EVALUATION OF PLANT AND ANIMAL PRODUCTS / BY PRODUCTS AS ALTERNATIVE PROTEIN SOURCES TO FISH MEAL IN TILAPIA DIETS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN TROPICAL ANIMAL PRODUCTION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

ABSTRACT

The study was conducted to develop practical diets in Tanzania for Nile tilapia using fish meal (FM), soybean meal (SBM), cotton seed meal (CSM), sunflower seed meal (SFSM), freshwater shrimp meal (FSM) and blood meal (BM). Eleven isonitrogenous (30g 100g⁻¹), isolipidic (10g 100g⁻¹) and isoenergetic (18 kJg⁻¹) test diets were prepared; the control diet (FM) used FM (22%) and SBM (30%) as the major sources of protein. In the test diets, FM was fixed at 5%, while inclusion of SBM, CSM and SFSM varied as follows; SBM diets contained SBM at 20(SBM20), 25(SBM25) and 30(SBM30). CSM diets contained CSM at 15(CSM15), 20(CSM20) and 25(CSM25) and SFSM diets contained SFSM at 10(SFSM10), 15(SFSM15) and 20(SFSM20). Blended diet (BLEND) contained CSM and SFSM at 10% and SBM at 5%. Chemical composition was determined using standard method (AOAC, 2002). A total of 330 fingerlings with an average weight of 3.5 g were stocked at rate of 10 fish per 20 L tank. Dietary treatments were in triplicates in complete randomized design. Fish were fed for eight weeks and once a week were weighed and ration adjusted accordingly. Collected data was analysed using descriptive statistics and one way ANOVA at p<0.05. SBM, CSM, SFSM, FSM and BM had 42.3/44.6/19.5/59.1/83.1% of CP and 11.9/14.4/35.5/7.0/0.20% of CF respectively. There was no significant difference in growth between FM and CSM diets (p<0.05). Higher growth was observed in CSM diets and least in SFSM diets. Optimum growth for each ingredient was observed at CSM20, SBM25 and SFSM20. Feed utilization followed the same trend. SFSM, BLEND and CSM diets were cost-effective than FM and SBM diets (p<0.05). Long-term feeding trials using the BLEND, CSM20, SBM25 and SFSM20 diets should be done to evaluate their biological and economical performance under actual farming conditions.

DECLARATION

Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution. Joseph Bazili (MSc. Tropical Animal Production Candidate) The above declaration is confirmed by;	I, JOSEPH BAZILI, do hereby declare to the Senate	of Sokoine University of
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ACKNOWLEDGEMENTS

I sincerely thank Dr. Nazael A. Madalla my research supervisor, for his guidance throughout my graduate study at Sokoine University of Agriculture (SUA) and his suggestion on the research title "Evaluation of plant and animal products / by products as alternative protein sources to fish meal in tilapia diets". I also thank him for financial support of part of this study through Tanzania Commission for Science and Technology (COSTECH) and for his lectures on Nutrition of Aquatic Organisms, feeds and feeding and Aquaculture Production Systems which were very useful during data collection and writing of this manuscript. Lastly is for everlasting help, comments, criticisms and continuous encouragement towards the successful completion of this study.

I am thankful to laboratory technicians Dominic Allute, Gadalia Muffui, Michael Kusaja and Khaji Hamad for their assistance during sample collection, preparation and proximate analysis. Evantuce Shirima, Martin James, Angelo Haule and Liliane Saba provide a valuable assistance during collection of tilapia fingerlings used in this study. I also thank my fellow graduates especially Jeremiah Sanka, Pantaleo Mushi, Charles Mpemba, Khamis Mohamed, Emil Hyera, Yusta Kashumba, Lusebo Nalishuwa, Walter Msangi and Alex Mrema for their help, comments and encouragement throughout the course and made my study at SUA more enjoyable.

I am particularly grateful to my parents the late Mwalimu Bazili Cosmas Tarimo and Mrs Bazili Cosmas Tarimo for their care, steadfast love, everlasting support and encouragement throughout the successful completion of this study. My wife Fausta Lamosay Tarimo for patience, love and prayers. Also to my beloved daughter Doreen for her welcoming smile. Lastly my thanks go to my sisters Catty, Prosister, Adeller,

Yubati and Mary for their support, love, encouragement and taking care of my wife and our daughter when I was away.

DEDICATION

I dedicate this work to my beloved father Mwalimu Bazili Cosmas Tarimo (Mwalimu B.C. Tarimo) who passed away on 26 August, 2014 at Huruma district hospital, Mkuu – Rombo, Kilimanjaro from heart attack exactly one week before the end of data collection.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADG Average daily gain

ANOVA Analysis of variance

AOAC Association of analytical chemist

APS Alternative protein source

BM Blood meal

CF Crude fibre

CL Crude lipid

COSTECH Tanzania Commission for Science and Technology

CP Crude protein

CRD Complete randomized design

CSC Cotton seed cake

CSM Cotton seed meal

DASP Department of animal science and production

df degree of freedom

DM Dry matter

DO Dissolved oxygen

EAA Essentials amino acids

EE Ether extract

F = Value

FAO Food and Agriculture Organization

FCR Feed conversion ratio

FI Feed intake

FM Fish meal

FNWT Final body weight

FO Fish oil

FSM Freshwater shrimp meal

g gram

GE Gross energy

i.e. that is

IMF International Monetary Funds

INWT Initial body weight

Kcal Kilocalories

Kg Kilogram

Kg⁻¹ Per kilogram

KJ Kilojoules

LTD Limited

ME Metabolizable energy

mg milligram

MIN/VIT Minerals/Vitamins

MJ Megajoules

MLFD Ministry of Livestock and Fisheries Development

MM Maize meal

MSc Master of Science

NFE Nitrogen free extract

NRC National Research Council

PER Protein efficiency ratio

PhD Doctor of philosophy

Pr Probability

Rep Replication

SBM Soybean meal

SD Standard deviation

Se Standard error

SFO Sunflower oil

SFSC Sunflower seed cake

SFSM Sunflower seed meal

SGR Specific growth rate

SoV Source of variation

spp Species

SPSS Statistical Package for the Social Sciences

SS Sum of square

SUA Sokoine University of Agriculture

TShs Tanzania shillings

USA United States of America

WM Wheat meal

WTGN Weight gain

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Overall global capture fisheries production continues to remain stable at about 90 million tons with exception to some marked changes in catch trends by country, fishing area and species (FAO, 2014). In Tanzania, fish production statistics show that there has been a decline of catch from 375 535 tons in 2005 to 335 674 tons in 2009 (MLFD, 2010). Research findings have shown that mean catch rates in the trawl survey decreased from 287.7 kg hour⁻¹ in December 1997 to 80 kg hour⁻¹ in March, 2010 (MLFD, 2010). This is an indication that many fishery resources in inland and marine ecosystems are declining despite fishery management efforts. The causes of decline include: illegal fishing methods, habitat alteration, over fishing and by–catch, pollutants from land and siltation among others (MLFD, 2010).

The major fishery management interventions include closure of prawn fishery, establishment of protected areas and collaborative fishing management areas, control of fishing efforts, fishing capacity and fish catches. These measures however, have not had much impact in reducing severity of declining inland and marine fish catches throughout the country. For instance, there has been a decline of fish exports from 57 289 metric tons in 2005 to 41 148 metric tons in 2009 and consequently the country imported a total of 527.12 metric tons of fish worth TShs. 109.68 billions in 2009 within the same timeframe (MLFD, 2010). Notwithstanding the strong increase in the availability of fish to most consumers, the growth in fish consumption differs considerably among countries and within countries and regions in terms of quantity and

variety consumed per head (FAO, 2014). For example, fish consumption was lowest in Africa (9.1 million tonnes, with 10.4 kg per capita), while Asia accounted for two – thirds of total consumption, with 89.8 million tonnes (21.6 kg per capita), of which 45.4 million tonnes was consumed outside China (16.1 kg per capita) (FAO, 2014). The current per capita fish consumption in the country is estimated at 8.0 kilogrammes, which is lower than the FAO recommended rate of 11 kilogrammes (MLFD, 2010). The most substantial increase in annual per capita fish consumption have occurred in East Asia (from 10.7 kg in 1961 to 35.4 kg in 2010), Southeast Asia (from 12.8 kg in 1961 to 33.4 kg in 2010) and North Africa (from 2.8 kg in 1961 to 12.2 kg in 2010). China has been responsible for most of the increase in world per capita fish consumption, owing to the substantial increase in its fish production, in particular from aquaculture. Per capita fish consumption in China has also increased dramatically, reaching about 35.1 kg in 2010, with an average annual growth rate of 4.5 percent in the period 1961–2010 and 6.0 percent in the period 1990 – 2010 (FAO, 2014).

Aquaculture on the other hand continues to grow more rapidly than all other animal food producing sectors (FAO, 2012). Freshwater fishes dominate global aquaculture production (56.4 percent, 33.7 million tonnes), followed by molluscs (23.6 percent, 14.2 million tonnes), crustaceans (9.6 percent, 5.7 million tonnes), diadromous fishes (6.0 percent, 3.6 million tonnes), marine fishes (3.1 percent, 1.8 million tonnes) and other aquatic animals (1.4 percent, 814 300 tonnes) (FAO, 2012). In 2008, global aquaculture production totalled 68.8 million tonnes, made up of 52.9 million tonnes of aquatic animals and 15.9 million tonnes of aquatic plants. The volume of farm–produced aquatic animals represented 46.7 percent of the global food fish supply, that is an average growth rate of 11 percent per year, with production expected to grow to 51.0 million tonnes by 2015 and to 71.0 million tonnes by 2020 (FAO, 2012).

Production from aquaculture has greatly outpaced population growth, with per capita supply of an average growth rate of 3.2 percent per year in the period 1961–2010, outpacing the increase of 1.6 percent per year in the world's population (FAO, 2014). World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 19.2 kg in 2012. Most of the fish produced in the country is consumed in the domestic markets, while exports for the period of five years (2005–2009) have oscillated between 15 and 12 percent of the total production (MLFD, 2010).

The aquaculture sub-sector has a great potential for expansion, especially due to the fact that demand for fish is increasing as a result of population growth and stagnant production from capture fisheries, both at global and domestic levels. To maintain the current level of per capita consumption, by 2030 the world will require at least another 23 million tonnes of aquatic animal food, which aquaculture will have to provide (FAO, 2012).

1.2 Problem Statement and Justification

The rapid development in aquaculture largely dependent upon increased production of aquafeeds, which traditionally rely on fish meal (FM) as the main protein source (Fitzsimmons, 2010). The increasing demand for FM in monogastric feeds, human food and fish diets has resulted in FM becoming scarce and expensive.

This makes FM to be one of the most expensive macro-ingredients in aquaculture, pushing prices to historic highs. The prices had increased by 206 percent between January 2005 and January 2013 i.e. US \$ 1919 per ton (FAO, 2014). Feed costs have tended to increase with the rising price of FM. Liti *et al.* (2005) reported higher costs of fish production with diets containing FM compared to those containing all plant protein

feedstuffs. In Tanzania for example, FM is periodically scarce due to stiff competition from human beings and monogastric animals such as pigs and poultry (Fitzsimmons, 2010). This is one of the reason why aquaculture is poorly developed as most farmers cannot afford fish meal based diets. There is a real need to continuously search for alternative protein sources to FM; especially agricultural by-products and try to minimize use of FM thus, decreasing feed costs without compromising diet quality for optimal tilapia production.

Soybean meal, cotton seed cake, sunflower seed cake, blood meal and freshwater shrimps (*Caridina nilotica*) are the leading candidates to supply this protein (and possibly oil). However, in more intensive fish farming using compound feeds, these diets should be formulated to spare expensive protein by using less expensive carbohydrates and to a more limited extent, fat. Fish meal (FM) if used, is added at low levels. Economical tilapia diets contain approximately 0–20% FM and 0–10% fish oil (1995 to 2008) (Tacon and Metian, 2008). Typically, tilapia diets in China contain 10% to 15% FM and mixture of vegetable proteins and oils, plus starchy root crop, for example, cassava/tapioca (Tacon and Metian, 2008). Therefore, optimization of alternative protein sources to accommodate changing requirements of fish due to age/size could significantly enhance the protein utilization, thereby reducing the cost of diet formulation and nutrient loading into the culture system.

1.3 Objectives

1.3.1 General objective

To develop practical diets for Nile tilapia (*Oreochromis niloticus*) using locally available ingredients in Tanzania.

1.3.2 Specific objectives

- To determine chemical composition of soybean meal, cotton seed cake, sunflower seed cake, blood meal and freshwater shrimp meal.
- ii. To evaluate effects of fixing fish meal to 5% with various inclusion levels of alternative protein sources in Nile tilapia diets on the growth performance and feed utilization.
- iii. To assess cost-effectiveness of fixing fish meal to 5% with various inclusion levels of alternative protein sources in Nile tilapia diets.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview

Fish meal has been a major component in fish diets due to its high protein quality, well balanced amino acid profile and high digestibility (Storebbaken *et al.*, 2000). Use of plant protein sources in fish feed is a response to scarcity and high price of fish meal. Though plant protein sources may be cheaper than the animal sources, they have several limitations (Fitzsimmons, 2010). The limitations include presence of inherent antinutritional factors, low protein content, poor amino acid profiles and high crude fibre content (Siddhuraju and Becker, 2003).

