**Universität für Bodenkultur**
University of Natural Resources and Applied Life Sciences, Vienna

**Department for Water – Atmosphere – Environment**
Institute of Hydrobiology and Aquatic Ecosystem Management

# Utilisation of locally available feedstuffs for Nile Tilapia (*Oreochromis niloticus* L.) production in small-scale cage culture in Kenya

A Thesis submitted to the University of Natural Resources and Applied Life Sciences Vienna, Austria, for the award of Doctor rerum naturalium technicarum (Doctor of Natural and Technical Sciences)

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***We have not inherited the world from our forefathers - we have borrowed it from our children.***

***Kashmiri proverb***

## Dedication

To my late mother Mary Mwelu Munguti; "Mwaitu" I'm grateful for your love, care and encouragement, I will forever cherish.

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# I. General introduction

## I.1. Thesis outline

This thesis is divided into six chapters; the first chapter is a general introduction, a description of the aquaculture status in sub-Saharan Africa and Kenya in particular, the history and the use of cage culture in tilapia farming and a brief review of tilapia feeds, the objectives and a description of the study area. The next three chapters are presented in form of publication papers, the first paper has been submitted for publication in "Die Bodenkultur", the *Austrian Journal of Agricultural Research* and has been accepted. The second paper is submitted to journal of *Aquaculture Nutrition* and is currently with the reviewers; the third paper is submitted to the journal of *Renewable Agriculture and Food Systems* and is also with reviewers. In these papers, I have presented the investigations carried out, the results and discussions on the various key aspects of nutritive value; feed formulation and Nile tilapia growth performance and diet digestibility through which my research questions were approached. These aspects include the study of the nutritive value of locally available agricultural by-products and other under-utilized protein sources, feed formulation and growth performance and apparent protein digestibility of the formulated diets.

The first paper is entitled "Proximate composition of selected potential feedstuffs for Nile tilapia (*Oreochromis niloticus* Linnaeus) production in Kenya". The second paper is "Effects of substitution of freshwater shrimp meal (*Caridina nilotica* Roux) with hydrolysed feather meal on growth and apparent digestibility in *Oreochromis niloticus* (L)" and the third paper "Use of different levels of hydrolysed feather meal and Pawpaw (*Papaya carica*) leaves – a contribution to increasing sustainability to Nile tilapia (*Oreochromis niloticus* L.) production".

In chapter five I present a general discussion for the study, a summary encompassing all the aspects discussed in the three papers, conclusions and recommendations for future studies. Chapter six is a summary of the study which is presented both in English and German; also I present my summarized Curriculum Vitae. In chapter seven I present the combined references list for chapter one and five, i.e. the general introduction and general discussion, respectively. The last chapter covers the appendices in which I have presented abbreviations/acronyms used in the thesis and also some selected pictures of the field study.

## I.2. Food situation in Africa and necessity of aquaculture

Chronic hunger is widespread in Africa where between two and four hundred million people in sub-Saharan Africa are affected by malnutrition (World Bank, 2006). According to the World Bank Report on reducing poverty and hunger, over 23 million African children are malnourished (World Bank, 2006). In Kenya as of 2005 approximately 42 percent of her population is living below poverty line (UNDP, 2005) and over 70 percent of the population (i.e. about 24 million people) live in the rural areas (UNDP, 2005). Fish are an important source of protein and income to many people in developing countries. In Africa, some 35 million people depend wholly or partially on the fishery resources for their livelihood (WorldFish, 2005). Fish yields from capture-based fisheries seem to have reached their natural limits (FAO, 1996). However, there is considerable potential for aquaculture expansion in Africa to improve food security (Kapetsky, 1994, 1995; Engle, 1997).

Most countries in sub-Saharan Africa face uneven water distribution, while others generally have water shortage; consequently land riparian to permanent water reservoirs where fish farming can be practiced throughout the year is limited. However, there are many under-utilized man-made water reservoirs, which retain water throughout or for most time of the year, which can be targeted for fish farming and help to supply the much needed fish protein to the poor rural communities. Cage culture is one of the most promising approaches in the utilization of these small and medium water reservoirs, which would rather not be conducive for fish farming.

In many Asian countries, tilapia, catfish and carp culture has been integrated with other agricultural systems – which include among others, livestock-fish, poultry-fish, and rice-fish culture in order to overcome the problem of land scarcity and competition from other agricultural activities (Pillay, 1992; Edwards, 1991). Lin & Diana (1995) and Yi *et al.* (1996) developed a system of fish-fish integration in Thailand and other Asian countries. In this system, two approaches are adopted. The first approach involves integration of Nile

tilapia (*Oreochromis niloticus*) with Catfish (*Clarias gariepinus*) (Lin, 1990; Lin *et al.*, 1993). The Catfish are stocked into cages and fed a high protein diet while the Nile tilapia are stocked in the open-pond and receive their nutrition directly from "cage waste" or indirectly from the phytoplankton stimulated by the "cage waste".

Aquaculture in Eastern Africa dates back to the early 1920s, these early attempts utilized earthen ponds (Vernon & Someren, 1960). However, the rate of development is still low and practiced mainly at subsistence level. Between 1960 and the 1970s, there was a fast development in aquaculture in eastern Africa. However, most of these fish ponds have been abandoned because of political unrest, drought, limited security of land tenures, labour shortage and reluctance by farmers to adopt new technologies (Coche *et al.*, 1994; Harrison, 1994). Other factors include an over-reliance on capture fisheries, limited availability of permanent water reservoirs, lack of quality seeds for the main culture species and unavailability of high quality, inexpensive fish feeds.

Semi-intensive culture of *O. niloticus* is the most common practice of fish farming in Kenya (Liti *et al.*, 2005a). According to FAO (2002), world annual tilapia production was about 1.461,239 metric tons, with a major focus in Far East and China. Bitterlich & Graiger (1984) and Getachew (1987) reported that *O. niloticus* is the preferred culture species because it relies mainly on planktons and the associated detritus for nutrition. However, lack of specific diets for semi-intensive production of *O. niloticus* in Kenya has necessitated the use of expensive diets designed for intensive production, which increases the operational costs (Liti *et al.*, 2005a). Expensive diets coupled with the low (2 fish $m^{-2}$) stocking densities limit the extent of acceptability by entrepreneurs to adopt commercial tilapia farming. Therefore, research in tilapia farming should be geared among others, towards formulating high quality diets based on locally available feedstuffs and increasing the stocking densities in semi-intensive systems.

## I.3. Fish cage culture

Cage culture of fish and other aquatic organisms was first reported in the Yangtze River delta in China dating back to the 1800s (Coche, 1976; Hu, 1994). In the late 19th and early 20th centuries it spread to Cambodia and Indonesia (Hickling, 1962; Ling, 1977). In Europe and North America, cage culture was introduced in the 1960's as a result of the fast growth of trout and catfish farming industries. Tilapia cage culture is relatively new with a short history dating back to the 1950's; the first trials were conducted in the United States of America with *Oreochromis aureus* (Pagan, 1969). In China, cage fish culture has been successfully integrated with other forms of aquatic food production (Li & Matthias, 1994). In Africa, cage culture of tilapia was first carried out in the Ivory Coast in 1974 in

Lake Kossou with *Tilapia nilotica* (Coche, 1974) and in Lake Victoria, Tanzania with *Tilapia zilli* (Ibrahim *et al.*, 1974) from where it spread to several other regions in Africa (Coche, 1974; 1982). However, these early attempts were not sustainable and most of them have since collapsed.

Nevertheless, more recent developments in Kariba dam situated at the border of Zambia and Zimbabwe reported success in small-scale cage culture of tilapia and carps (Gabriel, 1991; Fölster, 1994). In Kenya, a recent pilot study on small-scale cage culture in tilapia, which focused on utilizing small water reservoirs situated in semi-arid areas was a great success and an extension of the project is ongoing. The study was conducted in water reservoirs in the Machakos region by Sagana fish farm of the Department of Fisheries in conjunction with Kenya Marine and Fisheries Research Institute (KMFRI), Moi and BOKU Universities.

### I.3.1. Benefits and challenges in tilapia cage culture

Cage culture of tilapia has attracted interest due to its distinct advantages. Masser (1991) noted that many types of water resources can be utilized including lakes, reservoirs, ponds and rivers which otherwise were not suitable for fish farming due to difficulties in harvesting. McGinty & Rakocy (1989) reported that, if existing water bodies are used, relatively low initial investment is required. It has also been shown that the system enables one to have a better control over fish predators. Since the fish are confined in a small area, feeding can be monitored closely and this facilitates efficient use of feeds. Cages support high stocking densities and require less manpower, which reduces production costs. Additionally, a build-up of waste metabolites does not occur inside the cage, as there is a continuous water exchange (FAO, 1978). The use of floating cages in tilapia farming facilitates the culture of mixed sex without a reduced growth rate of the females since the mouthbrooding of the females is greatly reduced (Liti *et al.*, 2005b), because the eggs of the ripe fish which spawn pass through the cage netting and are lost out of the cage and therefore less fertilization occurs (FAO, 1978). Besides impeding reproduction, cages also limit the space available for each fish to move; this may reduce the amount of energy required for muscle activity, which may be utilized for growth.

McGinty & Rakocy (1989) reported that cage culture of tilapia is encountered with a number of challenges, among them loss from poaching and damage of cages from predators and/or storms; caged fish have a greater risk of disease outbreaks, because they have less tolerance to poor water quality as compared to those in open ponds; high mortality may occur especially during stocking due to handling stress. They also noted that it is expensive to grow fish in cages because they rely entirely on complete diets and

these systems demand a certain level of expertise. Other challenges facing cage farming of tilapias in tropical and sub-tropical Africa include the unavailability of high quality and locally available fish feeds and concerns regarding the use of cages in areas considered as public domain. In public waters, cage culture faces many competing interests and its legal status is not well defined in most parts of the world (McGinty & Rakocy, 1989). This aspect creates some uncertainty concerning the future of the culture system, which may discourage potential investors. Beveridge (1984) observed that due to the rich protein diets fed to caged fish, the surrounding water might have a high nutrient load. If such waters are discharged in natural eco-systems, this will accelerate the problem of water quality deterioration due to eutrophication. The choice of cage mesh size to be used in any water reservoir for optimum production is a major challenge. McGinty & Rakocy (1989) reported that the mesh size has a significant impact on production. Larger cage mesh size facilitates good water circulation through the cage to renew the Oxygen supply. However, the authors observed that the cultured fish could escape from the cage while wild fish can find their way into the cage. The fish, which escape cannot be easily accessed in the open waters, while the wild fish grows too large to swim out of the cage, but they don't grow large enough to reach market size, thereby representing a waste.

In an effort to solve problems associated with cage culture of tilapias, Lin (1990) tested the concept of integrated fish farming with polyculture of catfish-tilapia as well as tilapia-tilapia farming systems using cages in order to reduce the amount of waste discharged into the ecosystem. This system has been adopted and is widely practiced in south East Asia. Cage culture of tilapia has also been practiced in lakes and rivers where waste from cages is used as food to culture free swimming filter feeders in the cage neighbourhoods (Lin *et al.,* 1993; Yi & Lin, 2001). McGinty (1991) and Yi *et al.* (1996) demonstrated that growing of larger fish in cages, while nursing fingerlings in the open pond can help small-scale farmers with only a single pond to maximize fish production and profitability.

Yi & Lin (2001) realized 500g mean weight tilapia within 90 days, at 50 fish $m^{-3}$, in $4m^3$ cages. Stocking fingerlings at higher densities in cages and splitting of the stock at a later date can also be used to maximize production of smaller ponds. However, Yi *et al.* (1996) observed that increasing the density of the larger tilapia in cages increases waste generation for open pond tilapia but reduced fish production, partly due to exceeding the carrying capacity of cages.

Liti *et al.* (2005b) evaluated the option of increasing pond production capacity through small cage-cum-pond integration with *O. niloticus* in fertilized semi-intensive ponds. Feed was only offered to caged fish, while fish in the open-pond relied on natural pond food and "cage waste". The study revealed that cage-cum-pond fish integration increased

overall pond yields of *O. niloticus*. It was further observed that this system improves feed utilization, reducing feed input into semi-intensive culture ponds by as much as 47 percent as compared to systems where all the fish are fed. It was therefore concluded that small cage-cum-pond integration of *O. niloticus* is a viable system for production of *O. niloticus* by poor small-scale farmers in rural Kenya. Waidbacher *et al.* (2006) evaluated the effect of pond fertilization and feeding rate on growth, economic returns and water quality to develop a low-cost cage-cum-pond integrated system for production of *O. niloticus*. The authors reported that providing feed at 6 percent of body weight in fertilized ponds was the most effective feeding rate for small-scale production of tilapia in the cage-pond integrated system.

### I.3.2. Cage material, designs and implications

The design of fish cages is determined by the behaviour of the cultured species. For *O. niloticus,* which is less active and sometimes territorial in habitat, its mobility is not very much affected by the shape of the cage (Masser, 1988). In cage culture of *O. niloticus* both floating surface and standing surface cages are used for tilapia culture. Standing cages are tied to stakes driven into the bottom of the substrate, whereas floating cages require a floatation device to stay at the surface. Floatation can be provided by metal or plastic drums, sealed PVC pipe or Styrofoam and similar materials. McGinty & Rakocy (1989) recommended that cages should be constructed from materials that are durable, light-weight and inexpensive, such as galvanized and plastic coated welded wire mesh, plastic netting and nylon netting. Welded wire mesh is durable, rigid, more resistant to biological fouling and easier to clean than flexible material but it is relatively heavy and cumbersome. Plastic netting is durable, semi-rigid, light-weight and less expensive than wire mesh cages made of nylon netting. Cages made of Nylon netting are not subject to the size constraints imposed by other construction materials. Nylon mesh is inexpensive, moderately durable, lightweight and easy to handle. Nylon is susceptible to damage from predators such as turtles, otters, alligators and crabs. Therefore McGinty & Rakocy (1989) suggested an additional cage of larger mesh and stronger twine would be suitable around nylon cages. Mesh size has a significant impact on production. For cage culture of tilapia Masser (1988) recommended cages with mesh sizes of between 0.5 and 0.75 inches. These mesh sizes provide open space for good water circulation through the cage to renew the oxygen supply and remove waste, which is a key tool for the success of tilapia cage culture.

Cage sizes may vary from 1 to 1000 cubic meters (McGinty & Rakocy, 1989). However, cage handling is a challenge and labour intensive and at times requires complicated machinery. Therefore, for small-scale cage culture small, easy to handle cages are necessary in order to overcome the challenge of handling and to minimize labour. Waidbacher *et al.* (2006) designed a functional and easy to handle small net cage, which was made up of two rectangular frames, which formed the top (1.2 x 0.94 m) and base (0.9 x 0.9m) of the cage. The frames of the cages were constructed from 51-mm diameter polyvinyl chloride pipes and covered with nylon net of a mesh size of 15 mm. The cage has a height of 0.75 m when submerged in water, with a slight constriction at 0.40 m below the top frame, and a volume of 0.64 m3. The tubing of the upper frame was completely sealed to guarantee self-floatation, while the lower frame was open through two T-joints on the opposing sides of the frame. These openings allowed in water, which made the lower frame sink. The cage did not require external devices for aiding either floatation or sinking. This cage was collaboratively developed at Sagana fish farm and named BOMOSA, an acronym derived from the first two letters of the partners; **BO**KU (University of Natural Resources and Applied Life Sciences, Vienna), **Mo**i University and **Sa**gana fish farm.

## I.4. Feed components for tilapia

Among the farmed tilapias, Nile tilapia, *O. niloticus* is one of the commercially important species. The fry of *O. niloticus* feeds mainly on zooplankton, while the adults consume large quantities of plant material, which are largely dominated by live algae, detritus and the associated bacteria (Dempster *et al.*, 1993; Moriarty, 1973; Getachew 1987; Diana *et al.*, 1991; Getachew & Fernando 1989). However, despite the well documented herbivory in *O. niloticus*, commercial diets usually contain between 7 and 15 percent animal protein to supplement amino acids (Teichert-Cordington *et al.*, 1997). Fishmeal is a major source of protein in tilapia feeds, but increasing costs and scarcity stress the need for alternative protein sources. A number of studies have evaluated various alternative animal protein sources (Alceste & Jory 2000; EL-Saidy & Gaber, 2004; EL-Sayed, 1998; Fernandes *et al.*, 1999; Fasakin *et al.*, 1999; Rinchard *et al.*, 2002; Hossain *et al.*, 2002; Liti *et al.*, 2005a) and plant protein sources for fish feed (Liti *et al.*, 2006a; Olvera-Novoa *et al.*, 2002; Hughes 1991; Gomes *et al.*, 1995; McGoogan & Reigh, 1996). This has yielded quite considerable success in establishing alternative strategies for protein supply although the results have not always been consistent.

Different animal by-products have been tested as suitable substitutes for fishmeal in tilapia feeds. Among them are blood meal, poultry by-product meal, hydrolysed feather meal and meat and bone meal (NRC, 1993). Despite their usually high crude protein content, inclusion levels of most of these fishmeal substitutes are limited by their low contents of certain essential amino acids (EAA): blood meal is reported to be deficient in isoleucine, hydrolysed feather meal and most poultry by-product meals are low in lysine, while meat and bone meal, blood meal and to some extent hydrolysed feather meal are reported to be deficient in methionine (NRC, 1993; Tacon & Jackson, 1985). However, Davies *et al.* (1989) observed that these imbalances could be overcome to a large extent by mixing by-product meals with complementary amino acid patterns so as to obtain the desired EAA profile.

Tacon (1995a) reported that soybean meal is generally considered to be one of the most suitable plant protein sources in terms of its protein quality and EAA profile. He however observed that soybean meal is deficient in methionine. Like most other plant proteins it also contains a wide variety of endogenous antinutritional compounds, which require removal or inactivation through processing prior to usage within aquafeeds (Tacon, 1995a). Despite this, numerous studies have been conducted using processed soybean meal as a substitute for fishmeal in tilapia feeds. Most authors have reported that between 67 to 100 percent of the dietary protein could be supplied as soybean meal. However, the inclusion level depends on a variety of different factors, which include among others fish species and size, soybean source, processing method and culture system employed. Fagbenro (1998) and De la Pena *et al.* (1987) noted that although legume seeds are desirable in fish feeding owing to their nutritive value, low prices and market availability, the presence of anti-nutritional factors (ANFs) limit their level of inclusion in fish feeds. Furthermore and very important, in most developing countries leguminous seeds constitute part of the human diet and their use for aquafeeds would compete with the ultimate goal of securing human nutrition.

Other oil seed residues have also been widely tested for their suitability as replacements for fishmeal (Olvera *et al.*, 2002; EL-Sayed, 1990; Maina *et al.*, 2002; EL-Saidy & Gaber, 2004; Hossain *et al.*, 2002; Jackson *et al.*, 1982), but the results have not been consistent. Oil seed residues contain generally high CP levels but may be low in cystine, methionine and lysine, which are frequently lacking in plant protein sources (Jauncey & Ross, 1982). Rinchard *et al.* (2002) further reported that cottonseed meal contains a phenolic chemical compound that is found in the pigment glands of the cotton plant, which limits its inclusion in tilapia diets. Gossypol is toxic to fish and this has been reported to interfere with physiological processes of reproduction including inhibition of steroidogenesis in animals (Lin *et al.*, 1988; Coutinho *et al.*, 1985; Hadley *et al.*, 1981). Therefore the use of

diets based on cottonseed meal faces specific limitations; they are not ideal for farmers producing fingerlings, because they lead to reduced fingerling production (Liti *et al.*, 2005a). Hossain *et al.* (2002) also reported the presence of tannins, saponin and non-starch polysaccharides (NSP) in dhaincha (*Sesbania aculeate*) seed meal.

There have been attempts of utilizing aquatic plants such as *S. polyrrhiza* (Fasakin *et al.*, 1999; Wee 1991), *Azolla pinnata* (Almazan *et al.*, 1986; El-Sayed 1992), *Lemna* sp. (Edwards, 1987; Mbagwu *et al.*, 1990), *Eichhornia crassipes* (Riechert & Trede, 1977), *Elodea trifoliate*, *Myriophyllum spicatum* and *Potamogeton gramineous* (Okeyo, 1988) as ingredients of tilapia feeds. Moreover, as with the use of oilseeds, the results of feeding trials using aquatic plants often vary considerably and sometimes yield conflicting results. Almazan *et al.* (1986) and El-Sayed (1992) reported an extremely poor performance of *O. niloticus* fingerlings and adults fed *Azolla pinnata*-based diets. Similar results were reported by Micha *et al.* (1988) for *O. niloticus* and *T. rendalli* fed *Azolla microphylla*. In contrast to this, Santiago *et al.* (1988) found that *O. niloticus* fed rations containing up to 42% *A. pinnata* outperformed fish fed the fishmeal-based diet. However, many of the aquatic feedstuffs are limited by high levels of fibre resulting in low nutrient digestibility.

Cereal by-products such as maize, wheat, rice gluten meal, distiller's grains and brewery waste have also been successfully used as dietary protein sources for tilapia (Liti *et al.*, 2006b; Wu *et al.* 1994). Wu *et al.* (1994) and Tudor *et al.* (1996) reported better growth for *O. niloticus* fingerlings fed diets based on maize distiller's grains with gluten meal and gluten feed as main protein sources as compared to fish fed fishmeal based diets. Poumogne (1995) reported that brewery grains could be included up to 30 percent in diets of *O. niloticus* without any significant adverse effect on fish growth. Liti *et al.* (2006b) reported that cereal brans are a rich and inexpensive source of carbohydrates and with fertilization can be suitable choices in semi-intensive production. Several feeding trials utilizing cereal brans have been conducted in Kenya. Omondi *et al.* (1999) evaluated rice bran at two levels of application, 60 and 120 kg $ha^{-1}$ $day^{-1}$. They obtained higher fish yields at the higher rate of bran application. Liti *et al.* (2001) compared rice bran with two locally formulated feeds and observed lower growth in the rice bran as compared to the formulated feed treatment. In another experiment, Liti *et al.* (2005a) compared wheat bran, commercial pelleted pig feed and two locally formulated feeds; one with and the other without vitamins and observed better growth with the formulated feeds than with wheat bran. Nevertheless, the economic performance was better for the wheat bran treatment due to low costs of wheat bran. In a more recent feeding trial Liti *et al.* (2006b) evaluated growth performance of *O. niloticus* fed on maize, rice and wheat brans in semi-intensive earthen culture ponds; they reported good growth with maize and wheat bran but a poor growth performance of fish fed with rice bran. Maize bran has also been shown to support

good growth of tilapia in Malawi (Chikafumbwa *et al.*, 1993; Chikafumbwa 1996), maybe due to its high digestibility (Maina *et al.*, 2002).

