NUTRITIONAL EVALUATION OF PLANT INGREDIENTS FOR DIETS OF *TILAPIA RENDALLI* IN NKHATABAY, NORTHERN MALAŴI

MSc (Fisheries Science-Nutrition) Thesis

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MZUZU UNIVERSITY

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A THESIS SUBMITTED TO THE FACULTY OF ENVIRONMENTAL SCIENCES, DEPARTMENT OF FISHERIES SCIENCE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN FISHERIES

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DECLARATION

I, Kumbukani Mzengereza, declare that the work presented in this thesis is a result of my own research effort and that to the best of my knowledge; it has not been previously submitted to Mzuzu University or any other institution of higher learning for the award of any academic qualification. Where other sources of information have been used, acknowledgement has been made accordingly by means of references.

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CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the author's own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within Mzuzu University or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on **___________[day, month and year**]

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Date: ______________________________

DEDICATION

I dedicate this work to all fish farmers in Mpamba, NkhataBay District of northern Malawi.

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ABSTRACT

Modern fish culture requires the reduction of the cost of feeds which can partly be achieved by minimal use of dietary animal protein. This study assessed the nutritional potential of locally available plant based feedstuffs from Mpamba EPA in NkhataBay district; northern Malawi. The main objective was to isolate those that can be used as ingredients for formulation of affordable fish diets to increase pond based fish production in Malawi. The following plants were used in the study: cassava (*Manihot esculenta*) peels (CP) and leaves (CL), pawpaw (*Carica papaya*) leaves (PL), sweet potato (*Ipomea batatus*) leaves (SPL), peels (SPP), and tubers (SPM), jackfruit (*Artocarpus heterophyllus*) (JK), mexican fire plant (MFP) (*Euphorbia heterophylla*),cocoyam leaf meal (CYM) black jack (BJ) (*Bidens pilosa*), banana (*Musa balbisiana*) leaves (BL), maize (*Zea maize*) bran (MZB), and akee (*Blighia sapid*) leaves (AK). Proximate analysis was conducted to generate information for selection of potential plant ingredients to be used in the formulation of four diets of *Tilapia rendalli*. In addition, a digestibility experiment was also conducted on juvenile *Tilapia rendalli* to evaluate four diets formulated from the selected plants at NkhataBay fisheries Laboratory for 21 days. The four diets were designated as treatment 1 to 4. Treatment 1 comprised of (CL,BJ,MZB,SPM,SPL,CF,CO), treatment 2 (CL,CYM,MZB,SPM,SPl,CF,CO) treatment 3(CL, CYM,BJ,SPM,SPL,CF,CO) and treatment 4 (CL, CYM,BJ,MZB,SPL,CF,CO) .The experiment was laid out in a Completely Randomized Design (CRD) using glass aquaria with each diet replicated three times. Data for both proximate and digestibility experiments was analyzed using Analysis of Variance (ANOVA) at $P= 0.05$ using SPSS and R-software's respectively. Results showed that cassava (*Manihot esculenta*) leaves, black jack (*Bidens pilosa*) and cocoyam (*Caladium bicolor*) had the highest levels of crude protein recording 21.17±0.56%, 24.35±0.7 % and 24.28±0.11%, respectively which were significantly different ($P<0.05$) from each other and other plant ingredients.

Energy levels ranged from 8.78 kJ/g to 29.7 kJ/g for sweet potato leaves and cassava peels respectively. In general all plant feedstuffs had low levels of crude fiber ranging from 3.78±0.20% to 16.84±0.26%. Digestibility experiment results showed that there was a significant difference ($P<0.05$) in protein digestibility coefficients among different plant diets, however, diets 1 and diet 3 did not differ statistically ($P>0.05$) in digestibility coefficients. Apparent digestibility coefficients for energy (21.2 kJ/g to 43.44 kJ/g) and fat (54.29%-67.78 %) were higher than those of crude protein (24.15%-31.44). Depending on their availability and competition for other uses, most of the plant ingredients analyzed demonstrated potential for use in *Tilapia rendalli* feed. Information on nutritional and digestibility values of plant ingredients and diets will provide good nutritional indicators for the development of a system for selecting ingredients for inclusion in *Tilapia rendalli* diets.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ANF:	Anti- Nutritional Factors
FAO:	Food and Agriculture Organization
NAC:	National Aquaculture Center
NCFR:	Non-Conventional Feed Resources
LUANAR:	Lilongwe University of Agriculture and Natural Resources
RUFORUM:	Regional Universities Forum for Capacity Building in Agriculture
WFC:	World Fish Center
WVI:	World Vision International

CHAPTER ONE

GENERAL INTRODUCTION

1.1. The Challenge Facing Feed Development in Aquaculture

Fish feed is the most expensive input during aquaculture operations. The high cost of feed arises from extensive reliance on animal protein sources, such as fishmeal and shrimp meal (Omoregie, 2001). Shortage and high cost of pelleted feed severely constrains the development of low cost aquaculture systems suitable especially for small-scale farmers. Therefore, there is a need to assess the potential of non-conventional raw ingredients before use in fish diets. Good nutrition in animal production systems is essential to economically produce a healthy and high quality product. Fish nutrition has advanced dramatically in recent years Omoregie and Ogbemudia (1993) with the development of new, balanced commercial diets that promote optimal fish growth and health. However, as the cost of fish production continue to escalate due to soaring feed prices owing to extensive use of expensive animal protein like fish meal, aquaculture production becomes a less or non-profitable enterprise (El-Sayed, 2006). It is of primary importance for fish farmers to find affordable and high quality fish feeds through the use of locally available plant ingredients. Therefore, it is necessary to explore utilization of plant proteins in fish feeds as substitutes for expensive animal protein materials (Omoregie and Ogbemudia, 1993).

Fish meal has become the most essential protein for commercial aquaculture feeds. It provides the fish with high quality protein, an essential amino acid profile and has high palatability (Li *et al.*, 2006). However, fish meal is an expensive source of protein and is inaccessible to small scale fish farmers in Southern Africa because of other valuable competing uses including human consumption. Therefore, replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary because of rising costs and uncertain availability of fish meal (Higgs *et al*., 1995). Plant proteins are likely candidates because of local availability and low cost (Lim and Webster, 2006). However, substituting fishmeal with plant protein ingredients mostly results in reduction in fish growth (Francis *et al*., 2001). The current study, therefore, aims at evaluating the nutritional potential of plant ingredients despite its associated challenges.

The future development of small-scale aquaculture system depends on the use of available local ingredients which will reduce feed cost (Edwards and Allan, 2004). However, for plant ingredients to be incorporated into least cost-diets, an assessment of nutritional value is vital. It is imperative to systematically characterize the biological value of plant raw materials (Olele, 2011). In Malawi, the use of plant proteins in fish diets is sparingly practiced. Evidence exist that fish farmers are not fully aware of the potential of using plant protein in fish diets. The current study seeks to explore the nutritional potential of locally available plant and agriculture by products in Mpamba, NkhataBay. Proximate composition of plant ingredients is assessed to isolate those that have high protein and energy value. Secondly, a diet formulated from the nutritionally evaluated plant ingredients is assessed for its digestibility on *Tilapia rendalli.* The digestibility of nutrients and the feeds should be assessed to know the suitability of the feedstuffs in fish feeds. Digestibility trials and nutrient balance studies have utilized direct methods, force feeding, metabolism chambers, and various natural and artificial markers (Khan *et al*., 2003). Thus, digestibility, palatability of ingredients and nutrient utilization are the most important parameters to enable the optimal incorporation of particular ingredients in feed formulation (Glencross *et al*., 2007).

1.2. Global perspective on food security and fish production

World capture fisheries continue to steadily decline FAO, (2003) and as a result, fisheries scientists are grappling with an alternative source of fish and fish products for the global population. Aquaculture seems to be a readily available alternative to the provision of food fish eaten in the world. Fish is widely accepted because it cuts across social, cultural and religious backgrounds (Oresegun and Alegbeleye, 2001). Nutritionally, fish is one of the cheapest sources of protein and micro nutrient for millions of people in Africa (Bene and Heck, 2005). However, with the world's ever increasing population coupled with inadequate wild fish production patterns, fish supplies cannot sustain demand at 5 to 45 kg per person per/year (FAO, 2003). The United Nations predicted a population increase of 1188 million in Africa by 2010 (Muir *et al*., 2005). Chronic hunger is already prevalent in Africa where between two and four hundred million people in Sub-Saharan Africa alone are reportedly undernourished. It is estimated that over 23 million African children are malnourished (World Bank, 2006). Malawi has not been spared the scourge and there is need for stringent measures to reduce malnutrition and hunger. This dire situation would normally demand quick action and an aggressive approach tailored towards food production to feed the already high human population in order to prevent inadequate food supplies and the consequential malnutrition. Therefore, it is important to improve aquaculture feed production technologies to meet the high demand for fish and fish products. The current study therefore, aims at improving the aquaculture fish feed technology by developing an affordable and quality fish feed using local and available ingredients.

Access to quality and affordable fish feed would enhance fish production in Malawi aquaculture. As fish production increases, there would be an improvement in access to animal protein by the population. Eventually, the nutrition and health of the people would also be improved. At present, most fish famers in Malawi have a knowledge gap in feed formulation and therefore, the present study aims at empowering fish farmers to be able to mathematically formulate fish feed by combining different locally available plant ingredients into a mixed feed.

Fish provide a source of protein and sustainable income in many parts of Africa (FAO, 2003). Thirty five million people in Africa depend wholly or partially on the fishery resources for their livelihood (World Fish, 2005). In spite of aquaculture development and growth in Africa, production has been low despite the vast aquatic resources abound on the continent. Low access to affordable and quality fish feed is one of the significant challenges for the low fish production in Malawi (Figure 1) and other parts of Africa. Other significant challenges of fish farming in Malawi are; inherently slow growing fish species, poor aquaculture management and low credit facilities. According to Hecht (2000), the entire continent of Africa contributed only 0.4% to the total world aquaculture production for the period 1994 to 1995. In the year 2000, it contributed a mere 0.97% of the total global aquaculture (FAO, 2003). Since the introduction of aquaculture to Africa, some decades ago, there have been a lot of innovations, technological advancement and progress in the areas of genetics, seed propagation, pond construction and farm management in general. Despite breakthroughs recorded in these areas, most fish farmers in Africa still rely heavily on imported feed ingredients and fish feeds from European countries (Gabriel *et al.*, 2007). It is worthwhile for global efforts to be directed towards aquaculture feed technology advancement tailored at increasing fish production to cater for the ever-increasing world population.

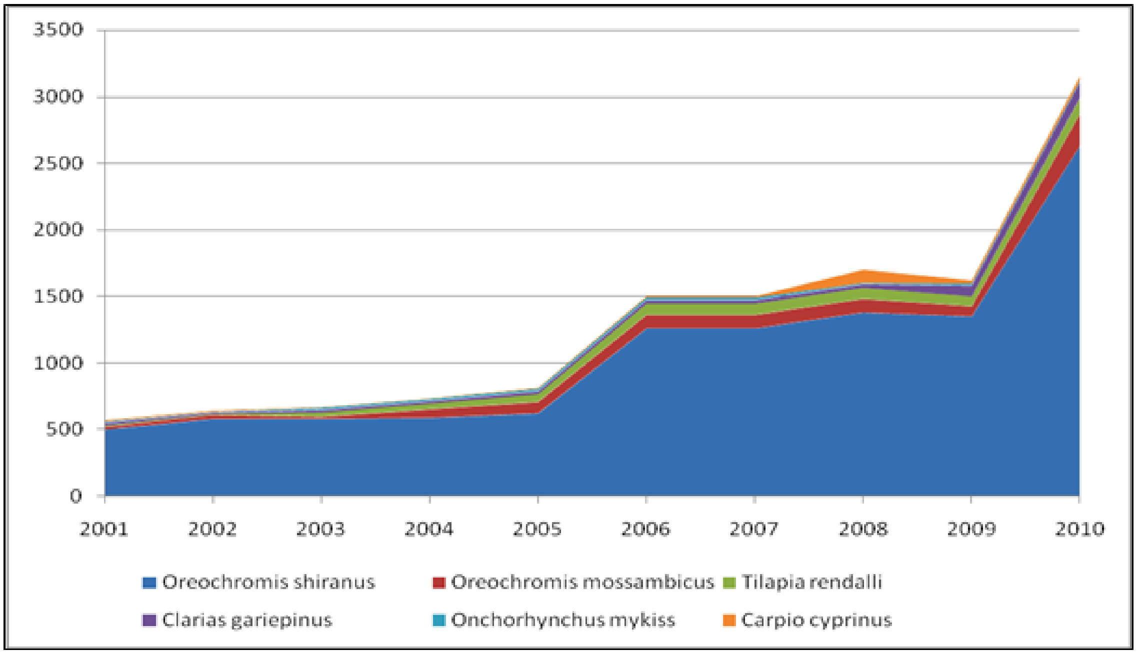

Figure 1: Production trend of aquaculture in Malawi from 2001 to 2010 (Source: FAO, 2013)

This will enhance food security and help eliminate malnutrition and hunger. The present study therefore, strives to provide information on the nutritional profile of locally available and affordable plant ingredients which can partially or wholly substitute the expensive animal-based feed ingredients like fishmeal. The development of the feed using local ingredients is also an important milestone towards improving aquaculture innovation. In addition, adoption of the plant diets can help ease the heavy importation of formulated feed, thereby reducing cost of fish production by both small and large scale fish farmers in Malawi and other parts of Africa.