Some anti-nutritional factors have nutrient binding effect in a form of complexes resulting low digestibility of diets (Watanabe *et al.*, 1997). Therefore, when they are used as fish feeds to replace fish meal care must be taken regarding their inclusion level and often they require some form of processing to reduce the anti-nutritional factors. However, a combination of different plant and animal protein sources is desirable because they provide adequate amino acids to fish and improve fish growth (Ogunji and Wirth, 2001). Thus, it is desirable to include several plant/animal protein sources when replacing fish meal in fish diets. This chapter will review existing information on tilapia fish, tilapia nutrition and alternative protein sources to tilapia fish with respect to their nutritional quality, limiting factors and inclusion levels which will be used as the basis to develop practical diets for Nile tilapia fingerlings in Tanzania.

2.2 Tilapia

Tilapia is an African fresh water cichlid finfish, introduced to many areas for food. They are endemic to freshwaters in Africa, Jordan, and Israel and are being cultured in virtually all types of production systems in both fresh and saltwater in tropical, subtropical and temperate climates. There are over 100 different species of tilapia, each with unique characteristics, behavior, and suitability to fish farming (El-Sayed, 2006). A few of the most commonly farmed are the Nile tilapia (*Oreochromis niloticus*), Blue tilapia (*Oreochromis aureus*), Mozambique tilapia (*Oreochromis mossambicus*), Redbelly tilapia (*Tilapia zillii*), and the Red breasted tilapia (*T. rendalli*). Nile tilapia (*Oreochromis niloticus*) is the predominant species that is cultured worldwide (FAO, 2010). The tilapia production grew from 1.0 million tonnes in 2001 to 2.5 million tonnes in 2010 (FAO, 2012).

Currently, tilapia is second most produced group of food fish globally (behind carps), and the growth trend is likely to continue (El-Sayed, 2006). According to FAO (2014) in 2012 global tilapia production was 4 507 002 million metric tons and will exceed 4 800 000 in 2014 (Fitzsimmons, 2014). Tilapia is a good fish for resource poor farmers to grow. It is often reffered to as "aquatic chicken" due to its rapid growth rates, ease in culture, ability to eat many types of feeds, feeding at low trophic levels, acceptance of artificial feeds immediately after yolk-sac absorption, ability to reproduce easily, short generation interval, disease resistance, hardiness and tolerance to a wide range of environmental conditions (temperature, salinity, low dissolved oxygen etc.) (Fitzsimmons and Naim, 2010). This explains why Tilapia have been, and still is, a target specie for aquaculture in Tanzania and many other Sub–Saharan Countries.

2.3 Tilapia Nutrition

Good quality feed to meet the nutritional requirements of tilapia for optimal aquaculture remains a major constraint (Fitzsimmons, 2000). However, sustenance and expansion of production is limited by the high cost of fish feeds, which comprise over 50% of the production costs (Craig and Helfrich, 2002). Nile tilapia, *Oreochromis niloticus*, requires all the nutrients such as proteins, energy, vitamins and minerals (Watanabe *et al.*, 1997). As a herbivore, tilapia requires 30 – 35% of dietary protein for their optimal growth and feeding efficiency (Ofojekwa *et al.*, 2003). Protein requirement at different life stages of various tilapia species have been studied extensively (El-Sayed, 2006). These values have generally been determined by measuring growth response of tilapia fed test diets containing graded levels of protein. Protein requirement of tilapia is depended on many factors such as species, size, protein source and quality, non-protein energy level in the test diets, feeding rate, water quality variables (temperature, dissolved oxygen, salinity, etc.), the presence and density of natural food (NRC, 1993).

Tilapia requires 25% to 56% protein of the diets depending on age (Table 1). For example *Oreochromis niloticus* fry grow best when fed diet containing 40% protein and juvenile tilapia when fed 30% protein diet (El-Sayed and Teshima, 1992). Protein requirements diminish as the fish grows older (El-Saidy and Gaber, 2003) and as size increases (Wilson, 2002). Large fish require only 25 to 35% dietary protein, depending on the rearing conditions (Al Halfedh, 1999). Tilapia are very efficient in utilizing natural food. At low stocking densities in earthen ponds, they obtain significant amount of protein from natural food and therefore lower dietary protein levels are sufficient (Lim and Webster, 2006). Natural food can provide up to 50% of amino acid requirement (Chowdhury *et al.*, 2006).

Table 1: Protein requirements of some cultured tilapia

Species and life stage	Weight (g)	Protein source	Requirement (%)	References
O. niloticus Fry	0.012	FM	45	El-Sayed and Teshima (1992)
	0.51	FM	40	Al Hafedh (1999)
Fingerlings	2.4	Casein/Gelatin	35	Abdelghany (2000)
	3.50	Casein	30	Wang et al. (1985)
	45-264	FM	30	Al Hafedh (1999)
Broodstock		FM/SBM	40	El-Sayed et al. (2003)
		FM	45	Siddiqui et al. (1998)
O.mossambicus	Fry	FM	40-50	Jauncey (1982)
	6-30	FM	30-35	Jauncey and Ross (1982)
O. aureus	0.30-0.50	SBM or FM	36	Davis and Stickney (1978)
	2.50	Casein/albumen	56	Winfree & Stickney (1981)
	7.5	Casein/albumen	34	Winfree & Stickney (1981)
T.zillii	1.35-1.80	Casein	35	Mazid et al. (1979)

FM = Fish meal, SBM = Soybean meal, CSM = Cotton seed meal

However, inadequate non-protein energy in the diet will lead to a higher dietary protein requirement because fish will utilize part of the protein as energy to meet their metabolic energy needs (Nguyen, 2008). Due to continous feeding pattern of tilapia, several meals per day produce higher or more efficient growth, particularly for young fish (De Silva and Anderson, 1995). Water quality variables such as temperature and dissolved oxygen (DO) have considerable effects on metabolic rate of fish and thus their dietary protein requirements. A higher dietary protein level is required at optimum water temperature and DO for growth than at lower temperature or DO (Gatlin, 2010).

Fish requires lipid for provision of essential fatty acids especially polyunsaturated fatty acids and concentrated source of energy (De Silva and Anderson, 1995). A normal lipid requirements for fish is 10% of the diet; this gives a normal growth rate without depositing excessive fat (De Silva and Anderson, 1995). Vitamins requirement by different fish species varies according to their feeding habit. Herbivores have low vitamin requirements than carnivores because they feed on vegetable based diets unlike

carnivores which are meat eaters. Also, vitamins requirements depends on capacity of fish to synthesize them in their gut (De Silva and Anderson, 1995).

2.4 Alternative Protein Sources (APS)

Tanzania being an agricultural country has a fairly good abundance of crop and animal products and by-products. These include soybean meal, cotton seed cake, sunflower seed cake and blood meal. Moreover, fishery industry also produces by-products such as *Caridina nilotica* and shrimp wastes which are usually not utilized for human consumption but may have a high potential as fish feed. All these are suitable as alternative protein sources to fish meal in tilapia diets.

2.4.1 Soybean (Glycine max) meal (SBM)

2.4.1.1 Nutritional quality

Soybean (*Glycine max*) meal (SBM) is a product remaining after extracting most of oil from whole soybeans. Heated, full-fat soybean meal is prepared by grinding heated soybeans that have not undergone the oil extraction process (Robinson *et al.*, 2001). SBM is one of the most interesting alternatives to fish meal for fish diets due to high protein content, satisfactory amino acid profile, fairly reasonable price and steady supply (Storebbaken *et al.*, 2000). The crude protein content of SBM is about 50% (Table 2). Levels of essential amino acids in SBM are comparable to those in fish meal required for meeting fish requirements, though it is limiting in Methionine, Lysine and Cystine (El-Sayed, 1999). SBM is less expensive and is more readily available than the fish meal (Hardy, 2006). However, it is relatively expensive to be affordable to resource poor farmers compared to other alternative plant protein sources due to its high demand food for both terrestrial livestock and human beings (Abraham, 2001).

SBM is known to vary in its amino acid composition. Geographical location of soybean production, soybean variety, and processing methods are factors known to influence crude protein and amino acid content of SBM (Baker *et al.*, 2011). The protein content can be improved by processing (Storebbaken and Refstie, 2000). Deffatted SBM contains 45–48% CP where as alcohol-washed protein concentrate contains 70–85% CP (Storebbaken and Refstie, 2000). SBM has low crude fibre content (less than 3% for the dehulled soybean meals) (McDonald *et al.*, 2010).

Table 2: Nutrient composition (%) of soybean (Glycine max) meal (SBM)

%DM	%CP	%CF	%EE	%Ash	%NFE	Author
94.2	52.9	4	1.4	6.7	35	Nyirenda et al. (2000)
93.0	44.4	5.9	4.8	6.0	38.9	El-Saidy and Gaber (2003)
89.1	53.5	-	1.4	6.4	-	Wang et al. (2006)
91	44.0	6.9	11.8	6.0	22.3	Gaber (2006a)
90.6	44.0	7.4	2.1	6.5	40.0	Abdel-Hakim et al. (2008)
89.2	44.7	3.4	0.9	5.3	35.1	Madalla (2008)
96.7	41.6	15.2	11.7	5.5	25.9	Shigulu (2012)
93.6	40.57	13.9	4.6	4.1	30.45	Fapohunda (2012)

DM = Dry matter, CP = Crude protein, CF = Crude fibre, EE = Ether extract, NFE = Nitrogen-free extract, % = percentage

2.4.1.2 Limiting factors

Soybean meal (SBM) is limited by the presence of anti-nutrients such as trypsin and chymotrypsin inhibitors, phytohaemagglutinins or lectins, indigestible carbohydrates, saponins, phytic acids and allergens (Nengas *et al.*, 1996). The phytohaemagglutinins comprise 1–3% of the protein of defatted soybean flour (Lim and Akiyama, 1992). SBM also contains non-starch polysaccharides (14–18%) like arabinan, arabinogalactan and acid polysaccharides which are less available to fish than starch (Francis *et al.*, 2001). Anti-nutritional factors in SBM can affect growth and health of tilapia

(Mbahinzireki et al., 2001). For example, Trypsin inhibitors cause reduction in growth and increased secretion of pancreatic enzymes which in turn causes pancreatic hypertrophy (Lim and Akiyama, 1992). The effects of the anti-nutritional factors in soybean meal (SBM) can be reduced by processing. Processing may involve heat treatment like roasting, cooking or boiling to remove heat labile anti-nutritional factors (Refstie et al., 1997). The heat treatment therefore is the best method as it deactivates and destroys the heat labile anti-nutritional factors present in raw soybean (Francis et al., 2001). It also helps rupture cellulose membrane surrounding the cell and release the cell contents making them more available to digestive enzymes (Goda et al., 2007).

Wee and Shu (1989) found that the quality of full-fat SBM boiled at 100° C for one hour was improved and trypsin inhibitor activity decreased for Nile tilapia. Autoclaving soybean at 105° C for more than 60 minutes was found to be more effective than the boiling procedure and removed 95–100% of the trypsin inhibitor (Viola *et al.*, 1983). However, the degree of heating soybean can affect its nutritional value (Borgeson *et al.*, 2006). Proper heating improves nutritional value by destroying anti-trypsin activity (Alexis, 1990) and overheating reduces the nutritional value of soybean by destroying Lysine content (Viola *et al.*, 1983). Another method is germinating of seeds (El-Sayed, 1999). This method can reduce the activity of protease inhibitor in soybean seeds (Wassef *et al.*, 1988).

2.4.1.3 Inclusion level

Soybean meal (SBM) can be used as a total or partial replacement of fish meal for farmed tilapia, depending on fish species, size, dietary protein level, soybean source, and processing methods. A study by El-Sayed (1999) indicated that SBM could replace between 67% and 100% of fish meal in tilapia diets depending on fish size, dietary

processed, solvent extracted SBM with or without Methionine supplementation, successfully replaced up to 75% of fish meal in the diet of Nile tilapia fry (El-Sayed, 2006). Inclusion level of SBM in fish diets has different effects on growth of different fish species. For example, higher inclusion level of SBM in Nile tilapia diets cause unpalatability resulting into poor growth performance (Ogunji and Wirth, 2001). Nguyen (2008) conducted an experiment to compare the performance of red tilapia (*Oreochromis spp*) fed SBM-based diet and control diet containing 6% FM, which was considered as a regular inclusion rate of FM in commercial diet for tilapia. He found that there were no significant difference in final mean weight, survival and FCR of the experimental fish fed these two diets.

It is also of interest to note that the dietary inclusion of SBM in tilapia feeds is affected by the dietary protein level. For example, Davis and Stickney (1978) fed *Oreochromis aureus* SBM-based diets at dietary protein levels ranging from 15 to 36%, and found that whilst SBM impared fish growth at 15% crude protein levels, that SBM could totally replace FM within diets containing 36% crude protein. Shiau *et al.* (1987) with *O.niloticus x O.aureus* hybrids reported that FM could be partially replaced by SBM within diets containing sub-optimal protein levels (24%), where as at optimum protein levels (32%) the dietary replacement of FM with 30% SBM significantly depressed fish performance.