In a fishmeal substitution review in tilapia feeds by El-Sayed and Tacon (1997), the authors reported the utilization of single cell protein (SCP) in tilapia diets. SCP are micro-organisms and includes bacteria, yeasts and algae. Viola and Zohar (1984) reported that a bacterial SCP (Pruteen, containing 70 percent crude protein) could replace up to 50 percent of the FM within a 30% crude protein diet with no loss in the growth of cage-reared tilapia hybrids (*O. niloticus* x *O. aureus*). However, fish performance was reduced when pruteen completely replaced fishmeal in the diets. In another experiment Chow and Woo (1990) used the filamentous alga *Spirulina sp.* to successfully replace 20 percent of a commercial eel diet without adversely affecting the growth and appetite. El-Sayed and Tacon (1997) reported that results obtained in feeding trials have sometimes been conflicting even for the same feedstuffs and species. To a large extent this has been due to the variability of processing methods, source of feedstuffs and the methodology employed by researchers for conducting their studies. Tacon (1995b) added that the changes are at times caused by differences in feed preparation and feeding methods, by different environments in indoor and outdoor experiments, different stocking densities and fish size.

El-Sayed and Tacon (1997) concluded that, although the majority of studies on tilapia feeds have been evaluated from a biological or nutritional viewpoint, little or no attention has been given to economic analyses within these studies. Since aquaculture is operated as an economic activity, studies concerning the development of feeds and feeding regimes should be also analyzed from an economic viewpoint.

## I.5. Objectives

This study aimed to contribute to the development of sustainable feed resources for Nile tilapia (*Oreochromis niloticus*), which are produced in a semi-intensive cage culture system in various regions of Kenya. In the first phase, the proximate composition of locally available agricultural by-products and other under-utilized protein sources from three eco-zones of Kenya was analysed as an indicator for their nutritional value. In a second phase, the effects of formulated diets containing previously identified feedstuffs on growth performance of *Oreochromis niloticus* were studied under different culture conditions. This also involved the investigation of the potential improvement of digestibility and fish growth performance by use of *Papaya carica* leaves. However, the studies did not include an analysis of the antinutritional factors in different feedstuffs, but in this respect relied on literature from previous studies.

To achieve these broad objectives, the investigations were focused on the following questions:

- determination of proximate composition of potential feedstuffs, thereby
- identification of feedstuffs which could be most suitable for tilapia production in the three eco-zones studied
- effects of diets containing feedstuffs identified as given above on growth performance and apparent digestibility in Nile tilapia
- estimate the level at which alternative feedstuffs could be used to replace costly protein sources of limited availability in diets of Nile tilapia without deleterious effects on growth performance

## I.6. Study hypothesis

- Feedstuffs are locally available and can be used in Nile tilapia diets to substitute other critical but scarce feed components which are currently used.
- Freshwater shrimps (*Caridina nilotica*), which are a limited resource can be substituted by hydrolysed feather meal in Nile tilapia diets.
- Feed components containing proteases can improve the nutritive value of hydrolysed feather meal.

## I.7. Experimental site, experimental conditions

The feedstuff proximate analysis and feeding experiments were undertaken at Sagana Fish Farm, which is under Kenya Department Fisheries. The farm is located 90 km northeast of Nairobi, altitude 1230 m, latitude 0°39′S and longitude 37°12′E; Figure I.1). The water supply to the farm comes from Ragati River through a 1.5 km long canal. It is surrounded by cultivated fields and settlement areas.

### I.7.1. Historical background of the farm

According to the farms reports, (Government of Kenya, FD, 1992), the Sagana fish farm was established in 1948 by the colonial British Government with primary objectives of providing extension services and producing both market size and fish seeds for the local people and fish farmers. Currently, the farm falls under the government of Kenya Fisheries Department with funding organizations to strengthen the research sector. Initially, the four species were tested for culture: the African catfish (*Clarias gariepinus*), Largemouth bass (*Mycropterus salmoides*), and two tilapia species; *Tilapia zillii* and

*Tilapia nigra*. The best performing fish species were recruited for culture. Currently, *C. gariepinus* and *O. niloticus* are produced to market size whereas the common gold fish (*Carassius auratus*) and Koi carp (*Cyprinus carpio*) are produced as ornamental fish. Integrated aquaculture is also practiced at the farm with chicken, sheep, cattle, food crops, agro forestry and vegetable production. Fish produced from the farm is mainly sold locally amongst the surrounding communities and to hotels in Nairobi.

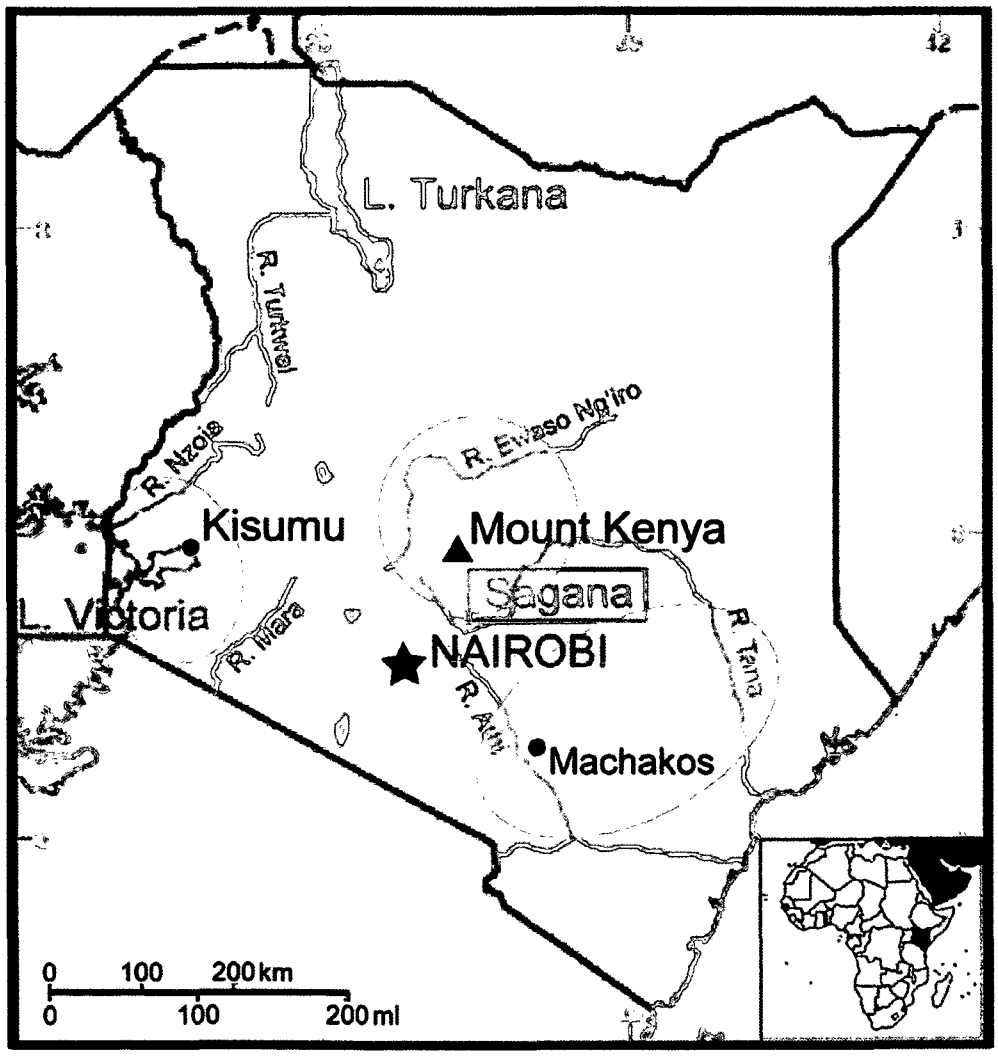


***Figure I.1**: Map of Kenya showing the locations where agricultural by-products and other feedstuffs were collected and Sagana fish farm where the experiments were conducted.*

### I.7.2. Vegetation and Climate

The area lies in a marginal area characterized by indigenous, cultivated fields and grassland areas. The major cultivated crops around the farm include maize, beans, arrowroots, sweat potatoes and peas. The climate is semi-arid with two rainfall periods from April-July (long rains) and from October-December (short rains). The amount of rainfall ranges from 1332mm $year^{-1}$ to 1612mm $year^{-1}$ (Veverica & Bowman, 1999). The air temperatures varies between an average minimum of 16.3°C and an average maximum of 26.9°C (Veverica & Bowman, 1999)

### I.7.3. Hydrology and soils

The catchment area originates from the slopes of Mount Kenya throughout Nyeri to Sagana. The soils are mainly black cotton type with about 80 percent clay. The principal source of nutrients in river Ragati is agricultural and domestic drainage since the catchment area is characterised by agricultural fields and settlement areas. There is, though, some input from municipal drainage since Nyeri town lies within the catchment area.

# II. Proximate composition of selected potential feedstuffs for Nile tilapia (*Oreochromis niloticus* Linnaeus) production in Kenya

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## II.1. Summary

Agricultural by-products were sampled depending on their local availability in three regions of Kenya. Proximate analysis was performed on 4 and 23 samples of animal and plant origin, respectively, to estimate their potential nutritive value for utilization as feedstuffs for tilapia grown in a low-input pond culture system, which greatly relies on local resources. Based on their availability, potential competition with other uses, content of protein and fibre and the feasibility of removal of antinutritional factors, feather meal, boiled tea leaves residues, leaves of *Ipomoea batatas*, *Manihot esculenta* and *Papaya carica* were identified as most promising potential feedstuffs. In addition, different seed cakes and cereal brans may be utilized where available and grain husks may serve as organic fertilizers in low-intensity aquacultures.

**Key words**: Tilapia, fish, nutrient, by-product, feed

## Zusammenfassung

Agrarische Nebenprodukte wurden nach ihrer lokalen Verfügbarkeit in verschiedenen Regionen Kenias beurteilt und beprobt. Vier Proben tierischer und 23 Proben pflanzlicher Herkunft wurden einer Futtermittelanalyse unterzogen, um ihren potenziellen Futterwert für die Erzeugung von Tilapia in Teichwirtschaft, die auf Nutzung lokaler Ressourcen basiert, abzuschätzen. Aufgrund der Verfügbarkeit, der Konkurrenz zu anderen Verwertungspfaden, dem Gehalt an Protein und Rohfaser sowie der Praktikabilität der Inaktivierung möglicher antinutritiver Inhaltsstoffe wurden folgende Nebenprodukte als

potenzielle Futtermittel eingestuft: Federnmehl, extrahierte Teeblätter sowie Blätter von *Ipomoea batatus*, *Manihot esculenta* und *Papaya carica*. Weiters können bei gegebener regionaler Verfügbarkeit verschiedene Kuchen von Ölsaaten sowie Getreidekleien verwertet werden, während Getreidespelzen allenfalls als organische Dünger in der Teichwirtschaft verwendet werden können.

**Schlagworte**: Aquakultur, Tilapia, Nährstoffe, Nebenprodukte, Futter

## II.2. Introduction

Nutrition is vital in fish farming because feed costs represent 40-50 % of the total variable production costs (SHANG, 1992; CRAIG and HELFRICH, 2002). For several decades, fishmeal has been used as the main source of protein in fish feeds (TACON and JACKSON, 1985; TACON, 1993; EL-SAIDY and GABER, 2004; ALCESTE and JORY, 2000). However, the periodically occurring low availability, competition and continuously fluctuating prices of fishmeal are affecting aquaculture feed production and consequently the profitability (WATANABE, 1988; WATANABE, and PONGMANEERAT, 1991; LIM and DOMINAY, 1990). As a result, a lot of effort has been focussed on feed alternatives to fishmeal both from plant and animal protein sources (EL-SAIDY and GABER, 2004; EL-SAYED, 1998; FERNANDES et al., 1999; FASAKIN et al., 1999; RINCHARD et al., 2002; HOSSAIN et al., 2002).

In order to enhance aquacultural production, improve food security, and reduce the level of poverty in developing countries, a search for cheap and locally available feedstuffs is required. Kenya is endowed with many by-products from agricultural processing, which are usually not utilized for human consumption, but may have a high potential in tilapia feeds. Nile tilapia, *Oreochromis niloticus* L., is one of the commercially important species among the farmed tilapias in Kenya and many other tropical and sub-tropical countries. This is due to its fast growth, resistance to diseases and the ability to feed on the lowest tropic level (PULLIN, 1988). The adults of this species consume large quantities of plant materials, which are largely dominated by live algae, detritus and the associated bacteria (MORIARTY, 1973; GETACHEW, 1987; DIANA, LIN and SCHNEEBERGER, 1991). Many of these agricultural by-products have been evaluated for inclusion in poultry and livestock feeds (GOMEZ, 1982; LAWRENCE and MUGERWA, 1974; LEDGER and TILLMAN, 1972; JACKSON and FULTON, 1971; BAUSTAD 1974); however only a few have been evaluated for their potential as tilapia feeds (WAIDBACHER et al., 2006; LITI et al., 2005; MAINA et al., 2002; LITI et al., 2006).

Development of a feed for fish production involves evaluation of proximate composition, digestibility and performance efficiency as well as cost implications and conditions of application. The current study was undertaken to determine and evaluate the potential of

selected by-products for use as feedstuffs in tilapia, using both laboratory analyses and information from the literature. Data from the current study are expected to form a basis for further evaluation of the effects of selected feed components on digestibility and fish growth under different culture conditions. One key element of the underlying concept of this work is, that the targeted feedstuffs are not directly consumed by human. Therefore it is anticipated that their transformation into high quality fish protein in low-input pond culture systems, which greatly rely on local resources, can be a major contribution to improving the protein supply for the local human population.

## II.3. Materials and methods

The present study surveyed selected potential feedstuffs with a specific focus on sources of plant origin. Selection of the feedstuffs was based on regional and temporal availability, and likely costs in Kenya. Analyses were conducted at Sagana laboratory of the Kenya Fisheries Department. Agricultural by-products available in Lake Victoria basin, central and eastern Kenya were targeted. Samples were collected during a period of three months from the different regions and seasonality did not affect their availability. They were sun-dried and ground to coarse particles using a blender liquidizer (model A989, Hampshire, UK). They were further ground into finer particles using an electric grinder fitted with a 1 mm seive (Thomas-Wiley intermediate mill, 3348-L10 series, USA) and dried in an oven to a constant weight at 60 °C. Poultry feathers were ground to smaller sizes by the use of a hammer mill prior to hydrolysing and cooked in an autoclave at a pressure of 1.1 bars and a temperature of about 105 °C, for 3 hours before being subjected to the processing described above.

Analyses of crude protein, crude fibre, ether extracts, ash and moisture content were done in triplicates. Dry matter (DM) was determined by drying 5 grams of sample in an oven for six hours to constant weight at 105 °C. The samples were cooled to room temperature in a desiccator and weighed. The difference in weight before and after drying expressed as a percentage of the initial weight was taken as the moisture content.

Crude protein was quantified by the standard micro-Kjeldahl Nitrogen method as described in AOAC (1995). Samples of 0.4 g were hydrolysed with concentrated sulfuric acid at 420 °C using Behroset InKje M digestion apparatus (Labor-Technik GmbH, Düsseldorf, Germany). The resulting solution was distilled under alkaline conditions using the Behr S 1 steam distillation apparatus (Labor-Technik GmbH, Düsseldorf, Germany). The distillate containing ammonia was trapped in 4 % boric acid solution and titrated with 0.1N HCl. Crude protein was estimated by multiplying the nitrogen content with a factor of 6.25. The result was expressed as a percentage of the original weight of the sample.

Ether extracts were assayed by extraction of samples of 2 g each in a soxhlet extractor for 6 hours with petroleum ether (boiling point 40-60 °C). After extraction, the thimble and sample were oven dried at 60 °C for 4 hours, cooled in a desiccator for one hour and weight was determined. Ether extracts were quantified by expressing the loss in weight as a percentage of the original weight of the sample. Crude fiber (CF) was determined by boiling 1 g of sample in a standard solution of 3.13 % $H_2SO_4$ for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with hot water followed by acetone. The residue was oven dried at 60 °C for 4 hours, cooled in a desiccator and weighed. The residue was ashed at 550 °C in a muffle furnace overnight. CF was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample. Ash was determined as the weight of the residue after 5 g of sample had been ashed at 550 °C in a muffle furnace overnight. Nitrogen Free Extracts were estimated by difference (DM-CP-EE-CF-Ash).

## II.4. Results

Data on proximate composition of selected feedstuffs are presented in Table II.1 (a-e). The proximate composition of feedstuffs from animal origin is presented in Table II.1a. Crude protein content of animal feedstuffs ranged between 551–808 g/kg DM. Among this group of feedstuffs, Omena fish (*Rastrineobola argentea*) had the lowest CP levels while hydrolysed feather meal (HFM) had the highest. Ether extracts (EE) were generally low in animal feedstuffs with the exception of R.argentea. The ash content was also quite variable (35-228g/kg DM) while CF and NFE contents were low.

***Table II.1a**: Proximate composition of locally available feedstuffs of animal origin, ($\bar{x}$ ±s); *DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE=N-free Extracts, BHFM= Broiler hydrolysed feather meal, IHFM= Indigenous hydrolysed feather meal.*

| Product | No of samples | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|---|
| | | g/kg | g/kg DM | | | | |
| Freshwater shrimp meal FSM | 4 | 877±1.7 | 635±3.3 | 13±1.3 | 50±1.8 | 67±2.1 | 228±2.5 |
| Hydrolysed feather meal BHFM | 3 | 891±1.0 | 797±2.1 | 24±1.5 | 48±1.5 | 96±2.0 | 35±1.5 |
| Hydrolysed feather meal IHFM | 3 | 897±1.5 | 808±1.7 | 19±2.1 | 31±1.0 | 43±1.6 | 100±1.0 |
| Omena (Rastrineobola argentea) | 4 | 879±0.6 | 551±1.7 | 187±1.5 | 13±0.6 | 68±1.0 | 182±1.5 |

Crude protein (CP) levels in feedstuffs of plant origin ranged from 72-353 g/kg DM (Table II.1b). Leaves of arrow root (*Maranta arundinacea*), cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*) had a CP content of over 308 g/kg DM, while leaves of leucaena (*Leucaena leucocephalus*), papaya (*Carica papaya*), boiled tea (*Camellia sinensis*) leaves residue (BTLR) and water fern (*Azolla pinnata*) had CP levels of between 230 and 282 g/kg DM. Banana (*Musa paradisiaca*) leaves, papaya fruit peels, pyrethrum (*Anacyclus* spp.) waste as well as whole water hyacinth (*Eichhornia crassipes*) had CP levels between 133-180 g/kg DM. Banana peels and stem as well as pyrethrum leaves showed CP levels below 100 g/kg DM. Papaya and banana leaves had a higher CP content than either their fruit peels or the stem.

***Table II.1b**: Proximate composition of different plant parts and by-products ($\bar{x}$ ±s); *DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE=N-free Extracts.*

| Product | No of samples | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|---|
| | | g/kg | g/kg DM | | | | |
| Arrow root leaves | 3 | 903±2.6 | 335±1.0 | 85±1.5 | 106±4.6 | 381±2.1 | 93±2.3 |
| Banana peel | 4 | 901±2.1 | 72±1.7 | 79±1.3 | 113±2.6 | 627±1.7 | 109±2.8 |
| Banana stem | 4 | 926±1.0 | 100±1.8 | 50±2.2 | 441±1.7 | 205±3.5 | 205±4.5 |
| Banana leaves | 4 | 899±1.0 | 170±1.8 | 127±1.4 | 241±1.8 | 337±1.3 | 124±3.6 |
| Boiled tea leaves residue | 4 | 919±1.7 | 279±2.2 | 149±1.3 | 148±1.7 | 377±1.9 | 47±1.9 |
| Casssava leaves | 5 | 919±3.6 | 308±4.8 | 86±4.1 | 156±4.0 | 368±2.1 | 82±5.2 |
| *Leucaena* leaves | 3 | 929±1.0 | 280±1.5 | 71±1.5 | 158±2.1 | 391±1.0 | 99±2.0 |
| Papaya peel | 4 | 839±1.3 | 179±2.4 | 18±3.1 | 194±2.2 | 456±4.0 | 154±3.4 |
| Papaya leaves | 4 | 903±2.9 | 282±5.0 | 105±2.5 | 130±1.3 | 329±3.3 | 154±1.2 |
| Pyrethrum whole | 2 | 890±0.7 | 150±1.4 | 45±0.7 | 282±3.5 | 420±1.4 | 104±4.2 |
| Sweet potato leaves | 5 | 892±1.6 | 353±3.6 | 43±3.7 | 105±3.6 | 388±1.1 | 104±3.6 |
| Water fern, whole | 4 | 888±2.4 | 232±1.9 | 49±0.8 | 302±3.6 | 239±1.3 | 179±3.4 |
| Water hyacinth, whole | 2 | 895±1.4 | 133±4.2 | 18±1.4 | 260±2.8 | 407±4.2 | 188±3.5 |

Ether extracts (EE) were generally low in all plant feedstuffs with exception of BTLR, banana and papaya leaves which contained over 100 g/kg DM. Crude fibre (CF) was generally high and variable among feedstuffs of plant origin and ranged between 105-441 g/kg DM; only arrowroot and sweet potato leaves contained CF levels below 110 g/kg DM (Table II.1b). Banana stem had the highest CF content. Nitrogen free extracts (NFE) were generally high in feedstuffs of plant origin with most of them in the range of 320-420 g/kg DM. Banana peel had NFE value above 480 g/kg DM while banana stem and water fern had NFE values below 250 g/kg DM. Ash content was generally low, banana stem had the highest ash values while BTLR had the lowest.

The nutrient content of the seeds and seed cakes was generally high and is presented in (Table II.1c): Cotton (Gossypium spp) seed cake (CSC) had the highest protein content among the seeds and seed cakes that were tested. Papaya (Carica papaya) seed meal (PSM) and sunflower (Helianthus annuus) seed cake (SFSC) had similar CP values while mango (Mangifera indica) seed embryo (MSE) had the lowest CP value. EE was generally low in CSC, SFSC and MSE but high in PSM. Crude fibre levels varied greatly with SFSC having the highest and MSE the least. Ash levels were generally low in all the seeds. MSE were especially rich in NFE.