1.3. Overview of aquaculture feed development in Malawi

Fish is an important source of both food and income to many people in developing countries (Gabriel *et al*., 2007). The fishing sector is important to both Malawi's economy and its overall food security, providing 300,000–450,000 jobs and 4% of GDP (FAO, 2008). Aquaculture is growing exponentially in other parts of the world as an answer to the stagnating fishery production against human population increase (Edwards and Allan, 2004). The aquaculture sector in Malawi contributes 2 % to nation's fish production with an average productivity of consumption (NAC, 2003) and per capita fish consumption has dropped from 12.9 Kg/year in 19702 to 7.3 Kg/ per in recent years (GoM, 2011). Availability of affordable quality feed is one of the most important problems that hamper aquaculture growth for both small scale as well as large scale aquaculture operators in Malawi. Most small scale fish farmers in Malawi are not able to buy animal based fish feeds, neither can they afford legumes like soybean as fish feed ingredients. This is because they are expensive since they are used as feed for humans as reported by Andrews *et al*. (2003) and also used as ingredients in livestock feed manufacturing. This has negatively affected production from and profitability of fish farming in Malawi (Chirwa, 2008). Results of a study by Chikafumbwa (1996), indicates that *Tilapia rendalli* seems to be a voracious and non-selective feeder on plants as it consumes *Cucurbita maxima*, *Tridax procumberns*, *Ipomea batatas*, *Bidens pilosa* and *Mucuna pruriens*. The study further showed that green grasses like leacuena could be presented whole into fish ponds since chopping and grinding does not present significant advantages in terms of fish growth and water quality. However, the study indicated that presentation of feed still required further studies some of which are fish growth, digestibility and anti- nutritional factors of using plant diets. Therefore, the present study contributes to the search for an affordable and quality feed for fish in Malawian aquaculture in a quest to improve the fish production at national level.

1.4. Potential of Tilapias in Malawi aquaculture

Tilapia has been identified as one of the species with greatest potential to contribute to fish production in Malawi. Previously, Tilapia was consumed mainly in Africa and Asia but nowadays it has been regarded as the "new white fish" replacing the depleted ocean stocks, leading to an increased worldwide demand (Yue and Zhou, 2008). In Malaŵi, Tilapia species are the most farmed fishes due to high people's preference to other species (Chirwa, 2008). Aquaculture fish production in Malaŵi consists of 93% Tilapia (*Oreochromis shiranus, Oreochromis karongae* and *Tilapia rendalli*), 5 percent catfish (*Clarias gariepinus*) and 2 percent exotic species such as common carp (*Cyprinus carpio*) and trout (*Onchorhychus mykiss*) (NAC, 2003). According to El-Sayed (2006) Tilapias generally display, under culture conditions, a 20% higher growth performance than in nature. In a quest to promote tilapias production, several studies have been conducted to enhance growth performance and health. Nutritional studies on species like *Oreochromis karongae, Oreochromis shiranus* and *Tilapia rendalli* (Kang'ombe *et al*., 2006; Kang'ombe *et al*., 2007) have been conducted in Malaŵi. Chikafumbwa (1996) reported on the utilization of napier *grass* (*Pennisetum perperium*) and maize bran in the polyculture of *Tilapia rendalli* and *Oreochromis shiranus* in ponds. The major determinant of a fish species to be used in aquaculture is its growth rate (especially in captivity), its acceptance of artificial feeds immediately after the yolk-sac absorption, resistance to handling stress, ease of reproduction, high fecundity and consumer acceptance (Chikafumbwa, 1996). However, its culture is limited in Malaŵi and a few neighboring countries since there other traditionally preferred fish species. Currently, *Oreochromis shiranus* is the most widely cultured Tilapia species in Malawi, despite its relatively poor growth rate (Hecht and Maluwa, 2003). However, its production has been low and this may be due to the poor husbandry practices and lack of affordable high quality formulated diet among many fish farmers in Malaŵi (Soko and Likongwe, 2002). *Tilapia rendalli* is a promising alternative candidate for aquaculture because of its ability to utilize plant protein more efficiently making it relatively cheap to raise (Chifamba, 1990).

The culturing of *Tilapia rendalli* in Malaŵi is often overlooked as an aquaculture candidate species because most farmers do not have adequate knowledge about its potential in aquaculture (Hecht and Maluwa, 2003). However, *Tilapia rendalli* can play an important role under extensive and semi-intensive fish culture systems as it feeds mainly on readily available macrophytes (Chifamba, 1990). *Tilapia rendalli* has many attributes that make it a good candidate for aquaculture and these include: feeding at low trophic levels as they feed largely on macrophytes, resistance to stress and disease, tolerance to a wide range of environmental conditions such as unfavorable temperatures, low dissolved oxygen levels, high ammonia levels and salinity, fairly fast growth and ability to reproduce readily in captivity and does not incubate eggs in the mouth, which implies that females do not stop feeding when breeding (El-Sayed, 2006). The paucity of information on the aquaculture potential of *Tilapia rendalli* prompted the current study whose main focus was to evaluate the nutritional potential of plant feed ingredients and the formulated diets fed to *Tilapia rendalli*. The present study, envisages that information generated will help fish farmers in finding an affordable and quality of diets of *Tilapia rendalli*.

1.5. Importance of digestibility in fish feed

Digestibility determination is one of the nutritional assessment tools that must be employed to deduce the availability of nutrients levels in the plant diets that can be turned into fresh. Nutritive value of feeds is determined by a number of factors, including composition, odor, texture and taste (Khan *et al.,* 2003). These factors are generally measurable in the case of the fish as digestibility and intake. Digestibility is simply a measure of the availability of nutrients. One of the most significant factors which determine the nutritive value of a feed is its digestibility (Khan *et al.*, 2003). Combination of feed intake data with digestibility data can make an accurate prediction of overall nutritive value. However, intake is relatively more important than digestibility in determining overall nutritive value because highly digestible feeds are of little value unless consumed by the animal in question (Allan *et al*., 2000). Only that portion of the feed which is soluble or is rendered soluble by hydrolysis or some other chemical or physical change can be taken up into the circulation to assist in supplying the animal body with material for building and repair of tissue or supplying the energy necessary for body functions (Glencross *et al*., 2007). In addition, measures of digestibility are somewhat easier to obtain than measures of intake and thus, considerable effort has been made by animal nutritionists to develop effective means of determining digestibility. The present study investigated the digestibility of plant diets as the first step in evaluating their potential for use in aquaculture production (Allan *et al*., 2000). The apparent digestibility of nutrients like protein and energy generated from the current digestibility study are of prime consideration for utilization in the fish feed formulation manufacturing industry (Khan *et al.*, 2003). Digestibility usually provides a fairly reliable index of nutritive value because more digestible feeds are normally consumed to a greater extent than less digestible feeds. Eventually, the generated digestibility index results from the current study will help farmers to have a better selection criterion for plant ingredients to be included in the diet of Tilapia species in captivity.

In Malaŵi and other developing countries, animal protein sources like fish and bone meal are not readily used in fish feeds manufacturing processes because they compete with human and livestock consumption and are thus expensive (Gabriel *et al*., 2007). Therefore, non-conventional plant ingredients like cassava leaf and peels could be used as an alternative to fish meal and other animal ingredients. However, it is necessary to conduct nutritional assessments of plant ingredients, agricultural-by products and plant diets. The evaluation of the digestible protein and energy value of feed ingredients is critical to the cost-effective formulation of modern aquaculture diets and is also an important part of the process in establishing their nutritional value (Glencross *et al*., 2007).

Therefore, the main objective of this study was to determine the nutritive value of different plant ingredients found in NkhataBay and to determine digestibility of different plant diets made from the plant ingredients fed to *Tilapia rendalli in* grass tanks. The current study therefore, determined digestibilities of different plant diets formulated and fed to *Tilapia rendalli*. It is anticipated that the generated information will be used for the formulation of affordable and quality feed by both large scale commercial and small scale fish farmers in Malaŵi and the rest of the world.

1.6. Objectives of the study

The main objective of this study was to evaluate the potential locally available feed ingredients from plant sources for the formulation of affordable diets for *Tilapia rendalli,* NkhataBay Northern Malaŵi.

1.6.1. *Specific objectives*

1.6.1.1. To determine the proximate composition of locally available feedstuffs from plant sources obtained from Mpamba, NkhataBay Northern, Malaŵi.

1.6.1.2. To determine the digestibility of diets formulated from locally available plant sources fed to *Tilapia rendalli.*

1.7. Research hypotheses

1.7.1. There are variations in proximate composition of different ingredients from locally available plant sources.

1.7.2. There is a variation in apparent digestibility of nutrients for different diets formulated from locally available plant sources.

1.8. Problem statement and Justification

Access to nutritional inputs is identified as a key constraint by all fish farmers in Malaŵi (Andrews *et al*., 2003). Over 90% of all fish farmers use primarily maize bran as fish feed. This feed ingredient has been recommended by extension services since the 1940s, but it has low protein content (5-12%) and a poor food-conversion ratio (FCR) of 12-20 (Hecht, 1999). While the availability of maize bran is usually good, it can vary by region or season, and when there is a general shortage of maize (the Malawian staple food), maize bran faces competing uses as it is a major source of feed for livestock such as pigs and may also be consumed directly by poorer families, hence may not be sustainable in production of fish feeds (Jamu and Costa-Pierce, 1993). In Mpamba, NkhataBay district in northern part of Malawi, aquaculture production is low (0.2 tons/ha) Kamangira unpublished (2015) and this is attributed to many reasons such as in availability of a high quality affordable feed, lack of information in formulated fish feed preparation, inadequate extension services as well as inherently slow growth of fish species currently being farmed in the area. Evidence exists that most fish farmers in Malawi are still not fully aware of the benefits of using alternative inputs such as composted maize stover, cassava leaves, sweet potato leaves, buffalo bean grass, antelope grass leaves, giant grass leaves, napier grass, mulberry leaves, *Leucanae* leaves, banana leaves, pawpaw leaves, cabbage leaves, leftover homestead food such as nsima (traditional maize porridge) (Hecht and Maluwa, 2003), and ash from kitchen fires (Jamu and Costa-Pierce, 1993).

Proximate analysis of locally available plant based ingredients was conducted to holistically assess the nutritional potential of the plant feed ingredients as feed for fish in Chingale area, Zomba district Southern part of Malaŵi (Kang’ombe *et al.*, 2009). It was noted from the analysis that plant based ingredients such as black jack and banana leaves which recorded 21% and 18% crude protein respectively can potentially be used in fish diets formulation. Additionally; Hecht (1999) reported the success in northern Malaŵi of an innovation brought by the Border Zone Development Project that promoted feeding cooked home-grown soybeans to fish. Hecht (1999) reported a food conversion ratio (FCR) of 3 and found that profit margins from the sale of fish fed on soybeans was 34% higher than for fish fed on maize bran.

It is from the foregoing discussion that development of an affordable fish feed using locally available a plant sources is imperative in Malawi. The present study focuses on utilization of affordable and locally available plant sources to replace fish meal, without reducing the nutritional quality of feed (EI-Sayed, 1999). This agrees favorably with observations by Hecht and Maluwa (2003) and Kang'ombe *et al.* (2009) that use of available plant based feed would be sustainable in Malaŵi fish industry. The present study, evaluated the nutritional potential of different plant ingredients to be used as fish feed ingredients for *Tilapia rendalli* in Mpamba, NkhataBay district northern Malawi.