2.4.2 Sunflower (Helianthus annuus) seed meal (SFSM)

2.4.2.1 Nutritional quality

Sunflower seed meal (SFSM) is a by-product of extraction of oil from sunflower seeds. It is prepared by grinding residues remaining after mechanical and/or solvent extraction of the oil from whole or decorticated sunflower seeds (Venou, 2006). SFSM is a relatively cheaper compared to other sources of vegetable proteins (Ahmad *et al.*, 2004). The crude protein content of the SFSM ranges from 22 to 42% (air dry basis) (Table 3). The quality of SFSM depends on the plant characteristics (seed composition, hulls/kernel ratio, dehulling potential, growth and storage conditions) and on the processing (dehulling, mechanical and/or solvent extraction) (Munguti *et al.*, 2012). For example, sunflower seed cake resulting from mechanical extraction of dehulled seeds has 37% CP while the one from un–dehulled seeds has 28% (Maina, 2001). The sunflower seed cake is rich in sulphur amino acids when compared to soybean meal protein but deficient in Lysine and Threonine (Maina, 2001).

Table 3: Nutrient composition (%) of sunflower (*Helianthus annuus*) seed meal (SFSM)

%DM	%CP	%CF	%EE	%Ash	%NFE	Author
98.5	25.3	22.9	15.3	5.3	31.2	Nyirenda et al. (2000)
96	29.6	26.9	4.4	8.7	30.5	Abraham (2001)
95.9	42.0	11.4	12.4	7.6	26.6	El-Saidy and Gaber (2003)
90.6	38.0	11.4	11.1	7.6	22.5	Gaber (2006b)
92.9	25.9	36.8	5.4	5.1	26.6	Munguti et al. (2006)
91	34.1	13.2	-	6.6	-	Ramachandran et al. (2007)
92.5	34.0	14.2	5.6	6.9	39.3	Abdel-Hakim et al. (2008)
92.9	25.9	36.8	5.4	5.1	26.6	Munguti et al. (2012)
97.5	22.0	38.1	10.9	7.2	21.7	Shigulu (2012)

DM = Dry matter, CP = Crude protein, EE = Ether extract, NFE = Nitrogen-free extract, % = percentage

2.4.2.2 Limiting factors

Sunflower seed cake has anti-nutritional factors such as arginase and trypsin inhibitors which are heat labile and potentially inactivated by heat treatment (Maina, 2001). Perhaps a major limiting is its high crude fibre content which is highly indigestible by most fish species (Maina, 2001). The crude fibre level in the cake generally varies

between 14% and 39% (air dry basis) (Villamide and Sun Juan, 1998). High levels of sunflower seed cake in fish diet tend to reduce specific growth rate (SGR). Diets based on the fibre-reduced cake had higher levels of all amino acids than the ones based on the high-fibre cake. Maina *et al.* (2007) found that fish fed diets based on anchovy fish meal had higher weight gains than those fed diets based on the high-fibre sunflower cake. They further stated that reducing the fibre content of sunflower cake improved growth rate and weight gain.

2.4.2.3 Inclusion level

The use of sunflower seed cake in fish diets is limited by level of crude fibre. High crude fibre in sunflower seed cake lowers feed intake, growth and feed efficiency (Olvera-Novoa *et al.*, 2002). Low fibre sunflower seed cake (10% CF) can replace 60% of the dietary protein and high fibre (24% CF) can replace 30% of the *Oreochromis niloticus* diet without affecting growth performance of this fish (Maina, 2001).

Sunflower meal (hulled meal) could replace 10 to 25% fish meal in the diets of Nile tilapia (*Oreochromis niloticus*) (El-Saidy *et al.*, 2002) and redbreast tilapia (*Tilapia rendalli*) fingerlings (Olvera-Novoa *et al.*, 2002). In the latter study, most cost-effective diet included 20% sunflower meal (Olvera-Novoa *et al.*, 2002). Good results on feed utilisation parameters like feed intake (FI), feed conversion ratio (FCR) and energy and protein efficiency were obtained when sunflower seed cake was included at 22% tilapia fingerlings diet (Olvera-Novoa *et al.*, 2002). L-Methionine and Lysine supplementation have been suggested in order to obtain optimal results (El-Saidy *et al.*, 2002).

2.4.3 Cotton (Gossypium spp) seed cake (CSC)

2.4.3.1 Nutritional quality

Cotton seed meal (CSM) is a by-product of the cotton processing industry (Apata, 2010). It is obtained by grinding the cotton seed cake (CSC) remaining after the oil has been extracted (Robinson *et al.*, 2001). CSC is one of the best plant protein sources for tilapia in developing countries due to its high availability, relatively low price, good protein content and amino acid profile (FAO, 1983) and it is very palatable to fish (Robinson and Li, 1995). Protein content is highly variable depending on the amount of dehulling and on the efficiency of oil extraction (Apata, 2010). Protein content goes from 26% DM for non dehulled cotton seed meal to up 50% DM for fully dehulled meals (Apata, 2010). CSC is deficient in some EAA such as Cystine, Lysine and Methionine. The fibre content varies accordingly, from 12.5% DM to 29.6% DM (Table 4).

Table 4: Nutrient composition (%) of Cotton (Gossypium spp) seed cake (CSC)

%DM	%CP	%CF	%EE	%Ash	%NFE	Author
89.3	38.8	24.9	10.7	6.3	19.2	Munguti et al. (2006)
89.8	34.9	25.8	12.8	6.0	19.4	Munguti et al. (2009)
93	41	12.5	1.5	6.3	-	Apata (2010)
92.3	20.1	16.7	3.53	4.2	-	Kumar et al. (2011)
92	33	23	7	21	16	Aanyu et al. (2012)
89.2	38.8	24.9	10.7	6.3	19.3	Munguti et al. (2012)
-	24.79	29.6	8.9	6.2	25.4	Babiker (2012)
93.5	35.7	17.6	12.1	7.2	20.9	Shoko et al. (2012)

DM = Dry matter, CP = Crude protein, EE = Ether extract, NFE = Nitrogen-free extract, % = percentage

2.4.3.2 Limiting factors

Cotton seed meal (CSM) contains gossypol, a poly-phenolic compound found in pigment glands of cotton seeds (Abowei *et al.*, 2011). Gossypol in whole cotton seed is

in the free (toxic) form. Free gossypol when present in large quantity in the diet, has been shown to be toxic to monogastric animals including fish (Soltan *et al.*, 2011). During processing into cotton seed meal, some free gossypol is bound to protein, rendering it non-toxic (Robinson and Li, 1995). Though free gossypol decreases during processing, more Lysine is bound, thereby decreasing its availability to the animal (Robinson and Li, 1995). Free gossypol also accumulates in muscles, kidney and liver tissues causing some disorders (Abowei *et al.*, 2011) such as anorexia and increased lipid deposition in fish liver when fed in excess (Robinson and Li, 1995).

Gossypol inhibits activity of digestive enzymes and reduces palatability of diet. Free gossypol also has been reported to increase incidence and growth of afflatoxin-induced liver tumors in rainbow trout (*Oncorhynchus mykiss*) (Robinson and Li, 1995). However, gossypol in commonly available cotton seed meal is generally well below toxic levels (Robinson *et al.*, 2001).

Concentrations of free gossypol in cotton seed cake may vary due to differences in crop variety, locality, and processing technique. Gossypol can be removed by mechanical pressing of seed followed by solvent extraction to reduce the gossypol content to 0.02 – 0.5% level. Diet with gossypol up to 0.015% levels are believed to be safer in poultry. Detoxification of cotton seed meal with solvent mixture containing hexane, and water were found to be useful after initial cooking of the meal. Gossypol toxicity also can be alleviated through the addition of iron salts (Ferrous Sulphate). Addition of Ferrous Sulphate at a rate of 1 part to 1part of free gossypol have been used to improve growth performance in Nile tilapia (El-Saidy *et al.*, 2004) including fingerlings, but not in channel catfish (Jiang Ming *et al.*, 2011). Tilapia appear to tolerate relatively high levels

of free gossypol. Mbahinzireki *et al.* (2001) reported that dietary free gossypol \leq 0.18% did not affect growth, feed conversion, and survival of fingerling *Tilapia aurea*.

2.4.3.3 Inclusion level

The presence of gossypol, the fibre content and the low availability of Lysine, Methionine and Cystine in CSC limits its use in fish farming. However, the actual limit depends on the fish species, on the type of cotton seed cake and on the level of gossypol. For example, pre-pressed, solvent extracted cotton seed meal with 300 mg/kg free gossypol replaced 50% of fish meal with no effect on *Oreochromis mossambicus* production (Jackson *et al.*, 1982). Dadgar *et al.* (2010) found that mechanically-extracted cotton seed meal can replace up to 100% soybean meal when fed to Rainbow trout (*Oncorhynchus mykiss*), juvenile. Mbahinzireki *et al.* (2001) reported that tilapia growth did not differ significantly with up to 50% substitution of FM with cotton seed meal.

Fish meal replacement above 50% resulted in significant growth decline with time in juvenile Nile tilapia. Fish fed 100% FM and diets including 50% CSM had significantly better daily weight gain, daily feed intake and feed efficiency ratio than those fed 100% cotton seed meal. Garcio-Abiado *et al.* (2004) reported that, fish fed 25–50% CSM protein replacement showed similar body weight and total lengths as the control at the completion of the 16 week trials and fish fed 75 and 100% CSM protein replacement showed a significant decline in body weight and total length. Robinson *et al.* (1984) found that *Oreochromis aureus* fed CSM-based diets yield poor performance. The authors attributed the poor performance to the gossypol in CSM. In the study of Barros *et al.* (2002) three basal diets containing 0, 27.5 or 55.0% solvent-extracted cotton seed meal as replacements of 0, 50 or 100% of solvent extracted soybean meal with three

levels of iron (40 336 671 mgk⁻¹) in 3x3 factorial experiment and fed to juvenile channel catfish for 10 weeks. Feed intake, feed conversion ratio and protein efficiency ratio was similar to diets containing 0 and 27.5% CSM but was significantly lower for diets containing 55.0% CSM.

2.4.4 Blood meal (BM)

2.4.4.1 Nutritional quality

Blood meal (BM) is a dry, inert powder made from blood used as a high protein supplement. It readily available in abattoirs as alternative cheaper protein source (Aladetohun and Sogbesan, 2013). It is prepared from clean, fresh animal blood, excluding hair, stomach belchings, and urine except in trace quantities that are unavoidable (Venou *et al.*, 2006). It is hygroscopic and needs to be dried to less than 10–12% moisture and stored in a dry place in order for it not to deteriorate (Aladetohun and Sogbesan, 2013). BM contains 42–87.6% crude protein (Table 5) and is an excellent source of Lysine (Venou *et al.*, 2006). It is deficient in Methionine and Isoleucine (Venou *et al.*, 2006). BM has been shown to be a satisfactory replacement for other protein sources in various animal production diets for dairy cattle, beef cattle, sheep, pigs, poultry and fish. However, it is not efficiently utilized by tilapia due to low digestibility and poor EAA profile (El-Sayed, 1999).

2.4.4.2 Limiting factors

Blood meal (BM) is characteristic smell, unpalatable at high inclusion levels, not readily digested and its use has reduced growth rates in poultry so that it is not recommended for young stock (McDonald *et al.*, 2010). BM contains small amounts of ash and oil. It is also deficient in Isoleucine and contains less Glycine than fish meal, meat, or meat and bone meals (McDonald *et al.*, 2010). Studies by Fetuga *et al.*, 1973

have indicated the disproportionate ratio of Leucine to Isoleucine of 12.8 g/16 g of N to 1.17 g/6 of N respectively.

Table 5: Nutrient composition (%) of blood meal (BM)

%DM	%CP	%CF	%EE	%Ash	%NFE	Author
93.0	87.6	0.0	1.5	6.0	-	DeRouchey et al. (2003)
92.0	77.1	0.6	0.6	-	-	Makinde and Sonaiya (2010)
84.6	73.8	-	1.4	5.8	-	Monentcham et al. (2010)
90	72	0	1	12	16	Aanyu et al. (2011)
90.8	42	1.1	1.8	9.8	45.3	Munguti et al. (2012)

DM = Dry matter, CP = Crude protein, EE = Ether extract, NFE = Nitrogen-free extract, % = percentage

2.4.4.3 Inclusion level

If the blood meal is included in the feed at the proper ratios, the EAA deficiencies can be overcomed and the quality of such diets is likely to improve (Aladetohun and Sogbeson, 2013). In African catfish (*Clarias gariepinus*) and tilapia, spray-dried blood meal can replace 50 to 75% of the fish meal (Goda *et al.*, 2007) and in rainbow trout (*Onchorhynchus mykiss*) up to 100% (Watanabe *et al.*, 1997).

