriate rather than using feeding stimulants to mask their negative taste characteristics. Inclusion of LMB also led to poor nutrient utilisation for a number of reasons such as antinutritional factors and poor digestibility which have been discussed in detail in chapters 3 and 4.

Soybean meal is a highly regarded plant protein source due to its high crude protein content and reasonably well balanced amino acid profile

(Kim *et al.,* 2007). However, high inclusion levels of soybean meal in fish diets are likely to be limiting in lysine, methionine and threonine which may result in poor performance as observed for diet SBM which contained 50% soybean meal. El–Saidy and Gaber (2002) reported that soybean meal could serve as a sole source of protein for *O. niloticus* without impairing growth when supplemented with methionine and lysine. Moreover, soybean meal contains several inherent antinutritional factors such as protease inhibitors, lectins, phytic acid and saponin which interfere with feed utilization (Krogdahl *et al.,* 1994). This however, may not be the case in the current study, protein digestibility of SBM diet was high (91%) suggesting minimal impacts of any trypsin inhibition.

There is also a possibility that the combination of soybean meal and cassava root meal may have contributed to poor performance of fish fed diets LMB and SBM. Both feedstuffs contain appreciable quantities of indigestible carbohydrates which include raffinose, stachyose and non–starch polysaccharides in soybean meal (Gatlin *et al.,* 2007) and amylopectin in cassava root meal (Khajarern *et al.,* 1977; Oke, 1978) which provide only marginal amounts of energy due to limited microbial fermentation.

Inclusion of leaf meal blends also affected whole body proximate composition. Fish fed LMB diets had higher moisture and ash contents while protein and lipid contents were lower. Hepatosomatic index was also lower in fish fed LMB diets which corroborated the observed lower lipid content. Whole body lipid is determined by feed supply, dietary energy

input and metabolic energy demands of fish (Shearer, 1994). Although the diets were isoenergetic, it is possible that fish could not efficiently utilise the dietary energy in LMB diets resulting in lower lipid deposition. Catabolism of stored lipid probably took place as final lipid content was lower than initial lipid content. Lower lipid content is not always desirable as it may lower the organoleptic quality of fish flesh (Grigorakis, 2007) .

Inclusion of feeding stimulants, betaine in particular, is responsible for the high feed price of the LMB diet compared to other diets. Betaine accounted for 50% of LMB diets prices. Hence, the anticipated economic advantages of using high inclusion of locally available/on farm produce which usually do not involve any tangible cost were not realised. Combination of high feed price and high FCR resulted in high incidence cost and low profit index. This was similar to the poor economic efficiency index reported by Olvera-Novoa (2002) when *Tilapia rendalli* were fed diets containing high inclusion levels of sunflower seed meal. The poor biological performance of LMB diets would require longer culture duration to attain a desirable market weight. This is likely to have an implication for production cost which could result in reducing the economic benefits usually anticipated when locally available feedstuffs are utilised (Omondi *et al.,* 2001). However, resource–poor farmers are more concerned with lowering feed cost even if that would lengthen the rearing period (Middendorp and Verreth, 1991). Improving the removal of antinutritional factors and avoiding feeding stimulants in LMB diets would significantly reduce cost and improve profitability. This will make the LMB diets potentially suitable

for resource poor fish farmers who characteristically have a strong desire to minimise risks rather than optimise returns.

The performance of LMB diets can further be enhanced with pond fertilisation to stimulate production of natural food. According to Albrecht and Breitsprecher (1969), as cited by De Silva and Davy (1992), natural food contains an average of 52.2% protein, 27.3% carbohydrate and 7.7% lipid on a dry weight basis. Furthermore, Shroeder (1980) reported that natural food accounted for 50–70% of total available food for tilapia in pond culture even when a complete diet was provided. Hence, it has been suggested that a combination of feeding and fertilisation is the most efficient way of growing Nile tilapia compared to complete feeding or fertilisation alone (Diana *et al.,* 1994).

Biological and economic performance of practical diets formulated from moringa and cassava products was poor compared to the fishmeal based diet but more or less similar to the soybean meal-based diet. Inclusion of feeding stimulants did not realise the anticipated improvement in feed intake but rather negatively affected the cost effectiveness. Hence, feeding stimulants should be avoided and instead more efforts should be directed towards removal of antinutritional factors. Further studies are recommended on long-term evaluation of the practical diets in fertilised ponds under farmer managed conditions.