Fish culture operations are being intensified and expensive conventional foodstuffs still in use, therefore, the present study aimed at developing nutritionally balanced and affordable diet that has good digestibility and provide the essential nutrients for optimal growth (Mokolensang *et al.*, 2003). To date, nutritionists and feed manufacturers have focused their trials on determining which of the wide variety of foodstuffs available to the livestock and fish feed industry may be used to produce a lower cost fish diet. Fish production sector provides not only animal protein and food security but also improve service and profits for poverty elimination in many developing countries (Sheikh and Sheikh, 2004). The current study further investigated the physical and chemical parameters of the plant ingredients and diets instead of constraining the focus on affordability. Thus, the proximate analysis and digestibility experiments were conducted to ascertain the nutritional value of the plant raw materials. Information generated from the present study is important into the contribution of knowledge on the bioavailability of nutrients and palatability of plant diets, parameters that affect feed intake and growth.

Plant byproducts are a promising source of protein and energy for the formulation of economical and nutritionally balanced fish feeds (Hardy, 2000). However, data on digestibility of most potentially available plant ingredients for fish feeds are not available in Malaŵi. Therefore, the present study was designed to determine the apparent nutrient digestibility of plant feed ingredients locally available in Mpamba, NkhataBay. The digestibility information will be used in the formulation of affordable feed for pond raised fish.

CHAPTER TWO

LITERATURE REVIEW

2.2. Current Challenges in Formulated Fish Feeds

Nutrition is central and a fundamental component in production of fish (Craig and Helfrich, 2002). In spite of this, it is also the most costly item in fish production because of protein source. In intensive aquaculture systems, feed is the most expensive item of all variable expenses irrespective of the intensity of the culture operation (Lim and Webster, 2006). This calls for deliberate and stringent measures to significantly reduce the fish feed costs. Therefore, different methodologies and approaches must be tried to find an affordable feed while growth and health of the fish is not significantly being compromised.

Fishmeal is the best protein source of fish feed globally (FAO, 2003). Among commonly used feed ingredients, fishmeal is considered to be the best ingredient due to its compatibility with the protein requirement of fish (Alam *et al*., 1996). Fishmeal is known to contain a complete essential amino acid profile that is needed for fish species (Abowei and Ekubo, 2011). It provides the fish with high quality protein, amino acid and has high palatability (Li *et al.*, 2006). Amino acids are the determining factor to meet the metabolic demands of a fish (Guimareas *et al*., 2010). In particular, essential or indispensable amino acids (EAAs) like methionine and lysine cannot be synthesized by fish and are often inadequate yet are needed for growth and tissue development (Fagbenro *et al*., 2000).

Despite being the main source of fish feed, fish meal is very expensive. As a result, most small-scale fish farmers in Malaŵi cannot afford it. This development is partly responsible for the slow growth of aquaculture in Malaŵi. Other limiting factors to aquaculture growth in Malawi are low level of advocacy on emerging technologies emanating from inadequate extension services, low capital to enable fish farmers embark on fish farming as a semi-commercial enterprise and slow growing aquaculture candidates just to mention a few. Since fishmeal is expensive as a feed ingredient, finding alternative protein sources to replace fishmeal in fish feed is important for growth of the aquaculture industry (Francis *et al*., 2001; Tacon, 2003). The use of non-conventional feedstuffs has been reported with good growth and better cost benefit values (Abowei and Ekubo, 2011). Non-conventional feed resources (NCFRs) are feeds that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production (Madu *et al*., 2003). NCFRs are noncompetitive in terms of human consumption, cheap to purchase; byproducts or waste products from agriculture. These include all types of feedstuffs from plant and animal by-products wastes.

2.3. Use of plants as ingredients in Tilapia diets

Literature indicates that several research studies have been conducted on the use of plants in fish diets. Experiments done in intensive culture systems where feeding regimes (glass, plastic or fibre glass tanks) are controlled have exhibited inconsistent results on use of plant diets by fish. Fish fed *Spirodela polyrhiza and Myriophyllum spicatum* lost weight and produced negative Specific Growth Rate (SGRs) of -1.75 and -1.71 and Feed Conversion Ratio (FCR) of -3.78 and -0.35 respectively (Setlikova and Adamek, 2004). However, *Oreochromis niloticus* exhibited slow growth and even lost weight when fed exclusively on aquatic vegetation. Fish fed *Potamogeton pectinatus* had a SGR of 3.18%/day whilst those fed *Elodea canadensis* had a slightly lower growth rate with an SGR of 2.54%/day and an FCR of 0.34.

Experiments have been done with success in feeding *Tilapia rendalli* with napier grass (*Pennisetum purpureum). Tilapia rendalli* fed on napier grass produced favorable growth rates (SGR, 1.29%/day) and was considered an effective low cost feed for African small holder farmers (Chikafumbwa, 1996). Therefore, it was recommended as supplementary feed in fish ponds. In a similar study, Adewulo (2008) reported that all the experimental plant diets were accepted by *Tilapia zilli* fingerlings, indicating that the levels of incorporation of sweet potato leaf meal did not affect the palatability of the diets. The study by Adewulo (2008) agrees favourably with Soko and Kang'ombe (2010) who reported on plant protein based diets made from locally available and low cost plant based ingredients formulated using least cost combinations. The plant diets were fed to *Tilapia rendalli* in tank based grow-out culture system and improved growth of *Tilapia rendalli*. This was evident by the good percentage increase in weight and acceptable feed utilization indices (PER and FCR). These results are consistent with a study by Kang'ombe and Brown (2008) who worked on low cost diets from plant sources administered to *Tilapia rendalli* reared in pond based cage put in ponds for 90 days. Findings of that study showed that fish growth was higher in the soybean meal-based diet, with final weight of 34.4 g, followed by sunflower cake, with final weight of 23.3 g from initial stocking weight of 4.8g an 4.9g respectively. The specific growth rate for *Tilapia rendalli* was 3.6%/day in Soybean meal based diet.

2.4. Limitations on use of Plant based Ingredient in fish Feeds

Non-conventional plant feed stuff (NCPF) are many and abundant, almost in every locality in Africa (Francis *et al*., 2001). NCPF potential and utilization in aquaculture feed have been extensively reviewed (Ugwumba, 2003). Their levels of inclusion in aquaculture feed varies and largely depends on their availability, nutrient level, processing technique, species of fish and cultural farming pattern prevalent in the locality (Oresegun and Alegbeleye, 2001). According to Nandeesha *et al*. (1991), there are so many factors which limit higher level of incorporation of plant ingredients diet. These include low protein content (Oresegun and Alegbeleye, 2001; Ibiyo and Olowosegun, 2004), amino acid imbalance (Eyo, 2001) and presence of anti-nutritional factors (Oresegun and Alegbeleye, 2001).

2.4.1. Effect of Anti-nutritional factors on feed utilization

When considering the nutrient content, some of the plant materials analyzed in various studies and the present study have showed relatively high protein levels and energy levels and relative low fiber content (Figure 2). However, it has been well known that most plant–derived nutrient sources contain a wide variety of anti-nutritional factors (ANF) (Francis *et al.*, 2011). Anti-nutritional factors include cell wall constituents, high levels of saponins, phenolic and phytic acid. Saponins are the main factors causing growth retardation when plant based protein sources are used (Oresegun and Alegbeleye, 2001). These are found in potential plant-derived feed sources and are considered to have a detrimental effect on fish. Anti-nutritional factors can adversely affect and physiological processes such as digestion absorption, and respiration (Murry *et al.*, 2010). The limiting factor of using plant–derived proteins is the presence of anti-nutritional factors or toxicants that may range from protease inhibitors, lectins, phytic acid, saponins, alkaloids, cyanogen's, tannins and gluccosinolates (Murray *et al*., 2010). These anti nutritional factors negates growth and other physiological activities when they are in high levels in the plant diets (Oresegun and Alegbeleye, 2001). On the other hand, as noted by Kays (1985), the sweet potato peel is devoid of most of these agents as the sweet potato plant usually stores these chemicals in its tubers. In the present study, the plant ingredients were first sundried primarily to rid them of anti-nutritional factors.

Processes like soaking and sun-drying of the plant ingredients before formulating the diets may reduce anti-nutritional factors and increase the growth performance of *Tilapia rendalli*. It has been reported that common processing techniques such as different cooking methods, soaking, drying, wet heating and adding feed supplements reduces the concentration of anti-nutritional factors in plant feeds and improve the feed intake. These processing techniques for plant ingredients have improved growth performance. Therefore, the quality of plant protein sources depends on the initial processing method used. Afuang *et al*. (2003) reported that solvent extracted *Moringa* leaf meal could replace up to 30% of fish meal in *Oreochromis niloticus* diets with no reduction in growth when compared with the control. In another study, Wassef *et al*. (1988) reported that germinating and defatting of soybean meal reduced the activity of protease inhibitors and consequently improved growth performance.

A better feeding management is necessary in order to achieve an optimal use of the feedstuff by the fish. In this context, mixing of different ingredients to make a diet ensure dilution of the different anti- nutrients present in individual ingredients making up the diet. Eventually, the resulting mixed diet could have beneficial effects on the nutrient utilization from the different feedstuffs available.

Thus, once the mixed plant feedstuffs are digested and the different anti-nutrients set free in the digestive tract, they can interact with each other and this could lead to a relative reduction of their individual detrimental effects (Dongmeza, 2009). A study by Olvera (2002), evaluated the effect of substituting animal protein (fishmeal) with a mixture of soybean meal and alfalfa leaf meal in diets of Nile tilapia (*Oreochromis niloticus*) fingerlings and results showed best growth performance resulting from the mixed diet.

Furthermore, it was reported that differences in climate, environmental conditions including soils in different geographical location, seasonal changes, growth conditions and agricultural practices as well as variations between individual plants can affect nutrient composition of plant materials (Harnly *et al*., 2009). Therefore, the present study was necessary to generate information that uses the local plant ingredients to formulate a diet whose digestibility was measured on an endemic species, the *Tilapia rendalli*.

It is evident from the foregoing discussion that, for the full nutritional potential of plant based ingredients and diets to be realized, the current study subjected the plant ingredients to sun drying and milling before use to rid them of anti-quality constituents such as cyanide, tannin and phytin. Thus, the present study corroborates with Francis *et al*. (2001) who reported that numerous anti-nutritional factors can be inactivated or reduced by heat treatment, de-hulling, germination and other processing steps.

2.5. The growth performance of *Tilapia rendalli* in aquaculture systems

Several researchers have reported on growth performance of *Tilapia rendalli* (Figure 2) in extensive culture systems especially ponds than intensive culture facilities. Mataka and Kang'ombe (2007) conducted a study to determine the effect of substituting maize bran with chicken manure on the production of *Tilapia rendalli* juveniles (10.71 g) in semi-intensive pond culture.

Figure 2: *Tilapia rendalli* showing external features

Results of the study by Mataka and Kang'ombe (2007) indicated a higher specific growth rate (SGR) of 1.18%/day in ponds where 75% maize bran and 25% chicken manure was applied than the 0.87%/day (SGR) where only maize bran was fed. It is recommended from the study that the fish farmers can use chicken manure combining with a supplementary feed like maize bran. The study by Mataka and Kang'ombe (2007) agrees favorably with a study by Ohashi (1998) who reported that maize bran produce better growth rates and FCR than rice bran in the *Tilapia rendalli* monoculture. The two studies are consistent with Mulumphwa and Kang'ombe (2010) who in a separate experiment reported that it is advantageous to use maize bran as a single energy source in a soybean-based diet than to use rice bran or a combination of rice bran and maize bran. Supplementation of soybean-based diets in fertilized ponds significantly improves fish growth.