Ogunji and Wirth (2001) in their study on alternative protein sources as substitute for fish meal in the diet of young tilapia (*Oreochromis niloticus*) observed that a proper combination of soybeans, blood meal (not more than 6%), groundnut cake and wheat bran could provide the 42 – 45% protein needed by the fish. In another experiments conducted with blood meal (boiled state) at varying levels as 50, 25 and 10% for *Oreochromis niloticus*, the feeds were accepted by the fish and utilized for growth. The feed containing the highest amount (50%) of blood meal gave the poorest performance in terms of growth and feed conversion ratio. The best performance was given by feed

containing 10% blood meal contributing 31.34% protein (Otubsin, 1987). Rainbow trout fed with a diet with 7% blood powder (freeze drying) and 35% anchovy meal had equivalent growth and feed conversion ratio to trout fed a diet with 45% anchovy meal and no blood powder (Priya Elizabeth and Davies, 2007). They attributed the poor performance due to poor digestibility of the blood meal. Low inclusion levels of fermented BM may improve growth and feed utilization of tilapia by boosting dietary Lysine levels of the respective diets (McDonald *et al.*, 2010).

2.4.5 Freshwater shrimp (Caridina nilotica) meal (FSM)

2.4.5.1 Nutritional quality

Caridina nilotica are common tropical freshwater shrimps growing to about 30 mm, are reported to be suitable for feeding fry and fingerlings of several food fish species, as well as to ornamental fish species (De Silva and De Silva, 1985). These shrimps are also suitable, in wet and dry form as high protein ingredient in suplementing feeds in fish culture (De Silva and De Silva, 1985). Freshwater shrimp meal (FSM) contains 35.3–63.5% crude protein (Table 6). They can easily be collected from the wild or can be cultured easily in high density in small ponds. FSM is a rich source of animal protein (60–65g/kg DM) (Jauncey and Ross, 1982).

Caridina nilotica has a high potential for inclusion in tilapia feeds because it is not used as human food and has high protein content and suitable amino acids composition (Munguti *et al.*, 2012). However, a large portion of the crude protein that is contained in shrimp meal is in the form of chitin, which is not readily digestible by fish (Hertrampf and Piedad-Pascual, 2000).

Table 6: Nutrient composition (%) of freshwater shrimp (Caridina nilotica) meal (FSM)

%DM	%CP	%CF	%EE	%Ash	%NFE	Author
-	47.6	-	7.14	16.88	-	Ibrahim <i>et al.</i> (1999)
22.1	35.3	7.04	8.6	22.2	28.4	Dong et al. (2005)
87.5	60.3	1.4	6.2	6.7	24.8	Munguti et al. (2009)
87.7	63.5	5.0	1.3	22.8	6.7	Munguti et al. (2012)
91.4	56.1	7.5	10.5	9.8	7.5	Mugo-Bundi et al. (2013)

DM = Dry matter, CP = Crude protein, EE = Ether extract, NFE = Nitrogen-free extract, % = percentage

2.4.5.2 Limiting factors

Cost is the major limitation of using *Caridina nilotica* as alternative protein source. In previous studies Liti *et al.* (2005) reported higher costs of fish production with diets containing FSM compared to those containing all plant protein feedstuffs. Furthermore, freshwater shrimps (*Caridina nilotica*) are scarce in Tanzania market due to competition as an ingredient in monogastric feeds. Also, because of its high level of chitin, shrimp meal has a poor pelletizing ability (Hertrampf and Piedad-Pascual, 2000).

2.4.5.3 Inclusion level

Fresh water shrimp meal may replace 100% fish meal without altering tilapia performances grown under semi-intensive conditions (Munguti *et al.*, 2006). Mugo-Bundi *et al.* (2013) found that fresh water shrimps can be effectively used to replace up to 75% of FM in the diets without compromising growth performance, survival, nutrient utilization and economic benefits in *Oreochromis niloticus*. In sex-reversed red tilapia (*Oreochromis niloticus*), shrimp meal can replace 50% to 100% fish meal without altering fish performances (El-Sayed, 1998). Moreover, El-Sayed (1998) reported that shrimp meal can replace FM in red tilapia (*O. niloticus x O. hornorum*) and Nile tilapia diets, at 50% and 100%, respectively, without significant retardation in weight gain and

feed efficiency. In Blue tilapia (*Oreochromis aureus*), inclusion of shrimp meal up to 6% dietary level had no negative impact on animal performances (Nwanna *et al.*, 2000). Liti *et al.* (2005) shown that diets containing whole freshwater shrimp (*Caridina nilotica* Roux) meal as the sole source of animal protein were more efficient in promoting fast growth of Nile tilapia than those containing plant sources only.

Different forms of shrimp by-products are also reported to give good results in fish feeding: Shrimp head silage protein hydrolysate can replace fish meal up to 10 - 15% in Nile tilapia (*Oreochromis niloticus*) and results in better performances and feed conversion ratio (Plascencia-Jatomea *et al.*, 2002). It is very palatable to tilapia (Plascencia-Jatomea *et al.*, 2002). In African catfish (*Clarias gariepinus*), formic acid preserved shrimp heads are reported to profitably replace fish meal at 20% dietary level (Nwanna, 2003) while lactic acid bacteria fermented shrimp meal can be included at 30% dietary level so that best profit margin is obtained (Nwanna *et al.*, 2004).

2.4.6 Blended diet (BLEND)

Combinations of plant proteins have been shown to be able replace fish meal totally (El-Saidy and Gaber, 2003). A blend of sunflower seed meal, cotton seed meal, linseed meal and soybean meal (1:1:1:1) could totally replace fish meal and gave better economical return without detrimental effect on animal performance and feed efficiency (El-Saidy *et al.*, 2003). Blending is more practical for small scale fish farmers in developing countries where amino acid supplementation may not be viable due to costs and availability (Fagbenro, 1999).

2.5 Summary of the Review

It is revealed from the literature that Nile tilapia is the predominant species that cultured worldwide. However, its production is limited by low quality feeds, high costs and limited supply of fish meal (FM) which is primarily used as source of protein in tilapia diets. Alternative protein sources (APS) from plants such as soybean meal (SBM), cotton seed cake (CSC) and sunflower seed cake (SFSC) were found to be nutritious, readily available ingredients in most parts of Tanzania and more cost-effective than FM. Though they are limiting in some amino acids such as Methionine, Lysine, Cystine, Threonine and Isoleucine. Presence of anti-nutritional factors were found to be another limitation of using the available APS in tilapia production. Proper processing of APS may deactivate anti-nutritional factors before they will be incorporated in feed formulations to improve protein content and utilisation. High crude fiber content in CSC and SFSC limits their inclusion levels due to reduced digestibility and nutrient bioavailability. However, proper processing and combination of these ingredients in the practical tilapia diets may enhance biological performance and cost-effectiveness in tilapia production.

On the other hand, alternative protein sources from animals such as freshwater shrimp meal (FSM) and blood meal (BM) had higher level of crude protein (CP) but limited in use due to large portion of chitin in FSM CP and significantly low percentage of digestible organic matter protein and digestible energy content in BM limits their inclusion levels due to reduced digestibility and nutrient bio-availability. However, inclusion of these APS were reported to enhance palatability and boosting protein levels in compounded fish diets.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at Sokoine University of Agriculture (SUA) in Morogoro which lies at latitude 6° 51'5" South and longitude 37° 39' 26" East with an altitude of about 600 m above sea level. The trial was done at Magadu aquaculture research facility belonging to Department of Animal Science and Production (DASP) situated in the Western part of the University along the Morogoro–Mzinga road. It is 3.0 km from the centre of Morogoro Municipality and about 200 km west of Dar es Salaam.

3.2 Source of Feedstuff and Preparation

The fresh cattle blood was collected from Morogoro Municipality abattoir. Efforts were made to ensure contaminants such as hair, stomach belchings and urine were in trace quantities that were unavoidable. The blood was allowed to ferment for four (4) days and cooked first before drying at 60°C to produce the blood meal (BM).

Fish meal (*Restrineobola argentea*) locally known as "Dagaa" from Lake Victoria were purchased from livestock feed shops in Morogoro Municipality. The dried "Dagaa" were milled into fine powder.

Soybean (*Glycine max*) meal was collected from a local market in Morogoro Municipality. Mature dry seeds of soybean seeds were sorted manually for any impurities. The seeds were then soaked for 24 hours in clean tap water at the seed: water ratio of 1:3 on a volume basis. There after the soaked seeds were boiled in water at the seed: water ratio of 1:2 by volume basis for 45 minutes. Boiling of soybeans was

meant to inactivate enzymes that inhibit protein digestion by fish. The soaked and boiled seeds were washed using clean tap water and spread on a polythene sheets for sun drying for 72 hours before being milled into fine powder.

The cotton and sunflower seed cake (CSC and SFSC respectively) were purchased from livestock feeds in Morogoro Municipality. The cakes were sun-dried and milled into fine powder.

Caridina nilotica purchased from livestock feeds in Morogoro Municipality were boiled and sundried. The dried Caridina nilotica were milled into fine powder.

3.3 Determination of Chemical Composition of Alternative Protein Sources (APS) and the Practical Diets

The chemical composition of the ingredients and diets were determined using standard methods (AOAC, 2002). Crude protein (CP) was determined using the Kjeldahl method through digestion (Digestion system 12 1009 Digester, Tecator, Sweden), distillation (2200 Kjeltic Auto Distillation, Foss Tecator, Sweden) and titration (Digitrate, Tecator, Sweden) to determine amounts of nitrogen. The quantified N was multiplied by a factor of 6.25 to get CP. Ether extract was determined using Soxtec extraction machine (Soxtec system HT 1043 Extraction unit, Tecator, Sweeden) using petroleum ether (40–60° C boiling range).

Crude fiber was determined using a moisture free defatted sample which was digested by a weak acid followed by a weak base using Ankom fiber analyzer (ANKOM ²²⁰, ANKOM Technology, USA). Ash was determined by incineration of a sample in a muffle furnace (N31R, Nabertherm, West Germany) at 550°C for 3 hours. The moisture

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content was determined by drying the samples in an oven (E 115, WTB binder 7200, Germany) at 105° C to constant weight gain for 24 hours. Nitrogen–free extract was determined by substracting the sum of moisture, crude protein, ether extract, crude fiber and ash from 100. Gross energy of the feed ingredients (Kcal) (1Kj = 4.186 Kcal) were estimated according to NRC (1993) equation as follows:

$$GE = (5.64 \times \%CP) + (9.44 \times \%EE) + (4.11Kcal \times \%NFE)$$
(1)

Where:

GE = Gross Energy,

 $CP = Crude\ Protein,$

 $EE = Ether\ Extract,$

Kcal = Kilocalories,

NFE = Nitrogen Free Extract.

3.4 Feed Formulation

Eleven (11) isonitrogenous (30g 100g⁻¹), isolipidic (10g 100g⁻¹) and isoenergetic (18kJg⁻¹) diets were prepared using fish meal (FM), soybean meal (SBM), cotton seed meal (CSM), sunflower seed meal (SFSM), blood meal (BM) and freshwater shrimp meal (FSM). The control diet (FM) used FM (22%) and SBM (30%) as the major sources of protein. In test diets, FM was fixed at 5%, while inclusion of SBM, CSM and SFSM varied as follows; SBM diets contained SBM at 20%(SBM20), 25%(SBM25) and 30%(SBM30). CSM diets contained CSM at 15%(CSM15), 20%(CSM20) and 25%(CSM25) and SFSM diets contained SFSM at 10%(SFSM10), 15%(SFSM15) and 20%(SFSM20). Blended diet (BLEND) contained CSM and SFSM at 10% and SBM at 5%. Other sources of protein (BM and FSM) were included at varying amounts in the test diets (6–15%) to boost protein levels and enhance palatability respectively. Wheat meal (8%) was included as a binder, maize meal (35–44%) as a source of carbohydrate, sunflower oil (3–5%) as a source of lipid and vitamin/mineral (2%) as a supplement of vitamins and minerals (Table7).

During diet preparation, the amount of feed ingredients obtained by feed formulation were weighed using electronic weight balance model BEB 61 (BOECO 20459, Hamburg, Germany) and mixed thoroughly by hand in a plastic container. The mixture were then mixed with 25% of water, pelleted using meat grinder, sundried, packed in airtight containers, labeled and kept frozen in a deep freezer until been fed to the experimental fish. The pellets were analysed for proximate composition as described in section 3.3.

Table 7a: Ingredient levels (% dry matter basis) of SBM diets

Ingredient (%)		Die	Diet					
	FM	SBM20	SBM25	SBM30				
FM	22	5	5	5				
SBM	30	20	25	30				
MM	35	42	39	37				
FSM	0	8	8	6				
BM	0	10	9	8				
SFO	3	5	4	4				
WM	8	8	8	8				
**VIT/MIN	2	2	2	2				
Total	100	100	100	100				
CP (30g 100g ⁻¹)	30	30	30	30				
*Cost(TShs/kg)	2216	1616	1641	1696				

FM = Fish meal, SBM = Soybean meal, MM = Maize meal, FSM = Freshwater shrimp meal, SFO = Sunflower oil, BM = Blood meal, WM = Wheat meal, VIT = Vitamins, MN = Minerals, CP = Crude protein, g = gram, kg = kilogram, TShs = Tanzania shilling.

^{*1} USD = 1 600 TShs (Accessed 20 April, 2013).