CHAPTER 8 Conclusions and Recommendations

Aquaculture in Tanzania has huge potential for improving farmer livelihoods through provision of high quality protein and income. This potential has, however, not yet been realised due to the lack of affordable fish feeds amongst other constraints. Most aquaculture in Tanzania is carried out in rural areas by resource poor peasants in small ponds stocked with Nile tilapia. The ponds are usually fertilised with compost and manure using animal droppings or tender leaves. Occasionally, fish are also fed kitchen leftovers, garden remains and bran (maize or rice). This is largely because formulated feeds containing conventional ingredients, such as fish meal, oilseed meals and cereals are not affordable. This has resulted in poor fish yields with aquaculture remaining underdeveloped and consequently failing to meet the challenge of providing much needed protein and income in rural areas. Therefore, this study evaluated suitability of processed moringa leaf meal (MLM), cassava leaf meal (CLM) and cassava root meal (CRM) as novel ingredients in Nile tilapia diets. The choice of these ingredients was based on their relatively high nutritional content, local availability, abundance, favourable agronomic characteristics such as minimum input requirements, drought resistance and hence potentially cost-effective solutions.

The ingredients are, however, known to contain several antinutritional factors which tend to interfere with palatability and nutrient utilisation.

Therefore, each of the ingredients was processed with the intention of removing the most significant antinutritional factor using methods deemed feasible for small-scale fish farmers. The following conclusions were drawn from the study:

1. Both moringa and cassava leaf meals had relatively high protein content (31 and 29g $100g^{-1}$ respectively). Their amino acid profles were fairly good with moringa leaf meal having higher amino acid content quantitatively than cassava leaf meal. Qualitatively, cassava leaf meal had higher content of sulphur amino acids (0.47 %CP) than moringa leaf meal (0.23 %CP). Cassava leaf meal had higher crude fibre content (10g $100g^{-1}$) than moringa leaf meal (6g $100g^{-1}$). Both leaf meals had a similar gross energy content of 20kJ g^{-1}. The leaf meals also contained appreciable amounts of minerals which could be beneficial for fish farmers unable to afford mineral supplements.
2. Grinding followed by sun drying of cassava leaves was more effective in reducing hydrogen cyanide content by 60% compared to overnight aqueous extraction of moringa leaves which removed only 0.3% of saponin. The contents of other antinutritional factors namely phenols, tannins and phytic acid remained largely unaffected.
3. Moringa leaf meal was more digestible than cassava leaf meal. Protein and energy digestibility of moringa leaf meal was 89% and

79% respectively while that of cassava leaf meal was 45% and 44% respectively. Consequently, digestible protein and digestible energy of moringa leaf meal was double (26g $100g^{-1}$/16kJ g^{-1}) that of cassava leaf meal (13g $100g^{-1}$/8kJ g^{-1}).

4. Inclusion of either of the leaf meals even at the lowest level of 15g $100g^{-1}$ of total dietary protein led to a significant reduction in feed intake, growth and feed utilisation. Poor fed intake due to inherent antinutritional factors was the main reason for poor performance in fish fed diets containing moringa leaf meal. On the other hand, poor digestibility and consequently poor intake of digestible energy and digestible protein was the main factor for poor performance of fish fed diets containing cassava leaf meal. Neither leaf meal caused any histopathological changes in liver or small intestine which suggested that the leaf meals were unlikely to have any long-term effect on fish.

5. Blending the two leaf meals generally improved biochemical composition due to additivity and synergism with few exceptions. Feeding stimulants led to marginal improvement in feed intake of a diet containing 20g $100g^{-1}$ of protein from LMB1 i.e. a blend containing a high proportion of poorly palatable moringa leaf meal. However, combination of blending and feeding stimulants allowed provision of up to 20g $100g^{-1}$ of protein from LMB3 i.e. a blend with

a high proportion of cassava leaf meal without significantly affecting growth and nutrient utilisation.

6. Cassava root meal was rich in starch as indicated by the high content of nitrogen free extract (84g $100g^{-1}$) and gross energy content of $16kJg^{-1}$.The crude fibre content was low (2.4g $100g^{-1}$) as was crude protein (1.5g $100g^{-1}$) and crude lipid (0.5g $100g^{-1}$) content. Grating followed by sun-drying of cassava roots was highly efficient with 90% of the hydrogen cyanide content removed. Cassava root meal was highly digestible with energy digestibility of 84% and digestible energy of $13kJ\ g^{-1}$. Growth trials showed that cassava root meal could replace up to 75% of wheat meal without significantly affecting fish performance.

7. Practical diets based on moringa and cassava products performed poorly both biologically and economically. The performance was, however, comparable to the soybean based diet. Feeding stimulants did not have any beneficial effect but rather led to an increase in feed production costs where betaine alone accounted for 50% of the feed price. In order to realise better biological and economical performance of diets based on moringa and cassava products, removal of antinutritional factors is more appropriate than masking using feeding stimulants.

Further studies are recommended on:

1. Conducting long-term growth trials using the LMB practical diets in fertilised ponds under farmer managed conditions to ascertain their potential in increasing current yield levels among small scale fish farmers
2. Exploring other simple and yet efficient methods of improving the nutritional profile of ingredients such as removal of antinutritional factors as well as reducing fibre content using methods like ensiling to improve digestibility
3. Identifying and evaluating other locally available materials from both plant and animal sources which have a potential to serve as novel ingredients in formulating cost effective diets

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