***Table II.1c**: Proximate composition of selected seed meals ($\bar{x}$ ±s); *DM=Dry matter in g/kg, CP=Crude Protein, EE=Ether Extracts, CF=Crude Fibre, NfE=N-Free Extracts.*

| Product | No of samples | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|---|
| | | g/kg | g/kg DM | | | | |
| Cottonseed cake | 5 | 893±2.0 | 388±7.2 | 107±1.0 | 249±4.5 | 192±2.6 | 63±4.6 |
| Mango seed embryo | 2 | 907±1.4 | 70±0.7 | 97±1.4 | 37±0.7 | 771±2.1 | 24±1.4 |
| Papaya seed meal | 4 | 945±1.7 | 264±21 | 316±1.3 | 119±1.0 | 203±1.6 | 98±1.3 |
| Sunflower seed cake | 5 | 929±0.4 | 259±0.1 | 54±0.8 | 368±0.2 | 266±0.8 | 51±0.1 |

Proximate composition data of the three cereal brans are presented in (Table II.1d). Wheat (*Triticum aestivum*) bran (WB) had the highest CP content while rice (*Oryza sativa*) bran (RB) had the lowest, EE in maize (*Zea mays*) bran (MB) was nearly double the amount recorded in WB, while RB registered the least. WB and MB had low ash contents but were high in NFE, while RB had the highest ash level and the lowest NFE among the brans. Crude fibre was nearly 2-6 times higher in rice bran than in wheat and maize bran, respectively.

***Table II.1d**: Proximate composition of selected cereal brans ($\bar{x}$ ±s); *DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE= N-free Extracts.*

| Product | No of samples | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|---|
| | | g/kg | g/kg DM | | | | |
| Maize bran | 5 | 894±3.0 | 118±4.6 | 107±2.7 | 55±0.7 | 691±1.9 | 29±1.3 |
| Rice bran | 5 | 923±4.2 | 70±3.8 | 41±1.6 | 309±2.4 | 349±3.5 | 229±2.2 |
| Wheat bran | 5 | 882±1.6 | 171±6.2 | 58±2.3 | 127±2.3 | 582±6.9 | 60±2.6 |

Data on proximate composition for seed husks are presented in (Table II.1e), The seed husks were generally of low nutritive value with exceptionally high crude fibre content and low CP content, coffee (Coffea arabica) pulp and cotton husk had moderate CP levels of 172 and 173 g/kg DM respectively. Coffee pulp and husks had NFE values of over 320 g/kg DM. The ash content was low in cotton husk but high in coffee pulp and coffee husk.

***Table II.1e**: Proximate composition of selected seed husks ($\bar{x}$ ±s); *DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE= N-free Extracts.*

| Product | No of samples | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|---|
| | | g/kg | g/kg DM | | | | |
| Coffee husks | 4 | 893±1.9 | 47±1.8 | 36±0.6 | 383±2.6 | 418±3.6 | 115±2.8 |
| Coffee pulp | 4 | 874±1.9 | 172±2.2 | 60±1.3 | 281±2.2 | 320±1.9 | 168±1.3 |
| Cotton husks | 3 | 906±4.9 | 173±4.4 | 55±1.0 | 587±1.5 | 153±1.5 | 36±0.6 |

## II.5. Discussion and Conclusions

The present results indicated that the protein content of feedstuffs from animal origin was higher than that of plant by-products. Nevertheless, the costs of these feed components from animal origin are generally high and therefore they are unlikely to be cost-effective for semi-intensive tilapia production systems. The exception in the category of the animal feedstuffs was the hydrolysed feather meal, which also had the highest level of CP (Table II.1a, II.3). Utilization of HFM in tilapia feeds is economically feasible since the costs involved for procurement were essentially transport costs. However, despite the high CP level and low costs, feather meal is an uncommon ingredient in the fish feed industry. Among others, its unbalanced amino acid profile is probably one reason for its limited exploitation. TACON et al. (1984) reported high CP in HFM but also cautioned on the deficiency in some essential amino acids, which included methionine, lysine, histidine and tryptophan. Some fish feeding trials indicated that HFM could only be included up to 20% in diets for *Labeo rohita* without adverse effects on growth (HASAN et al., 1997). Nevertheless, HFM could possibly be included in tilapia feeds at even higher levels under semi-intensive culture conditions, where nutrient deficiencies might be supplemented by natural pond food. LI and YAKUPITIYAGE (2002) reported that pond fertilization provides exogenous elementary nutrients that enhance natural food productivity for omnivorous fish like tilapia.

***Table II.3**: Comparison of nutritive levels of common selected animal and plant by-products of the current and previous studies (ADCP, 1983; 1987); *DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE= N-free Extracts.*

| Product | *DM | CP | EE | CF | NfE | Ash |
|---|---|---|---|---|---|---|
| | g/kg | g/kg DM | | | | |
| Sweet potato (*Ipomoea batatas*) leaves | | | | | | |
| Current study | 902 | 358 | 34 | 86 | 95 | 466 |
| Israel | 892 | 194 | 37 | 259 | 105 | 408 |
| Malaysia | 913 | 188 | 23 | 113 | 188 | 488 |
| Trinidad | 877 | 219 | 34 | 150 | 180 | 417 |
| Cotton (*Gossyium* spp.) seed cake | | | | | | |
| Current study | 902 | 393 | 81 | 485 | 217 | 301 |
| Egypt | 879 | 264 | 57 | 66 | 242 | 371 |
| USA | 989 | 461 | 7 | 71 | 151 | 310 |
| Israel | 923 | 477 | 54 | 66 | 125 | 278 |
| Fresh water shrimp (*Caridina nilotica*) | | | | | | |
| Current study | 878 | 638 | 12 | 179 | 51 | 120 |
| India | - | 455 | - | 221 | - | - |
| Madagascar | - | 736 | 66 | 186 | - | - |
| Malaysia | 795 | 455 | 21 | 400 | - | 124 |
| Hydrolysed feather meal (Indigenous) | | | | | | |
| Current study | 893 | 807 | 18 | 70 | 31 | 74 |
| Hydrolysed feather (Broiler) | | | | | | |
| Current study | 893 | 796 | 4 | 15 | 23 | 178 |
| USA | 930 | 914 | 39 | 38 | 4 | 5 |
| Sunflower (*Helianthus annuus*) cake, | | | | | | |
| Current study | 931 | 259 | 44 | 44 | 345 | 326 |
| Uganda | 910 | 341 | 143 | 66 | 132 | 318 |
| Nigeria | - | 411 | - | - | - | - |
| Rice (*Oryza sativa*) bran | | | | | | |
| Current study | 916 | 74 | 34 | 194 | 309 | 395 |
| India | 913 | 137 | 54 | 181 | 200 | 488 |
| Malaysia | 899 | 109 | 108 | 136 | 169 | 454 |
| Maize (*Zae mays*) bran | | | | | | |
| Current study | 887 | 120 | 82 | 22 | 51 | 738 |
| Tanzania | 890 | 106 | 48 | 13 | 19 | 814 |
| Thailand | 880 | 109 | 50 | 34 | 29 | 768 |
| Wheat (*Triticum eastivum*) bran | | | | | | |
| Current study | 876 | 174 | 43 | 44 | 108 | 651 |
| Tanzania | 876 | 169 | 38 | 64 | 113 | 616 |
| Malaysia | 881 | 188 | 46 | 54 | 97 | 616 |
| India | 907 | 139 | 83 | 46 | 131 | 601 |

Consequently, depending on the actual conditions, phytoplankton and zooplankton may be an important source of nutrients, supplementing diets of fish raised in pond culture (RAKOCY and McGINTY, 1989). The ash content in HFM was markedly higher in the feathers of indigenous chicken than in those of broiler chickens (Table II.1a). The difference might be attributed to contamination of the feathers with inorganic soil particles during dust bathing. Broilers are usually confined in pens and do not have access to inorganic substrate for dust bathing (LACY, 2002).

Freshwater shrimp meal (FSM) ranked second to HFM in terms of CP (Table II.1a, II.3), which was within the range of 490-740g/kg DM as reported by JAUNCEY and ROSS (1982). Although FSM has a high potential for inclusion in tilapia feeds because it is not used as human food, it suffers several limitations. In previous studies LITI et al. (2005) reported higher costs of fish production with diets containing FSM compared to those containing all plant protein feedstuffs. In addition, FSM is periodically scarce in the Kenyan market due to seasonal closures of the Omena (*Rastrineobola argentea*) fishery in Lake Victoria, in which FSM is a by-product. Besides this, there is usually stiff competition from other feedmills such as the poultry feed industry. These limitations make FSM a less competitive candidate in tilapia feeds.

The protein content of *R. argentea* is high (Table II.1a, II.3), and from a nutritional point of view it may be a suitable source of dietary protein in fish feeds. However, *R. argentea* is directly used as human food and thus inclusion in tilapia feeds might imply direct competition with the ultimate target. This, coupled with cost implications, reduces its feasibility for utilization in low-input pond fish production systems.

All plant leaves with the exception of banana leaves contained crude protein levels above 25% (Table II.1b, II.3, II.4) and thus may have a high potential for inclusion in tilapia feeds. However, no plant protein can on its own support good growth of fish due to deficiency in at least one essential amino acid (JAUNCEY and ROSS, 1982).

*Table II.4: Sample size and the locality where it was collected*

| Product | No of samples | Sampling site and no. of samples per site |
|---|---|---|
| Freshwater shrimp meal | 4 | Lake victoria Kisumu (4) |
| Broiler chicken feathers | 3 | Sagana-1, Machakos-1, Kisumu-1 |
| Indigenous chicken feathers | 3 | Sagana-1, Machakos-1, Kisumu-1 |
| Omena fish | 4 | Lake victoria Kisumu-4 |
| Arrow root leaves | 3 | Sagana-1, Machakos-1, Kisumu-1 |
| Banana peel | 4 | Sagana-2, Machakos-1, Kisii-1 |
| Banana stem | 4 | Sagana-2, Machakos-1, Kisii-1 |
| Banana leaves | 4 | Sagana-1, Machakos-1, Kisii-2 |
| Boiled tea leaves residue | 4 | Sagana-4, Machakos-1, Kisumu-1 |
| Casssava leaves | 5 | Sagana-2, Machakos-2, Kisumu-1 |
| Leucaena leaves | 3 | Sagana-2, Machakos-1 |
| Papaya peel | 4 | Sagana-2, Athiriver-2 |
| Papaya leaves | 4 | Sagana-2, Athiriver-2 |
| Pyrethrum whole | 2 | Naivasha-1, Nakuru-1 |
| Sweet potato leaves | 5 | Sagana-2, Machakos-3 |
| Water fern, whole | 4 | Sangoro fishponds-2, Sagana fishponds-2 |
| Water hyacinth, whole | 4 | Lake Victoria-2, Nairobi dam-2 |
| Cottonseed cake | 5 | Nairobi-2, Makueni-2, Kisumu-1 |
| Mango seed embryo | 2 | Sagana-1, Machakos-1 |
| Papaya seeds | 4 | Sagana-,2 Nairobi-2 |
| Sunflower seed cake | 5 | Kitale-2, Nairobi-2, Machakos-1 |
| Maize bran | 5 | Kitale-1, Sagana-2 Machakos-2 |
| Rice bran | 5 | Sagana-2, Kisumu-3 |
| Wheat bran | 5 | Nyeri-3, Eldoret-2 |
| Coffee husks | 4 | Machakos-2, Sagana-2 |
| Coffee pulp | 4 | Machakos-2, Sagana-2 |
| Cotton husks | 3 | Machakos-1, Makueni-1, Sagana-1 |

However, utilization may be feasible in semi-intensive production systems, where autotrophic and heterotrophic food material may supply the deficient amino acids (RAKOCY and McGINTY, 1989; LI and YAKUPITIYAGE, 2002). Based on their proximate composition, leaf meals with exception of banana have a high potential for inclusion in tilapia feeds, as they all had protein contents above 250 mg $kg^{-1}$ feed, which was close to the value recommended for inclusion in the grow-out diets for *O. niloticus* (SANTIAGO and LOVELL, 1988). The suitability of these feedstuffs for use in tilapia feeds is further made feasible by the fact that with the exception of arrowroot, they grow well in low rainfall areas, which form a greater portion of Kenya. Cassava, arrowroot and sweet potato are tuber plants and their roots are commonly used as human food. Their leaves are rarely consumed by human in many regions of Kenya, and thus may be available for use in tilapia feeds. Compared to values from previous research (ADCP,

1983; 1987; TAN, 1970), sweet potato leaves registered higher CP levels than reported from other countries (Table II.3). All the other feedstuffs were in the same range as results from previous studies (Table II.3). Several of the by-products mentioned herein are likely to contain components, which may affect their nutritive value. In the case of Cassava, a toxic component known as Linamarin has to be considered (Table II.2). Linamarin causes cyanide poisoning, but the toxicity may be removed by boiling and/or sun drying (JAUNCEY and ROSS, 1982; TEWE, 1991).

Literature on the utilization of *papaya carica* leaf meals in fish feeds is scarce. The limited available information (REYES and FERMIN, 2003) indicates that *papaya carica* leaf meal could be a good protein source because of its amino acid profile (GERPACIO and CASTILLO, 1979). In Kenya, papaya leaf meals are not used for human food. The papaya leaf and the unripe fruit contain papain, which degrades protein into amino acids (CHAPLIN, 2005). BUCHANAN (1969) reported that papain promotes proteolytic digestion and thereby increases the protein digestibility of papaya leaf meal. Therefore, future studies on papaya leaves may be directed towards improvement of feed digestibility. *Papaya carica* peels contain lectins, which are toxic compounds relevant to fish and other animals (MAKKAR & BECKER, 1997; CANO ASSELIECH et al., 1989), but, can be destroyed by heat treatment followed by aqueous methanol extraction or soaking in water for 24 hours under refrigerated conditions (Table II.2; MAKKAR & BECKER, 1999). If antinutritional components can be inactivated, papaya leaves may be a valuable feed component in tilapia production systems, which are based on local resources, due to their high protein content and proteolytic properties.

***Table II.2**: Selected toxic constituents for the analysed components.*

| Product | Toxic Factor | Effect | Preventative Treatment |
|---|---|---|---|
| *Manihot esculenta* | Linamarin | Cyanide poisoning when bruised in water | Peeling (fresh), washing, boiling (JAUNCEY and ROSS, 1982; TEWE, 1991) |
| *Leuceana leucocephalus* | Mimosine | Disruption of reproductive disorders, teratogenic effects (D'Mello 1991) | Boiling, addition of ferrous sulphate solution (DUKE, 1983), soaking (WEE and WANG, 1987) |
| *Papaya carica* leaves and green fruit | Benzyl isothiocyanate (BITC) Lectins | Irritation of mucus epithelial membrane | Heat treatment plus extraction or soaking (MAKKAR and BECKER, 1999). |
| *Gosspyium spp* | Gossypol | Complex formation with lysine, growth depression | Screw-pressing or solvent extraction (JAUNCEY and ROSS, 1982 |
| *Zae mays* | Dhurrin | HCN due to hydrolysis of cyanogenetic glycoside | Proper storage (JAUNCEY and ROSS, 1982) |

Information on the use of *Leucaena* leaf meal in fish feeds is limited (SANTIANGO et al., 1988), but it is widely used in livestock feeds (DUKE, 1983). The plant is rich in the amino acid leucine, which enhances its potential for inclusion in fish feeds. However, the presence of mimosine (Table II.2), which is toxic to most animals, may limit its application in fish feeds (D'MELLO, 1991). Difference in growth response of male and female tilapia has been observed when fed a diet containing *Leucaena* leaf meal: males seemed to tolerate it better than females. However, the production of fry was significantly reduced beyond the 40% inclusion level (SANTIAGO et al., 1988). D'MELLO (1991) noted that mimosine causes disruption of reproductive processes and teratogenic effects in animals. Mimosine toxicity can be removed through boiling in an open vessel or by addition ferrous sulphate solution (DUKE, 1983) and/or soaking in water at 30°C for 48 hours (Table II.2; WEE and WANG, 1987).

Boiled tea leaves residue (BTLR) is a by-product of a popular beverage in Kenya and is readily available in all parts of the country. Due to the usual way of preparing milk tea in Kenya, BTLR is likely to contain remnants of milk, which will improve its nutritive value. BTLR contains high levels of CP and EE (Table II.1b), which make it a potential ingredient for inclusion in tilapia feeds. There are no reports on antinutritional factors in BTLR and as they were not analysed in the present study, a critical evaluation of antinutritional compounds prior to utilization is essential.

The nutritional quality of oilseed by-products has been extensively evaluated (OLVERA et al., 2002; EL-SAYED, 1990; MAINA et al., 2002; EL-SAIDY and GABER, 2004). Seed residues have generally high CP levels but may be low in cystine, methionine and lysine, which are frequently lacking in plant protein sources (Table II.1c, II.3; JAUNCEY and ROSS, 1982). The levels of nutrients and toxic compounds in seed residues depend largely on the methods of processing and may also vary between strains (LIENER, 1980). The limit in inclusion levels of CSC is determined by the level of gossypol (LIN et al., 1988; RINCHARD et al., 2002; EL-SAIDY and GABER, 2004), a toxic phenolic compound that is found in the pigment glands of the cotton plant (BERARDI and GOLDBLATT, 1980). Gossypol has been associated with reduced fingerling recruitment in *O. niloticus* (LITI et al., 2005).

The suitability of sunflower seed cake (SFSC) as a fish feed has been evaluated (OLVERA et al., 2002; JACKSON et al., 1982; MAINA et al., 2002). SFSC contains a high level of protein (Table II.1c, II.3), which may vary according to the quality of the original seed and the method of processing (JAUNCEY and ROSS, 1982). A wide variety of products are available on the Kenyan market, ranging from low quality straw to high quality meals. Among the different by-products of sunflower seed, GOHL (1975) recommended that dehulled cakes are the by-product to be included in tilapia feeds

because of their high protein and relatively low CF levels. Sunflowers are widely grown in many parts of Kenya; therefore their by-products have a high quantitative potential for use in fish feeds. The oilseed meal of papaya (PSM) contains high amounts of protein (Table II.1c). Although information on the potential inclusion levels in fish feeds is rare or even missing, PSM may quantitatively have a high potential in the fish feed industry throughout Kenya, where papaya plants are abundant. Mango stone seeds are also available in great amounts, mainly in the drier areas of Eastern Kenya. However, due to the low CP content of the seed (Table II.1c), it is unlikely to become an important food component for tilapia in the future.

The use of cereal brans in Kenya has recently been evaluated: LITI et al. (2006) fed wheat, maize and rice brans to *O. niloticus* and evaluated the growth performance in fertilized ponds. Both wheat bran (WB) and maize bran (MB) promoted good growth of Nile tilapia and can substitute each other, depending on whichever of the two is locally available (LITI et al., 2006). The authors reported that rice bran (RB) was nutritionally inferior to WB and MB. The low nutritive quality of RB was attributed to poor processing methods. RB is reportedly mixed with hulls (VEVERICA et al., 1998; LITI et al., 2006) resulting in high levels of crude fibre and a low protein content (Table II.1d, II.3). GOHL (1975) reported a general deficiency of lysine in cereal by-products, but deficient nutrients might be supplemented by natural pond food in semi-intensive culture systems (NRC 1993). Cereal brans are generally cheap and readily available in most Kenyan regions, and may therefore be an important feed component in semi-intensive tilapia production.

Seed pulp/husks are quite cheap and abundantly available from processing factories. However, most seed pulps and husks are of low nutritive quality, due to high fibre contents (Table II.1e) and eventually their low acceptability by fish (ULLAO and VERRTH, 2002). Nevertheless, they may be utilized as feed components in semi-intensive production of tilapia, where they may be either consumed directly by the fish or serve as organic fertilizers and thereby indirectly enhance the food basis for tilapia (NRC, 1993).

From the data presented here and from information provided by the literature, it can be concluded that the currently underutilized leaves of *Camellia sinensis* (residues), *Ipomoea batatas*, *Manihot esculenta*, *Papaya carica* and hydrolysed feather meal may have a high potential as feedstuffs for tilapia grown in semi-intensive pond culture which relies greatly on local resources. This estimation is mainly based on the actual contents of protein and crude fibre, the possibility and practicability of removing antinutritional constituents, and on the local availability and the potential competition with other uses. However, before wholescale utilization there is need for further research to evaluate among others the amino acid profile, digestibility and antinutritional factors.

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# III. Effects of substitution of freshwater shrimp meal (*Caridina nilotica* Roux) with hydrolyzed feather meal on growth performance and apparent digestibility in Nile tilapia (*Oreochromis niloticus* L.) under different culture conditions.

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**Journal of *Aquaculture Nutrition***

## III.1. Abstract

The effects of substituting freshwater shrimp meal (FSM) (*Caridina nilotica,* ROUX) with hydrolysed feather meal (HFM) on growth, digestibility and survival in Nile tilapia (*Oreochromis niloticus* L.) were evaluated under laboratory and practical culture conditions. Fingerlings averaging 26 and 36g were held indoor in aquaria with recirculating water, and in cages that were installed in 800 $m^2$ fertilized earthen pond, respectively. Five isonitrogenous (250g $kg^{-1}$) and isocalorific (2.94 kca $g^{-1}$) diets were prepared by substituting FSM with HFM at rates of 0, 25, 50, 75 & 100% and fed to fish in aquaria; those in cages were fed diets at substitution levels 0, 50 and 100% All fish were fed at 10% body weight $day^{-1}$ in three replicates for 84 days. Results indicated that substitution of FSM at HFM levels above 50% in aquaria led to significant ($P<0.05$) growth reductions. However, substitution at 100% with HFM did not significantly ($P>0.05$) affect growth of fish in the cages. In both experiments, survival was similar among treatments, but protein digestibility decreased with increasing levels of HFM in the diet. In conclusion, 100% substitution of FSM with HFM may be possible in semi-intensive culture of *O. niloticus*, where natural food is available.

**Key words**: Hydrolyzed feather meal, shrimp meal, *O. niloticus*, growth, digestibility.

## III.2. Introduction

Production of farmed tilapia is among the fastest expanding food sectors in the world with a record of 599,135 mt in 1994 (FAO 1996). Nile tilapia is the most cultured freshwater species among the farmed tilapias and contributes about 71 % of the total tilapia production (FAO 1996). However, the sustenance and expansion of this food sector is limited by the high cost of fish feeds, which comprise 50% of the production costs. Protein is the most expensive nutrient in fish feeds, and the cost of fish feeds is usually based on the level of dietary protein. Moreover, the cost of fish feeds including those of tilapias is dependent on the quality and source of dietary protein (Tacon 1993).

Most fish feeds contain a proportion of animal protein, which is considered superior to that of plant sources in terms of palatability and nutrient availability to the targeted animal (Tacon 1993; Hardy & Tacon 2002). In the majority of fish feeds, ish meal has been incorporated as the main source of dietary protein. However, due to worldwide decline in fishery products, this source of protein is increasingly becoming scarce and expensive (Mohsen & Lovell 1990; Wu *et al.* 2004; Abdelghany 2003). Consequently, fish nutritionists and feed manufactures have been searching for alternative dietary protein from non-conventional sources, including plants to replace fish meal in Nile tilapia feeds and reduce production costs (El-Sayed 1998; Falaye 1982; Fernandes *et al.* 1999; Viola *et al.* 1988; Rinchard *et al.* 2002; El-Saidy & Gaber 2004). However, almost all plant protein sources are deficient in at least one of the essential amino acids, and besides this, some contain anti-nutritional factors, which reduce their efficiency of utilization (Tacon 1997; Lovell 1980; Hossain *et al.* 2003; Bureau *et al.* 1998; Shao *et al.* 2002; Fagbenro 1998). Thus, only a few of the plant protein sources can support good fish growth on their own (Jauncey & Ross 1982).