^{**}Vitamin A 25 500 000 IU, Vitamin D3 5 000 000 IU, Vitamin E 5 050 IU, Vitamin B2 mg 4750, Vitamin B6mg 2750, Vitamin B12 mcg 11 750, Vitamin K3 mg 4850, CAL PAN mg 5750, Niacinamide mg 16 500, Vitamin C 10 000 mg, IRON 5250 mg, MANGANESE 12 760 mg, COPPER 13 250 mg, ZINC 13 250 mg, SODIUM CHLORIDE 48 750 mg, MAGNESIUM 12 750 mg, POTASSIUM ACETATE 73 750 mg, LYSINE 15 000 mg, METHIONINE 12 000 mg, antioxidant and anticaking qsf 1 kg.

Table 7b: Ingredient levels (% dry matter basis) of CSM diets

Ingredient (%)		Diet		
	FM	CSM15	CSM20	CSM25
FM	22	5	5	5
SBM	30	0	0	0
CSM	0	15	20	25
MM	35	44	42	39
FSM	0	10	10	10
BM	0	11	8	6
SFO	3	5	5	5
WM	8	8	8	8
**VIT/MIN	2	2	2	2
Total	100	100	100	100
CP (30g 100g ⁻¹)	30	30	30	30
*Cost(TShs/kg)	2216	1351	1346	1336

FM = Fish meal, SBM = Soybean meal, CSM = Cotton seed meal, MM = Maize meal, FSM = Freshwater shrimp meal, SFO = Sunflower oil, BM = Blood meal, WM = Wheat meal, VIT = Vitamins, MN = Minerals, CP = Crude protein, g = gram, kg = kilogram, TShs = Tanzania shilling.

Table 7c: Ingredient levels (% dry matter basis) of SFSM diets

Ingredient (%)		Diet		
, ,	FM	SFSM10	SFSM15	SFSM20
FM	22	5	5	5
SBM	30	0	0	0
SFSM	0	10	15	20
MM	35	43	38	35
FSM	0	12	13	12
BM	0	15	14	14
SFO	3	5	5	4
WM	8	8	8	8
**VIT/MIN	2	2	2	2
Total	100	100	100	100
CP (30g 100g ⁻¹)	30	30	30	30
*Cost(TShs/kg)	2216	1341	1321	1266

FM = Fish meal, SBM = Soybean meal, SFSM = Sunflower seed meal, MM = Maize meal, FSM = Freshwater shrimp meal, SFO = Sunflower oil, BM = Blood meal, WM = Wheat meal, VIT = Vitamins, MN = Minerals, CP = Crude protein, g = gram, kg = kilogram, TShs = Tanzania shilling.

^{*1} USD = 1 600 TShs (Accessed 20 April, 2013).

^{**}Vitamin A 25 500 000 IU, Vitamin D3 5 000 000 IU, Vitamin E 5 050 IU, Vitamin B2 mg 4 750, Vitamin B6mg 2750, Vitamin B12 mcg 11 750, Vitamin K3 mg 4850, CAL PAN mg 5750, Niacinamide mg 16 500, Vitamin C 10 000 mg, IRON 5 250 mg, MANGANESE 12 760 mg, COPPER 13 250 mg, ZINC 13 250 mg, SODIUM CHLORIDE 48 750 mg, MAGNESIUM 12 750 mg, POTASSIUM ACETATE 73 750 mg, LYSINE 15 000 mg, METHIONINE 12 000 mg, antioxidant and anticaking qsf 1 kg.

^{*1} USD = 1 600 TShs (Accessed 20 April, 2013).

^{**}Vitamin A 25 500 000 IU, Vitamin D3 5 000 000 IU, Vitamin E 5 050 IU, Vitamin B2 mg 4 750, Vitamin B6mg 2 750, Vitamin B12 mcg 11 750, Vitamin K3 mg 4 850, CAL PAN mg 5 750, Niacinamide mg 16 500, Vitamin C 10 000 mg, IRON 5 250 mg, MANGANESE 12 760 mg, COPPER 13 250 mg, ZINC 13 250 mg, SODIUM CHLORIDE 48 750 mg, MAGNESIUM 12, 750 mg, POTASSIUM ACETATE 73 750 mg, LYSINE 15 000 mg, METHIONINE 12 000 mg, antioxidant and anticaking qsf 1 kg.

Table 7d: Ingredient levels (% dry matter basis) of blended diet

Ingredient (%)	Diet		
	FM	BLEND	
FM	22	5	
SBM	30	5	
CSM	0	10	
SFSM	0	10	
MM	35	36	
FSM	0	10	
BM	0	10	
SFO	3	4	
WM	8	8	
**VIT/MIN	2	2	
Total	100	100	
CP (30g 100g ⁻¹)	30	30	
*Cost(TShs/kg)	2216	1346	

FM = Fish meal, SBM = Soybean meal, SFSM = Sunflower seed meal, MM = Maize meal, FSM = Freshwater shrimp meal, SFO = Sunflower oil, BM = Blood meal, WM = Wheat meal, VIT = Vitamins, MN = Minerals, CP = Crude protein, g = gram, kg = kilogram, TShs = Tanzania shilling.

3.5 Experimental Procedures

3.5.1 Source of fingerlings

The study used Nile tilapia (*Oreochromis niloticus*) fingerlings (Plate 1) with an average weight of 3.5 g per fingerling. The fingerlings were obtained from Fish Ponds located in the Aquaculture Research facility in Magadu.

3.5.2 Description of the experimental facility

Feeding trials were done using an indoor recirculating aquaculture system (RAS), comprised of thirty three (33) 20 L capacity circular self-cleaning plastic tanks (Plate 2) that drained into five (5) biofilters and one (1) settling tank. The system comprised of a header tank which supplied dechlorinated tap water from SUA's independent water source from Mount Uluguru to culture tanks through a series of pipes as shown in Plate

^{*1} USD = 1 600 TShs (Accessed 20 April, 2013).

^{**}Vitamin A 25 500 000 IU, Vitamin D3 5 000 000 IU, Vitamin E 5050 IU, Vitamin B2 mg 4750, Vitamin B6mg 2750, Vitamin B12 mcg 11 750, Vitamin K3 mg 4850, CAL PAN mg 5750, Niacinamide mg 16 500, Vitamin C 10 000 mg, IRON 5,250 mg, MANGANESE 12 760 mg, COPPER 13 250 mg, ZINC 13 250 mg, SODIUM CHLORIDE 48 750 mg, MAGNESIUM 12 750 mg, POTASSIUM ACETATE 73 750 mg, LYSINE 15 000 mg, METHIONINE 12 000 mg, antioxidant and anticaking qsf 1 kg.



Plate 1: Nile tilapia (Oreochromis niloticus) fingerlings used in this study



Plate 2: Internal appearance of circular plastic tanks of the recirculating system



Plate 3: Indoor recirculating aquaculture system showing the arrangement of circular plastic tanks at Magadu aquaculture research facility

3.5.3 Experimental layout

Three hundreds and thirty (330) Nile tilapia (*Oreochromis niloticus*) fingerlings were stocked in circular plastic tanks at a density of ten (10) fingerlings per each tank. Fish were individually weighed prior to the start of the experiment in order to obtain fish of uniform size. Weighing were done using electronic weight balance model BEB 61 (BOECO 20459, Hamburg, Germany). Fingerlings were acclimatized to experimental conditions for one week. During this period, they were fed on control diet to remove any managemental differences from their source of origin. Then, the eleven (11) diets were randomly allocated in a complete randomized design (CRD). Each diet was replicated three times making a total of 330 fingerlings for the whole study. Fish were

fed to apparent satiation but not exceeding 5% of their body weight in two portions per day at 0930 h and 1530 h for a period of 8 weeks.

3.6 Data Collection

Data were collected for a period of 8 weeks from the month of July 2014 through September 2014. Feed residues for each replicate were weighed and recorded weekly. Fish in each treatment were group weighed and recorded after every one week and the amount of diet fed was adjusted accordingly for the entire experiment. The variables measured / recorded were weekly weights, feed residues and ingredient costs. These were used to determine the following:

3.6.1 Growth and nutrient utilisation

Growth and nutrient utilisation were determined in terms of feed intake (FI), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) as follows:

$$FI(g fish^{-1} day^{-1}) = \frac{Total feed intake}{Number of days};$$
(2)

$$ADG (g fish^{-1} day^{-1}) = \frac{Final weight - initial weight}{Number of days}; \qquad (3)$$

$$SGR\ (\%\ day^{-1}) = \frac{100 \times (In[final\ body\ weight] - In[initial\ body\ weight])}{Number\ of\ days}; \qquad(4)$$

$$FCR = \frac{Feed\ intake\ (gram\ dry\ weight)}{Live\ weight\ (g)}; \qquad \qquad (5)$$

PER $= \frac{Live\ weight\ gain(g)}{Crude\ protein\ intake(g)}.$ (6)

3.6.2 Cost-effectiveness analysis

Feeding cost for producing a kilogramme of fish = fish feed price (TShs.) per kilogramme × feed conversion rate (FCR).

3.7 Data Analysis

The data collected were tested for homogeneity of variance using Levene's test. Then analysed using one-way analysis of variance (ANOVA) and where there were significant differences between treatments means, Post-hoc analysis was done using Tamhane's Significant Difference Test due to unequal variances. In all cases differences were considered significant at p <0.05. The analysis were done using the SPSS Software Version 20 (SPSS Inc.). The model used to test the effect of the diets on Performance indicators was as follows:

$$Y_{ijk} = \mu + D_i + \varepsilon_{ijk} \tag{7}$$

Where:

 Y_{ijk} = Performance indicators (final weight, weight gain, average daily gain, specific growth rate, feed conversion ratio, protein efficiency ratio),

 $\mu = Overall mean,$

 D_i = Effect of experimental diets on the performance indicators of Oreochromis niloticus,

 $\varepsilon_{ijk} = Random\ error.$

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Composition of Feedstuffs

Chemical composition of the ingredients is shown in Table 8. Blood meal (BM) had the highest crude protein while the lowest crude protein was found in sunflower seed cake (SFSC). Soybean meal (SBM) had the highest crude lipid and BM the lowest. In terms of crude fibre content, SFSC had the highest crude fibre followed by cotton seed cake (CSC) and BM the least. SFSC had highest nitrogen free extract and fish meal (FM) had the lowest. Ash content was highest in FM followed by freshwater shrimps (FSM) and lowest in SBM. Dry matter content was highest in CSC and lowest in FSM. SBM had highest energy content followed by BM and lowest in SFSC.

Table 8: Chemical composition, gross energy and price of the individual ingredients used in the study (as % DM)

		Ingredient				
Item	BM	FSM	FM	CSC	SBM	SFSC
Moisture	6.8	9.7	8.8	2.5	5	4.2
Crude protein	83.1	59.1	58.9	44.6	42.3	19.5
Crude lipid	0.4	4.3	10.1	10.9	24.7	13.9
Crude fibre	0.2	7.0	1.1	14.4	11.9	35.5
Ash	3.9	21.1	22.5	6.7	3.5	5.8
NFE	5.6	8.5	7.4	23.4	17.6	25.3
$GE(kJg^{-1})$	21.98	17.17	19.22	18.97	22.86	14.55
*Cost (TShs/kg)	500	1000	4000	600	2000	500

BM = Blood meal, FSM = Freshwater shrimp meal, FM = Fish meal, CSC = Cotton seed cake, SBM = Soybean meal, SFSC = Sunflower seed cake, NFE = Nitrogen free extract, GE = Gross energy, g = gram, Kcal = Kilocalories, kg = kilogram, % = Percentage, DM = Dry matter, TShs = Tanzania shilling.

*1 USD = 1600 TShs (Accessed 20 April, 2013).

4.2 Soybean Meal (SBM) Diets

4.2.1 Chemical composition of SBM diets

Chemical composition of SBM diets is shown in Table 9. Moisture content was lowest in control diet but increased in SBM diets with increasing SBM levels. Control diet had highest crude protein (CP) compared to SBM diets. The CP increased with increasing SBM levels in SBM diets. Crude fibre was lowest in control diet but increased as the inclusion levels of SBM were increased in the SBM diets. The control diet had the highest ash content compared to SBM diets but ash content decreased with increasing SBM levels in SBM diets. Energy and crude lipid followed the same trend (Table 9).

Table 9: Chemical composition and gross energy of the SBM diets (as % DM)

Item	FM	SBM20	SBM25	SBM30
Moisture	4.20	5.00	6.89	7.04
Crude protein	34.6	31.50	31.70	33.50
Crude lipid	10.52	10.36	10.00	10.00
Crude fibre	3.52	3.69	3.81	3.84
Ash	7.77	6.05	5.92	5.83
NFE	43.59	48.4	48.57	45.02
GE(kJg ⁻¹)	19.82	19.81	19.78	19.68

NFE = Nitrogen free extract, GE = Gross energy, kJg⁻¹ = kilojoules per gram, % = Percentage, DM = Dry matter, FM=Fish meal, SBM=Soybean meal

4.2.2 Effects of SBM diets on growth, feed utilization and cost-effectiveness

Weight gain (WTGN) during the growth trial is shown in Figure 1. The control diet had the highest values of WTGN compared to SBM diets. SBM30 followed by SBM25 had the highest values of WTGN while the lowest values were in SBM20.