Soko and Likongwe (2002) reported a SGR of 0.87%/day in *Tilapia rendalli* fed maize bran in ponds and that the addition of chicken manure increased the SGR to 1.18%/day. Soko and Kang'ombe (2010), worked on another experiment that established a potential of using a combination of local plant protein sources which was relatively cheap, readily available and easily accessible to make least- cost feed for tilapia species in tank based grow out system. Juveniles of *Tillapia rendalli* of average weight 9.5 ± SD 0.5g were stocked in outdoor concrete tanks and fed on diets of different plant protein sources formulated at different crude protein (CP) levels. The results of the study by Soko and Kang'ombe (2010) indicated that diets formulated from plant protein ingredients had significant effect on growth and survival of *Tilapia rendalli*. Fish fed on Diet 1 had an average final weight 25.64±0.79g, Diet 2 had final average weight of 23.31±0.71g, Diet 3 had final average weight of 21.71 ±0.80g and Diet 4 had an average final weight of 23.00±1.12g and differed significantly among treatments. (Diets 1, 2 3).

In another related study, Kang'ombe and Brown (2008) reported that fertilization with chicken manure alone produced low growth rates in *Tilapia rendalli*. The highest growth and SGR was realized in treatments where chicken manure was supplemented with soybean based diets, followed by sunflower based diets and cottonseed cake based diets. The SGRs were 3.6; 2.9; 2.5; and 2.1 for soybean, sunflower, cottonseed and chicken manure respectively. The lowest Feed Conversion Ratio (FCR) of 1.2 was obtained in soybean based diets followed by sunflower (1.6) and cottonseed (1.9). These researchers suggest that the use of low protein diets having soybean would produce better results and increased yield of *Tilapia rendalli* when combined with fertilization of the aquaculture system. The findings corroborates those by Chifamba (1990) whole indicated that Tilapias including *Tilapia rendalli* grow 86% better in captivity than in nature. These studies point out that *Tilapia rendalli* has a potential as an aquaculture candidate because it has the ability to utilize plant diets. Therefore, the present study further explores the ability of *Tilapia rendalli* to use other locally available plant diets like cassava leaf meal, sweet potato leaf meal and cocoyam, which are locally available in Mpamba, NkhataBay because of the interest of farmers in fish farming. In addition, it is also imperative to conduct further studies on the plant diets in various aquaculture systems e.g. raceways, tanks and cages.

2.6. Feed digestibility studies-an important parameter for feed quality

Fish meal is known for its high digestibility due to high essential amino acids and fatty acid contents, low carbohydrates and low anti-nutritional factors contents (Naylor *et al*., 2009).There is a general consensus that as aquaculture production will increase to meet expected demand for fisheries products in the next 7–10 years, and, annual fish meal supply will not meet increasing demand (Allan *et al.,* 2000). Therefore, the price of fish meal is going to increase steadily since supply for fish meal will be lower than demand (Cheng *et al*., 2004). This calls for better knowledge of the nutritional value of non-conventional ingredients that could replace fish meal particularly for small-scale farmers in developing countries (Edwards and Allan, 2004; Naylor *et al*., 2009). In recent years, research on the use of locally available feed resources, such as agricultural by-products, industrial waste and animal by-products, in fish feed has increased (Allan *et al.,* 2000; Sklan *et. al*., 2004; Gatlin *et al*., 2007). The nutritional value of feed components depends on ability of the animal to digest and absorb the nutrients (Falaye and Jauncey, 1999; Riche *et al.,* 2001). Therefore, determination of digestibility is an important first step in the evaluation process of an ingredient for use in diets for different fish species.

Many studies have reported the digestibility of various plant feed ingredients for Atlantic salmon (*Salmo salar*) (Storebakken *et al*., 2000; Glencross *et al*., 2004; Refstie *et al.,* 2005; Aas *et al*., 2006; Refstie *et al*., 2006; Denstadli *et al*., 2007; Kraugerud *et al*., 2007). Digestibility of plant proteins was lower for the plant feed ingredients compared to fishmeal; except for bacterial protein meal, extracted soybean meal, oat, and rapeseed and sunflower. There have been variable results reported for soybean meal with some studies showing decreased protein digestibility compared to fishmeal while other studies did not detect any change in protein digestibility (Refstie *et al.*, 2005, 2006; Kraugerud *et al*., 2007). When the plant feed stuff was further chopped to make protein concentrates, digestibility was unaffected (Glencross *et. al*., 2004; Denstadli *et al*., 2007).

The present study, conducted to generate information on digestibility of *Tilapia rendalli* fed plant diets is an important step in provision of data for the formulation of affordable and quality diets that uses locally available agriculture by products and plant ingredients.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Location of the study area

The study was conducted in Mpamba Extension Planning Area (EPA) (Figure 3) located in the north-western part of Nkhatabay district in the area of Traditional Authority (TA) Timbiri and sub TA Mnyaluwanga. It is accessed via the Mzuzu-Nkhatabay tarmac road, which runs through the southern part of the Mzuzu Agricultural Development Planning area at a distance of about 20km to the east of Mzuzu City. Mpamba (Figure 3) predominantly consists of lithosoils especially in steep slopes and receives an average monthly rainfall of 380mm. The area is warm since it is located in the tropics with average temperature range of 30^0C in October to 22^0C in June or July. Mpamba (Figure 3) lies in the vicinity of a wet grassland traditionally called Limphasa dambo named after a perennial Limphasa river that runs through Mpamba. Water availability is therefore abundant throughout the year and 500 farmers practice fish farming with an average pond size of $275m^2$ in the area.

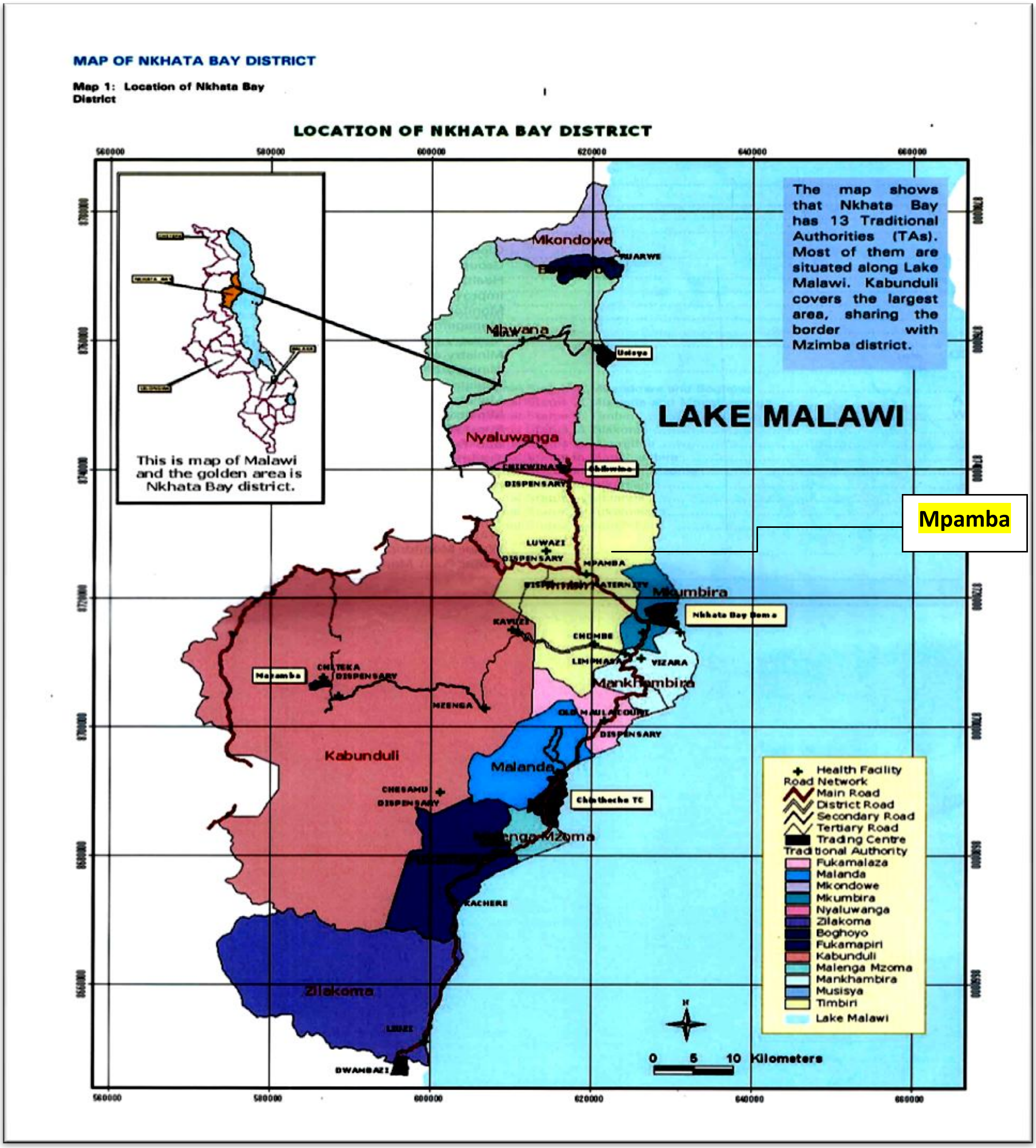

Figure 3: Map of Nkhatabay district

3.2. Collection of locally available plant feed ingredients

An observational survey by farmers and researchers was conducted in Mpamba, Nkhata-bay northern Malaŵi, to identify locally available plants for assessment of their nutritional value .The aim was to ascertain the plant's potential for inclusion into diets for pond raised *Tilapia rendalli*. Selection of the plant ingredients was based on both seasonal availability in areas where they are present, quantity; competition for other uses like human and livestock consumption as well as compost fertilizer, nutritional value reported in literature by previous researchers and cost of purchasing the plant ingredients. The following fresh plant feed ingredients were collected (Figure 4a) for the experiment by hand picking, use of pangas and as well as slashers: cassava (*Manihot esculenta*) peels and leaves, pawpaw (*Carica papaya*) leaves, sweet potato (*Ipomea batatus*) leaves, and tubers, jackfruit (*Artocarpus heterophyllus*), mexican fire plant (*Euphorbia heterophylla*), black jack (*Bidens pilosa*), cocoyam (*Colocasia esculenta*) leaves, banana (*Musa balbisiana*) leaves, maize (*Zea mais*) bran, and akee (*Blighia sapid*) leaves. The leafy plant ingredients like cassava leaf, sweet potato leaf, cocoyam leaf, banana leaf were chopped in small sizes while tubers like sweet potato tuber and cassava were first peeled to get the peels when sliced into smaller portions for storage. The collected plant ingredients were kept in sacks and stored in the well ventilated dry rooms for a day before being sundried.

Figure 4: Collection (a) and drying (b) of plant feed ingredients

3.3. Processing of plant feed ingredients

All plant ingredients were dried (Figure 4b) in the sun for three days as recommended by Adewulo, (2008). Drying (Figure 4b) of plant ingredients was done not only to reduce toxicity but also to prepare them for milling into powder suitable for proximate analysis. Ingredients were milled using a mortar and a pestle (Figure 7a). Finally, the milled plant samples were sieved using a wooden framed 2mm mesh sized sieve to remove debris to remain with the powder (Figure 7b).

3.4. Proximate Analysis of plant feed ingredients

Proximate analysis (Figure 5) of the plant ingredients was done at Department of Aquaculture and Fisheries Science laboratory Lilongwe University of Agriculture and Natural Resources (LUANAR), Bunda Campus. The milled plant feed samples were analyzed for crude protein, crude fiber, crude fat, ash, moisture content and gross energy in triplicate, following the procedure outlined by the Association of Official Analytical Chemists AOAC (2003).