Due to inadequate amino acid profiles in plants, research on potential substitutes in Nile tilapia feeds, is being focused on non-conventional animal protein feedstuffs (Fowler 1982; Soliman 2000; El-Sayed 1998). Among these studies, whole and various body parts of shrimp meal have been evaluated as potential sources of animal protein (Jauncey & Ross 1982). A more recent study has shown that diets containing whole freshwater shrimp (*Caridina nilotica* Roux) meal as the sole source of animal protein were more efficient in promoting fast growth of Nile tilapia than those containing plant protein sources only (Liti *et al.* 2005). However, the diets were economically inferior due to scarcity and the high cost of shrimp meal. In Kenya, FSM is a by-product of the Omena (*Rastrineobola argentea*) fishery in Lake Victoria, but the supply is increasingly becoming scarce in the

market due to frequent closures of the lake's fishery. Therefore, replacement of FSM with cheaper and reliable animal protein feedstuffs is necessary in order to reduce the cost of *O. niloticus* feeds.

Poultry farming is widespread across Kenya and generates large volumes of feathers as a by-product. The protein content of feather meal is high (Tacon *et al.* 1984), and utilization of hydrolyzed feather meal protein in tilapia feeds could economically be feasible. Although studies conducted so far have indicated that HFM could only substitute fish meal up to levels between 20 and 50% without growth reductions in several fish species (Hasan *et al.* 1997; Falaye 1982; Steffens 1994; Abdel-Warith *et al.* 2001; Mendoza *et al.* 2001; Hasan *et al.* 1997), a slightly higher substitution level of 66% was reported for Nile tilapia (Bishop *et al.* 1995). Investigations to improve the nutritive value of feather meal have focused mainly on two approaches; direct supplementation of limiting amino acids (ref), and modification of processing techniques (Moran *et al.* 1966; Mendoza *at al.* 2001; Tiews *et al.* 1979; Hughes 1990; Steffens 1994). However, direct amino acid supplementation may not be economically viable given their high cost and the low prices fetched by tilapias in developing sub-Saharan countries. Furthermore, many investigators have expressed doubts on the viability of supplementing free amino acids in diets for tilapias since free amino acids are assimilated long before the accompanying polypeptides, and whole proteins are broken down; therefore, they are not presented at the same time for tissue formation (Jauncey 1982). On the contrally, tilapias are reported to utilize free dissolved amino acids in natural waters (Bowen 1982). Since *O. niloticus* feed continuously under semi-natural conditions, it is possible that a countinuous supply of amino acids from the natural food may have a potential in improving the nutritive value of feather meal. The current study was conducted to compare the efficacy of hydrolyzed feather meal protein on growth, apparent digestibility and survival of *O. niloticus* under intensive and semi-intensive culture conditions, and also to evaluate whether there are any additional nutritional benefits derived from natural food.

# III.3. Materials and Methods

## III.3.1. Site and facility descriptions

The study was conducted at Sagana Fish Farm (90 km northeast of Nairobi, altitude 1230 m, latitude 0°39′S and longitude 37°12′E). Two experiments were conducted in laboratory aquaria each with a dimension of 0.45m x 0.3m x 0.3m, and net cages with a top frame of 1.2 x 0.94 m and a base (0.9x0.9m) and a height of 0.75m that were installed in a 20m x 40m x 0.75m earthen pond. The aquaria were set in a thermoregulated recirculating system, comprising a settling tank for solids removal and an aerobic bio filter (tickling filter) to remove ammonia. All cages were installed in a single pond.

## III.3.2. Pond and water quality management

The experimenta pond was fertilized weekly at a rate of 20 kg N and 8 kg P $ha^{-1}$ with Urea and diammonium phosphate (DAP), respectively, and limed once at 2500 kg $ha^{-1}$ with $CaCO_3$ at the beginning of the experiment. Key water quality parameters, which included temperature, pH, dissolved oxygen (DO) and chlorophyll *a* were measured three times a week in the aquaria and cage experiments. DO was measured using model 57 oxygen meter (YSI industries, Yellow springs, OH, USA), while a glass electrode pH meter, Hi-9024 microcomputer (Hanna Instruments Ltd., Chicago, IL., USA), was used to take pH measurements. Chlorophyll *a* was determined as described in APHA (1990)

## III.3.3. Sources and processing of feed ingredients

Chicken feathers were collected from hotels around Sagana town and sun-dried. They were reduced to smaller sizes with a hammer mill, and thereafter hydrolysed in a cooking autoclave at 16 PSI (pounds per square inch) and about 110° C for 3 hours. The hydrolysed feather was sun-dried dried and further reduced to a fine powder using a blender model A989 liquidizer (Hampshire, UK). Freshwater water shrimp meal was purchased from Kisumu fish supply stores, while cotton seed cake was sourced from a local feed manufacturer. Wheat bran was purchased from a nearby wheat-processing factory. Carboxyl methyl-cellulose was used as a filler material in the diet formulations. All the ingredients were ground in to fine powder before being subjected to proximate analysis.

### III.3.4. Proximate analysis and diet formulation

The nutritional composition of the ingredients was analysed prior to formulations. The proximate analysis of the feeds was carried out in triplicates as described in AOAC (1995). The analyses involved the following nutrients: crude protein (CP), ether extract (EE), ash, nitrogen free extracts (NFE) and crude fibre (CF). Crude protein was estimated from Kjedahl nitrogen, while crude lipid was quantified as the loss in weight after extraction of the sample with petroleum ether (40-60 °C). Ash was determined by burning dry samples in a muffle furnace at 550 °C for 4 hours. Crude fibre was determined by alkaline/acid digestion, which was followed by ashing the dry residue at 550 °C in a muffle furnace for 4 hours. NFE was determined by the difference method (DM-CP-EE-CF-Ash). The proximate composition of experimental diets is shown in Table III.1. The indigenous inert mark for digestibility was determined using the method described by Bowen (1981).

***Table III.1**: Proximate composition of the feedstuffs used in diet formulation (g $kg^{-1}$); DM=Dry matter, SD=standard deviation, CP=Crude protein, EE=Ether extracts*, CF=Crude Fiber, NFE=Nitrogen Free Extracts. [1)] FSM = Freshwater shrimp meal [2)], HFM = Hydrolysed feather meal, * All substances soluble in petroleum ether.*

| Ingredients | Nutrient (mean SD) | | | | | |
|---|---|---|---|---|---|---|
| | **DM** | **CP** | **EE** | **CF** | **NFE** | **Ash** |
| FSM [1)] | 875±0.8 | 603±0.3 | 14±0.4 | 62±0.0 | 67±1.3 | 248±0.2 |
| HFM[2)] | 899±0.8 | 807±0.2 | 18±0.2 | 32±0.2 | 42±1.3 | 101±0.1 |
| Cotton seed meal | 898±0.5 | 349±0.2 | 128±0.1 | 258±0.4 | 194±0.3 | 60±0.1 |
| Wheat bran | 880±1.2 | 140±0.1 | 59±0.1 | 136±0.3 | 602±0.6 | 63±0.3 |

### III.3.5. Diet formulation and preparation

Five experimental diets, each with 25% CP and 2.94 kcal $g^{-1}$ energy were formulated by substituting FSM protein with HFM at 0, 2.25, 4.5, 6.75 and 9.4 % of HFM, representing substitution rates of 0, 25, 50, 75 & 100%, respectively. Each ingredient was homogeneously ground and passed through a 200µm sieve. Carboxyl methyl-cellulose was used as a filler material to top formulations to 100%. The formulations were made by mixing the necessary ingredients into a homogenate, which was moistened before passing through a modified meat mincer. The resulting expeller-like strands were sun-dried and stored at room temperature. The composition of experimental diets and their biochemical proximate composition are shown in Table III.2.

***Table III.2**: Composition and results of proximate analysis of experimental diets; DE, digestible energy; GE, gross energy; NFE, Nitrogen Free Extracts; SD, standard deviation.*

| Ingredients | Control (0%) | Content (%) 25% | 50% | 75% | 100% |
|---|---|---|---|---|---|
| Freshwater shrimp meal | 12 | 9 | 6 | 3 | 0 |
| Hydrolysed feather meal | 0 | 2.25 | 4.5 | 6.75 | 9.4 |
| Cotton seed cake | 25 | 25 | 25 | 25 | 25 |
| Wheat bran | 63 | 63 | 63 | 63 | 63 |
| Carboxyl methyl cellulose | 0 | 0.75 | 1.5 | 2.25 | 2.6 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Proximate analysis (± SD) | | | | | |
| Moisture (%) | 88.5±0.8 | 88.7±0.5 | 90.1±0.9 | 89.2±0.7 | 90.3±0.6 |
| Protein (%) | 28.5±0.2 | 29.1±0.1 | 29.8±0.4 | 30.4±0.3 | 31.0±0.5 |
| Ether extract (%) | 5.6±0.7 | 6.6±0.5 | 4.6±0.8 | 5.6±0.2 | 6.6±0.6 |
| Crude fibre (%) | 14.8±1.2 | 16.7±1.0 | 16.6±2.2 | 15.5±1.4 | 14.4±2.0 |
| NFE (%) | 43.2±2.3 | 40.1±3.1 | 42.0±2.6 | 41.9±3.3 | 41.8±2.7 |
| Ash (% | 7.9±0.1 | 7.5±0.2 | 7.0±0.4 | 6.6±0.1 | 6.2±0.3 |
| DE (kcal $g^{-1}$) | 2.94 | 2.94 | 2.94 | 2.94 | 2.94 |

## III.3.6. Experimental animals, design and feeding

The diets were randomly allocated to groups of hand-sexed male *O. niloticus* fingerlings that were held in aquaria and cages. Those in aquaria were fed all the five diets in five treatments, while the caged fish were fed diets at substitution levels of 0, 50 and 100% in three treatments; both experiments had three replicates per treatment. The initial average stocking weight in aquaria and cage experiment was 26, 32g, respectively, at densities of 6 and 15 fish, respectively. Fish were acclimatised for two weeks prior to start of each experiment. Feed was offered at 10% of body weight per day for 84 days. Sampling was done on a bi-weekly basis to monitor growth and adjust feed rations. Fish were fed using the automated feeders described in Waidbacher *et al.* (2006). The feeders were calibrated to deliver feed continuously between 8.00 and 18.00 hrs. At the end of the study, all fish from cages and aquaria were harvested, weighed and counted.

### III.3.7. Evaluation of dietary performance

The growth performance of the experimenta diets was evaluated by measuring growth performance parameters (final harvest mean weight, weight gain and specific growth rate) and apparent protein digestibility coefficient. Specific growth rate (SGR) was calculated using the following equation:

$$SGR(\%) = \frac{(\ln Wf - \ln Wi)x100}{t}$$

Where Wi and Wf are the initial and final mean body weights, respectively and t is time in days from stocking to harvest.

The apparent digestibility coefficient of protein (ADCp) was calculated using the following formula:

$$ADCp(\%) = \frac{100x(1-(\%MD)x(\%NF)}{(\%MF)x(\%ND)}$$ (Maynard & Loosli 1962)

Where NF is the nutrient in faeces; ND is the nutrient in the diet; MD, marker in the diets, and MF is marker in the faeces.

### III.3.8. Data analysis

Data were subjected to a one-way analysis of variance (ANOVA). Where appropriate, data were transformed to conform to the requirements of the test. Duncan multiple (Duncan 1955) range tests was applied to identify means that were significantly different from each other. The relationship between HFM inclusion and ADCp, and comparison of regression coefficients were evaluated by applying linear regression analysis and analysis of covariance as described in Sokal & Rohlf (1981). A type I error of 0.05 was used to declare significance.

# III.4. Results

## III.4.1. Water quality

Water quality did not vary significantly (P>0.05) among treatments, both in cages and in aquaria over the culture periods. The mean value for chlorophyll *a* was 162.4±17 mg $m^{-3}$. The ranges of DO values were 3-4 mg $L^{-1}$ for morning and 8-10 mg $L^{-1}$ for afternoon. Water temperature ranged from 24.3 to 28.9 °C (mean= 27.0±0.5 °C) in the pond, and was maintained between 26 and 28 °C (mean=27.0±0.043 °C) in the aquaria. The pH values ranged from 7.8 to 8.3 (mean=8.1±0.03) in the experimental pond and 7.0-7.5 (mean=7.2±0.01) in the aquaria experiment. Water quality parameters were also within the recommend values for tilapia culture.

## III.4.2. Growth performance

Data on fish growth performance in aquaria and cages are presented in Table III.3 & III.4, respectively. Fish that were fed diets substituting 0, 25 and 50% FSM in aquaria had similar mean weight, specific growth rate and percent weight gain but grew significantly better (P<0.05) than those fed diets in which 75 and 100% of FSM was substituted by HFM. There were no significant differences (P>0.05) in growth performance among treatments of fish that were fed in the cages. Survival rate was high and very similar (P>0.05) among treatments both in aquaria and cages (Table III.3 & III.4).

***Table III.3**: Growth performance of O. niloticus fed diets in aquaria containing increasing levels of HFM in place of FSM; values with the same superscript are not significantly different at α=0.05.*

| Parameter | Treatment<br>Rate of FSM substitution | | | | | |
|---|---|---|---|---|---|---|
| | 0 % | 25 % | 50 % | 75 % | 100 % | SE |
| Initial body weight (g) | 26.5a | 26.0a | 26.2a | 26.5a | 26.2a | 0.35 |
| Final body weight (g) | 43.4a | 41.9a | 41.2a | 34.2b | 33.4 b | 0.91 |
| Weight gain (%) | 67.1a | 61.3a | 58.3a | 31.4b | 28.4b | 3.51 |
| Specific growth rate (% day-1) | 0.61a | 0.57a | 0.54a | 0.32b | 0.30b | 0.03 |
| Survival (%) | 97.6a | 95.3 a | 95.3a | 95.3a | 100a | 1.91 |

***Table III.4**: Growth performance of O. niloticus fed diets in cages containing decreasing levels of hydrolysed feather meal in place of freshwater shrimp meal; values with the same superscript are not significantly different at α=0.05; SE, standard error of mean.*

| Parameter | Treatments | | | |
|---|---|---|---|---|
| | 0 % | 50 % | 100 % | SE |
| Initial mean weight (g ) | 37.0a | 35.4a | 36.0a | 1.00 |
| Final harvest mean weight (g ) | 83.2a | 82.2a | 77.5a | 3.40 |
| Mean weight gain (% ) | 131.2a | 128.2a | 115.7a | 9.44 |
| Specific growth rate (% day-1) | 0.99a | 0.98a | 0.91a | 0.05 |
| Survival (%) | 97.6a | 95.3a | 100a | 1.91 |

Growth trend curves for *O. niloticus* in cages and aquaria are presented in Figures III.1 & III.2, respectively. In aquaria, fish receiving diets substituting up to 50% of FSM separated from the other treatments two weeks after stocking. Similarly, growth curves in cages in the control diet (0% HFM) registered the highest growth, while diet 5 (100% HFM substitution of FMS) resulted in the lowest growth. The growth of fish that fed on diet 3 was intermediate, although the differences were not significant (P>0.05).

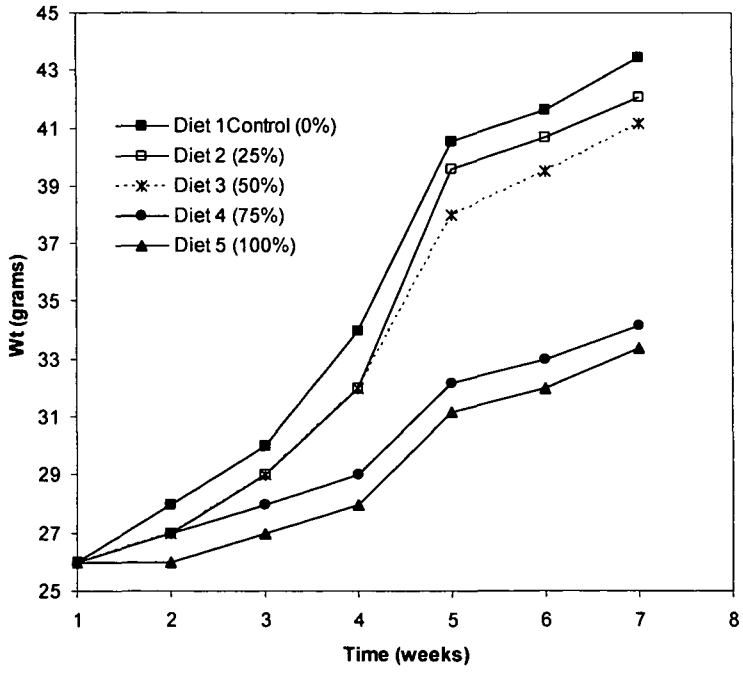


***Figure III.1**: Growth curves for O. niloticus receiving formulated diets with varying levels of HFM in aquaria during 84 days culture period.*

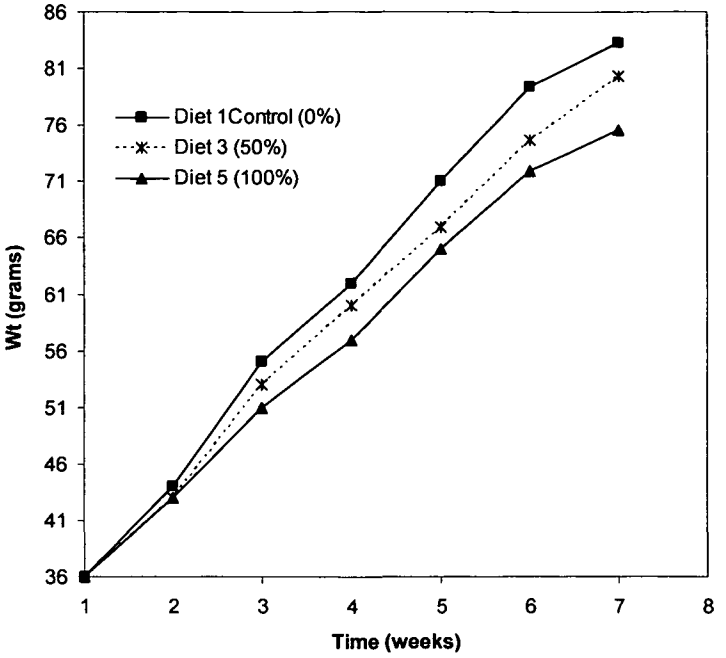


***Figure III.2**: Growth curves for O. niloticus receiving formulated diets with varying levels of HFM in cages installed in a fertilized earthen pond during 84 days culture period.*

### III.4.3. Digestibility performance and trends

Data on apparent protein digestibility ($ADC_p$) in aquaria and cages are shown in Table III.5 and Figure III.3. There were significant differences ($P<0.05$) in $ADC_p$ among treatments in the aquaria experiment. The $ADC_p$ values decreased with increasing levels of HFM both in cages and aquaria, with regression slopes of -0.26±0.02 and -0.098±0.02, respectively. The regression coefficient for $ADC_p$ was significantly ($P<0.05$) more positive for caged than aquaria fish.

***Table III.5**: Percent apparent protein digestibility coefficient (APDC) in cages and aquaria; Values with the same superscript are not significantly different at α=0.05; ADCp Apparent Protein Digestibility Coefficient.*

| Parameter | 0% | 25% | 50% | 75% | 100% | SE |
|---|---|---|---|---|---|---|
| ADCp( cage) | 64.5a | - | 64.0a | - | 54.6b | 0.39 |
| ADCp (aquaria) | 62.2a | 58.0b | 52.2c | 46.5d | 34.7e | 0.96 |

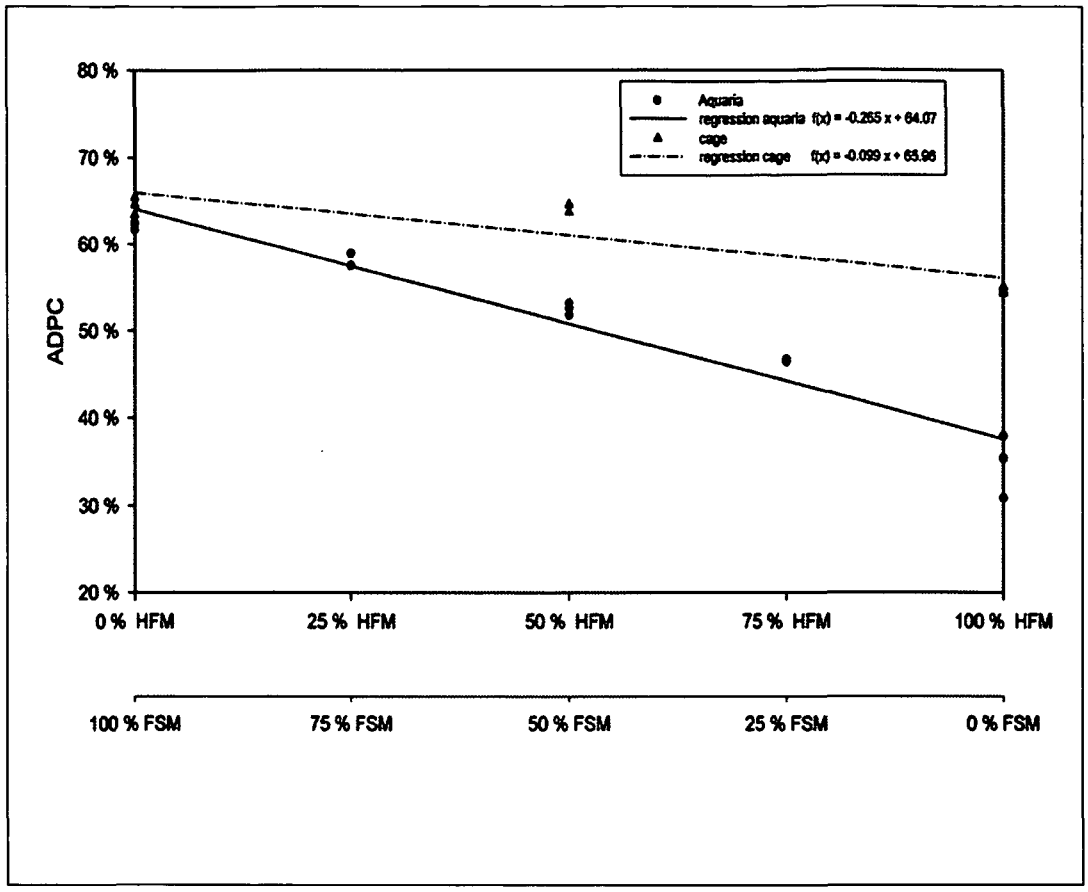


***Figure III.3**: Regression lines showing the trends of apparent protein digestibility coefficient of O. niloticus diets and level of HFM.*

## III.5. Discussion

Data from the present study indicated that growth of groups of *O. niloticus* that were fed diets containing a mixture of freshwater shrimp (FSM) and hydrolyzed feather meal (HFM) protein in cages was similar to that of the control treatment. However, there was a significant decline in fish growth when fish were fed diets containing over 4.5 % of HFM (50% protein substitution level) in aquaria.