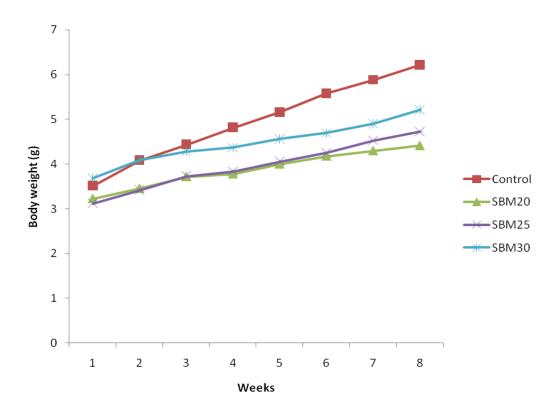


Figure 1: Change in body weight of *Oreochromis niloticus* fed diets containing graded levels of soybean meal (SBM)

Table 10 shows growth performance, feed utilization and cost-effectiveness of Tilapia fingerlings fed soybean meal (SBM) based diets.

Table 10: Growth performance, nutrient utilization and cost-effectiveness of O.

niloticus fed soybean meal diets* (mean \pm SE, n=3)

	Diet				
Parameter	FM	SBM 20	SBM25	SBM30	
Initial weight (g)	3.32 <u>+</u> 0.19 ^a	3.34 ± 0.06^{a}	3.19 <u>+</u> 0.29 ^a	3.41 <u>+</u> 0.06 ^a	
Final weight (g)	6.22 <u>+</u> 0.15 ^a	4.43 ± 0.38^{c}	5.09 ± 0.39^{bc}	5.31 <u>+</u> 0.16 ^a	
Weight gain (g)	2.90 ± 0.08^{a}	1.09 <u>+</u> 0.32 ^c	1.89 ± 0.10^{b}	1.91 <u>+</u> 0.11 ^b	
Average daily gain (g fish-1 day-1)	0.05 ± 0.001^{a}	0.02 <u>+</u> 0.01 ^c	0.03 ± 0.002^{b}	0.03 ± 0.001^{b}	
Feed intake (g fish ⁻¹ day ⁻¹)	0.01 ± 0.001^{a}	0.03 ± 0.02^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	
Feed conversion ratio	2.01 ± 0.10^{a}	2.49 ± 0.52^{a}	2.44 ± 0.08^{a}	2.50 ± 0.09^{a}	
Protein efficiency ratio	0.002 ± 0.00^{a}	0.001 ± 0.00^{b}	0.001 ± 0.00^{b}	0.001 ± 0.00^{b}	
Specific growth rate (% day ⁻¹)	3.00 <u>+</u> 0.19 ^a	1.32 <u>+</u> 0.32 ^c	2.22 ± 0.06^{b}	2.11 ± 0.06^{b}	
Cost effectiveness (TShs/kg)	4444 <u>+</u> 230 ^a	4038 <u>+</u> 841 ^a	4011 <u>+</u> 133 ^a	4166 <u>+</u> 165 ^a	

Means with different superscript letters within a row are significantly (p<0.05) different

The control diet had a significant influence on final weight (FNWT), weight gain (WTGN), average daily gain (ADG), protein efficiency ratio (PER) and specific growth rate (SGR) compared to SBM diets (p<0.05). The SBM25 and SBM30 had significantly (p<0.05) higher values on FNWT, WTGN, ADG and SGR as compared to SBM20 (p<0.05). The results also showed that, the SBM diets had no significant (p<0.05) effects on PER.

Moreover, the control and SBM diets had no statistical significant effect on feed intake (FI), feed conversion ratio (FCR) and cost-effectiveness (p<0.05). However, SBM diets were more cost-effective by Tshs. 400 less compared to the control diet. The control diet had insignificant lowest values on FI followed by SBM25 and SBM30 while SBM20 recorded the highest values. The control diet had insignificantly lowest values on FCR followed by SBM25, SBM20 and the highest values was in SBM30. SBM25 was the most cost-effective diet (TShs. 433) followed by SBM20 (TShs. 406) and least in SBM30 (TShs. 278) while the highest cost to produce a kilogram of fish was observed in control diet. In general, SBM25 with an inclusion level of 25% SBM had comparable performance to the control diet but more cost-effective compared to other SBM diets used in this study.

4.3 Cotton Seed Meal (CSM) Diets

4.3.1 Chemical composition of CSM diets

Chemical composition of CSM diets is shown in Table 11. Moisture content was lowest in control diet but increased in CSM diets with increasing CSM levels. The control diet had highest crude protein (CP) followed by CSM20 and CSM15 while CSM25 had the lowest CP. The crude lipid followed the same trend. The crude fibre was lowest in control diet but increased in the CSM diets as the CSM levels were increasing. The

control diet had highest ash content compared to CSM diets but ash increased in the CSM diets with increasing CSM levels. The control diet had highest energy content though decreased in the CSM diets with increasing CSM levels.

Table 11: Chemical composition and gross energy of the CSM diets (as % DM)

Item	FM	CSM15	CSM20	CSM25
Moisture	4.20	8.32	8.39	8.48
Crude protein	34.6	28.5	28.6	26.2
Crude lipid	10.52	10.10	10.23	9.95
Crude fibre	3.52	5.1	5.71	6.43
Ash	7.77	6.25	6.54	6.79
NFE	43.59	50.05	48.92	50.63
GE(kJg ⁻¹)	19.82	19.32	19.20	18.82

NFE = Nitrogen free extract, GE = Gross energy, kJg⁻¹ = kilojoules per gram, % = Percentage, DM = Dry matter, FM=Fish meal

4.3.2 Effects of CSM diets on growth, feed utilization and cost-effectiveness

Weight gain (WTGN) during the growth trial is shown in Figure 2. The control diet had lowest values on WTGN compared to CSM diets. CSM20 followed by CSM25 had highest values on WTGN and the lowest in CSM15.

The control and CSM diets did not differ significantly (p<0.05) on final weight (FNWT), weight gain (WTGN), average daily gain (ADG), protein efficiency ratio (PER) and specifc growth rate (SGR). The control diet had lowest values on FNWT compared to CSM diets. CSM20 however displayed higher values on FNWT followed by CSM25 and lowest values in CSM15 diet. The weight gain (WTGN), average daily gain (ADG) and specifc growth rate (SGR) followed the same trend.

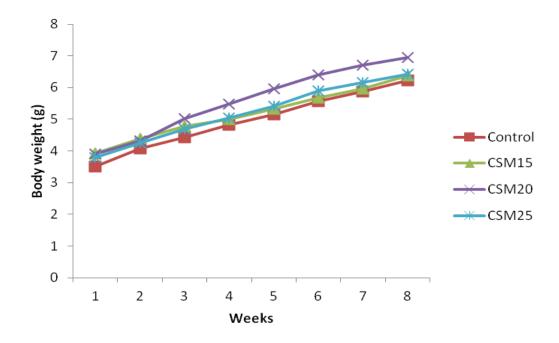


Figure 2: Change in body weight of *Oreochromis niloticus* fed diets containing graded levels of cottonseed meal

Table 12 shows growth performance, feed utilization and cost-effectiveness of tilapia fingerlings fed cottonseed meal (CSM) based diets.

Table 12: Growth performance, nutrient utilization and cost-effectiveness of *O. niloticus* fed cottonseed meal diets (mean <u>+</u> SE, n=3)

		Die	t	
Parameter	FM	CSM15	CSM20	CSM25
Initial weight (g)	3.32 <u>+</u> 0.19 ^a	3.58 <u>+</u> 0.15 ^a	3.78 <u>+</u> 0.14 ^a	3.57 <u>+</u> 0.19 ^a
Final weight (g)	6.22 <u>+</u> 0.15 ^a	6.38 ± 0.24^{a}	6.94 <u>+</u> 0.63 ^a	6.43 ± 0.26^{a}
Weight gain (g)	2.90 ± 0.08^{a}	2.80 <u>+</u> 0.21 ^a	3.16 <u>+</u> 0.49 ^a	2.85 <u>+</u> 0.16 ^a
Average daily gain (g fish-1 day-1)	0.05 ± 0.001^{a}	0.05 ± 0.003^{a}	0.06 ± 0.01^{a}	0.05 ± 0.003^{a}
Feed intake (g fish ⁻¹ day ⁻¹)	0.014 ± 0.001^{a}	0.017 ± 0.003^{a}	0.019 ± 0.003^{a}	0.016 ± 0.002^{a}
Feed conversion ratio	2.01 ± 0.10^{b}	2.30 ± 0.09^{ab}	2.32 ± 0.17^{ab}	2.45 ± 0.07^{a}
Protein efficiency ratio	0.002 ± 0.00^{a}	0.002 ± 0.00^{a}	0.002 ± 0.00^{a}	0.002 ± 0.00^{a}
Specific growth rate (% day ⁻¹)	3.00 ± 0.19^{a}	2.75 <u>+</u> 0.19 ^a	2.87 ± 0.26^{a}	2.80 <u>+</u> 0.15 ^a
Cost effectiveness (TShs/kg)	4444 <u>+</u> 230 ^a	3111 <u>+</u> 124 ^b	3120 <u>+</u> 224 ^b	3275 <u>+</u> 97 ^b

Means with different superscript letters within a row are significantly (p<0.05) different.

Moreover, the control and CSM diets tended to differ significantly (p<0.05) on feed conversion ratio (FCR). The control diet had significantly (p<0.05) lower values on FCR as compared with CSM25. However, the control diet did not differ significantly (p<0.05) with other CSM diets, it had insignificantly (p<0.05) lower values than CSM15 and CSM20. The results also showed that, the control and CSM diets did not differ significantly (p<0.05) on feed intake (FI). The control diet had insignificantly lower values on FI followed by CSM25, CSM15 and highest in CSM20 (Table 12).

The control and CSM diets tended to differ significantly (p<0.05) on cost-effectiveness. The control diet had significantly higher values on cost-effectiveness compared to CSM diets (p<0.05). However, CSM15 had insignificantly (p<0.05) lower values on cost-effectiveness followed by CSM20 and CSM25. In general, cotton seed meal (CSM) diets at 15–25% inclusion levels had comparable performance interms of growth and feed utilization to the control diet but more cost-effective.

4.4 Sunflower Seed Meal (SFSM) Diets

4.4.1 Chemical composition of SFSM diets

Chemical composition of SFSM diets is shown in Table 13. The SFSM15 and SFSM20 had the lowest moisture content while SFSM10 followed by control diet had the highest. The SFSM10 had the highest crude protein followed by control diet while SFSM15 had the lowest. The control diet had the highest crude lipid compared to SFSM diets. Crude fibre was lowest in control diet but increased in the SFSM diets as SFSM levels were increasing. The control diet had the highest ash content compared to SFSM diets but ash content increased in the SFSM diets with increasing SFSM levels. The control diet had highest energy content but energy levels decreased with increasing SFSM levels.

Table 13: Chemical composition and gross energy of the SFSM diets (as % DM)

		Diets		
Item	FM	SFSM10	SFSM15	SFSM20
Moisture	4.20	4.44	3.76	3.81
Crude protein	34.6	34.7	29.4	32.7
Crude lipid	10.52	9.96	10.11	9.97
Crude fibre	3.52	6.53	8.41	9.81
Ash	7.77	7.01	7.15	7.28
NFE	43.59	41.8	44.93	40.24
$GE(kJg^{-1})$	19.82	19.31	18.66	18.59

NFE = Nitrogen free extract, GE = Gross energy, kJg⁻¹ = kilojoules per gram, % = Percentage, DM = Dry matter, FM = Fish meal, SFSM = Sunflower seed meal

4.4.2 Effects of SFSM diets on growth, feed utilization and cost-effectiveness

Weight gain (WTGN) during the growth trial is shown in Figure 3. The control diet had the highest values on weight gain compared to SFSM diets. SFSM15 followed by SFSM10 had the highest values on weight gain and the lowest in SFSM20.

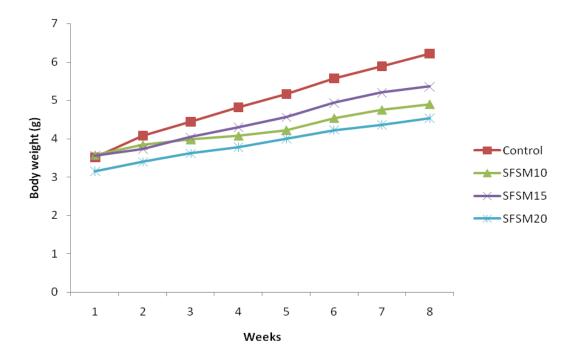


Figure 3: Change in body weight of *Oreochromis niloticus* fed diets containing graded levels of sunflower seed meal

Table 14 shows growth performance, feed utilization and cost-effectiveness of tilapia fingerlings fed sunflower seed meal (SFSM) based diets.