3.4.1. Determination of moisture (dry matter basis)

Moisture content was determined by the standard method where samples were dried at 105^{o}C for about six hours. The difference between the initial weight of the sample and that of the final weight of the sample constituted the moisture content while the final weight was the dry matter.

$$MC(\%) = \frac{\{(W_1 - W_0) - (W_2 - W_0)\}}{(W_1 - W_0)} * 100 \qquad (Eq. \quad 1)$$

Where:

MC (%) = Moisture content of sample (MC %)

W_0 = Weight of the dish

W_1 = Weight of the dish + wet sample

W_2 = Weight of the dish + dry sample

DM (%) =Dry matter content of the sample

DM (%) = 100 – MC (%)

3.4.2 Determination of crude protein

Crude protein content in the plant ingredients was determined following the Kjeldhal method Samples were digested in sulphuric acid, distilled and titrated against standard 0.05N sodium hydroxide solution. To quantify the crude protein%, the nitrogen was converted to protein by multiplying with a conversion factor of 6.25. Protein contains 16% nitrogen hence 6.25 are obtained from dividing 100 by 16.

$$CP(\%) = (Wt_r / Wt_s) * 100 \qquad (Eq. \quad 2)$$

Where:

CP (%)= % Crude Protein

Wt_r =Weight of residue

Wt_s =Weight of sample

3.4.3. *Determination of Crude fat*

The lipid content was determined by Soxhlet Method. Ether extracts were analyzed using a sample size of 2 g digested in a Soxhlet extractor with petroleum ether (boiling point 40–60 ^{o}C). Crude fat (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^{o}C for 4 hours, cooled in desiccators and weighed. The lipid content was determined by the following formula:

$$LC(\%) = \frac{(W_4 - W_3)}{(W_2 - W_1)} * 100 \qquad (Eq. \quad 3)$$

Where:

LC (%)= Lipid content (%)

Weight of the filter paper	=	W_1
Weight of the filter paper + sample	=	W_2
Weight of the cup + boiling chips	=	W_3
Weight of the cup + chips + lipid	=	W_4

3.4.4. Determination of Crude fibre

Crude fibre was determined by acid-base digestion using 1.25% H2S04 (w/v) and 1.25% NaOH (w/v) solution. A 5 g sample was boiled in weak acid of 0.1 M HCl and afterwards placed in weak base, 0.313 M Sodium hydroxide. Samples were further subjected to heating at 550^{o}C temperature for 2 hours using a muffle furnace and then cooled. Crude fibre was then quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample

$$C_f(\%) = \frac{W_2 - W_3}{W_1} * 100 \qquad (Eq. \quad 4)$$

C_f (%)	=	Crude fibre in (%)
Sample weight	=	W_1
Weight of the crucible + Dry residue	=	W_2
Weight of the crucible + Ash	=	W_3

3.4.5. Determination of Ash

Ash is the inorganic material that remains after a sample is burnt at 550^{o}C . The ash was determined by heating the sample in the muffle furnace at 550^{o}C for 5 hours. The temperature was used to prevent loss of certain volatile minerals. Ash (%) was calculated by dividing weight of ash (g) of the sample and of dry matter (g) of the sample multiplied by 100.

$$Ash(\%) = \frac{W_2 - W_0}{W_1 - W_0} * 100 \qquad \textbf{(}\mathit{Eq}\textbf{.} \quad 5\textbf{)}$$

Where

Ash (%) =Ash Content of the Sample (%)

W_0 =Weight of clean, dry crucible

W_1 = Weight of clean, dry crucible + dry sample

W_2 =Weight of clean, dry crucible + ash

3.4.6. Determination of Gross energy

Gross energy was determined by igniting the samples in a Gallenkamp Ballistic bomb calorimeter CB-370 .Total heat of combustion of the sample was determined by completely oxidizing the compound to carbon dioxide, water and other gases and measuring the heat released.

Figure 5: Proximate analysis

3.5. Digestibility Experiment

3.5.1. Methods of determining Apparent Digestibility Coefficients (ADCs)

There are several methods for determining apparent digestibility. These are: direct method which can either be gravimetric or total collection and indirect method which uses chromic oxide or inorganic matter marker, indigestible fiber, *in vitro* enzyme digestion, nutrient composition correlations (i.e., fiber, soluble/insoluble sugar ratio or nitrogen) and radiolabeled tracers (Watts *et al*., 2010). The gravimetric method for estimating the digestibility of food by direct calculation is based on the difference in quantity of food eaten and feces produced. The major problems are the large amount of food and feces necessary for weighing, difficulty in complete recovery of feces, variation in individual ingestion rate and prolonged gut retention time. An additional problem is the continuous-flow, stirred-tank reactor nature of the gut that results in mixing of food ingested over time and prolonged defecation of that food. Fernandez and Boudourn esque (2000), combined feces collected daily for three days from ten *P. lividus* while Otero-Villanueva *et al*. (2004) made daily estimates of the food provided one day and feces collected the following morning by individual *P. miliaris* for each month of their experiment.

To avoid the problems associated with complete recovery of feceas and the amount of feceas necessary for direct calculation of digestibility, an indirect method based on the difference in concentration of a marker (McGinnis and Kasting,1964) or ash (Conover, 1966) in the food and feces has been used. The marker is presumed to be non-digested and non-absorbed in the gut. Otero-Villanueva *et al*. (2004) found digestibility in *P. miliaris* measured indirectly with ash as a marker was much higher than that measured directly. Klinger *et al*. (1994) estimated apparent dry matter digestibility of *L. variegatus* calculated indirectly with ash as a marker was significantly less (12.5%) than with chromic oxide as a marker, interpreted to mean loss of ash.

In the present study, determination of Apparent Digestibility Coefficient (ADC) was performed by the indirect method, using 1% chromic oxide $_{III}$ (Cr_2O_3) as inert marker following the procedure set out by (Bremer-Neto *et al*., 2005).

3.5.2. Digestibility Experiment Design and fish used

The digestibility experiment was conducted at the Department of Fisheries Science Laboratory, Mzuzu University located in NkhataBay district, northern Malaŵi. The experiment was laid out in Completely Randomized Design (CRD) (Figure 6). Each treatment was replicated three times, 10 *Tilapia rendalli* per replicate, with mean initial fish weights of (25 ± 2 g). Fingerings were procured from fish farmers around Mpamba area in NkhataBay. Each treatment was randomly assigned to three glass aquaria (Figure 6) which were 35cm long, 30cm wide and 30cm high. Water for the experiment was supplied from Lake Malaŵi and had average temperature (21 ± 2 °C), dissolved oxygen (7.3 ± 0.3 mg/ L and pH 7.1 ± 0.2). Fish were exposed to natural daily light regime.

3.6.1 *Experimental Design Layout*

The experiment was laid out in a Completely Randomized Design as illustrated in Figure 6.

Figure 6: Layout of glass aquaria for digestibility experiment

Figure 7: Processing of plant feed ingredients

3.5.3. Plant ingredients and diet preparation for Digestibility Experiment

Locally available plant feed ingredients obtained from Mpamba NkhataBay were evaluated for proximate composition (Table 2). The selected ingredients for diet formulation were cassava leaf and flour, sweet potato leaf and meal, black jack and cocoyam leaf. They were dried in the sun for three days before being milled using a traditional mortar and pestle except the cassava and sweet potato flour. The experimental diets were formulated using the trial and error method. Chromic oxide (Cr_2O_3) was used as an inert marker in reference diet (Table 1). Formulated plant diets (Table 1) were mechanically mixed with warm water to make dough which was later used to produce pellets. The resultant moist pellets were then dried under a shade for approximately 12 hr. After that, the diets were reduced in size and sieved into 2–3 mm pellet sizes (Figure 8).

Figure 8: Pelleted plant diets

Table 1: Percentage ingredient composition of experimental diets fed to *Tilapia rendalli*

Ingredients(Kg)	Diet 1 (18%) CP	Diet 2 (18%)CP	Diet 3 (18%)CP	Diet 4 (18%)CP
Cassava leaf meal	31.4	31.4	23.4	23.4
Cocoyam meal	-	31.4	19.4	19.4
Black jack	31.4	-	19.4	19.4
Maize bran	11.4	11.4	-	17.4
Sweet potato meal	11.4	11.4	17.4	-
Sweet potato leaf meal	11.4	11.4	17.4	17.4
Cassava Flour	2	2	2	2
Chromic oxide	1	1	1	1
Total	100	100	100	100

Table 1 shows the percentage composition of experimental diets fed to *Tilapia rendalli* whose Apparent Digestibility Coefficients were calculated. Diet 1 (CL, BJ, MZB, SPM, SPM, CF and Chromic oxide) was designated as treatment 1 during digestibility experiment), Diet 2 (CL, CYM,, MZB, SPM, SPM, CF and Chromic oxide) was designated as treatment 2 during digestibility experiment), Diet 3 (CL, CYM, BJ, SPM, SPM, CF and Chromic oxide) was designated as treatment 3 during digestibility experiment) and Diet 4 (CL, CYMBJ, MZB, SPM, CF and Chromic oxide) was designated as treatment 4 during digestibility experiment).

3.5.4. Digestibility trial procedure

The fish (each 25g on average) were acclimatized for 7 days prior to the beginning of fecal collection during which they were fed a combination of experimental plant diets. During the 21 day experimental period, fish were fed two times at 4 h intervals from 09:00 to 1:00 h at the daily rate of 4% of their body weight. One hour after the feed was administered; any feed and feceas present in the aquaria were removed.

3.5.5. Feacal collection during the digestibility trial

Faecal matter (Figure 10) was collected from the aquaria by using a siphon and a small hand net (Figure 9) and then placed into a beaker. Feacal collection was done within 2 hours of voiding during the day and the fecal material voided during the night was collected next morning at 07:00 hours. Feacal collection (Figure 9) was done for 21 days. Samples of feacal material from each treatment replicated twice were pooled and kept in beakers to dry until analysis of feceal matter. Prior to the analysis, feceal samples (Figure 10) from the rest of the days from fish on each experimental diet were pooled together and analyzed.

Figure 9: Feacal collection by siphoning

3.5.6. Biochemical analysis of fecal material

The feceal material (Figure 10) and plant diets (Figure 8) for the entire experimental period were pooled in triplicates and then analyzed for crude protein.

Figure 10: Feacal matter for biochemical analysis

Crude fat, fiber, and ash, following the procedures stipulated by the Association of Official Analytical Chemists (AOAC, 2003). Gross energy was analyzed using a bomb calorimeter and chromic oxide was determined according to Fenton & Fenton (1979). Total nitrogen (N) was determined by the Kjeldahl method and CP content was calculated as N × 6.25. Ether extract was determined by Soxhlet extraction without acid hydrolysis. Ash was the residue after ashing the samples at 550^0C. Fiber content determined using acid–base digestion.

3.6. Digestibility determination

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

$$ADCN_{dt} = 100 - \{100[(Cr_d / Cr_f)(F_n / D_n)]\}$$ (Eq. 6)

Where:

$ADCN_{dt}$ = Apparent Digestibility Coefficient of Nutrients in the diets

Cr_d = % Chromic Oxide in the diet

Cr_f =% Chromic Oxide in the feaces

F_n = % nutrient in feaces

D_n = % nutrient in feaces

3.7. Data Analysis

3.7.1 Statistical analysis for proximate composition of plant ingredients and Apparent Digestibility Coefficients for the diets(ADCs)

Data for crude protein (%), crude fat (%), crude fiber (%), ash (%), moisture content (%) and gross energy (kJ/g) from both proximate and digestibility experiments were subjected to a one-way Analysis of Variance (ANOVA) using the Statistical Package for Social Scientists (SPSS) and R statistical software respectively. Differences in proximate composition and Apparent Digestibility Coefficients among samples were determined according to Duncan's multiple range test mean comparison Duncan (1955) and were considered significant at P <0 05.

3.7.3. Statistical Model used in the experiment is as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \qquad \text{(Eq. 7)}$$

Where Y_{ij}= Percent nutrient digestibility

μ= Overall mean

τ= Treatment effect i = 1….4

ε_{ij} = Residual error

CHAPTER FOUR

RESULTS

4.1. Proximate Composition of plant ingredients

Data on proximate composition of selected plant feedstuffs are presented in Table 2:

Table 2: Proximate composition of plant feed ingredients from Mpamba (Mean±SE) expressed as percent (%) dry matter.