Specific growth of fish in cages was higher than in the aquaria experiment in the present study. The differences in growth between the caged and aquaria fish in the present study may be attributed to differences in culture conditions. The aquaria experiment in the present study took place in indoor laboratory environments, while the caged experiment was conducted in a fertilized earthen pond. This setup offered fish extra nutrition from natural food in the pond, which may have supplemented the deficit of essential amino acids in the diets. HFM has been reported to be deficient in some essential amino acids (Falaye 1982; Mendoza *at al.* 2001; Tacon *et al.* 1984); therefore, the interaction between the components of pond's natural food and feather meal may have improved the essential

amino acid profiles of the latter, thus resulting in better utilization and growth performance of fish in the cages.

The findings from the aquaria experiment are similar to those reported by other investigators. Falaye (1982) did not observe a significant decrease in growth parameters (SGR, weight gain, mean weight) of *O. niloticus* when 50% of FM was replaced with HFM. Similar results were reported by Viola & Zohar (1984), who reported that up to 50% of FM could be replaced by poultry by-products in diets of tilapia hybrids without deleterious effects on fish growth. However, Bishop *et al.* (1995) reported a slightly higher substitution level (66%) of fish meal protein with HFM protein in diets for *O. niloticus* without significant reduction in growth. The higher substitution level reported by Bishop *et al.* (1995) compared to that of the present study in the aquaria experiment may be attributed to differences in culture conditions. The present experiment was conducted in aquaria, which were cleaned every day, while that of Bishop *et al.* (1995) was conducted in concrete tanks where development of natural food was possible.

The decrease in growth observed in aquaria in the present study when fish were fed diets containing over 50% HFM may be due to sub-optimal levels of essential amino acids (Jauncey & Ross 1982; NRC 1983; Santiago & Lovell 1988) in HFM, which might have hindered proper utilization of dietary HFM protein and its subsequent synthesis to body protein. Attempts have been made to improve the nutritive value of feather meal-based diets for some aquatic species by supplementation of deficient amino acids (Tiews *et al.* 1979, Hughes 1990, Steffens 1994). However, Falaye (1982) reported decreased performance of fish growth when HFM contributed over 50% of the animal protein even after some deficient amino acids were supplemented. The author suggested that growth inhibition might have involved factors other than amino-acid imbalances.

Reports on utilization of supplementary amino acids are varied and conflicting, with some authors reporting improved growth of fish after supplementation (Webster *et al.* 1991; Murai *et al.* 1982), while others report no response in growth (Ng & Wee 1979; El-Sayed 1990). Other investigators have suggested that utilization of amino acids is dependent on the meal frequencies (Webster *et al.* 1991). When fish are fed once a day, the added amino acids were converted into other compounds, and are thus not utilized for protein synthesis (Thebault 1985). However, when channel catfish was fed diets containing L-lysine twice a day, growth was improved to the level of the control diet (Webster *et al.* 1991). Under natural or semi-natural conditions, *O. niloticus* feed continuously during day time (Moriarty 1973; Getachew & Fernando 1989; Zenebe & Getachew 1998); thus based on the hypothesis of meal frequency, utilization of free amino acids might be enhanced. Bowen (1980) reported that tilapias could thrive well on naturally occurring dissolved

amino acids. This mode of feeding ensures that free amino acids are supplied continuously at the site of tissue formation. The observation by Bowen (1980) and the lack of differences in growth performance between the control and the diets containing HFM in the cages in the present study suggest that supply of amino acids from natural food may be an effective and economical strategy of supplementing the limiting amino acids in tilapia diets.

The method used to process feathers in the present study produces two categories of essential amino acids, a batch of free amino acids and those contained in the remaining polypeptides and intact protein. The set of free essential amino acids are irreversibly absorbed before those in the polypeptides and intact protein are liberated for tissue protein synthesis (Yamada *et al.*, 1981; Cowey & Walton 1988), leading to poor utilization of feather meal. However, the continuous supply of amino acids from natural food could enhance utilization of feather meal by upgrading the profile of both free essential amino acids, and those contained in units of polypeptides and protein after liberation.

In the present study, $ADC_p$ declined with increasing levels of HFM the diets. This trend was similar to that reported by Degani *et al.* (1997) and Yang *et al.* (2006), who observed a decrease in $ADC_p$ with increasing levels of feather meal in Carp (*Cyprinus carpio* L.) and gibel carp (*Carassius auratus gibelio* Bloch). However, the values of APDp calculated in the present study are lower than those reported for *O. niloticus* by Hanley (1987). The discrepancy may be attributed to the composition of the test diets. Hanley (1987) tested hydrolyzed feather meal as a single ingredient diet, while in the present study, the diets consisted of mixtures of ingredients, which may have contributed to the low apparent digestibility coefficient of protein in the feather based diets.

The regression slope for the apparent protein digestibility (b= -0.098) of feather meal based diets in the cage experiment was significantly higher than the values calculated for fish in aquaria (b=-0.26). Since fish in the cages had access to natural food, there was an added component from the natural food, which was resistant to digestion. Such a component would elevate the level of inert marker relative to that in the food, thus increasing the digestibility coefficient. However, this would occur across the treatments without necessarily changing the slope from that for in the aquaria experiment; therefore, the two slopes would be similar. It appears from the present study that the presence of natural food improved the protein digestibility in feather meal based diets in the cage experiment. The mechanism for the improvement may be linked to the presence of algal based diets. Algae or algal based diets are reported to stimulate secretion of copious amount of gastric acid in *O. niloticus* and *O. mossambicus* (Getachew 1987, 1989; Bowen

1981). Therefore, under the highly acidic medium, further degradation of HFM protein is likely to occur with enhancement in digestibility.

In conclusion, the present study demonstrated that hydrolyzed feather meal could substitute freshwater shrimp meal up to 50% in the diets of *O. niloticus* under intensive culture conditions, while 100% substitution was possible without compromising growth under semi-intensive culture conditions.

## III.6. Acknowledgements

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# IV. Use of different levels of locally available hydrolysed feather meal and Pawpaw (*Papaya carica*) leaves – a contribution to increasing sustainability of Nile tilapia (*Oreochromis niloticus L.*) production

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## IV.1. Abstract

A study was conducted to evaluate the effects of incorporating varying levels of hydrolysed feather meal (HFM) and papaya leaf meal (PLM) into diets for Nile tilapia (Oreochromis niloticus L.) on growth, digestibility and survival rate under laboratory and cage-cum-pond culture conditions. Fingerlings averaging 22 and 30g were held indoor in aquaria with recirculating water, and in cages that were installed in a fertilized earthen pond of 800 m2, respectively. Five isonitrogenous (250g CP kg-1) and isocaloric (2,940 kcal kg-1) diets were formulated using different levels of wheat bran and carboxyl methyl cellulose for balancing CP and energy contents: three diets contained 6 % of freshwater shrimp meal (FSM) and 4.5 % HFM (control), supplemented either with papain (treatment 2) or 4.5% PLM (treatment 3). In two diets FSM was substituted by 8.6% HFM plus papain (treatment 4) or 8.6% HFM plus 8.6% PLM (treatment 5). All fish were fed at 10% body weight $day^{-1}$ in three replicates for 58 days. Results indicated that in aquaria including of HFM and PLM at levels above 4.5% each led to significant ($P<0.05$) growth reductions. However, substituting FSM with HFM at 8.6% did not significantly ($P>0.05$) affect growth of fish in the cages. In both experiments, survival was similar among

treatments, but protein digestibility decreased with increasing levels of HFM in the diet. In conclusion, a combination of the protein sources FSM, HFM and PLM tends to give the highest growth performance in both aquaria and cages the growth depression observed for treatments 4 and 5 in aquaria was not observed in the cages, where the natural food may have provided an important nutrient supplement.

**Key words**

Hydrolyzed feather meal, *Papaya carica* leaves, *O. niloticus*, growth, digestibility.

## IV.2. Introduction

Aquaculture production in Africa is relatively low and represents about 1.1 percent of the total world production (FAO, 2006). Most of the African aquaculture production is located in the Mediterranean region, representing over 65% of the total African production (Pedini & Shehadeh, 1997). A review conducted by FAO (2005) reported that sub-Saharan countries are behind in aquaculture development, mainly due to lack of a well developed aquaculture fish feed production. Furthermore, Shang (1992) and Craig & Helfrich (2002) emphasized the importance of inexpensive and efficient feeds in fish farming, because fish feeds represent over 50 % of the total variable production costs.

Tilapia culture is one of the fastest growing forms of aquaculture worldwide with more than 1,265,000 tons produced in 2000 (FAO, 2002). Among the tilapias, Nile tilapia (*Oreochromis niloticus*) is the principle culture species and represents 44% of total global tilapia (Rana, 1997). Stickney (1986) observed that *O. niloticus* are excellent candidates for semi intensive aquaculture production because of their rapid growth, tolerance to poor water quality, high reproductive rates and relatively few problems with diseases. Furthermore, *O. niloticus* feed low on the trophic level in nature and is therefore accustomed to gaining much of their nutritional needs from ponds natural food (Fitzsimmons, 1997; Philippart & Ruwet, 1982). These attributes makes *O. niloticus* a suitable choice for fish farmers within sub-Saharan Africa where semi-intensive culture in fertilized earthen ponds is the main aquaculture practice.

Despite this, fishmeal still remains an important protein component in diets for *O. niloticus* (Abdelghany, 2003; Tacon, 1993). A number of studies have evaluated various plant protein sources as substitutes for fishmeal in fish feed (El-Saidy & Gaber, 2003; Gaber, 2006; Bureau *et al.*, 1998; Liti *et al.*, 2006; Olvera-Novoa *et al.*, 2002; Hughes, 1991). However, complete replacement of fishmeal with plant protein has generally resulted in a decrease in *O. niloticus* growth performance (Mbahinzirek *et al.*, 2001; Sklan

*et al.*, 2004). This has been attributed to sub-optimal supply with essential amino acids, and besides this, some plant feedstuffs contain anti-nutritional factors, which reduce their efficiency of utilization (Tacon 1997; Lovell 1980; Hossain *et al.* 2003; Bureau *et al.* 1998; Shao *et al.*, 2002; Fagbenro 1998).

To overcome these limitations of unbalanced essential amino acids and antinutritional factors, research on feed substitutes in *O. niloticus*, has focused more on non-conventional animal protein and a mixture of plants and animal feedstuffs (Robinette, & Dearing, 1978; Borgeson *et al.*, 2006; Bishop *et al.*, 1995; El-Sayed, 1998; Fowler, 1982; Liti *et al.*, 2005; El-Saidy & Gaber, 2003). Among the studies Liti *et al.* (2005) evaluated diets which contained whole freshwater shrimp (*Caridina nilotica*) meal as the sole source of animal protein and realised that they were more efficient in promoting fast growth of *O. niloticus* than those containing plant protein sources. In another study, Jauncey & Ross (1982) evaluated the use of various body parts of shrimp meal as potential sources of animal protein in fish feeds and reported good growth. In Kenya, however, fresh water shrimps which are mainly from lake Victoria fishery is increasingly becoming scarce in the market due to competition from livestock feed manufactures and frequent closures of the lake's fishery during breeding season. Therefore, there is urgent need to find inexpensive, effective and locally available replacements of FSM in *O. niloticus* feeds.

*Papaya carica* plants are widespread in Kenya, and their leaves are not used for human food. Proximate analysis in a recent study revealed a good crude protein profile in the green leaves (Munguti *et al.*, in press). Chaplin (2005) reported that the papaya leaf and the unripe fruit contain papain, which degrades protein into amino acids. Further, Buchanan (1969) reported that papain promotes proteolytic digestion and thereby increases the protein digestibility of papaya leaf meal. On the other hand, poultry farming is a common practice and is widespread across Kenya and generates large volumes of feathers as a by-product, which can be obtained at no cost. The protein content of feather meal is high (Hasan *et al.*, 1997; Tacon *et al.*, 1984), and utilization of hydrolyzed feather meal protein in tilapia feeds could economically be feasible, but studies conducted to evaluate the use of hydrolysed feather meal (HFM) in fish diets indicated low substitution levels due to low digestibility and sub-optimal levels of essential amino acids (Jauncey & Ross, 1982; Hasan *et al.*, 1997; Falaye, 1982; Steffens, 1994; Santiago & Lovell, 1988; Mendoza *et al.* 2001). A combination of HFM and papaya leaf meal (PLM) may promote the feeding value of HFM and by extension promote its use in *O. niloticus* feeds. Liti *et al.* (2005) reported that in Kenya lack of feeds specifically designed for semi-intensive culture of *O. niloticus* has forced farmers to utilize feeds designed for intensive culture systems and this has resulted to increased production costs.

Therefore the current study was conducted to evaluate the effects of a varying rate of inclusion of *papaya carica* leaves and hydrolyzed feather meal on growth performance and apparent digestibility of *O. niloticus* under intensive (aquarium) and semi-intensive (cage-cum-pond) culture conditions.

## IV.3. Materials and Methods

### IV.3.1. Site and facility descriptions

A pre *in vitro* digestibility test was conducted to determine the effect of pure papain obtained from CARLROTH - GMBH, Karlsruhe, Germany on hydrolysed feather meal. 15 ml of distilled water and 20 mg of Papain were added to each of two batches of 30 g feathers, which have been hydrolysed either at pH 5 or at pH 9. After stirring this mixture it was maintained at room temperature for 19 hours. An aliquot of 2 g was subjected to *in vitro* N-digestibility analysis: after addition of 480 ml distilled water and 10 ml HCl (25 %), the samples were incubated at 40°C for 24 hours. After adding 10 ml HCl (25 %) for the second time, incubation continued for another 24 hours, after which the suspension was filtrated and washed with hot water until pH 7 was reached. The filters, together with the residue were subjected to Kjeldahl analysis (AOAC, 1995). The result was interpreted as "indigestible" N and i*n vitro* digestibility was calculated as the relative difference between total and "indigestible" N and expressed as percentage.

The *in vivo* studies were conducted at Sagana Fish Farm (90 km northeast of Nairobi, altitude 1230 m, latitude 0°39′S and longitude 37°12′E). Two experiments were conducted in laboratory aquaria each with a dimension of 0.45m x 0.3m x 0.3m; net cages with a top frame of 1.2 x 0.94m and a base (0.9 x 0.9m) and a height of 0.75m were installed in a 20m x 40m x 0.75m earthen pond. The aquaria were set in a thermoregulated recirculating system, comprising a settling tank for solids removal and an aerobic bio filter (tickling filter) to remove ammonia.

### IV.3.2. Pond and water quality management

The experimental pond was fertilized weekly at a rate of 20 kg N and 8 kg P $ha^{-1}$ with Urea and diammonium phosphate (DAP), respectively, and limed once at 2500 kg $ha^{-1}$ with $CaCO_3$ at the beginning of the experiment. Key water quality parameters, which included temperature, pH, dissolved oxygen (DO) and chlorophyll *a* were measured three

times a week in the aquaria and cage experiments. DO was measured using model 57 oxygen meter (YSI industries, Yellow springs, OH, USA), while a glass electrode pH meter, Hi-9024 microcomputer (Hanna Instruments Ltd., Chicago, IL., USA), was used to take pH measurements. Chlorophyll *a* was determined as described in APHA (1990)

### IV.3.3. Feed ingredients and diet formulation

Green papaya leaves were collected in gardens at the neighbourhood of Sagana aquaculture centre while chicken feathers were sourced from hotels around Sagana town and sun-dried. They were reduced to smaller sizes with a hammer mill, and thereafter hydrolysed by cooking them in an autoclave at mean pressure of 40-50 psi and a mean temperature of 140-150° C for one hour at a pH of 9. After feather hydrolysation, part of the wet hydrolyte was mixed with green papaya leaves using a blender-liquidizer (model A989, Hampshire, UK) and left overnight. The hydrolysed feathers as well as the mixture of papaya leaves and the remaining part of hydrolysed feathers were sun-dried dried and further reduced to a fine powder using the blender- liquidizer as given above. Freshwater water shrimp meal was purchased from Kisumu fish supply stores, while cotton seed cake was supplied by Goldstar Company, Nairobi. Wheat bran was purchased from Maisha millers, Nyeri. Carboxyl methyl-cellulose was used as a filler material in the diet formulation. All the ingredients were ground to fine powder before being subjected to proximate analysis (Table IV.1).

***Table IV.1**: Proximate composition of the feedstuffs used in diet formulation (g $kg^{-1}$); SD=Standard deviation, DM=Dry matter, CP=Crude protein, EE=Ether extracts*, CF=Crude Fiber, NFE=Nitrogen Free Extract, FSM=Freshwater shrimp meal, HFM=Hydrolysed feather meal.*

| Ingredients | Nutrient (mean ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | DM | CP | EE | CF | NFE | Ash |
| FSM | 875±0.8 | 603±0.3 | 14±0.4 | 62±0.0 | 67±1.3 | 254±0.2 |
| HFM | 899±0.8 | 807±0.2 | 18±0.2 | 32±0.2 | 42±1.3 | 101±0.1 |
| Cotton seed meal | 898±0.5 | 349±0.2 | 128±0.1 | 258±0.4 | 205±0.3 | 60±0.1 |
| Papaya leaf meal | 901±0.5 | 253±0.2 | 97±0.1 | 116±0.4 | 395±0.3 | 138±0.1 |
| Wheat bran | 880±1.2 | 140±0.1 | 59±0.1 | 136±0.3 | 602±0.6 | 63±0.3 |

Five experimental diets, each containing 250 g $kg^{-1}$ of CP and 2,940 kcal $kg^{-1}$ energy were formulated: three diets contained 6% of freshwater shrimp meal (FSM) and 4.5% HFM (control), supplemented either with papain (treatment 2) or 4.5 % PLM (treatment 3). In two diets FSM was substituted by 8.6% HFM plus papain (treatment 4) or 8.6% HFM plus 8.6% PLM (treatment 5). In order to keep energy and CP levels constant, diets contained different levels of wheat bran and carboxyl methyl-cellulose as a filler.

Each ingredient was homogeneously ground and passed through a 100µm sieve. The formulations were made by mixing the ingredients into a homogenate, which was moistened before passing through a modified meat mincer. The resulting expeller-like strands were sun-dried and stored at room temperature. The composition of experimental diets and their proximate composition are shown in Table IV.2.

***Table IV.2**: Composition and results of proximate analysis on dry matter basis of experimental diets; DE=digestible energy, NFE=Nitrogen Free Extracts, SD=standard deviation.*

| Ingredients | Treatment 1 (control) | Treatment 2 (control + papain) | Treatment 3 (control + papaya leaves) | Treatment 4 (substitution of FSM + papain) | Treatment 5 (substitution of FSM + papaya leaves) |
|---|---|---|---|---|---|
| Freshwater shrimp meal, % | 6.0 | 6.0 | 6.0 | 0.0 | 0.0 |
| Hydrolysed feather meal, % | 4.5 | 4.5 | 4.5 | 8.6 | 8.6 |
| Cotton seed cake, % | 25 | 25 | 25 | 25 | 25 |
| Wheat bran, % | 60 | 60 | 53 | 60 | 45 |
| Papaya leaves, % | 0.0 | 0.0 | 4.5 | 0.0 | 8.6 |
| Carboxyl methyl cellulose, % | 4.5 | 4.5 | 7.0 | 6.4 | 12.8 |
| Papain (g/kg) | - | 0.5 | - | 0.5 | - |
| Proximate analysis (± SD) | | | | | |
| Moisture (%) | 90.2±0.7 | 89.9±0.4 | 90.1±0.8 | 89.7±0.5 | 90.5±0.3 |
| Protein (%) | 25.2 ±0.1 | 25.6 ±0.3 | 25.7±0.1 | 26.1±0.3 | 25.9±0.3 |
| Ether extract (%) | 6.1±0.4 | 6.0±0.5 | 6.2±0.1 | 5.8±0.1 | 5.9±0.5 |
| Crude fibre (%) | 14.9±1.1 | 15.3±1.2 | 16.9±1.3 | 15.9±1.1 | 16.7±1.7 |
| NFE (%) | 46.1±1.3 | 45.5±1.9 | 43.8±1.5 | 46.1±1.7 | 45.6±1.8 |
| Ash (%) | 7.7±0.3 | 7.6±0.2 | 7.4±0.1 | 6.1±0.3 | 5.9±0.2 |
| DE (kcal $g^{-1}$) | 2.94 | 2.94 | 2.94 | 2.94 | 2.94 |

### IV.3.4. Proximate analysis

The nutritional composition of the ingredients was analysed prior to diet formulation. The proximate analysis of the feeds was carried out in triplicates as described in AOAC (1995). The analyses involved the following nutrients: dry matter (DM), crude protein (CP), ether extract (EE), ash, nitrogen free extracts (NFE) and crude fibre (CF). CP was estimated from Kjedahl nitrogen, while EE was quantified as the loss in weight after extraction of the sample with petroleum ether. Ash was determined by ashing dry samples in a muffle furnace at 550 °C for 4 hours. CF was determined by a consecutive alkaline-acid digestion, which was followed by ashing the dry residue at 550 °C in a muffle furnace for 4 hours. NFE was determined by the difference method (DM-CP-EE-CF-Ash; ALVA, 1983). Amino acid were analysed using HPLC after a 20 hour hydrolysation process with 6 molar HCl and a previous stabilisation with $Ba(OH)_2$ of tryptophan. Separation of amino acids was made using a einer hyperphil ODS 250 x 4 mm-column after pre-column derivatisation with OPA (orthophtalaldehyde) (ALVA, 1983; Degussa, 1986; Altmann, 1992; Commission of the European Union, 1998). Amino acid compositions for the different feed ingredients are shown in Table IV.6. The indigenous inert marker (insoluble ash) for digestibility was determined using the method described by Bowen (1981).