Table 14: Growth performance, nutrient utilization and cost-effectiveness of *O. niloticus* fed Sunflower seed meal diets (mean ± SE, n=3)

	Diet			
Parameters	FM	SFSM10	SFSM15	SFSM20
Initial weight (g)	3.32 <u>+</u> 0.19 ^a	3.32 ± 0.09^{a}	3.43 <u>+</u> 0.28 ^a	3.05 ± 0.76^{a}
Final weight (g)	6.22 ± 0.15^{a}	5.02 ± 0.14^{ab}	4.90 <u>+</u> 1.06 ^b	4.65 ± 0.04^{b}
Weight gain (g)	2.90 ± 0.08^{a}	1.70 <u>+</u> 0.05 ^b	1.47 ± 0.78^{b}	1.60 <u>+</u> 0.09 ^b
Average daily gain (g fish-1 day-1)	0.05 ± 0.001^a	0.03 ± 0.001^{b}	0.03 <u>+</u> 0.01 ^b	0.03 ± 0.003^{b}
Feed intake (g fish ⁻¹ day ⁻¹)	0.01 ± 0.001^{b}	0.02 ± 0.003^{b}	0.03 ± 0.004^{a}	0.01 ± 0.005^{b}
Feed conversion ratio	2.01 ± 0.10^{a}	2.37 ± 0.09^{a}	2.29 ± 0.27^{a}	2.19 <u>+</u> 0.13 ^a
Protein efficiency ratio	0.002 ± 0.00^{a}	0.001 ± 0.00^{b}	0.001 ± 0.00^{b}	0.001 ± 0.00^{b}
Specific growth rate (% day ⁻¹)	3.00 <u>+</u> 0.19 ^a	1.97 <u>+</u> 0.02 ^b	1.60 <u>+</u> 0.66 ^b	2.01 <u>+</u> 0.13 ^b
Cost effectiveness (TShs/kg)	4444 <u>+</u> 230 ^a	3182 <u>+</u> 130 ^b	3021 <u>+</u> 351 ^b	2779 <u>+</u> 159 ^b

Means with different superscript letters within a row are significantly (p<0.05) different.

The control diet had significantly (p<0.05) higher values on FNWT as compared to SFSM15 and SFSM20 but insignificant to SFSM10. However, SFSM10 had insignificantly (p<0.05) higher values on FNWT as compared to SFSM15 and SFSM20. Weight gain (WTGN) and average daily gain (ADG) followed the same trend with the exception of control diet which differed significantly (p<0.05) on these variables. The control diet had significantly (p<0.05) higher values on SGR as compared to the SFSM diets. SFSM20 had insignificantly (p<0.05) higher values on SGR followed by SFSM10 and SFSM15.

The control and SFSM diets differ significantly (p<0.05) on feed intake (FI) and protein efficiency ratio (PER). SFSM15 had significantly (p<0.05) higher values on FI as compared to the rest of the diets. The control diet and SFSM20 followed by SFSM10 had insignificant (p<0.05) lower values on FI. The control diet had significantly

(p<0.05) higher values on PER compared to the SFSM diets. Feed conversion ratio (FCR) in all diets did not differ significantly (p<0.05) though the control diet had insignificantly lower values followed by SFSM20, SFSM15 and the highest in SFSM10. The control diet had significantly (p<0.05) higher values on cost-effectiveness as compared to SFSM diets. SFSM20 had insignificantly (p<0.05) lower values on cost-effectiveness followed by SFSM15 and SFSM10. In general, this study realized that SFSM20 with 20% SFSM level had comparable performance to the control diet but more cost-effective.

4.5 Blended Diet (BLEND)

4.5.1 Chemical composition of blended diet

Chemical composition of blended diet is shown in Table 15. The control diet had highest levels of crude protein and crude lipid compared to blended diet. Crude fibre (CF) content of blended diet was more than double that of the control. The control diet had highest ash content compared to blended diet. The control diet also had highest energy content compared to blended diet.

Table 15: Chemical composition and gross energy of the blended diet (as %DM)

	Diets		
Item	FM	BLEND	
Moisture	4.20	4.44	
Crude protein	34.6	33.5	
Crude lipid	10.52	9.96	
Crude fibre	3.52	6.53	
Ash	7.77	7.01	
NFE	43.59	41.8	
GE(kJg ⁻¹)	19.82	19.31	

NFE = Nitrogen free extract, GE = Gross energy, kJg⁻¹ = kilojoules per gram, % = Percentage, DM = Dry matter, FM=Fish meal

4.5.2 Effects of blended diet on growth, feed utilization and cost-effectiveness

Weight gain (WTGN) during the growth trial is shown in Figure 4. The control diet had highest values on weight gain compared to blended diet.

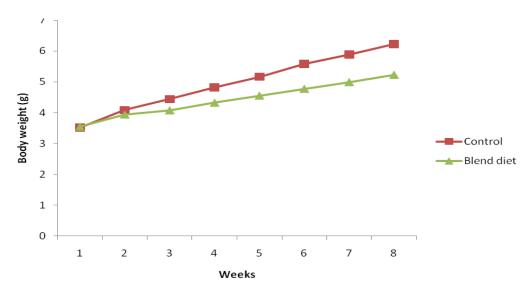


Figure 4: Change in body weight of Oreochromis niloticus fed blended diet

Table 16 shows growth performance, feed utilization and cost-effectiveness of tilapia fingerlings fed on blended diet.

Table 16: Growth performance, nutrient utilization and cost-effectiveness of O.

**niloticus* fed blended diet (mean \pm SE, n=3)

	Diets		
Parameters	FM	BLEND	
Initial weight (g)	3.32 <u>+</u> 0.19 ^a	3.37 ± 0.03^{a}	
Final weight (g)	6.22 <u>+</u> 0.15 ^a	5.23 ± 0.07^{b}	
Weight gain (g)	2.90 ± 0.08^{a}	1.87 ± 0.09^{b}	
Average daily gain (g fish-1 day-1)	0.05 ± 0.001^{a}	0.03 ± 0.002^{b}	
Feed intake (g fish-1 day-1)	0.01 ± 0.001^{a}	0.01 ± 0.002^{a}	
Feed conversion ratio	2.01 ± 0.10^{a}	2.11 ± 0.05^{a}	
Protein efficiency ratio	0.002 ± 0.00^{a}	0.001 ± 0.00^{b}	
Specific growth rate (% day-1)	3.00 ± 0.19^{a}	2.10 ± 0.09^{b}	
Cost effectiveness (TShs/kg)	4444 ± 230^{a}	2842 ± 70.8^{b}	

Means with different superscript letters within a row are significantly (p<0.05) different.

The control diet had significant (p<0.05) effect on final weight (FNWT), weight gain (WTGN), average daily gain (ADG), protein efficiency ratio (PER), specific growth rate (SGR) and cost-effectiveness. The control diet had significantly (p<0.05) higher values on FNWT than the blended diet. Weight gain (WTGN), average daily gain (ADG), protein efficiency ratio (PER), and specific growth rate (SGR) followed the same trend.

The blended diet did not differ significantly (p<0.05) on feed intake (FI) and feed conversion ratio (FCR) as compared to the control diet. The control and blended diet had similar FI values. The control diet had insignificantly (p<0.05) lower FCR values than the blended diet. The results also depicted that, the blended diet had significantly (p<0.05) better values on cost-effectiveness as compared to the control diet.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Composition of Feedstuffs

The ether extract, ash and nitrogen free extract of blood meal (BM) obtained in this study were found to be lower than those obtained by Aanyu *et al.* (2011); DeRouchey *et al.* (2003); Makinde and Sonaiya (2010); Monentchan *et al.* (2010) and Munguti *et al.* (2012). Similarly, the crude fibre content compared well by these workers. The level of crude protein (CP) was higher than those obtained by these workers except for DeRouchey *et al.* (2003) who found slightly higher levels. The high level of CP in BM is useful in boosting CP levels in fish compounded diets even at small inclusion levels.

The high level of crude fibre (CF) observed in SBM corroborate that of Shigulu (2012) who reported higher CF in SBM than that obtained in the respective ingredient by other researchers. This was because the soybean seeds were undecorticated (Shigulu, 2012). However, the level of CF of SBM in this study was higher than that obtained by Nyirenda *et al.* (2000); Abdelhany (2000); El-Saidy and Gaber (2003); Wang *et al.* (2006); Gaber (2006a); Abdel-Hakim *et al.* (2008) and Madalla (2008). The higher CF level in SBM obtained in this study limited higher inclusion level in diets. This is because increased inclusion level of SBM led to increased crude fibre content in the diet. This is undesirable in fish diets because it is undigestible (Maina, 2001) and lowers feed intake, growth and feed efficiency (Maina, 2001; Olvera-Novoa *et al.*, 2002).

Crude fibre is usually considered indigestible as tilapia do not possess the required enzymes for crude fibre digestion. Although some cellulase activity from microbes has been found in the gut of *O. mossambica* (Saha *et al.*, 2006). For this reason, and to

attain maximum growth performance and nutrients utilization, crude fibre level in tilapia diets should not exceed 5% of the total amount of diets (NRC, 1993). Dehulling of the soybean seeds can reduce the crude fibre content and thereby increase feed intake, acceptability, digestibility and protein utilization efficiency (El-Sayed, 1999; Ogunji and Wirth, 2001). Soybean can reduce growth of fish due to poor palatability caused by its bitter off-flavour especially if fishes are not adapted to it (Francis *et al.*, 2001).

The crude fibre of cotton seed cake (CSC) found in this study was lower than that reported by Munguti *et al.* (2006); Munguti *et al.* (2009); Kumar *et al.* (2011); Aanyu *et al.* (2012); Munguti *et al.* (2012) and Babiker (2012). Contrary, sunflower seed cake (SFSC) was found to have higher crude fibre than those reported by Nyirenda *et al.* (2000); Abraham (2001); El-saidy and Gaber (2003); Ramachandran *et al.* (2007) and Abdel-Hakim *et al.* (2008). The difference observed in the present study may be due to difference in species, origin and processing conditions of these oil seed cakes. The big limitation to these protein sources (CSC and SFSC) is their higher level of crude fibre. The higher crude fibre level in CSC and SFSC had the same effect as that of SBM fibre level. Reducing the fibre content of sunflower seed cake has been found to improve growth rate and weight gain (Maina *et al.*, 2007).

5.2 Effects of Diets on Growth, Feed Utilization and Cost-effectiveness

5.2.1 Soybean meal (SBM) diets

The control diet displayed improved weight gain (WTGN) and specific growth rate (SGR) followed by SBM30, SBM25 and lowest in SBM20 likely due to increased crude protein levels in the respective diets (Table 9). WTGN and SGR increased significantly

with increasing dietary protein levels from 21% to 37% (Loum *et al.* 2013). The author attributed the difference in improved WTGN and SGR to increase in protein utilization and digestibility.

The control diet depicted increased weight gain (WTGN) of *Oreochromis niloticus* but decreased in the SBM diets with increasing SBM levels due to increased crude fibre content in the given diets (Table 9). High crude fibre (CF) reduced feed intake which led to fewer nutrients available for fish growth (El-Sayed, 1999). Viola *et al.* (1982) showed a linear decrease in ralative weight gain of carp in cages with increasing SBM inclusion levels. This resulted into higher feed conversion ratios (FCR) indicative of poor feed utilization as more food was required for a unit gain in body weight which is also not cost-effective.

Ogunji and Wirth (2001) observed similar results when they incorporated high levels of soybean meal (SBM) in Nile tilapia diets. They observed unacceptability as the level of soybean meal increased in the diets. Rawles *et al.* (2010) attributed the poor growth performance due to the presence of residual levels of tripsin inhibitors caused by inadequate heating during processing. Similar findings were reported by Robaina *et al.* (1995) showed a general reduction of weight gain with increasing inclusion levels of soybean meal from 10 to 30%. The authors suggested that some other anti-nutritional factors such as oligosaccharides excluding trypsin inhibitors might limit the use of high level of SBM.

Increase with increasing rate in body weight gain observed at first was due to low crude fibre content led to increased feed intake and utilization by *Oreochromis niloticus* (Ogunji and Wirth, 2001; Maina, 2001; Olvera-Novoa *et al.*, 2002). The average daily

gain followed the same trend. The increase in body weight gain of *Oreochromis niloticus* as the inclusion levels of SBM increased at first, is likely due to combination of different amino acid sources thus, promoting fish growth (Ogunji and Wirth, 2001; El-Saidy and Gaber, 2003). Variations in nutrients values of the local ingredients necessities the combination of two or more of the feed resources in order to improve quality of fish diets. The control diet attained highest growth performance and feed utilization over the SBM diets attributed to higher inclusion levels of fish meal (22%) in the control diet. Fish meal is a more readily digested and assimilated ingredient than SBM, FSM and BM because they have high crude fibre, anti-nutritional factors and poor palatability. Though, the control diet had 30% SBM, its negative effects were masked by the high inclusion level of fishmeal. Similar results were reported by Wu *et al.* (2004) who worked with hybrid tilapia (*O.niloticus x O. aureus*) offered diets devoid of FM and found significantly (p<0.05) lower growth values than those fed diets containing FM.

The relief of about TShs. 400 in producing a kilogram of fish using SBM diets compared to control diet was due to reduced levels of fishmeal in the respective diets (Table 7a). FM is the most costly ingredient in fish diets. SBM30 was the least cost effective SBM diet due to higher inclusion level of SBM (Table 7a). Khattab (2001) reported that SBM is expensive because of the growing world wide demand for human and animal feeding and increasing the cost of processing techniques for elimination of inhibition factors.

5.2.2 Cotton seed meal (CSM) diets

In the present study, the control and CSM diets had no significant (p<0.05) effect on growth and feed utilization values (Table 11). These results were similar to reports

obtained by Garcia-Abiado *et al.* (2004) who found similar body weight and total lengths of tilapia fed 25–50% CSM protein replacement at the completion of the 16 week trial. El-Sayed (1990) found that dietary inclusion levels of 20–30% CSM were safe and useful for *Oreochromis niloticus*; and Tilapia offered feeds with or without FM grew at similar rates (Nguyen, 2008).