Ingredient Analyzed	Moisture Content (%)	Ash (%)	Crude Fiber (%)	Crude Protein (%)	Crude Fat (%)	Energy kJ/g
CL	11.97±0.75^{c}	13.6±0.65^{c}	16.35±0.75^{a}	21.17±0.56^{b}	3.16±0.00^{c}	20.59±0.08^{b}
CP	6.70±0.09^{d}	46.6±0.40^{b}	16.84±0.26^{a}	7.40±0.34^{d}	5. 92±0.1^{c}	8.78±0.97^{d}
SPL	10.89±0.31^{c}	85.75±0.0^{a}	9.16±0.70^{b}	8.40±0.10^{c}	2.98±0.25^{c}	29.7±0.23^{a}
SPP	25.95±4.29^{a}	6.04±0.45^{c}	3.26±0.20^{c}	8.40±0.80^{c}	5.01±1.64^{c}	15.21±0.12^{c}
CYL	7.08±1.56^{c}	14.84±0.45^{c}	3.95±0.15^{c}	24.28±0.11^{a}	7.23±1.52^{b}	19.54±0.21^{b}
BL	7.80±1.56^{c}	16.8±3.50^{c}	6.95±0.15^{c}	7.65±0.23^{d}	2.22±0.10^{c}	19.06±0.92^{b}
PPL	10.95±0.10^{c}	13.5±0.47^{c}	5.50±0.20^{c}	2.78±0.14^{e}	16.07±0.10^{a}	15.21±0.23^{c}
BJ	20.79±0.71^{b}	23.1±0.91^{b}	6.40±0.75^{c}	24. 35±0.7^{a}	5.65±0.93^{c}	12.4±0.00^{c}
MZB	8.87±0.90^{c}	3.72±0.32^{c}	3.40±0.15^{c}	11.81±0.11^{c}	7.28±1.90^{b}	15.72±0.00^{c}
MFP	10.05±1.00^{c}	11.9±0.21^{c}	6.35±0.25^{c}	11.40±0.11^{c}	4.64±1.49^{c}	12.22±0.00^{c}
AK	10.37±0.43^{c}	7.06±0.05^{c}	5.5±0.35^{c}	12.07±0.18^{c}	10.58±1.00^{b}	19.63±0.17^{b}
JF	8.44±0.20^{c}	9.05±0.15^{c}	7.0±0.20^{c}	4.77±0.45^{d}	7.83±0.25^{b}	19.27±0.24^{b}
SPM	9.67±0.11^{c}	85.7±0.15^{a}	3.19±0.30^{c}	11.97±0.45^{c}	3.2±0.45^{c}	15.32±0.50^{c}
P-value	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)	48.69	92.81	61.15	57.47	59.12	74.33

Values (Mean±SE) in a column with different superscript letters are significantly different ($P<0.05$); Where; CL: Cassava Leaf, CP: Cassava Peels, SML: Sweet Potato Meal, SPP: Sweet Potato Peel, CYL: Cocoa yam, BL: Banana Leaf, PPL: Papaya Leaf Meal, BJ: Black Jack, MZB: Maize Bran, MFP: Mexican fire plant, AK: Akee, JF: Jackfruit, SPM: Sweet potato meal,P-value:0.05 ,CV(%): percentage Coefficient of Variation

4.1.1. *Moisture Content*

Moisture content differed significantly (P=0.00) among the plant ingredients (Table 2), with CL, SPP, SPL, AK, MZB, AK, CYL, JF PPL, BL, BJ and SPM. Among the plant ingredients, sweet potato (*Ipomoea batatus*) peels had the highest (25.95%) moisture content whilst cassava (*Manihot esculenta*) peels had the lowest (6.70%). Multiple comparisons tests (Table 2) further shows that CL, SPL, BL, PPL, MFP, AK, MZB and were not significant (share same superscript letters) among themselves. Coefficient of variation (CV %) shows that 48.69% of the data on moisture content has been dispersed. Ash contents were generally variable with the lowest being in maize bran (*Zea maize)* recording 3.7% whist the highest was in sweet potato peels with 85%.

4.1.2. Gross energy

Energy levels were ranged from 8.7 kJ/g to 29.7 kJ/g (Table 2) in cassava peels (*Manihot esculenta*) and sweet potato (*Ipomoea batatus)* peels respectively and an average of 17 kJ/g for all ingredients analyzed. In that context, sweet potato (*Ipomoea batatus*) peels, maize (*Zea maize*) bran and papaya (*Carica papaya*) leaves had the same amount of energy levels, i.e. almost 15 kJ/g on average. Similarly, banana (*Musa acuminata*) leaves, leaves of akee (*Blighia sapid*), jackfriut (*Artocarpus heterophyllus*), and cocoyam (*Caladium bicolor*) leaves gave almost a uniform amount of energy level of average of 19 kJ/g. Energy levels of black jack (*Bidens pilosa*), and (*Euphorbia heterophylla*), Mexican fire plant were lower at 12 kJ/g for both plant ingredients (Table 2).

4.1.3. Crude Protein

Analysis of variance (ANOVA) show that crude protein in the present study differed significantly (P=0.00) among the plant ingredients (Table 2). Multiple comparisons tests (Table 2) further shows that CYL and BJ were the highest (24.28% and 24.35%) and were not significantly different (share same superscript) among themselves. On the other hand, PPL registered the lowest (2.78%) CP% and the CV% show that the data on CP% was 57.47% spread.

4.1.4. Crude fat

Analysis of variance (ANOVA) show that crude fat differed significantly (P=0.00) among the plant ingredients (Table 2). Multiple comparisons tests (Table 2) further shows PPL (16. 07 %) had the highest whilst BL show the lowest (2.22%) crude fat (%) . Coefficient of variation show that 59.12 % of the data for Crude fat is distributed.

4.1.5. Crude fiber

ANOVA showed that cassava (*Manihot esculenta*) leaf meal and cassava (*Manihot esculenta*) peels had 16.35% and 16.84 % respectively (Table 2) and were statistically different (P=0.00) from sweet potato (*Ipomoea batatus*) leaf, sweet potato (*Ipomoea batatus*) peels, cocoyam (*Caladium bicolor*) leaf meal and maize (*Zea maize*) bran had 3.19%, 3.26%, 3.96% and 3.4% respectively. On the other hand, banana (*Musa acuminate*) leaf meal, mexican fire plant and black jack (*Bidens pilosa*) had 6.95%, 6.35% and 6.45% crude fiber content respectively. Coefficient of variation shows that 61.57% of the data has been dispersed.

Table 3: Selected mineral and vitamin C composition of plant feed ingredient (Mean±SE) from Mpamba expressed as percent (%) dry matter

		Mineral Composition		
Ingredient Analyzed	Calcium (%)	Potassium (%)	Phosphorus (%)	Vitamin C (%)
CL	1.62±0.04[c]	1.11±0.01[a]	0.29±0.02[b]	5.55±0.75[c]
CP	0.57±0.02[d]	0.89±0.01[a]	0.12±0.01[b]	3.63±0.15[c]
SPL	21.1±0.29[a]	1.33±0.01[a]	0.88±0.03[b]	12.3±0.05[b]
SPP	14.8±0.12[b]	0.98±0.01[a]	14.8±0.12[a]	4.75±0.15[c]
CYL	0.23±0.10[d]	0.19±0.02[c]	0.55±0.00[b]	12.4±0.15[b]
BL	0.33±0.00[d]	0.26±0.23[c]	0.12±0.00[b]	3.00±0.20[c]
PPL	1.05±0.05[d]	0.89±0.01a	2.23±0.04[b]	21.2±0.15[a]
BJ	4.66±0.00[c]	2.20±0.14b	7.01±0.00[c]	5.07±0.75[c]
MZB	0.55±0.01[d]	0.33±0.00c	0.56±0.02[a]	1.30±0.00[c]
MFP	2.80±0.00[c]	1.70±0.00a	5.30±0.10[b]	13.7±5.15[b]
AK	2.30±0.06[c]	1.48±0.14[a]	4.02±0.01[b]	13.7±0.00[b]
JF	3.33±0.03[c]	2.60±0.25[d]	2.44±0.38[b]	12.6±0.20[b]
SPM	19.0±0.16[b]	1.64±0.00[e]	1.04±0.06[b]	2.80±0.00[c]
P-value	0.00	0.00	0.00	0.00
CV (%)	75.0	60.02	74.56	68.64

Values (Mean±SE) in a column with different superscript letters are significantly different ($P<0.05$); Where; CL: Cassava Leaf, CP: Cassava Peels, SML: Sweet Potato Meal, SPP: Sweet Potato Peel, CYL: Cocoa yam, BL: Banana Leaf, PPL: Papaya Leaf Meal, BJ: Black Jack, MZB: Maize Bran, MFP: Mexican fire plant, AK: Akee, JF: Jackfruit, SPM: Sweet potato meal.P- value: 0.05, CV: Coefficient of Variation(%).

4.1.6. Mineral composition

ANOVA show that mineral contents (Table 3) were statistically different (P=0.00) among the plant ingredients ranging from 0.12% to 5.3% for phosphorus, 0.05% to 2.6% for potassium and 0.03% to 19.05% for calcium. However, big variations were observed in calcium levels between sweet potato (*Ipomoea batatus*) leaf and sweet potato (*Ipomoea batatus*) peel had 19.05% and 14.08% respectively, and banana (*Musa acuminate*) leaf meal which recorded 0.03%.1. The Coefficient of variation CV%, (Table 3) show that the data was adequately dispersed in all mineral contents with calcium 75%, potassium 60. 02%, phosphorus 74.56% and vitamin C 68.64.

Table 4: Proximate composition (%) of the whole body carcass of *Tilapia rendalli* fed on pelleted diets containing different plant feeds ingredients

Element Analyzed	Crude protein (%)	Energy kJ/g	Crude Fat (%)	Crude fibre (%)	Ash (%)	Moisture (DM) (%)
Treatment 1	61.88±0.25[a]	15.25±0.05[a]	24.04±0.14[a]	0.32±0.006[a]	0.33±0.03[a]	36.78±0.03[a]
Treatment 2	63.21±0.03[ab]	14.69±0.02[b]	22.69±0.10[a]	0.37±0.006[b]	0.35±0.08[b]	34.91±0.03[b]
Treatment 3	62.71±0.07[ab]	15.78±0.06[c]	27.2±0.28[b]	0.38±0.00[b]	0.34±0.03[c]	34.76±0.03[b]
Treatment 4	62.38±0.03[b]	15.36±0.04[a]	26.64±0.08[b]	0.36±0.003[ab]	0.34±0.03[c]	32.43±0.03[a]

Values (Mean±SE) in a column with different superscript letters are significantly different ($P<0.05$)

Whole body protein concentration differed significantly ($P>0.05$) among the treatments (Table 4). Amongst the nutrients analyzed, crude protein showed higher values than all nutrients ranging from 63.38% to 61.88% for treatment 2 and 1 respectively. Crude fiber and ash concentrations in the whole body carcass of *Tilapia rendalli* showed the least contents among the nutrients analyzed ranging from 0.35% to 0.34% for crude fibre and 0.33%-0.34% for ash (Table 4).

Table 5: Proximate composition (%) of pelleted diets containing different plant feedstuffs fed to *Tilapia rendalli*

Element Analyzed	Crude protein (%)	Gross energy kJ/g	Crude fat (%)	Crude fibre (%)	Ash (%)	Moisture (DM) (%)
Diet 1	29.52±0.07[a]	10.99±0.09[a]	9.57±0.12[a]	14.28±0.04[a]	14.76±0.05[ab]	92.52±0.06[a]
Diet 2	30.5±0.05[b]	10.81±0.09[b]	10.95±0.05[b]	14.31±0.09[a]	14.3±0.05[ab]	93.14±0.02[b]
Diet 3	29.31±0.19[a]	11.18±0.02[a]	11.5±0.06[c]	14.02±0.07[b]	14.1±0.03[b]	92.64±0.04[a]
Diet 4	30.82±0.81[c]	10.38±0.02[c]	9.5±0.11[c]	14.49±0.02[c]	15.06±0.01[a]	93.55±0.34[c]

Values (Mean±SE) in a column with different superscript letters are significantly different ($P<0.05$)

Crude protein (Table 5) for the pelleted diets containing different plant ingredients were significantly different at P<0.05 with diet 4 showing 30.82% and the lowest 29.31% CP recorded for diet 3. Similarly, gross energy (Table 5) differed significantly at P<0.05 with diet 4 showing the highest level at 11.18kJ/g and diet 3 showing the lowest gross energy content at 10.38kJ/g. Crude fibre contents for diets 1 and 2 did not differ significantly ranging from 4.28% and 4.31 % respectively. However, diets 1 and 2 differed significantly P>0.05 with diets 3 and 4 that had 4.02% and 4.49% respectively.

4.2. Apparent Digestibility Coefficients (ADCs)

The apparent digestibility coefficients determined by indirect method of digestibility as reported in Table 6.

Table 6: Apparent digestibility coefficients (%) of pelleted diets containing different plant feedstuffs fed to *Tilapia rendalli*

Element Analyzed	Crude protein (%)	Energy kJ/g	Crude fat (%)	Crude fiber (%)	Ash (%)
Diet 1	30.44±0.29[b]	43.44±0.59[a]	67.78±1.35[a]	23.77±0.95[c]	3.82±0.66[c]
Diet 2	31.45±0.34[a]	31.50±0.36[b]	61.54±0.91[b]	39.57±0.84[a]	4.34±1.35[b]
Diet 3	30.04±0.06[b]	31.04±0.34[b]	61.65±0.71[b]	26.93±0.76[b]	4.82±0.72[b]
Diet 4	24.15±0.28[c]	21.56±0.34[c]	54.29±2.22[c]	24.29±0.59[c]	11.80±1.61[a]

Values (Mean±SE) in a column with different superscript letters are significantly different (P<0.05).