***Table IV.6**: Amino acid requirements for O. niloticus and digestible amino acid composition of feed ingredients (g $kg^{-1}$ DM) AA=Amino acid, FSM=freshwater shrimp meal, HFM=Hydrolysed feather meal, PLM=Papaya leaf meal, WB=wheat bran, CSM=Cotton seed meal. Amino acid requirements for* Nile tilapia *(Santiago & Lovell, 1988).*

| | | Experimental diet ingredients | | | | |
|---|---|---|---|---|---|---|
| **Amino acid (%)** | ***O. niloticus* AA requirements (g/kg DM)** | **HFM** | **PLM** | **FSM** | **WB** | **CSM** |
| Lysine | 51.2 | 14.9 | 12.7 | | | |
| Methionine | 26.8 | 3.9 | 4.1 | | | |
| Threonine | 37.5 | 38.4 | 10.5 | | | |
| Tryptophan | 10.0 | 5.5 | 4.5 | | | |
| Arginine | 42.0 | 70.2 | 12.0 | | | |
| Phenylalanine | 37.5 | 46.4 | 11.8 | | | |
| Histidine | 17.2 | 4.7 | 6.4 | | | |
| Isoleucine | 31.1 | 46.4 | 8.4 | | | |
| Leucine | 33.9 | 82.9 | 15.5 | | | |
| Valine | 28.0 | 74.5 | 11.2 | | | |

### IV.3.5. Experimental animals, design and feeding

The diets were randomly allocated to groups of hand-sexed male *O. niloticus* fingerlings that were held in aquaria and cages. The experimental diets were tested both in aquaria and cages, using three replicates per treatment. The initial average stocking weight in the aquaria and cage experiment was 23 and 30g, respectively, at densities of 6 and 10 fish, respectively. Fish were acclimatised for two weeks prior to start of each experiment. Feed was offered at 10% of body weight per day for 58 days. Sampling was done on a bi-weekly basis to monitor growth and adjust the amount of feed offered. Fish in aquaria were hand fed four times a day while those in cages were fed using the automatic feeders described by Waidbacher *et al.* (2006). The feeders were calibrated to deliver feed continuously between 8.00 and 18.00 hrs. At the end of the study, all fish from cages and aquaria were harvested, weighed and counted.

### IV.3.6. Evaluation of growth performance

The growth performance of the experimental fish was evaluated by measuring the parameters final mean weight, weight gain and specific growth rate. Specific growth rate (SGR) was calculated using the following equation:

$$SGR(\%) = \frac{(\ln Wf - \ln Wi)x100}{t}$$

Where Wi and Wf are the initial and final mean body weights, respectively and t is time in days from stocking to harvest.

The apparent digestibility coefficient of protein (ADCp) was calculated using the following formula:

$$ADCp(\%) = \frac{100x(1-(\%MD)x(\%NF)}{(\%MF)x(\%ND)}$$ (Maynard & Loosli, 1962)

Where NF is the nutrient content of faeces; ND is the nutrient content of the diet; MD, is the marker content of the diet, and MF is the marker content of the faeces.

### IV.3.7. Data analysis

Data were sorted and subjected to a one-way analysis of variance (ANOVA Duncan multiple range test (Duncan, 1955) was applied to identify means that were significantly different from each other. A type I error of 0.05 was used to declare significance.

## IV.4. Results

### IV.4.1. Water quality

Water quality did not vary significantly ($P>0.05$) among treatments, both in cages and in aquaria over the culture periods. The mean value for chlorophyll *a* was 173.4±15 mg $m^{-3}$. The ranges of DO values were 2.5-4.5 mg $L^{-1}$ for morning and 6.7-11 mg $L^{-1}$ for afternoon. Mean water temperature in the experimental pond was 27.5 ±1.0 °C, and the same temperature was maintained in the aquaria. The pH values ranged from 7.7 to 8.7 (mean=8.2±0.04) in the experimental pond and 7.6-8.6 (mean = 8.1±0.01) in the aquaria experiment.

### IV.4.2. Growth performance

Data on fish growth performance in aquaria and cages are presented in Table IV.3 & IV.4, respectively. Fish that were fed diets containing 4.5 % HFM, eventually supplemented with papain or PLM (diets 1, 2, 3) in aquaria had similar mean weight, specific growth rate and percent weight gain, but grew significantly better ($P<0.05$) than those fed diets in which FSM was substituted by either HFM plus papain (diet 4) or HFM plus PLM (diet 5). Fish eating diets 4 and 5, in which FSM was substituted by HFM plus papain or HFM and PLM weighed significantly less than fish from treatments 1 to 3 at the termination of the experiment. There was a tendency towards lower weight gain in treatments 4 and 5 as compared to treatments 1 to 3, with the difference between treatments 5 and 3 being significant. However, specific growth rate was not significantly different between treatments. Survival rate was high and similar ($P>0.05$) among treatments both in aquaria and cages (Table IV.3 & IV.4). Growth trend curves for *O. niloticus* in cages and aquaria are presented in Figures IV.1 & IV.2, respectively. In aquaria, fish receiving diets containing 6.0% FSM (treatments 1, 2 & 3) separated from treatments in which FSM was substituted by HFM or HFM and PLM (treatments 4 & 5) three weeks after stocking. In cages, a similar pattern was observed: diet 3 (containing 4.5% each of HFM and PLM) resulted in the highest growth, while diet 5 (containing 8.6% each of HFM and PLM)

resulted in the lowest growth. The growth of fish that fed on diet 1, 2 and 4 was intermediate, although the differences were not significant (P>0.05).

***Table IV.3**: Growth performance of O. niloticus fed diets in aquaria containing different levels of HFM and papaya leaf meal in place of FSM; values with the same superscript are not significantly different at α=0.05, SD=standard deviation of mean.*

| Parameter | Treatment 1 (control) | Treatment 2 (control + papain) | Treatment 3 (control + papaya leaves) | Treatment 4 (substitution of FSM + papain) | Treatment 5 (substitution of FSM + papaya leaves) | SD |
|---|---|---|---|---|---|---|
| Initial body weight (g) | $23.1^{a}$ | $23.0^{a}$ | $23.1^{a}$ | $23.5^{a}$ | $23.0^{a}$ | 0.17 |
| Final body weight (g) | $37.6^{bc}$ | $36.7^{b}$ | $39.3^{c}$ | $31.1^{a}$ | $34.4^{a}$ | 3.48 |
| Weight gain (%) | $62.8^{bc}$ | $59.6^{bc}$ | $70.1^{c}$ | $38.2^{a}$ | $49.6^{a}$ | 3.51 |
| Specific growth rate (% $day^{-1}$) (G) | $0.84^{b}$ | $0.81^{b}$ | $0.92^{b}$ | $0.56^{a}$ | $0.69^{a}$ | 0.15 |
| Survival (%) | $99.0^{a}$ | $98.0^{a}$ | $98.7^{a}$ | $96.1^{a}$ | $95.1^{a}$ | 0.92 |

***Table IV.4**: Growth performance of O. niloticus fed diets in cages containing decreasing levels of hydrolysed feather meal and papaya leaf meal in place of freshwater shrimp meal; values with the same superscript are not significantly different at α=0.05; SD=standard deviation of mean.*

| Parameter | Treatment 1 (control) | Treatment 2 (control + papain) | Treatment 3 (control + papaya leaves) | Treatment 4 (substitution of FSM + papain) | Treatment 5 (substitution of FSM + papaya leaves) | SD |
|---|---|---|---|---|---|---|
| Initial body weight (g) | $30.5^{a}$ | $30.0^{a}$ | $30.2^{a}$ | $30.5^{a}$ | $30.2^{a}$ | 0.17 |
| Final body weight (g) | $49.2^{b}$ | $50.2^{b}$ | $51.2^{b}$ | $48.0^{a}$ | $46.6^{a}$ | 2.42 |
| Weight gain (%) | $61.3^{ab}$ | $67.3^{ab}$ | $69.5^{b}$ | $57.4^{ab}$ | $54.3^{a}$ | 2.41 |
| Specific growth rate (% $day^{-1}$) (G) | $0.86^{a}$ | $0.88^{a}$ | $0.92^{a}$ | $0.81^{a}$ | $0.75^{a}$ | 0.09 |
| Survival (%) | $98.2^{a}$ | $97.1^{a}$ | $96.7^{a}$ | $98.1^{a}$ | $97.1^{a}$ | 0.42 |

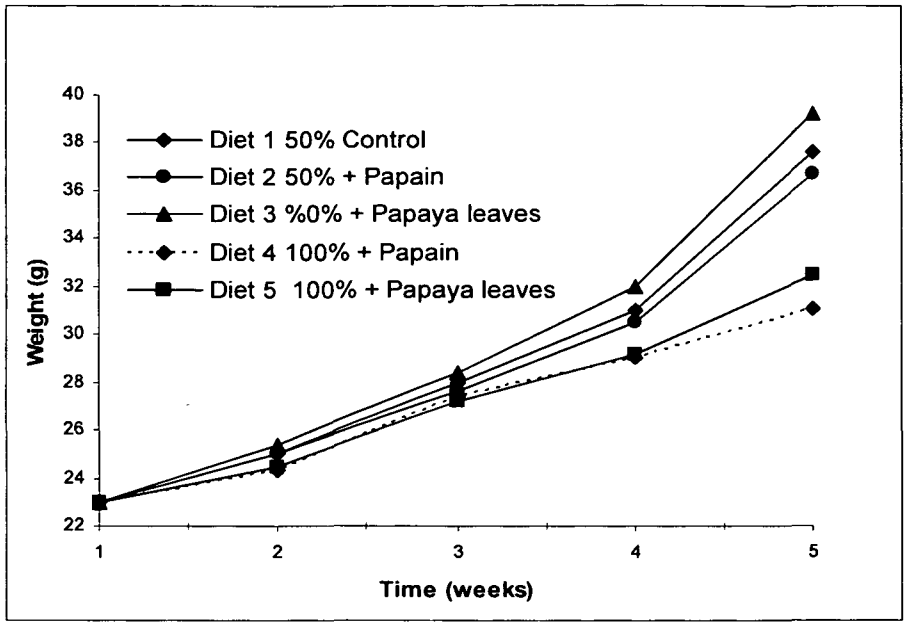


***Figure IV.1**: Growth curves for O. niloticus receiving diets with varying levels of HFM and papaya leaf meal in aquaria during 58 days culture period.*

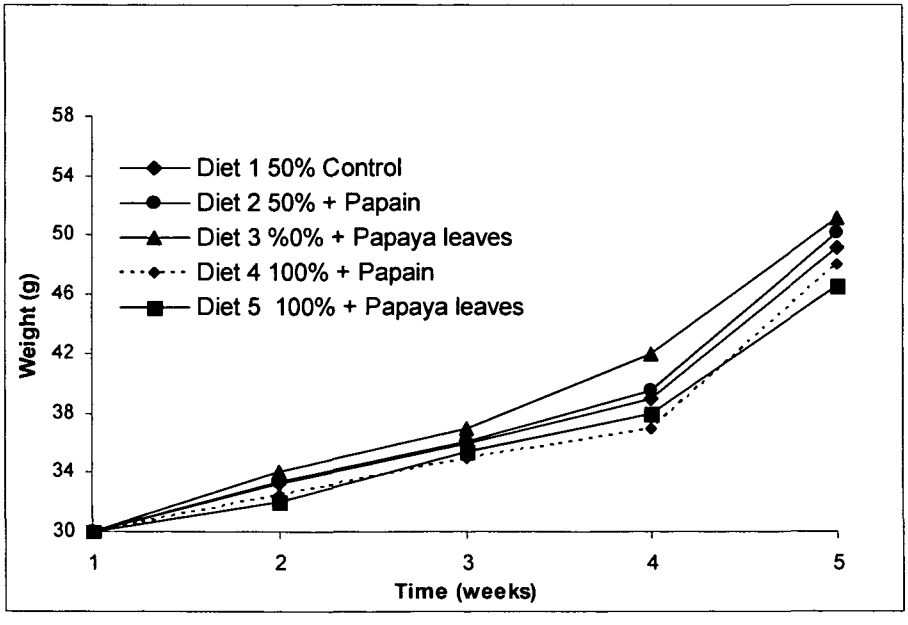


***Figure IV.2**: Growth curves for O. niloticus receiving diets with varying levels of HFM and papaya leaf meal in cages installed in a fertilized earthen pond during 58 days culture period.*

### IV.4.3. Protein digestibility

Results from *in vitro* digestibility indicated that *in vitro* digestibility coefficient of protein ($IDC_p$) for hydrolysed feathers was best at an alkaline pH (9) which had 35 percent compared to acidic pH (5) which had $IDC_p$ of 27 percent. Feathers hydrolysed without adjustment of the water pH had $IDC_p$ of 29 percent. The results from *in vivo* experiment for apparent protein digestibility ($ADC_p$) in aquaria and cages are shown in Table IV.5. Especially in the aquaria experiment, significant differences ($P<0.05$) in $ADC_p$ were observed among treatments. The $ADC_p$ values decreased with increasing levels of HFM both in cages and aquaria (Figure IV.3).

***Table IV.5**: Percent apparent protein digestibility coefficient (APDC) in cages and aquaria; values with the same superscript are not significantly different at $\alpha=0.05$; $ADC_p$=Apparent Protein Digestibility Coefficient.*

| Parameter | Treatment 1 (control) | Treatment 2 (control + papain) | Treatment 3 (control + papaya leaves) | Treatment 4 (substitution of FSM + papain) | Treatment 5 (substitution of FSM + papaya leaves) | SD |
|---|---|---|---|---|---|---|
| $ADC_p$( cage) | $68.5^a$ | $71.5^a$ | $72.0^a$ | $64.5^a$ | $65.6^a$ | 0.39 |
| $ADC_p$ (aquaria) | $67.1^b$ | $68.2^b$ | $69.8^b$ | $56.1^a$ | $57.5^a$ | 0.96 |

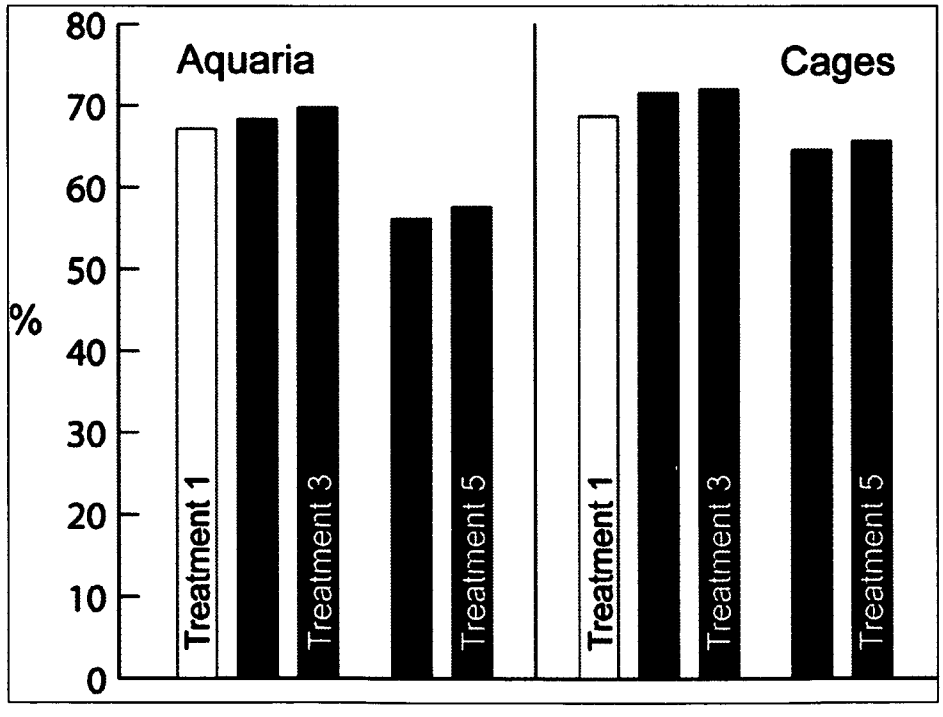


***Figure IV.3**: A bar graph showing apparent protein digestibility of the test diets in cages and aquaria*

## IV.5. Discussion

Results on growth performance parameters from the present study indicated that growth of *O. niloticus* that were fed diets containing FSM and a mixture of HFM and papain or PLM in cages were similar to that of the control treatment. However, there was a significant and distinct decline in fish growth when fish were fed diets containing 8.6% of HFM protein (100% protein substitution level) in aquaria experiments. All the water quality parameters monitored were within the recommend values for tilapia culture (Popma & Masser, 1988).

Specific growth rate (SGR) of fish in cages was high and similar among all treatments; however SGR in aquaria differed significantly between diets with 4.5% and 8.6% FSM substitution with HFM. The differences between the SGR in aquaria and in cages may be attributed to the culture conditions. The aquaria experiment in the current study was conducted in indoor laboratory environments, while the caged experiment was conducted in a fertilized earthen pond. Therefore it is possible that the fish in the cage experiment got some extra nutrients from the natural pond food, a specific potential strong point of *O. niloticus* (Kaliba *et al.*, 2006; Rakocy & Mcginty, 1989). This source of nutrients may have supplemented the deficit of essential dietary amino acids. Furthermore, Bowen (1980) reported that tilapias can thrive well on naturally occurring dissolved amino acids. Hydrolyzed feather meal is often considered to be an inferior source of protein for fish because of its poor digestibility and unbalanced essential amino acid profile (Falaye, 1982; Hasan *et al.*, 1997; Mendoza *et al.*, 2001; Roley *et al.*, 1977; Tacon *et al.*, 1984). We therefore postulate that the interaction between the natural pond food and HFM may have resulted in an improved dietary amino acid profiles, thus leading to better utilization and growth performance of fish in the cages.

Falaye (1982) did not observe a significant decrease in growth parameters (SGR, weight gain, mean weight) of *O. niloticus* when 50% of fish meal (FM) was replaced with HFM, which can be seen as similar to our findings, when HFM was used to substitute FSM. In relation to Tilapia diets, which are commonly used in the study area, treatments 1, 2 and 3 also represent diets in which 50% of FSM are successfully substituted by HFM. In another study similar results were found by Viola & Zohar (1984), who reported that up to 50% of FM could be replaced by poultry by-products in diets of tilapia hybrids without deleterious effects on fish growth. The results in the present study were also close to those of Abdel-Warith *et al.* (2001); from experiments with African catfish (*Clarias gariepinus*) he could replace 40% of fish meal protein with poultry by-products without a significance reduction in growth. In another study with *O. niloticus*, Bishop *et al.* (1995) reported a slightly higher level of substitution (66%) of FM-protein with HFM-protein without a significant reduction in growth. This relatively high substitution level has to be related to the specific

culture conditions: While part of the present experiment was conducted in aquaria indoors, Bishop *et al.* (1995) used concrete tanks in which additional nutrients from natural food may have been available. However, in the experiment with cages installed in the earthen pond, complete substitution of FSM by HFM and papain or PLM only moderately reduced growth. This may have been due to the extra source of nutrients from the natural pond food, as the pond was fertilized weekly.

Previous research on the use of hydrolysed feather meal in fish diets reported biased amino acid profiles (Santiago & Lovell, 1988; Jauncey & Ross, 1982; NRC, 1983). Therefore the reduced growth recorded in the aquaria experiment in the present study when fish were fed diets containing over 4.5% HFM may be attributed to sub-optimal levels of essential amino acids. This in part, might have hindered a proper utilization of protein from HFM for body protein synthesis. Fisher *et al.,* (1981) and Harrap & Woods (1964) reported that the major component of feathers is ß-keratin and had a high degree of cross-linking of disulfide bonds, hydrogen bonding and hydrophobic interactions. Fraser *et al.,* (1969) noted that keratin in its natural form is insoluble and difficult to digest by humans and animals.

The nutritional inferiority and insolubility of native feather protein derive from the composition and molecular configuration of constituent amino acids that ensure the structural rigidity of the feathers (Parry & North, 1998). Fisher *et al.,* (1981) and Harrap & Woods (1964) reported that the major component of feathers is ß-keratin and has a high degree of cross-linking of disulfide bonds, hydrogen bonding and hydrophobic interactions (Fraser *et al.,* 1969). Use of physical and chemical treatments to convert feathers to feather meal can destroy certain amino acids and decrease protein quality and digestibility (Moritz & Latshaw, 2001; Wang & Parsons, 1997). Therefore to improve feather digestibility research has focused on microbial proteolytic systems; *Streptommyces fradia* (Nickerson *et al.,* 1963; Noval & Nickerson 1958), *Streptommyces fradia* supplemented with methionine (Elmayergi & Smith (1971), *Bacillus licheniformis* (Williams & Shih, 1989). However these feather degradation mechanisms are rather complicated for rural farmers specifically in developing countries who may want to utilize feathers inclusion at farm made diet level. Therefore studies have been conducted to evaluate feather hydrolysation through cooking the feathers under high pressure and temperature (Hasan *et al.,* 1997; Bishop *et al.,* 1995; Papadopulos *et al.,* 1985).

Gohl (1981) reported that feathers are insoluble due to their high content of keratin and that by autoclaving disulfide bonds are broken thereby making the feathers more soluble and digestible. However some essential amino acids such as cystine are reduced from about 10 to 3.5 %. The highest *O. niloticus* weight gain reported in this study for treatment

3 in both the aquaria and cage-environment may have been due to increased digestibility and nutritive value of the complete diet because green papaya leaves were mixed with the hydrolysed feathers plus ponds natural food. Green *papaya carica* leaves are known to contain proteases of the papain superfamily and bleomycin hydrolases, which potentially improves protein digestion (Croall & Dermartino, 1991; Enenkel & Wolf, 1993; Brocklehurst *et al.,* 1987). However, a positive effect of the inclusion of PLM could not be shown for a situation in which FSM was completely substituted by HFM and PLM; treatments 4 and 5). To improve the nutritive value of feather meal based diets, some researchers have suggested to specifically supplement deficient amino acids after hydrolysation in diet formulations (Webster *et al.*, 1991; Murai *et al.*, 1982; El-Sayed 1990), but given the low market prices for tilapia in developing countries, this approach does not seem economically viable in a situations which this study was related to. Miller *et al,* (1989) reported that papain has very broad specialties and therefore indiscrminatively breaks down major muscle (connective tissue, collagen and myofibrillar proteins). Middlebrook & Philips (1941) further, reported that in presence of an alkaline (sodium bisulphate) solution, wool, which is mainly keratin, was rapidly attacked by papain. Therefore the use of *papaya carica* leaves, which contain papain, may be a viable way of promoting the quality of feather meal based diets.