These findings are however contrary to that by Robinson *et al.* (1984) who reported poor performance by *Oreochromis aureus* fed cotton seed meal (CSM) based diets. Ofojekwu and Ejike (1984) reported a much lower weight gain and feed efficiency of *Oreochromis niloticus* fed a CSM diet as compared to the tilapia fed a fish meal control diet. The authors attributed the poor performance to the gossypol and low available Lysine content in CSM diets. Gossypol interferes with protein digestion, bind Lysine and reduced growth rate and productivity (Apata, 2010; Robinson and Li, 1995). Cotton seed cake used in this study was obtained by mechanical pressing of cotton seeds which may have reduced free gossypol to the level tolerable by tilapia fish (Apata, 2010).

Feed conversion ratio (FCR) was observed to increase with the increasing CSM levels in the CSM diets (15–25% CSM). FCR is the amount of flesh gained per quantity of feed consumed which adequately reflects the quality of the feed and the ability of the fish to digest it (Adeyemo and Longe, 2007). The increase in FCRs as CSM levels increased in the CSM diets indicated decreased feed utilization likely due to increased crude fibre content in the CSM diets (Table 11). The lowest feed conversion ratio indicated better-feed utilization by the fish (Adikwu, 2008) and this obviously accounted for better growth performance of tilapia fingerlings fed CSM diets as comparable to those fed control diet.

The highest cost of producing a kilogram of fish observed in the control diet was due to high inclusion levels of fish meal (22%) and soybean meal (30%) which attracted more cost. This is in harmony with similar study by Foturoti and Lawal (1986) who stated that high inclusion of fish meal increase cost of feeds. The high cost of fish meal in tilapia diets warrants the potential use of cotton seed meal (CSM) as an alternative source of high quality protein (Soltan *et al.*, 2011). It is noticed that the incorporation of cotton seed meal herein reduced the price of one kilogram diet as compared to the control diet (Table 11). Similar result was observed by Fagbenro (1999) who recommended inclusion of locally available feedstuffs especially agricultural by-products to reduce the cost of a complete feed.

5.2.3 Sunflower seed meal (SFSM) diets

The increased sunflower seed meal (SFSM) levels from 15 to 20% in SFSM diets improved specific growth rate (SGR) and feed conversion ratio (FCR) due to increased feed utilization. The optimum growth and feed utilization was observed when SFSM was included at 20% (Table 12). The findings were similar to previous studies that reported SFSM diets as a suitable feed ingredient for tilapia complete diets when it constitutes up to 20% of the dietary protein (Olvera-Novoa *et al.*, 2002; Olvera-Novoa *et al.*, 2004).

The cost of feed per kg weight gain followed a similar trend as the cost per kg of feed, both variably decreased as the SFSM level increased in the SFSM diets (Table 7c & 14). This was due to the fact that the cost per kg of SFSM was lowest as compared with all other feed ingredients used in this study. Thus, cost-effectiveness of the current study indicated that the SFSM diets were more profitable (El-Saidy *et al.*, 2002; Olvera-Novoa *et al.*, 2002). SFSM at an inclusion level of 20% SFSM (SFSM20) was more

cost-effective than the control and other SFSM diets. These results were also similar to reports by El-Saidy and Gaber (2003) on a mixture of different plant protein sources for Nile tilapia. Another study by Oduro-Boateng and Bart-Plang (1988) on brewery wastes for *Tilapia busumana* indicated that these sources were more cost-effective than FM, even at total replacement levels. Furthermore, El-Sayed (2004) found that high quality protein diet may not be cost-effective as low quality protein diet which may lead to poor growth, but more profitable.

5.2.4 Blended diet

In the current study, the control diet had significantly (p<0.05) higher values on growth performance and feed utilization than the blended diet. These results may be attributed to higher level of crude fibre content in the blended diet (Table 15). Related study by Shoko *et al.* (2012) with *Oreochromis variabilis* fry fed on composite diet contained a mixture of nutrients from fish meal, soybean meal, cotton seed cake and sunflower seed cake reported poor growth performance. They attributed that presence of fibrous material in SFSM diets might be the reason contributed to the recorded poor growth performance of *Oreochromis variabilis* on composite diet.

The control diet had highest values on cost-effectiveness twice that of blended diet. This is caused by higher inclusion levels of fish meal (FM) (22%) in the control diet (Table 7d). Costs of compounded diets containing fish meal as a primary protein source can be expected to rise as FM prices increase in response to static supply and growing demand (Lech and Reigh, 2012). Reduction of FM to 5% in blended diet provide economic benefits by reducing feed costs for fish producers (Table 16). The findings were similar to previous studies that reported better economical return of fish on blended diet without detrimental effect on animal performance and feed efficiency (El-Saidy *et al.*, 2003).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has been able to establish the following:

- Protein contents of SBM and CSM used in this study were fairly high, at 42.3 and 44.6% respectively, which qualifies them as good alternative protein sources to FM, but that of SFSM was fairly low at 19.5%. Crude fibre contents of SBM, CSM and SFSM varied considerably with high level (35.5%) observed in SFSM compared to that of CSM (14.4%) and SBM (11.9%). High protein content of BM (83.1%) and FSM (59.1%) makes them suitable in boosting protein levels and enhancing palatability in diets containing SBM, CSM and SFSM.
- ii) Growth of fish fed on CSM diets containing 5% FM was comparable to those fed FM control diet. Among test diets, higher growth was observed in CSM diets and least in SFSM diets. Optimum growth for each ingredient was observed in diets CSM20, SBM25 and SFSM20. Feed utilization followed the same trend.
- iii) SFSM, BLEND and CSM diets were more cost-effective than FM and SBM diets.

Therefore, CSM20, SBM25, SFSM20 and BLEND diets had comparable performance to FM diet but more cost-effective to other diets used in this study.

6.2 Recommendations

Further studies should be done to:

- i) Conduct long-term feeding trials using the CSM20, SBM25, SFSM20 and BLEND diets to evaluate their biological and economical performance under actual farming conditions (i.e. within outdoor tanks, ponds or cages).
- Develop efficient method of improving the nutritional profile of ingredients such as reducing fibre content of sunflowerseed cake using method like ensiling to improve digestibility and feed intake.
- iii) Assess digestibility on all the practical diets to analyse their quality to fish productivity.

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APPENDICES

Appendix 1: Analysis of variance for growth performance, nutrient utilization and cost-effectiveness of *Oreochromis niloticus* fed Soybean meal diets

Parameter	Source of Variation	Sum of Squares	df	Mean Square	F	Pr>F
INWT	Between Groups	.054	3	.018	.257	.854
	Within Groups	.423	6	.070		
	Total	.477	9			
FNWT	Between Groups	4.128	3	1.376	9.504	.011
	Within Groups	.869	6	.145		
	Total	4.996	9			
	Between Groups	4.128	3	1.376	25.590	.001
WTGN	Within Groups	.323	6	.054		
	Total	4.451	9			
	Between Groups	.001	3	.000	25.590	.001
ADG	Within Groups	.000	6	.000		
	Total	.001	9			
	Between Groups	.001	3	.000	1.679	.269
FI	Within Groups	.001	6	.000		
	Total	.001	9			
	Between Groups	.448	3	.149	1.321	.352
FCR	Within Groups	.678	6	.113		
	Total	1.125	9			
	Between Groups	.000	3	.000	25.590	.001
PER	Within Groups	.000	6	.000		
	Total	.000	9			
SGR	Between Groups	3.464	3	1.155	15.372	.003
	Within Groups	.451	6	.075		
	Total	3.915	9			
	Between Groups	305021.682	3	101673.894	.315	.814
Cost	Within Groups	1934892.036	6	322482.006		
effectiveness	Total	2239913.718	9			

INWT = Initial weight, FNWT = Final weight, WTGN = Weight gain, ADG = Average daily gain, FI = Feed intake per fish per day, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR = Specific growth rate, df = degree of freedom, Pr = Probability, F = F-value

Appendix 2: Analysis of variance for growth performance, nutrient utilization and cost-effectiveness of *Oreochromis niloticus* fed Cotton seed meal diets

Parameter	Source of Variation	Sum of Squares	df	Mean Square	F	Pr>F
	Between Groups	.269	3	.090	1.014	.442
INWT	Within Groups	.620	7	.089		
	Total	.889	10			
	Between Groups	.658	3	.219	.919	.479
FNWT	Within Groups	1.670	7	.239		
	Total	2.328	10			
	Between Groups	.171	3	.057	.419	.745
WTGN	Within Groups	.952	7	.136	1.014 .919 .419 .419 .896 3.625 .419	
	Total	1.123	10			
	Between Groups	.000	3	.000	.419	.745
ADG	Within Groups	.000	7	.000		
	Total	.000	10		.419 .419 .896 3.625	
	Between Groups	.000	3	.000	.896	.489
FI	Within Groups	.000	7	.000		
	Total	.000	10			
	Between Groups	.317	3	.106	3.625	.073
FCR	Within Groups	.204	7	.029		
	Total	.521	10			
	Between Groups	.000	3	.000	.419	.745
PER	Within Groups	.000	7	.000		
	Total	.000	10			
	Between Groups	.107	3	.036	1.014 .919 .419 .419 .896 3.625 .419 .353	.788
SGR	Within Groups	.707	7	.101		
	Total	.814	10		1.014 .919 .419 .419 .896 3.625 .419	
	Between Groups	3560011.166	3	1186670.3 89	1.014 .919 .419 .419 .896 3.625 .419	.002
Cost effectiveness	Within Groups	570612.875	7	81516.125		
	Total	4130624.041	10		1.014 .919 .419 .419 .896 3.625 .419	

INWT = Initial weight, FNWT = Final weight, WTGN = Weight gain, ADG = Average daily gain, FI = Feed intake per fish per day, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR = Specific growth rate, df = degree of freedom, Pr = Probability, F = F-value

Appendix 3: Analysis of variance for growth performance, nutrient utilization and cost-effectiveness of *Oreochromis niloticus* fed Sunflower seed meal diets

Parameter	Source of variation	Sum of Squares	df	Mean Square	F	Pr>F
	Between Groups	.208	3	.069	1.031	.435
INWT	Within Groups	.471	7	.067		
	Total	.679	10			
	Between Groups	4.327	3	1.442	4.041	.058
FNWT	Within Groups	2.499	7	.357		
	Total	6.826	10			
	Between Groups	3.720	3	1.240	6.547	.019
WTGN	Within Groups	1.326	7	.189		
	Total	5.046	10			
	Between Groups	.001	3	.000	6.547	.019
ADG	Within Groups	.000	7	.000		
	Total	.002	10			
	Between Groups	.000	3	.000	4.725	.042
FI	Within Groups	.000	7	.000		
	Total	.001	10			
	Between Groups	.218	3	.073	6.547	.316
FCR	Within Groups	.358	7	.051		
	Total	.576	10			
	Between Groups	.000	3	.000	4.725 1.417 6.547	.019
PER	Within Groups	.000	7	.000		
	Total	.000	10			
	Between Groups	2.912	3	.971	5.676	.027
SGR	Within Groups	1.197	7	.171		
	Total	4.110	10			
	Between Groups	4852300.670	3	1617433.557	13.801	.003
Cost effectiveness	Within Groups	820385.448	7	117197.921		
	Total	5672686.118	10			

INWT = Initial weight, FNWT = Final weight, WTGN = Weight gain, ADG = Average daily gain, FI = Feed intake, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR = Specific growth rate, df = degree of freedom, Pr = Probability, F = F-value

Appendix 4: T – test for growth performance, nutrient utilization and costeffectiveness of *Oreochromis niloticus* fed blended diet

Parameter	Source of variation	Sum of Squares	df	Mean Square	F	Pr>F
	Between Groups	.003	1	.003	.047	.839
INWT	Within Groups	.240	4	.060		
	Total	.242	5			
	Between Groups	1.463	1	1.463	37.359	.004
FNWT	Within Groups	.157	4	.039		
	Total	1.620	5			
	Between Groups	1.595	1	1.595	75.966	.001
WTGN	Within Groups	.084	4	.021		
	Total	1.679	5			
	Between Groups	.001	1	.001	75.921	.001
ADG	Within Groups	.000	4	.000		
	Total	.001	5			
	Between Groups	.000	1	.000	.672	.458
FI	Within Groups	.000	4	.000		
	Total	.000	5			
	Between Groups	.017	1	.017	.835	.412
FCR	Within Groups	.082	4	.020		
	Total	.099	5			
	Between Groups	.000	1	.000	54.000	.002
PER	Within Groups	.000	4	.000		
	Total	.000	5			
	Between Groups	1.215	1	1.215	18.208	.013
SGR	Within Groups	.267	4	.067		
	Total	1.482	5			
	Between Groups	3846408.111	1	3846408.111	44.098	.003
Cost effectiveness	Within Groups	348898.849	4	87224.712		
	Total	4195306.960	5			

INWT = Initial weight, FNWT = Final weight, WTGN = Weight gain, ADG = Average daily gain, FI = Feed intake, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR = Specific growth rate, df = degree of freedom, Pr = Probability, F = F-value