4.2.1. Crude Protein Digestibility

The protein digestibility coefficients ranged from 31.45% to 24.15% (Table 6). There were significant differences among the digestibility of protein in different diets P (<0.05). But protein digestibility between diets 1 and 3 was not significantly different P (>0.05) .However, diet 2 (31.45%) had the highest digestibility coefficient, followed by diet 1 (30.44%), then diet 3 (30.04%) and lastly diet 4 (24.15%) (Table 6).

4.2.2. Crude fat Digestibility

The fat digestibility coefficient ranged from 67.78% to 54.29% (Table 6). There were significant differences among the digestibility of fat in different diets P (<0.05). But fat digestibility between diets 2 and was not significantly different P (>0.05). However, diet 1 (67.78%) had the highest digestibility coefficient, followed by diet 3 (61.65%), then diet 2 (61.54%) and lastly diet 4 (54.29%) (Table 6).

4.2.3. Ash Digestibility

The fat digestibility coefficient ranged from 11.80% to 3.82%. There were significant differences among the digestibility of ash in different diets P (<0.05). But ash digestibility for diets1, 2 and 3 was not significantly different P (>0.05). However, diet 4 (11.80%) had the highest digestibility coefficient, followed by diet 3 (4.82%), then diet 2 (4.34%) and lastly diet 4 (3.82%) (Table 6).

4.2.4. Crude fibre Digestibility

The fat digestibility coefficient ranged from 39.57% to 23.77% (Table 6). There were significant differences among the digestibility of fat in different diets P (<0.05). But fibre digestibility between diets 1 and 4 was not significantly different P (>0.05). However, diet 2 (39.57%) had the highest digestibility coefficient, followed by diet 3 (26.93%), then diet 4 (24.29%) and lastly diet 1 (23.77%) (Table 6).

4.2.5. Gross Energy Digestibility

The gross energy digestibility coefficient ranged from 43.44% to 21.56% (Table 6). There were significant differences among the digestibility of energy in different diets P (<0.05). But gross energy digestibility between diets 2 and 3 was not significantly different P (>0.05). However, diet 1 (43.44%) had the highest digestibility coefficient, followed by diet 2 (31.50%), then diet 3 (30.04%) and lastly diet 4 (21.56%) (Table 6).

Water quality parameters

Table 7: Water quality parameters during digestibility experiment

Water quality parameters			
Treatment	Temperature ^{0}C	PH	Conductivity**(µmhos/cm)**
A	20.9	7.5	323
B	20.7	7.9	310
C	21.0	6.7	332
D	20.7	7.1	319

Water quality parameters (Table 7) recoded shows that mean temperature ranged from 21.0^{0}C to 20.7^{0}C. PH values were within allowable range from 7.9 to 6.7 while conductivity ranged from 332 µmhos/cm to 310 µmhos/cm.

CHAPTER FIVE

DISCUSSION

Experimental proximate composition results (Table 2) of the present study show that cassava leaf meal registered 21.17% crude protein which is comparable to complete fish diets that have been reported to have a range of 18%-50% crude protein (Craig and Helfrich, 2002). This suggests that cassava leaf meal can be used as a plant protein source by fish famers in Mpamba, NkhataBay. Findings from previous studies indicate that all experimental diets in which cassava was an ingredient were accepted by fish. For instance, cassava leaf meal in fish diets did not have adverse effect on the palatability of experimental diets (Ekenam *et al*., 2010). These results (Table 2) are in agreement with findings by Omoregie *et al*. (1991) who included cassava peelings and mango seeds in the diet of *Oreochromis niloticus.* Furthermore, cassava has been successfully used to replace maize bran in raising *Clarias gariepinus* fingerling and advanced fry (Abou *et al*., 2010). Therefore, the use of cassava leaf meal by fish farmers in Mpamba NkhataBay would help ease the cost of fish production and lead to an active and sustainable development of fish farming because it is the staple food in the area and is found in abundance. Globally, cassava is one of the top foodstuffs with high calorific content and is generally grown without fertilization of soils and can survive prolonged water deficits. It tolerates acid soils, but the yields are generally limited by low soil phosphorus content.

The nutritional limitations of cassava leaves were not assessed in the present study; however, they include cyanide content, low digestible energy, bulkiness and the high tannin content. The inherent cyanogenetic glycosides effects on animals may limit its use as fish feed. These parameters were not measured in this study but their effects could be inferred from other studies (Abou *et al*., 2010). The amount of cyanogenetic glycosides is influenced by the nutritional status, species and age of the plant while cyanide content decreases with the maturity of the leaves. Therefore, use of relatively mature cassava leaf meal can avoid running the risk of compromising growth and survival of the fish. To counter the negative attributes of using cassava in fish and livestock feeds, processing methods could reduce the cyanogenic effects. According to Francis *et al*. (2001) the two most widely used processing methods are sun drying and ensiling. Sun drying must be done thoroughly especially during the wet season because it may result in the production of low quality product with severe *Aspergillus* and *Aflatoaxin* contamination if the moisture is high. Ensiling the leaves entails chopping into small pieces (average of 2 cm), adding additives and common salt at 0.5% and storage in sealed air tight plastic bags for two months; this is reported to reduce cyanide content of up to 90% of the original concentration. Although cassava leaf protein is deficient in methionine, and has marginal tryptophan content, it is rich in lysine which is important in skeletal and skin formation. Besides, it also improves the body immune system by inducing the production of antibodies (Francis *et al*., 2001).

Sweet potato leaf meal and sweet potato peels registered 11.17% and 8.4% crude protein content respectively (Table 2), these are lower than 23.57% CP reported by Adewulo (2008) and 14.59% by Kang'ombe *et al*. (2009). However, sweet potato leaf meal has potential for use as a protein source in *Tilapia zilli* diet substituting up to 15% level without compromising growth (Omoregie *et al*., 2009). Expanded use of sweet potato as an animal feed appears to be promising both for agro-biological and socio-economic reasons. Sweet potato is grown widely at diverse altitudes (up to

2,000 m above sea level) and tolerates wide temperature ranges. Under the conditions it is grown in Malawi, sweet potato requires practically less cash inputs and minimal horticultural practices. According to Ishida *et al*. (2000) the above-ground parts of sweet potato, such as leaves, stalks and stems have a high nutritive value. In particular, leaves contain a large amount of protein with a high amino acid score (Ishida *et al.,* 2000). Both roots and vines are used as a protein and vitamin source for fish and livestock (Ali *et al*., 1999). The energy levels for sweet potato leaf meal observed in present study were 29.7 kJ/g and 15.21 kJ/g for sweet potato peel, respectively. Therefore, results (Table 2) of the present study demonstrated that incorporating the sweet potato in diets of fish would help to maintain a low protein energy ratio thereby allowing all dietary protein to be channeled towards somatic growth. This will ensure that there is good growth performance and high survival rate due to the protein sparing effect facilitated by high energy formulations.

Pawpaw recorded 2.23% (Table 2) crude protein, which is lower than that reported by other workers. For instance, Onyimonyi and Ernest (2009) reported crude protein values of 30.12% (PLM), while Esonu *et al*. (2002) showed 17.3% crude protein value, and Kang'ombe *et al.* (2009) found 9.45% crude protein for pawpaw leaf meal. The disparity may be due to differences in strains, soil types and age (Lola, 2009). Onyimonyi and Ernest (2009) reported that when pawpaw leaves are incorporated at 2% level in the diets of Tilapias, growth performance improves, carcass and organoleptic indices are favorable. In addition, the pawpaw plant is high in vitamins (A, B1, B2, and C) and minerals (Ca, K, P, and Fe) content (Yadava *et al*., 1990). Furthermore, pawpaw contains papain which aids digestion thereby releasing free amino acids which enhance growth (Chaplin, 2005; Onyimonyi and Ernest 2009). The inclusion of pawpaw leaves in the fish diets by fish farmers in NkhataBay might benefit the fish through several attributes other than protein.

Cocoyam registered 24.28 % and 19.54 kJ/g (Table 2) values for crude protein and energy levels in the present study, respectively. Therefore, the high energy levels suggest that the use of cocoyam as both protein and energy source in fish production in Mpamba is feasible. Cocoyam is non-conventional feedstuff recognized as cheaper and easily digestible carbohydrate source than grains or other tuber crops. In addition, it has high caloric yield per hectare, low production cost and relatively low susceptibility to insect and pest attack (Abduralshid and Agwunob, 2012). It is almost non-competitive with humans in most places of Malawi as it is eaten as food of last resort. In the present study, palatability and digestibility were low (Table 6). However, 10% cocoyam inclusion level is the best in terms of daily weight gain feed conversion ratio and cost effectiveness (Abduralshid and Agwunob, 2012).

In the present study black jack (*Bidens pilosa*) had 24.3% crude protein, 12.4 kJ/g energy, 23.1 % ash, 4.66% calcium, 2.20% phosphorus and 7.0% potassium. Comparative studies done elsewhere, Alikwe *et al.* (2012) reported that black Jack (*Bidens pilosa*) leaf meal (BPLM) had crude protein of 15.86%, ash 12.31% calcium 0.39%,0.31% and potassium 1.21%, methionine 0.54 % and, lysine 1.07% among others. The high protein (24.3%) level of black jack (*Bidens pilosa*) and the presence of methionine and lysine amino acids reported in previous studies is an indication of the potential for inclusion in animal diet as a protein source. Secondly, the high ash (23.1%) level is an indication of a good mineral profile in black Jack (*Bidens pilosa*). The results of the present study, therefore, suggest a moderate deposition of mineral elements in the leaves which could be made available to the fish (Antia *et al*., 2006). Thus, black jack (*Bidens pilosa*) is a potential plant diet of high protein (24.3%) and is a good mineral (4.66% calcium, 2.20% phosphorus) source. The use of the black jack (*Bidens pilosa*) could contribute to the development of an affordable fish feed for fish farmers in Mpamba who largely depend on non-protein household hold wastes and maize bran.

Cassava leaf meal and cassava peels registered 16.35% and 16.86% (Table 2) fiber respectively in the current study. However, crude fiber content in sweet potato leaf meal was 3.19% which was lower than those in the studies referred to earlier. Crude fiber content for papaya leaf meal was 5.6% (Onyimonyi and Ernest, 2009), 5.99% was reported by Kang'ombe *et al*. (2009) and both results are in agreement with the current study which recorded 5.5%. On the other hand, crude fiber level for cocoyam leaf meal reported by Kang'ombe *et al*. (2009) was 5.10% and that by Onyimonyi and Ernest (2009) was 8.8%, both of which were higher than what was found in the present study (3.96%). The crude fiber content for black jack in the current study was 6.45% (Table 2) which is very close to 6.84% that Kang'ombe *et al*. (2009) reported. Finally, crude fiber contents for jackfruit (7%), mexican fire plant (6.35%) and akee (5.5%) are in any case considered moderate for inclusion in fish diets. Low concentrations of dietary fibre (3–5%) may have a beneficial effect on fish growth with respect to improving digestion. On the other hand, high dietary fibre (>8%), may decrease dry matter digestibility of the diet and reduce the availability of other nutrients (Altan and Korkut, 2011). High dietary carbohydrate contents reduce the activity of proteolitic enzyme in fish. In addition, high crude fiber levels in fish diets do not only impede digestibility of diets but also jeopardize the binding capacity of the feed. (Ekenam *et al*., 2010). According to the present study, sweet potato leaf had crude fibre at 3.19%, mexican fire plant (6.35%), black jack at 6.45% and akee (5.5%) are some of plant sources being advocated to fish farmers for inclusion in fish diets on the basis of low crude fibre content (Table 2).