Webster *et al.* (1991) suggested that utilization of amino acids is dependent on the meal frequencies. Thebault (1985) reported that, added amino acids were converted into other compounds he cited a case in which methionine was converted into methionine sulfoxide. The author further reported that in fish diets supplemented with methionine reached peak level in plasma substantially sooner than the rest of the EAA, and thus are not utilized for protein synthesis when fish are fed once a day. However, in the current study the good growth recorded in the cages may have been due to amino acid utilization from the natural environment, because tilapias feed continuously in a semi-intensive environment (Getachew & Fernando, 1989; Moriarty, 1973; Zenebe & Getachew, 1998). Moreover, Bowen (1980) reported that tilapias could do well on naturally occurring dissolved amino acids. Therefore based on the hypothesis of meal frequency, utilization of free amino acids might be enhanced in the cages were *O. niloticus* feeds continuously from the feed delivered by automated feeders and also from natural food. This mode of feeding ensures that free amino acids are supplied continuously at the site of tissue formation. Since no significant differences ($P>0.05$) were recorded in *O. niloticus* growth among all the cage treatments in the present study the observation by Bowen (1980) would suggest that supply of amino acids from natural food may be an effective and economical strategy of supplementing the limiting amino acids in *O. niloticus* diets in a fertilized pond setup.

Apparent protein digestibility coefficient ($ADC_p$) in the present study was generally high for feather meal based diets this may have been to better hydrolysation by adjusting the pH to 9, However the $ADC_p$ declined if the inclusion rate of HFM increased from 4.5% to 8.6% in the diets. A similar trend was reported by Yang *et al.* (2006) and Degani *et al.* (1997), who observed a decrease in $ADC_p$ with increasing levels of feather meal in carp (*Cyprinus carpio* L.) and gibel carp (*Carassius auratus gibelio* Bloch). However, the APC values calculated in the present study are generally slightly lower than those reported for *O. niloticus* by Hanley (1987). The difference may be due to the composition of the test diets. Hanley (1987) tested hydrolyzed feather meal as a single ingredient diet, while in the present study; the diets consisted of mixtures of ingredients. It appears from the present study that the presence of natural food improved the protein digestibility in feather meal based diets in the cage experiment. The mechanism for the improvement may be linked to the presence of algae. Algae or algal based diets are reported to stimulate secretion of copious amount of gastric acid in *O. niloticus* and *O. mossambicus* (Getachew, 1987, 1989; Bowen 1981).

In conclusion, a combination of the protein sources FSM, HFM and PLM tends to give the highest growth performance in both aquaria and cages the growth depression observed for treatments 4 and 5 in aquaria was not observed in the cages, where the natural food may have provided an important nutrient supplement.

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# V. General discussion

## V.1. The potential role of aquaculture in food security in sub-Sahara Africa

Aquaculture is the fastest expanding food-production sector, complementing and, in some cases substituting capture fisheries (Watanabe, 2002; Yang *et al.*, 2006). The potential for aquaculture to tackle challenges of food security and generate employment and economic gains has been demonstrated by its rapid growth, which has been at a rate of 10 percent per annum since the mid 1980s compared with 1.6 percent for capture fisheries (Watanabe, 2002). A report by the United Nations Food and Agriculture Organization (FAO, 1998) predicted that the sharp increase in scale that the aquaculture industry has experienced in the 1990s will continue until 2015. The same FAO report predicted that global fish consumption could increase from the current per capita rates of approximately 16 kg per year to approximately 20 kg per year by the year 2030, raising the total food use of fish to approximately 150 million metric tonnes. The annual sustainable yield of marine capture fisheries is no more than 100 million metric tonnes; therefore the increase has to come from aquaculture (Watanabe, 2002).

Aquaculture development in sub-Sahara Africa (SSA) has been sporadic and in many cases has failed to live up to expectations in the 50 years since it was first introduced in the region (Hecht, 2000; FAO 2006; Moehl *et al.*, 2005). Aquaculture in SSA currently accounts for less than one percent of world production, and while there are many causative factors that have retarded its development, one is lack of suitable aquafeeds. FAO (2006) reported that in SSA feed availability, quality, distribution and acceptable feed conversion ratios remain major constrains to both non-commercial and commercial producers. The report further observed that, with the exception of South Africa, most non-commercial farmers use diets limiting in protein, while manufactured feeds are generally of a low quality. This observation may be attributed to little or lack of proper knowledge on the proximate composition and amino acid profiles of the locally available feed ingredients

and as result poor diet formulations and subsequent low fish yields. Aguilar-Manjarrez & Nath (1998) reported that SSA has the bio-physical resources to increase production significantly and estimated that small-scale fish farmers on only 0.5 percent of the total area potentially available, could produce 580 000 tonnes or 35 percent of Africa's increased fish demand by 2010.

All major feedstuffs that are used in livestock feed production are generally available across the region and are also available for aquafeed FAO (2006). However, the growth of aquafeed production and the necessity to produce complete feeds will place increased competition on some resources (FAO, 2005). The situation is worsened by a potential competition between human and fish nutrition for foods such as "Omena" fish (*Rastrineobola argentea*); this not only applies to Kenya, but also to many other SSA countries, which already have a major problem with food security. To have a sustainable fish feed development it is important for research to focus on those feedstuffs, which are not used as human food. It is against this background that the present study was conducted to reduce the foreseen competition for nutritional resources between fish, livestock and humans. The study focuses on identification of mainly non-convectional feedstuffs and their utilization for sustainable fish feed development in three eco-zones of Kenya. Further proximate analysis and amino acid analysis of certain feedstuffs identified as high potential for use in *O. niloticus* diets were performed, and feeding and digestibility trials were conducted using *O. niloticus* in laboratory aquaria and in cages installed in fertilized earthen pond.

## V.2. The Kenyan perspective

A welfare monitoring survey indicated that in Kenya between 1994 and 1997 the poverty level rose from 47 to 53 percent in rural areas and from 29 to 49 percent in urban areas. As of 2005 approximately 42 percent of the population still lives below the national poverty line (UNDP, 2005). This finding calls for urgent measures to find solutions and to save future generations from severe malnutrition. Since Kenya has many under - utilized water bodies, aquaculture can serve as one of the measures. In Kenya aquaculture is typically a smallholder operation undertaken by a few thousand farmers (FAO, 2005). It is normally integrated into other farming activities. Extensive smallholder fish farming comprises one or more earthen ponds of about 130-1,000 $m^2$. Most of Kenya fish farming is located in western and central province; Nile tilapia is the species, which is mostly stocked. Typically, formulated feeds are rarely used, and thus production levels are comparatively low and are in the range of 500-2000 kg/ha/year (FAO, 2005). As feedstuffs used in the culture of the fish are similar to those used for livestock production, small scale fish

farming is predominantly viewed in the context of agricultural production and not as a separate operation. In addition to the small-scale operators there are a few semi-intensive and intensive aquaculture installations using formulated feeds. These semi-intensive culture systems produce mostly tilapia, carp, catfish, trout and freshwater ornamental fish.

In Kenya aquafeeds are supplied as either unprocessed or semi-processed natural or formulated feeds. While semi-processed natural feeds such as cereal brans and oil seed cakes are used in extensive low cost conditions, formulated feeds are used in the semi-intensive and intensive operations. Although Kenya has a reasonably robust livestock feed industry, no established aquafeed industries are currently operating in the country. However, lack of information on the nutritional value of most feedstuffs, raw material costs and lack of appropriate equipment and technical expertise are also important factors currently restricting the development of the sector (FAO, 2006).

## V.3. Nutritional value of potential alternative feedstuffs

To develop a feed for sustainable fish production, the evaluation of proximate and amino acid composition, digestibility and performance efficiency as well as cost implications and conditions of application is necessary (Watanabe, 2002). Therefore in part one of the present study agricultural by-products were sampled depending on their local availability in three eco-zones (Lake Victoria basin, Central and parts of Eastern provinces of Kenya). Proximate analysis was performed on 4 and 23 samples of animal and plant origin, respectively, to estimate their potential nutritive value for utilization as feedstuffs for *O. niloticus* grown in an intensive culture system (indoor aquaria) and in a semi-intensive pond culture system, which greatly relies on local resources. Among the analysed feedstuffs for proximate and amino acid analysis, underutilized residues of tea leaves (*Camellia sinensis*), sweet potato leaves (*Ipomoea batatas*), cassava leaves (*Manihot esculenta*), pawpaw leaves (*Papaya carica*) and hydrolysed feather meal were identified as high potential feedstuffs for tilapia grown in semi-intensive pond culture which relies greatly on local resources. Hydrolysed feather meal was identified as the feedstuffs with the highest potential in *O. niloticus* diets due to it high crude protein and availability in all the three eco-zones of this study. In addition, different seed cakes and cereal brans may be utilized where available and grain husks may serve as organic fertilizers in low-intensity forms of aquacultures. It should however be noted that this estimation is mainly based on the actual contents of crude protein, amino acids and crude fibre, the feasibility of removing antinutritional constituents, and on the local availability and the potential competition with other uses. However, we recommend that, before large scale utilization of these feedstuffs, there is need for further research to evaluate among others,

digestibility, antinutritional factors and processing methods. A review by the United Nation Food and Agricultural Organization (2005) observed that most of the less conventional feed sources are mainly available in small quantities, may only be seasonally available and often moist. As such, they may be unsuitable for commercial aquafeed production, whereas they have a potential for incorporation into farm made feeds for small-scale tilapia production. We therefore recommend that future research should focus on proper post-harvest handling, including drying and processing, to ensure that the formation of harmful substances such as afflotoxins can be avoided.

## V.4. Aspects of the use of hydrolyzed feather meal as a protein source

In formulating efficient fish diets, knowledge on the digestibility of the feed ingredients is a basic requirement (Cho & Kaushik, 1990). In Kenya, however information on the digestibility of many of the potential feedstuffs is scarce or missing. Sklan *et al.* (2004) observed that the nutrient requirements and nutrient concentrations of a feedstuff should be expressed in units of availability so that least-cost formulations can optimize the nutrient requirements minimizing the cost of feeds. In a previous experiment Liti *et al.* (2005) had reported that diets formulated using freshwater shrimp meal, as a source of animal protein is efficient in promoting fast growth of *O. niloticus*. However, in Kenya, freshwater shrimp meal is a by-product of the Omena (*Rastrineobola argentea*) fishery in Lake Victoria, but the supply is increasingly becoming scarce in the market due to frequent closures of the lake's fishery during fish breeding season and competition from livestock feed industry. Therefore, there is an urgent need for replacement of freshwater shrimp meal with cheaper and more constantly available animal protein feedstuffs in order to reduce the cost of *O. niloticus* feeds.

Feathers are widely available, cheap and are rich (over 80%) in crude protein; however feathers have unbalanced amino acid profiles and low digestibility (Hasan *et al.*, 1997; Roley *et al.*, 1977; Falaye, 1982; Mendoza *et al.*, 2001; Tacon *et al.*, 1984), and besides disposal is a problem in many developing countries (Mendoza *et al.*, 2001). However feathers are insoluble due to the high levels of keratin, and therefore show a low digestibility (Gohl, 1975). To overcome this limitation, feathers are hydrolysed by cooking them under pressure prior to inclusion in the diets. Gohl (1975) reported that when feathers are autoclaved, keratin is broken down, thereby making the feathers more soluble and digestible. On the other hand, cystine is reduced from about 10 to 3.5% by this processing method.

Therefore in part two of this study the effects of substituting freshwater shrimp meal (*Caridina nilotica*) with hydrolysed feather meal were evaluated under laboratory (indoor aquaria) and practical culture conditions (cages installed in a fertilized earthen pond) on growth; digestibility and survival in Nile tilapia (*Oreochromis niloticus* L.).

Fingerlings with mean weights of 26 and 36 g were held indoor in aquaria with recirculating water, and in cages that were installed in 800 $m^2$ fertilized earthen pond, respectively. Five isonitrogenous (250g $kg^{-1}$) and isocaloric (2.94 kcal $g^{-1}$) diets were prepared by substituting freshwater shrimp meal with hydrolysed feather meal at rates of 0, 25, 50, 75 & 100 % and fed to fish in aquaria. 0, 50 and 100 % diets were used in cages. All fish were fed at 10 % body weight $day^{-1}$ in three replicates for 84 days. Results indicated that substitution of freshwater shrimp meal by hydrolysed feather meal at levels above 50% led to significant ($P<0.05$) growth reductions in aquaria. However, substitution at 100% with hydrolysed feather meal did not significantly ($P>0.05$) affect growth of fish in the cages. In both experiments, survival was similar among treatments, but protein digestibility decreased with increasing levels of hydrolysed feather meal in the diet. In conclusion, 100 % substitution of freshwater shrimp meal with hydrolysed feather meal may be possible in semi-intensive culture of *O. niloticus*, where additional natural food is available. However, the calculated values for apparent protein digestibility in the present study are lower than those reported for *O. niloticus* by Hanley (1987). The discrepancy may be attributed to the composition of the test diets. This therefore emphasizes the need for more research towards efficient and affordable processing methods for feather meal to improve its digestibility.

As a follow up to the second experiment, a study was designed which was geared towards improving the nutritive value and digestibility of feather meal by blending the wet hydrolysed feathers with green use of *papaya carica* leaves and left overnight to soak. Green *papaya carica* leaves are known to contain proteases of the papain superfamily and bleomycin hydrolases, which may contribute to an improved protein digestion (Croall & Dermartino, 1991; Enenkel & Wolf, 1993; Brocklehurst *et al.*, 1987). To improve the nutritive value of feather meal based diets some researchers have suggested to supplement deficient amino acids in diet formulations (Webster *et al.*, 1991; Murai *et al.*, 1982; El-Sayed, 1990), but given the low market prices for tilapia from small-scale subsistence fish farming in developing countries this approach does not seem economically viable. Therefore, based on the results from feeding trials with diets containing feather meal within this study, we designed an experiment to evaluate whether green *papaya carica* leaves or papain additives improve the digestibility of hydrolysed feather meal in diets for *O. niloticus*: in the third part the effects of the inclusion of papaya leaves or papain to diets containing different amounts of hydrolysed feather meal on growth digestibility and

survival in Nile tilapia (*Oreochromis niloticus L.*) were evaluated in laboratory aquaria and cages installed in an earthen pond. Fingerlings averaging 22 and 30 g were cultured indoor in aquaria with recirculating water and in cages that were installed in an 800 $m^2$ fertilized earthen pond, respectively. Five isonitrogenous (250g $kg^{-1}$) and isocaloric (2,940 kcal $g^{-1}$) three diets contained 6 % of freshwater shrimp meal (FSM) and 4.5 % hydrolysed feather meal (HFM; control), supplemented either with papain (treatment 2) or 4.5 % papaya leaf meal (PLM; treatment 3). In two diets FSM was substituted by 8.6 % HFM plus papain (treatment 4) or 8.6 % HFM plus 8.6 % PLM (treatment 5). All fish were fed at 10% body weight $day^{-1}$ in three replicates for 58 days. Results indicated that complete substitution of FSM by a mixture of HFM and PLM led to significant ($P<0.05$) growth reductions in aquaria. However, in the cage environment, this did not significantly ($P>0.05$) affect growth of fish. In both experiments, survival was similar between treatments, but protein digestibility decreased with increasing levels of HFM in the diet. In conclusion, 100% substitution of FSM with a mixture of HFM and PLM may be possible in semi-intensive culture of *O. niloticus*, where additional natural food is available but digestibility improved in comparison to the experiments where no papain additives were used.

## V.5. Conclusions and recommendations

Besides four potential plant feedstuffs, hydrolysed feather meal is considered to have a high potential as a dietary ingredient in *O. niloticus*. HFM has a high crude protein content and its availability throughout the year in areas where fish farming has a high potential were the most important factors in this context. Hydrolysed feather meal can at least partially replace protein feedstuffs such as fish meal or freshwater shrimp meal in diets of Nile tilapia produced in semi-intensive systems. However, if hydrolysed feather meal is to be included in greater proportions, protein digestibility of the diet and growth performance of fingerlings may be reduced. The addition of papain is unlikely to improve the nutritive value of hydrolysed feather meal to the extent that it can be used as the major source of protein. However, the combination of different protein sources seems to be a feasible way for partial substitution of critical components such as freshwater shrimp meal and at the same time maintain a high level of performance.

Other resources such as leaf meals of pawpaw sweat potato, cassava and extracted tea leaves may also be utilized as non-conventional ingredients in diets of *O. niloticus*, and may contribute to improved sustainability of the production system. However, future research should focus on proper post-harvest handling and processing to remove antinutritional factors, avoid nutrients loss and contamination.

# VI. Summary

## VI.1. Summary

The present study was conducted to identify sustainable alternative protein sources using locally available feedstuffs for Nile tilapia (*Oreochromis niloticus* L) production in three eco-regions in Kenya. The first step was geared towards identifying feedstuffs which were locally available and which were not used as human food to avoid direct competition between fish and human food. Agricultural by-products and other underutilized protein sources were sampled depending on their local availability in three eco-zones of Kenya (Lake Victoria basin, Eastern & Central provinces). Proximate analysis was performed on a total of 27 feedstuffs of both animal and plant origin to estimate their potential nutritive value for utilization as feedstuffs for tilapia grown in a low-input pond culture system, which greatly relies on local resources. Based on their availability, content of protein and fibre and the feasibility of removal of antinutritional factors, hydrolysed feather meal, boiled tea leave residues, leaves of *Ipomoea batatas*, *Manihot esculenta* and *Papaya carica* were identified as most promising potential non-conventional feedstuffs.

In step two of the study diets for *O. niloticus* were formulated using hydrolysed feathers as animal protein source. Five isonitrogenous (250g $kg^{-1}$) and isocaloric (2,940 kcal $kg^{-1}$) diets were prepared by substituting freshwater shrimp meal (FSM) (*Caridina nilotica*) with hydrolysed feather meal (HFM) at rates of 0, 25, 50, 75 & 100% and fed to fish in aquaria; diets at substitution levels 0, 50 and 100% were also fed to fish in cages. Fingerlings averaging 26 and 36g were cultured in indoor aquaria with recirculating water, and in cages that were installed in an 800 $m^2$ fertilized earthen pond, respectively. All fish were fed at 10% body weight $day^{-1}$ in three replicates for 84 days. Results indicated that substitution of FSM at HFM levels above 50% in aquaria led to significant growth reductions. However, substitution at 100% with HFM did not significantly affect growth of fish in the cages. In both experiments, survival was similar among treatments, but protein digestibility decreased with increasing levels of HFM in the diet. In conclusion,

100% substitution of FSM with HFM may be possible in semi-intensive culture of *O. niloticus*, where natural food is available. Both in cage and aquaria setup, the 50% diet gave the best performance overall and therefore the highest potential for *O. niloticus* production.

Step three was based on the results of the second experiment; in this set up the 50% diet as given above was used as a control diet. The aim was to improve the nutritive value and digestibility of feather meal by use of *papaya carica* leaves, which contain proteases of the papain superfamily and bleomycin hydrolases, which potentially improves protein digestion. To evaluate this hypothesis, a study was conducted to analyse the effects of different levels of hydrolysed feather meal and pawpaw (*Papaya carica*) leaves (PLM) on growth, digestibility and survival rate of *O. niloticus* under laboratory aquarium and cage-cum-pond culture conditions. Fingerlings averaging 22 and 30g were cultured indoor in aquaria with recirculating water, and in cages that were installed in a fertilized earthen pond of 800 $m^2$, respectively. Five isonitrogenous (250g CP $kg^{-1}$) and isocaloric (2,940 kcal $kg^{-1}$) diets were formulated using different levels of wheat bran and carboxyl methyl cellulose for balancing CP and energy contents: three diets contained 6 % of freshwater shrimp meal (FSM) and 4.5 % HFM (control), supplemented either with papain (treatment 2) or 4.5 % PLM (treatment 3). In two diets FSM was completely substituted by 8.6 % HFM plus papain (treatment 4) or 8.6 % HFM plus 8.6 % PLM (treatment 5). All fish were fed at 10% body weight $day^{-1}$ in three replicates for 58 days. Results indicated that in aquaria dietary levels of HFM and PLM above 4.5% each led to significant ($P<0.05$) growth reductions. However, substituting FSM with HFM at 8.6% did not significantly ($P>0.05$) affect growth of fish in the cages. In both experiments, survival was similar among treatments, but protein digestibility decreased with increasing levels of HFM in the diet. In conclusion, a combination of the protein sources FSM, HFM and PLM tends to give the highest growth performance in both aquaria and cages. Locally available non-convectional feedstuffs can sustainably be utilized in *O. niloticus* production. Further research is to be directed towards analysing the effects of the feedstuffs identified herein on production traits of *O. niloticus* grown to market size.

## VI.2. Zusammenfassung

Die vorliegende Untersuchung sollte Beiträge zur Entwicklung von Futterressourcen für eine nachhaltige low-input-Teichwirtschaft zur Produktion von Nil-Tilapia (*Oreochromis niloticus* L.) in drei Regionen Kenias leisten. In einer ersten Stufe sollten Futterkomponenten identifiziert werden, die nicht in der Humanernährung Verwendung finden: 27 Proben von landwirtschaftlichen Nebenprodukten und anderen lokal verfügbaren Produkten pflanzlicher oder tierischer Herkunft wurden in drei Regionen Kenias (um den Viktoriasee, Ost- und Zentralkenia) gezogen und auf ihren Gehalt an Rohnährstoffen untersucht.

Federnmehl, extrahierte Teeblätter, Blätter von Süßkartoffel (*Ipomoea batatas*), Maniok (*Manihot esculenta*) und Papaya (*Papaya carica*) wurden aufgrund ihrer Verfügbarkeit, ihrem Gehalt an Rohprotein und Rohfaser sowie der Möglichkeit der Entfernung antinutritiver Inhaltsstoffe als potenzielle Futtermittel identifiziert.

In der zweiten Stufe der Untersuchung wurde ein Fütterungsversuch mit Rationen für *O. niloticus* durchgeführt, die hydrolysiertes Federnmehl (HFM) als alternative Eiweißquelle enthielten. Fünf isonitrogene (250 g $kg^{-1}$) und isokalorische 2940 kcal $kg^{-1}$) Rationen wurden formuliert, in denen sogenanntes Süßwasser-Shrimpmehl (*Caridina nilotica*, FSM) in mehreren Abstufungen durch HFM substituiert wurde. Substitutionsraten von 0, 25, 50, 75 und 100 % wurden in Aquarien untersucht, die Varianten 0, 50 und 100 % wurden außerdem in einem Fütterungsversuch mit Fischen in Käfigen in einem Versuchsteich getestet. Dabei wurden Setzlinge mit einer Lebendmasse von 26 g (Aquarien) bzw. 36 g (Käfige) verwendet, die täglich angebotene Futtermenge betrug 10 % der Lebendmasse. Die Käfige befanden sich in einem gedüngten Teich mit einer Fläche von 800 $m^2$. Sowohl im Aquarien- als auch im Käfigversuch wurde jede Behandlung in drei Wiederholungen über einen Zeitraum von 84 Tagen geprüft. In den Aquarien führte eine Substitution von mehr als 50 % des FSM durch HFM zu einer signifikanten Wachstumsreduktion. Im Teich (Käfige) trat demgegenüber auch bei einer Substitutionsrate nur eine tendenzielle Verschlechterung des Wachstums ein. Die Überlebensrate war sowohl im Aquarien- als auch im Käfigversuch nicht durch die Behandlung beeinflusst; allerdings verschlechterte sich die Proteinverdaulichkeit der Ration mit zunehmendem HFM-Anteil. Es kann gefolgert werden, dass eine vollständige Substitution von FSM durch HFM bei low-input-Produktion und dem Vorhandensein einer natürlichen Futterbasis in Teichen von *O. niloticus* möglich ist. Sowohl in Aquarien als auch in den Käfigen erbrachte die 50 % Substitutionsstufe die beste Zuwachsleistung und stellt daher jedenfalls eine empfehlenswerte Variante dar.