The amount of energy for most plant feedstuffs registered in the present study agreed favorably with those reported by other scientists. Kang'ombe *et al*. (2009) reported the following energy contents: cassava leaf meal (12.95 kJ/g), black jack (12.4 kJ/g), and banana leaf meal (19.4 Kj/g) whilst cocoyam leaf meal and papaya leaf meal registered (15.21kJ/g). In other previous studies, Onyimonyi and Enerst (2009) found energy contents for cassava leaf meal (14.3kJ/g), sweet potato leaf meal (12.5kJ/g), pawpaw leaf (15.21 kJ/g) and cocoyam leaf (33.18kJ/g). This study (Table 2), reported the following energy contents: cassava leaf meal (20.59 kJ/g), sweet potato leaf (15.34 kJ/g), pawpaw leaf (15.21 kJ/g), banana leaf meal (12.4 kJ/g), maize bran (10 kJ/g) and cocoyam leaf meal (19.54 kJ/g) among others plant ingredients. Energy is the second limiting nutrient in fish diets and the deficiency of energy hence protein is used for provision of energy. As a result, growth is minimal or slow since the protein is not used in somatic cells. The energy levels registered in the present study would suggest that incorporating these plant feedstuffs in fish diet would have a protein sparing effect where the fish would not need to convert some of the protein into energy.

In the present study, ash content (Table 2) showed substantial variation amongst ingredients from a high 85.75% to a low 3.72% for sweet potato leaf meal and maize bran respectively. In a study by Kang'ombe *et al*. (2009), the ash contents were 6.64% for cassava leaf meal, 6.48% for sweet potato leaf meal, 6.49% for cocoyam leaf meal, 7.27% for banana leaf meal and 5.54 % and 6.59% for pawpaw leaf meal and black jack meal respectively. It has been found that the concentration of minerals was proportional to the ash content in plant materials from aquatic weeds, sweet potato and cassava leaves, guinea and napier grasses (Dongmeza, 2009). Therefore, the intake of these plant feed ingredients could contribute to a large proportion of calcium, phosphorus and iron requirement of fish when they are used as supplementary feed in the fish ponds as well as in low cost formulated feed in semi-intensive aquaculture systems like ponds (Dongmeza, 2009). In addition, where vitamin and mineral premixes are not incorporated especially in rural communities like Mpamba, the use of plant feedstuffs would assist in formulation of feeds with high nutritive value and also affordable.

Moisture content of pawpaw leaf meal in the present study was 10.95% (Table 2) and this corresponds to those reported by Onyimonyi and Ernest (2009) who found 10.20% whilst Kang'ombe *et al*. (2009) reported 48% moisture content for pawpaw leaf meal. The disparity between the moisture contents in the present study and that of Kang'ombe *et al*. (2009) could be attributed to the different processing techniques used. In the present study, plant ingredients were sundried to rid them of anti- nutritional factors whilst in the study by Kang'ombe *et al*., (2009) plant ingredients were air dried on the shade to avoid loss of nutrition value.

The crude fat levels ranged from 2.22% to 16.07% (Table 2) in the present study. Kang'ombe *et al*. (2009) reported 2.56% crude fat level for cocoyam, 0.01% for PPM, 0.12% and 1.0% for cassava leaf meal and black jack respectively. A study by Abowei and Ekubo (2011) show that the crude fat contents reported were 1.0% for cassava leaf meal, 3% for sweet potato leaf meal which is also similar to that reported by Adewulo (2008) and 0.8% for pawpaw leaf meal. It is evident from the results that fat content for most ingredients in this study were higher than those observed in other studies. Commercial Tilapia feeds for grow out operations usually contain 5 to 6% total lipid, but high lipid levels of 10 to 12% are used in diets containing higher protein (Lim *et al*., 2009). Therefore, the crude fat contents of ingredients analyzed in this study are within the allowable range and can easily be used in *Tilapia rendalli* diets. Fat is important in provision of energy and helps in protein sparing thereby allowing protein deposition for somatic growth.

The differences in the proximate compositions (Table 2) could be attributed to different processing techniques, variations in climate, strain of the plant species and soil chemistry, corroborating with study findings of Lola (2009). In the same perspective, Onyimonyi and Ernest (2009) reported that disparity in nutritive value of plants may be attributed to differences in environmental conditions, such as soil chemistry, harvesting method, ingredients varieties and temperature. In the present study (Table 2) however, the differences in nutrient with previous studies could be attributed to varying soil types, strain types and climate.

Fish use around 80% of Apparent Nutrient Digestibility of dietary dry matter (ADC_{DM}) which describes how efficiently the feeds or feed ingredients are digested, and how much of their nutrient contents can be made available to fish for maintenance and growth (Altan and Korkut, 2011). In addition, (ADC_{DM}) generally provides a better estimate of the quantity of indigestible materials in the feeds or feed ingredients, rather than that of the individual nutrient (Eusebio *et al*., 2004).

Nutritional values of proteins and protein sources vary as a function of amino acids profile and digestibility (Altan and Korkut, 2011). The proportion (%) of crude protein, (i.e. nitrogen) in a feed ingredient can either originate from protein or non protein nitrogen. Therefore, ingredients with high CP from non-protein nitrogen will not contribute adequate amino acids in tandem with the nutritional requirements of fish. Thus, such ingredients will on the other hand, lead to increased production ammonia and other nitrogenous wastes by the fish thereby lowering productivity and water quality of production systems (Cho, 1990; Altan, 2002; Koprucu and Ozdemir, 2005).

The digestibility coefficients (Table 6) in the present study (24.15%-31.44% crude protein, 21.26 kJ/g to 43.44 kJ/g energy, 54.29%-67.78% fat, 23.77%-39.57% and ash 3.82-11.80%) were generally lower compared with previous research on plant diets. For example, protein digestibility values for sunflower cakes are 86–89% and wheat bran 75% (Maina *et al*., 2002). This is in agreement with the findings of Fontainhas-Fernandes *et al*. (1999), who reported defatted soybean meal 94.4%, full-fat soybean meal 90% and micronized wheat 88.6%. In corroboration , Mbahinzireki *et al*. (2001) reported values that ranged from 70 to 89% in tilapia for cottonseed meal and corn gluten meal 89% (Koprucu¨and Ozdemir, 2005), corn meal 83–84% (Hanley, 1987), and cottonseed meal 81.8% (Guimaraes *et al.*, 2008) for Nile tilapia, *Oreochromis niloticus*.

Water quality parameters have a profound effect on the performance of fish in aquaculture and may affect feed intake as well as digestion (Cho, 1990). In the present study, water temperature records ranged from 20-21% (Table 7) which is absolutely low as the optimum temperature range for Tilapia is 20 -30^{0}C with better results being recorded at 26-30^{0}C (Cho, 1990). Thus, the water temperatures recorded in the present study (Table 7) may have contributed to low protein digestibility due to low metabolic and slow enzymatic activity. PH and conductivity (Table 7) readings reported in this study were within allowable range and thus could not have influenced the feeding or digestibility trial results.

Earlier studies have shown that Tilapias have the capacity to utilize large number of alternative plant and animal protein sources (Ogunji, 2004). Tilapia has a digestive tract that is relatively long, just as other herbivorous fish, and shows morphological and physiological adaptations for the utilization of diets high in fibre (Maina *et al*., 2002; Stone, 2003). This is consistent with previous work that has shown that Tilapias are capable of digesting and absorbing relative large amounts of carbohydrates (Stone, 2003). Although Tilapias have an adaptation to digest plants diet, individual proximate composition of various nutrients of the diet influences digestibility. The source of the ingredients making up a diet can either be animal or plant based and has an effect on digestibility due to varying nutrient make up. Most plant based sources have high fibre contents especially the leafy parts (Altan and Korkut, 2011). In the present study, the diets (Table 1) were exclusively formulated from plant sources and these may be responsible for the low crude protein digestibility coefficients (23.7%-39.57%) (Table 6). Dietary plant ingredients can affect gastrointestinal transit time of feed as a result of the presence of fibres and complex sugars, and alter the digestibility of nutrients ingested by the fish (Eusebio *et al*., 2004; Zhou *et al*., 2004). According to Eusebio *et al*. (2004), dietary fibre is part of the carbohydrate component of plant ingredients, and most fish cannot utilize it. However, low dietary concentrations of dietary fibre (3–5%) may have a beneficial effect on fish growth. On the other hand, high dietary fibre (>8%), may decrease dry matter digestibility of the diet and reduce the availability of other nutrients (Altan and Korkut, 2011). The present study however, reported crude fibre (Table 5) for plant diets ranging from 14.02%-14.78% which in outside threshold range. In addition, selected individual plant ingredients used in the diet like CL, SPL and BJ had crude fibre contents of 16.35%, 9.16% and 6.40% (Table 2) respectively, which could have significantly contributed to low digestibility levels in the plant diets fed to *Tilapia* *rendalli* .by among other ways reducing the activity of proteolitic enzyme (Altan and Korkut, 2011).

Dietary energy is the second most important factor after protein affecting the utilization of feeds by fish. Fish are known to feed to satisfy their energy requirements, and if the diet does not contain sufficient energy levels, protein is used for energy rather than for growth (Cowey and Sargent, 1979). Protein is the most expensive component in fish feeds and plays an important role in growth of fish (NRC, 1993). Previous studies demonstrated that providing properly balanced ratios of protein to non-protein energy in diets can spare dietary protein from energy metabolism and then increase its utilization for fish growth (Nankervis *et al*. 2000; Morais *et al*. 2001; Wang *et al*. 2006; Schulz *et al*. 2008; Ahmadr, 2008). In the current study, the Apparently Digestibility Coefficient of gross energy (ADC_{GE}) range from 43.44% to 21.56% (Table 6); which are slightly higher than the Apparent Digestibility Coefficient (ADC_{CP}) range for crude protein (31.45%-24.15%). Inadequate dietary protein dietary protein to energy ratio may result in lower growth as well as low protein and energy utilization in fish (Ali *et al*., 2008). In diets with low protein to energy ratio, the use of dietary protein for growth and maintenance of body protein is maximized, while in diets with high protein to energy ratio, more protein is used for energy or stored as fat (Ali *et al*., 2008). The protein sparing effect of energy occurs only if the minimum protein requirements are met, including adequate amounts of amino acids. In the present study, Apparently Digestibility Coefficient of gross energy (ADC_{GE}) range from 43.44% to 21.56% (Table 6) and the Apparent Digestibility Coefficient for crude fat (ADC_{F}) range from 67.78%-54.29 % (Table 6) both higher than the Apparent Digestibility Coefficients for crude protein (ADC_{CP}) that ranged from31.45%-24.15% (Table 6). Providing adequate energy from dietary lipid can minimize the use of protein as an energy source (Takeuchi *et al*., 1992). Therefore, the presented study (Table 6) demonstrates that there was protein sparing effect in plant diets. Therefore, the formulated plant diets (Table 1) could lead to maximum use of crude protein for somatic cells and subsequent growth of fish since the minimum energy requirements would be met by non-protein sources.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The potential of plant feedstuffs such as leaf meal in fish diets was evaluated on the basis of its proximate chemical composition, comprising the moisture content, crude protein, crude fiber, crude lipid, total ash and gross energy. Results of the present study show that the nutritional value of different plant ingredients were significantly different thereby accepting the first hypothesis. Similarly, the study has also showed that there were variations in the Apparent Digestibility Coefficients (ADCs) for different plant diets thereby accepting the second hypothesis. Crude protein content of cocoyam, cassava leaf and black jack were higher than all other plant ingredients analyzed. Apparent Digestibility Coefficients indicate diets exclusively formulated from plant sources tested on *Tilapia rendalli* have a low digestibility potential and subsequently less nutrient available for growth and energy. Therefore, knowledge of the nutritional values of plant feed ingredients and the diets generated in the current study would help the farmer to deduce whether or not feed is meeting the optimum requirements of fish.

Based on proximate composition of the ingredients and the Apparent Digestibility Coefficients (ADCs) of different diets fed to *Tilapia rendalli*, the current study suggests that inclusion of plant feedstuffs in Tilapia diets would improve the quality and affordability fish diets for small scale fish farmers in Malawi. The study further advocates application of heat through boiling, drying or roasting as some of the methods before using the ingredients. To this effect, fish farmers are advised to include the plant ingredients with high crude protein (cassava leaf meal, black jack and cocoyam leaf meal) and energy levels since they are the limiting nutritional elements of fish diets. Secondly, the study recommends that further studies must be tailored at comparing the use of a diet comprising of mixture of plant ingredients and use of individual plant ingredients. Furthermore, studies on quantifying levels of antinutitional factors the plant feed ingredients should be the next step after the current study. In addition, efforts should be made to teach farmers how to formulate feeds based on the present study findings.

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