Als dritte Stufe der Untersuchung wurde ein weiterer Fütterungsversuch, der auf dem vorhergehenden aufbaute, angestellt. Dieser hatte zum Ziel, eine mögliche Verbesserung des Futterwertes von Rationen mit HFM durch den Einsatz von Papaya (*Papaya carica*)-Blättern (PLM) zu untersuchen. Diese enthalten Proteasen der Papain-Gruppe sowie Bleomycin-Hydrolasen, die die Proteinverfügbarkeit gegebenenfalls erhöhen könnten. In diesem Versuch wurde die oben erwähnte 50 % Substitutionsstufe als Kontrollgruppe gewählt. Es wurden die Effekte von Rationen, die unterschiedliche Anteile von HFM und PLM enthielten, auf Wachstum und Überlebensrate von *O. niloticus* und die Verdaulichkeit der Ration in Aquarien und Käfigen (Teich) untersucht. Der Versuchsansatz entsprach dem im vorhergehenden Versuch. Die Lebendmasse der verwendeten Setzlinge betrug 22 g (Aquarien) bzw. 30 g (Käfige), die Versuchsdauer lag bei 58 Tagen. Im Versuch standen fünf isonitrogene (250 g $kg^{-1}$) und isokalorische (2490 kcal $kg^{-1}$) Rationen, Weizenkleie und Carboxymethyl-Cellulose wurden zur Nährstoff- und Energie-Verdünnung verwendet. Drei Rationen enthielten 6 % FSM und 4,5 % HFM (Kontrolle) bzw. zusätzlich synthetisches Papain (Gruppe 2) oder 4,5 % PLM (Gruppe 3). In zwei weiteren Rationen erfolgte eine vollständige Substitution von FSM durch 8,6 % HFM plus Papain (Gruppe 4) bzw. 8,6 % HFM plus 8,6 % PLM (Gruppe 5).

In den Aquarien führten Anteile von über 4,5 % HFM bzw. PLM zu einer signifikanten Wachstumsreduktion. In den Käfigen resultierte auch bei vollständiger Substitution von FSM durch HFM nur eine tendenzielle Wachstumsdepression. Die Überlebensrate wurde durch die Behandlung nicht beeinflusst, die Proteinverdaulichkeit der Ration sank bei steigendem Anteil von HFM in der Ration. Die Kombination der Proteinquellen FSM, HFM und PLM ergab die höchste Zuwachsleistung in Aquarien und Käfigen.

Aus der vorliegenden Untersuchung kann geschlossen werden, dass lokal verfügbare, alternative Futtermittel in der Produktion von *O. niloticus* eingesetzt werden können und zu einer Verbesserung der Nachhaltigkeit der Erzeugung beitragen. Die höchsten Leistungen in der frühen Wachstumsphase sind bei einer Kombination von HFM mit FSM und allenfalls PLM zu erwarten. Weiterführende Untersuchungen zu den Effekten der hier getesteten Futtermittel bei *O. niloticus* in späteren Wachstumsphasen bzw. bis zur Marktreife sind notwendig.

# VII. Curriculum Vitae

Jonathan Mbonge MUNGUTI born on the 26th, September 1974 in Machakos, Kenya. Worked as a research assistant between 2002 & 2003 at Sagana Aquaculture Center in an aquaculture project. Moved to Kenya Marine Fisheries Research Institute, Sangoro Aquaculture Station and have worked as a Research officer since 2003. Attained a bachelor of education Science second upper Honours in Zoology and Chemistry from Moi University, Eldoret Kenya in 1999, then a Master of Science in Environmental science and technology (Limnology & Wetland Ecosystems) at UNESCO-IHE Institute for Water Education, (IHE) Delft the Netherlands in 2001.

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## VII.1. Publications

### VII.1.1. Peer reviewed Journals

1. **Munguti J.M.,** Liti D.M., Waidbacher H., Straif M. & Zollitsch W. (2006) Proximate composition of selected potential feedstuffs for fish production in Kenya. ***Die Bodenkultur Austrian Journal of Agricultural Research*** (in press)
2. D.M. Liti, R.M. Mugo, **J.M. Munguti** & H. Waidbacher (2006). Growth and economic performance of Nile tilapia (*O. niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. ***Aquaculture Nutrition,*** **12** 1-7

3. Waidbacher H., Liti D.M., Fungomeli M., Mbaluka R.K., **Munguti J.M.** & Straif M. (2006) Influence of feeding rates and pond fertilization on growth performance measures, economic returns and water quality in a small-scale cage-cum-pond integrated system for production of Nile tilapia (*O. niloticus* L.) ***Aquaculture Research*, 37,** 594-600.
4. Liti D.M., Waidbacher H., Straif M., Mbaluka R.K., **Munguti J.M.** & Kyenze M.M (2006) Effects of partial and complete replacement of freshwater shrimp meal (*Caridina nilotica* Roux) with a mixture of plant protein sources on growth performance of Nile tilapia *(O. niloticus* L.) in fertilized ponds. ***Aquaculture Research*, 36,** 746-752.
5. Liti D.M., Fulanda B., **Munguti J.M.**, Straif M., Waidbacher H. & Winkler G. (2005) Effects of open-pond density and caged biomass of Nile Tilapia (*Oreochromis niloticus* L.) on growth, feed utilization, economic returns and water quality in fertilized ponds. ***Aquaculture Research*, 36,** 1535-1543.
6. Liti D.M., **Munguti J.M.,** Kreuzinger N., & Kummer H. (2005) Effects of sodium chloride on water quality and growth of *Oreochromis niloticus* L. in earthen ponds, ***African Journal of Ecology,* 43**, 170-176
7. Liti D.M, Cherop. L., **Munguti J.M.** & Chhorn, L. (2005) Growth and economic performance of Nile tilapia (*Oreochromis niloticus*) fed on two formulated diets and two locally available feeds in fertilized ponds. ***Aquaculture Research*, 36,** 746-752
8. Liti D.M. & **J.M. Munguti**; 2003. Utilization of organic resources in fish farming. In Savala C. E. N. Omare, Woomer P.L **(Eds)** ***Organic resource management In Kenya Perspectives and Guidelines.*** **6**:43-50
9. Liti D.M and **J.M. Munguti.** (2002) Use of local technology to promote aquaculture development in Sagana Kenya and Aquaculture development in Kenya. ***Aquanews.* 17 (4)** pp 1-5 USA.

### VII.1.2. Submitted papers

1. **Munguti J.M.**, Waidbacher H., Liti D.M., Straif M. & Zollitsch W. (submitted) Effects of substitution of freshwater shrimp meal (*Caridina nilotica* Roux) with hydrolyzed feather meal on growth performance and apparent digestibility in Nile tilapia (*O. niloticus* L.) under different culture conditions. ***Aquaculture nutrition***.
2. Munguti **J.M.**, Liti D.M., Waidbacher H., Straif M. & Zollitsch W. (Submitted) Use of different levels of locally available hydrolysed feather meal and Pawpaw (*Papaya carica*) leaves – a contribution to increasing sustainability of Nile tilapia (*Oreochromis niloticus* L.) production. ***Renewable Agriculture and Food Systems***.

### VII.1.3. International technical reports

1. Liti D.M, Baraza J.E, **Munguti J.** & Phelps R. (2005) Evaluation and Reproduction Capacity of Three Strains of Nile tilapia, (*O. niloticus)*, Found in Kenya for use in Aquaculture In: *J. Burright, C. Flemming and H.Egna (editors), Twenty-second Annual Technical Report*. Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp 327-336.
2. Liti D.M, Kerongo L. Lim C., & **Munguti J.** (2005) Development and Evaluation of the Nutritive and Economic Potential of Formulated Fish feeds and Comparison with locally Available feeds for Semi-Intensive production of Nile tilapia, (*O. niloticus*) In: J. Burright, C. Flemming and H.Egna (editors), Twenty-second Annual Technical Report. Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp 327-336.

### VII.1.4. Master Science thesis

1. Munguti J. M. (2001) The effect of salt (NaCl) addition on abundance and species composition of phytoplankton, zooplankton and macrozoobenthos, and subsequent fish growth in Sagana, Kenya. MSc Thesis, IHE- Delft, the Netherlands.

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# IX. Appendix

## IX.1. Abbreviations/ Acronyms

| | |
|---|---|
| ADC | Apparent digestibility coefficient |
| ADCp | Apparent digestibility coefficient for protein |
| ANFs | Anti-nutritional factor(s) |
| ALVA | Arbeitsgemeinschaft Landwirtschaftlicher Versuchsanstalten in Österreich |
| ANOVA | Analysis of variance |
| AOAC | Official Methods of Analysis of the Association of Official Analytical Chemists |
| APHA | American Public Health Association |
| BHFM | Broiler hydrolysed feather meal |
| BITC | Benzyl isothiocyanate |
| BOKU | Universität für Bodenkultur Wien, University of Natural Resources and Applied Life Sciences, Vienna |
| BOMOSA | **Bo**ku University-**Moi** University-**Sa**gana Fish Farm; mostly used in the context of a specific small-cage production system |
| BTLR | Boiled tea leaves residue |
| *C.gariepinus* | *Clarias gariepinus* |
| CaCO3 | Calcium bicarbonate |
| CF | Crude fibre |
| CMC | Caboxyl methyl-cellulose |
| CP | Crude protein |
| CSC | Cotton seed cake |
| SCP | Single cell protein |
| DAP | Diammonium phosphate |
| DM | Dry matter |
| EAA | Essential amino acid (s) |
| EE | Ether extracts |
| FAO | Food and Agriculture Organisation |
| FM | Fish meal |
| FSM | Freshwater shrimp meal |
| HFM | hydrolysed feather meal |
| IHFM | hydrolysed feather meal from indigenous chickens |
| KMFRI | Kenya marine fisheries and research institute |
| MB | Maize bran |

| | |
|---|---|
| MD | Mark in the diet |
| MF | Mark in |
| MSE | Mango seed embryo |
| NaOH | Sodium hydroxide |
| ND | Nutrient in diet |
| NF | Nutrient in feaces |
| NFE | Nitrogen free extracts |
| NRC | National Research Council |
| *O. niloticus* | *Oreochromis niloticus* |
| OPA | Orthophtalaldehyde |
| PLM | Papaya leaf meal |
| PSI | Pounds per square inch (*14.5 psi is equivalent to 1 bar*) |
| PSM | Papaya seed meal |
| RB | Rice bran |
| SFSC | Sunflower seed cake |
| SGR | Specific growth rate |
| UNDP | United Nations Development Programme |
| WB | Wheat bran |

## IX.2. Photograph plate

### Cage experimental set-up

***Photo IX.1**: 15 BOMOSA cages installed in 800m$^2$ fertilized earthen pond*

***Photo XII.2**: Handling of a BOMOSA cage*

## Aquaria experimental set-up

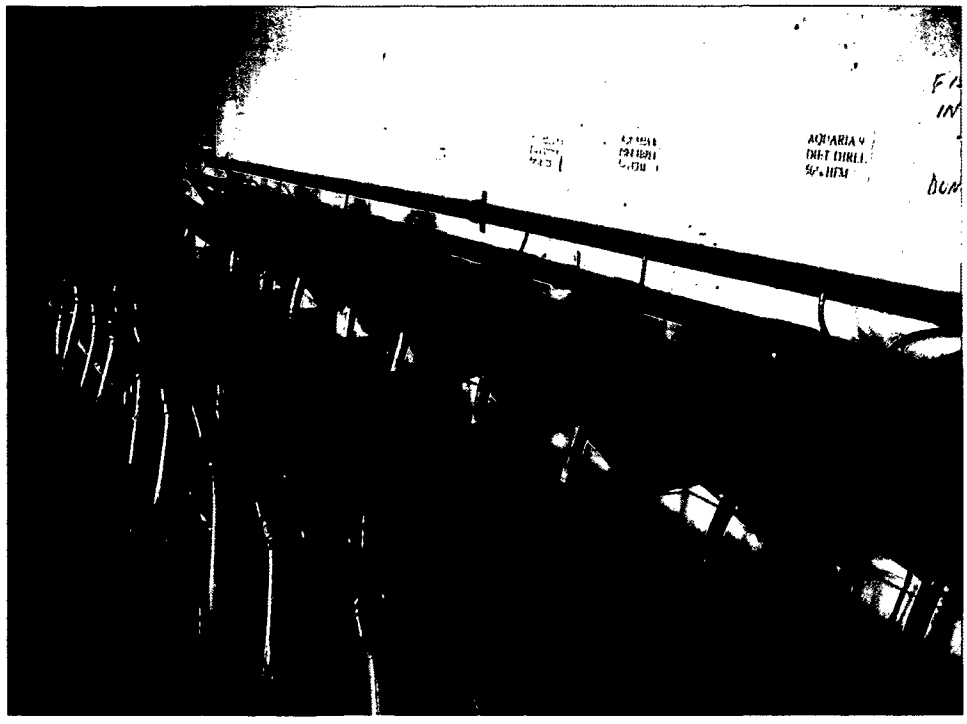

***Photo XI.3**: Series of Aquaria used for in the feeding experiments*

***Photo XI.4**: A close view to O. niloticus in the aquaria*

**Some of the key equipments used for feed analysis in the study**

***Photo XI.5**: Kjendahl analyser digester unit*

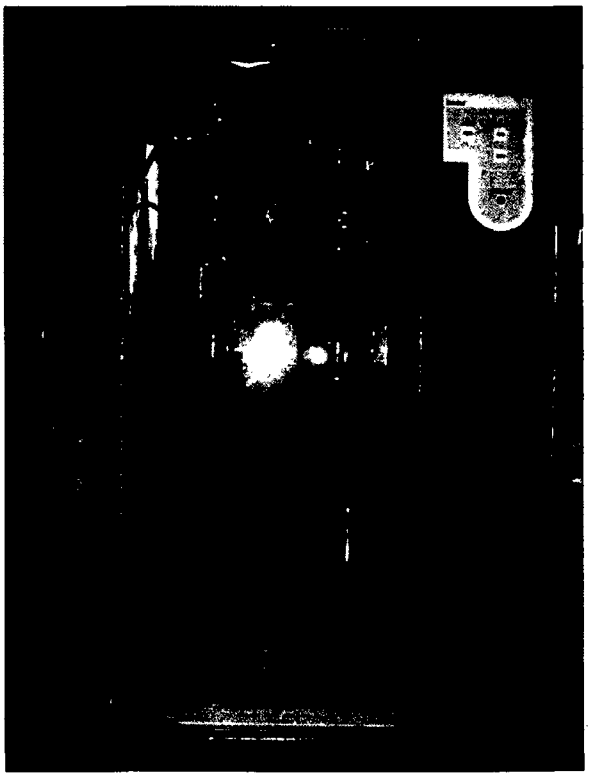

***Photo XI.6**: Kjendahl protein analyser a semi-automated distiller unit*

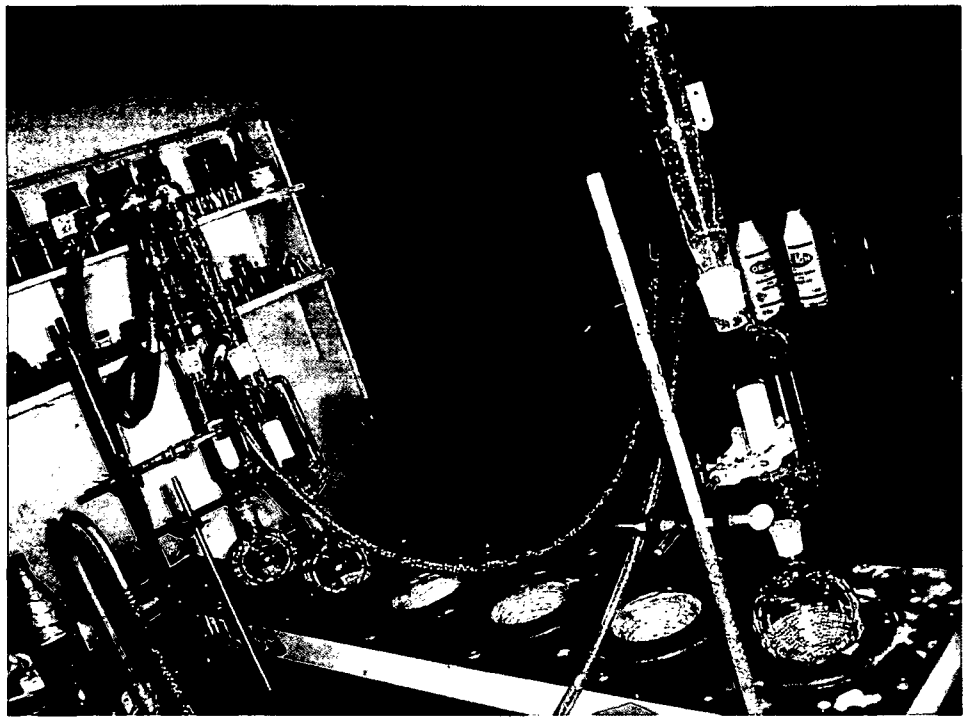

***Photo XI.7**: Soxhlet extractor for ether extraction*

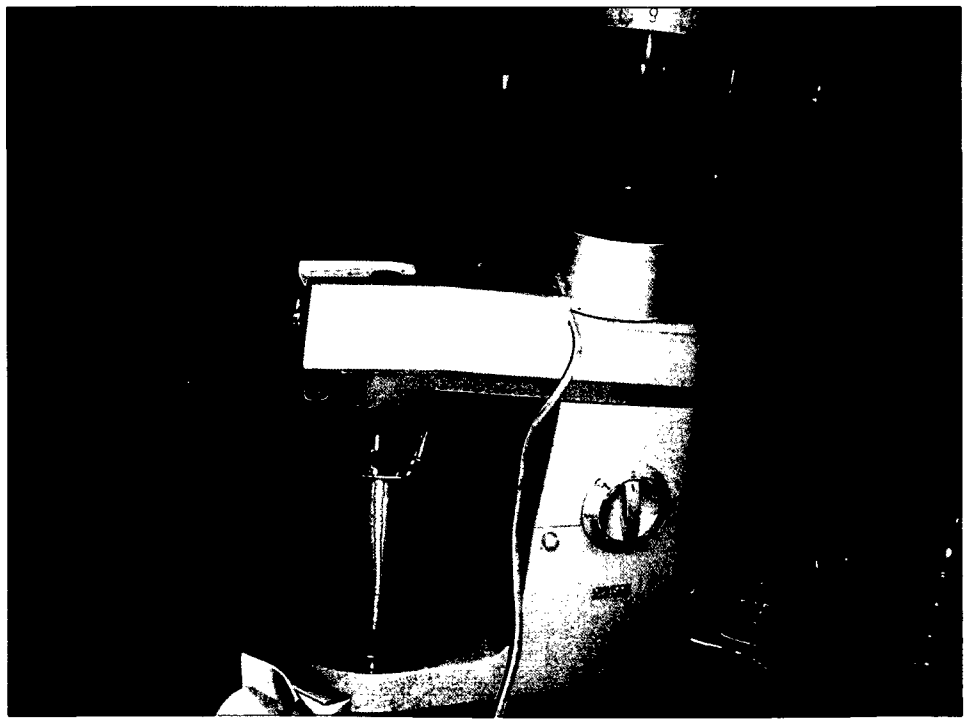

***Photo XI.8**: Blender liquidizer (model A989, Hampshire, UK)*

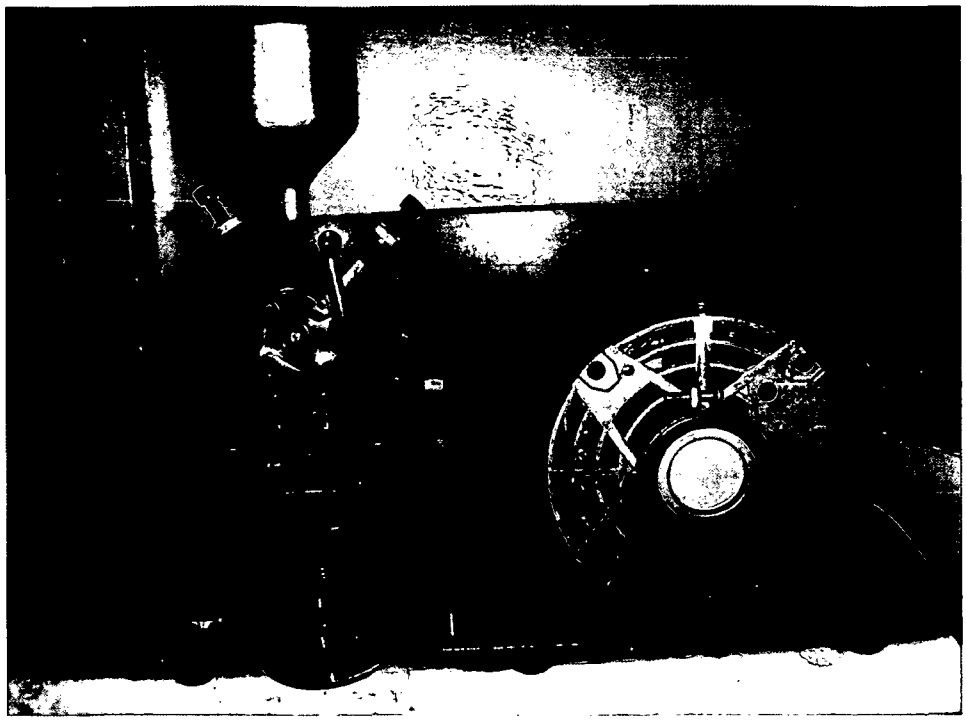

***Photo XI.9**: Electric grinder (Thomas-Wiley intermediate mill, 3348-L10 series, USA)*

**Feedstuff with the highest potential in Nile tilapia feeds**

***Photo XI.7**: Wet hydrolysed chicken feather*

***Photo XI.8**: Market size Nile tilapia (Oreochromis niloticus)*