DIETARY EFFECTS OF PLANT PROTEIN SOURCES ON THE GROWTH PERFORMANCE AND FEED UTILIZATION OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*, RAISED IN CONCRETE TANKS

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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.

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ABSTRACT

The study was conducted to evaluate practical diets for Nile tilapia *Oreochromis niloticus* composed of fishmeal, soybean meal, cotton seed cake meal and sunflower seed cake meal. Five isonitrogenous (30 g 100 g^{-1}), isolipidic (10 g 100 g^{-1}) and isoenergetic (18 kJ g^{-1}) test diets were formulated. The control diet composed of fish meal (22%) and soybean meal (30%) as the major sources of protein. In the other test diets, fish meal was fixed at 5%, while inclusion of soybean meal, cotton seed cake meal and sunflower seed meal varied as follows; soybean meal 25 (SBM25), cotton seed cake meal 20 (CSM20), sunflower seed cake meal 20 (SFSM20) and blend diet (BLEND) contained CSM and SFSM were included at 10% and SBM at 5% respectively. A total of 120 fingerlings with an initial average weight of 13.96 ± 0.06 were stocked at a rate of eight fish per tank with a diameter of two meters and depth of one meter. Dietary treatments were in triplicates in complete randomized design. The experiment was conducted for six month and fish were weighed once a month to monitor growth and adjust feed ration. Data was analyzed using descriptive statistics and one way analysis of variance (ANOVA). There was significant difference in growth performance between control and other tested diets ($p<0.05$). Higher growth performance and feed utilization occurred in control diets followed by cotton seed cake meal, soybean meal, sunflower seed cake meal and finally blend diet. Cotton seed cake meal and sunflower seed cake meal diets were significantly more cost-effective than the control, BLEND and soybean meal diets ($p<0.05$). The study indicated that cotton seed cake meal could replace at least 20% as a protein source in the diet of *Oreochromis niloticus* without affecting growth performance and feed utilization under farmer managed condition.

DECLARATION

I, BEATA WILLIAM BALINDILE, do hereby declare to the Senate of Sokoine University of Agriculture that the work presented in this dissertation is my own original work and has neither been submitted nor concurrently submitted for a similar degree award in any other institution.

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DEDICATION

I dedicate this research work to my lovely mother Fortunate A. Balindile for her support to every stage of my education, my husband Antony J. Mosha and my beloved children Joseph, Fortunate, Joel, Jackson and Isabella for their persevere during the whole period of my study.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADG	-	Average daily gain
AOAC	-	Association of analytical chemist
ANOVA	-	Analysis of variance
BM	-	Blood meal
COSTECH	-	Tanzania Commission for Science and Technology
CP	-	Crude protein
CF	-	Crude fibre
CL	-	Crude lipid
CRD	-	Complete randomized design
CSC	-	Cotton seed cake
CSM	-	Cotton seed meal
DAARS	-	Department of Animal, Aquaculture and Range Sciences
DM	-	Dry matter
DO	-	Dissolved oxygen
df	-	degree of freedom
EAA	-	Essentials amino acids
EE	-	Ether extract
et al	-	and others
F	-	F = Value
FAO	-	Food and Agriculture Organization
FCR	-	Feed conversion ratio
FI	-	Feed intake

FNWT	-	Final body weight
FM	-	Fish meal
FO	-	Fish oil
FSM	-	Freshwater shrimp meal
g	-	gram
GE	-	Gross energy
i.e.	-	that is
IMF	-	International Monetary Funds
INBWT	-	Initial body weight
Kcal	-	Kilocalories
Kg^{-1}	-	Per kilogram
Kg	-	Kilogram
KJ	-	Kilojoules
l	-	litre
LTD	-	Limited
ME	-	Metabolizable energy
mg	-	milligram
MIN/VIT	-	Minerals/Vitamins
MJ	-	Mega joules
MLF	-	Ministry of Livestock and Fisheries
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
SFSC	-	Sunflower seed cake
SFSM	-	Sunflower seed meal
SGR	-	Specific growth rate
SFO	-	Sunflower oil
SoV	-	Source of variation
spp	-	Species
SPSS	-	Statistical Package for the Social Sciences
SS	-	Sum of square
SUA	-	Sokoine University of Agriculture
TZS	-	Tanzania shillings
USA	-	United States of America
WM	-	Wheat meal
WTGN	-	Weight gain

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Fish and fish products are very good source of protein and essential micronutrients for nutrition wellbeing (FAO, 2012). On the other hand, fish oils have high value in abating cholesterol and hence minimizing cardiac arrests as well as treating nutritional disorders such as Marasmus and Kwashiorkor (MLF, 2014; Anand *et al*., 2008; Artham *et al*., 2008).

Overall world capture fisheries production has dwindled since late 1980s (FAO, 2016). In 2014, fishery exports from developing countries were valued at US$80 billion, and their fishery net export revenues (exports minus imports) reached US$42 billion, higher than other major agricultural commodities such as meat (FAO, 2016).). On other hand Aquaculture continues to grow faster than other major food production sectors, the total contribution in the world reaching 46.8 percent in 2016, up from 25.7 percent in 2000 (FAO, 2018). In 2014, fish harvested from aquaculture approximate to 73.8 million tonnes, with an estimated first-sale value of US$160.2 billion, consisting of 49.8 million tonnes of finfish (US$99.2 billion), 16.1 million tonnes of molluscs (US$19 billion), 6.9 million tonnes of crustaceans (US$36.2 billion), and 7.3 million tonnes of other aquatic animals including frogs (US$3.7 billion) (FAO, 2016).

Fisheries sector in Tanzania plays an important socio-economic role in terms of employment creation, income generation and food security. Tanzania is among the top 10 countries in capture fisheries production (MLF, 2014). On contrary, aquaculture production is still low and currently dominated by tilapia species and to a lesser extent

African catfish (*Claries garipineus*), trout (*Onchorynchus mykiss*), seaweed (*Eucheuma cottonii, E. spinosum*) and milkfish (*Chanos chanos*). The industry is dominated by small scale fish farmers producing mostly for household consumption and little surplus for domestic market. In the year 2012 there were about 20,134 grow-out earthen ponds which were in operation. Arusha, Mbeya, Iringa, Morogoro, Kilimanjaro, Ruvuma, Tanga, Coast, Dar es Salaam, Lindi and Mtwara regions are most active in fish farming (MLF 2012). It was estimated that in year 2013 Tanzania had 17,726 freshwater fish farmers. Mariculture farmers were estimated at 1,306 for milkfish, 51 for prawn, 188 for crabs, 98 for pearl culture and 2826 for seaweed farmers (MLF, 2014). Inadequate quality feeds and their high cost is one of the major constraints facing fish farming industry in Tanzania. Other constraints include poor management practices, use of inappropriate technology, insufficient extension services, and poor market services (Rice *et al.*, 2006).

1.2 Problem Statement and Justification

High cost of the feed is attributed to fish meal, a high quality protein source which currently cost between TZS 4,000.00 and 4,500.00 per kg. In order for aquaculture to grow, it has to reduce its dependence on fish meal and search for other alternatives sources of feed which are affordable, easily available and yet do not compromises fish performance. Therefore the present study evaluate cotton seedcake meal, soybean meal and sunflower seedcake meal as alternative sources of protein in raising Tilapia fish in Tanzania.

1.3 General objectives

To evaluate cotton (*Gossypium spp*) seed cake meal, soybean (*Glycine max*) meal and sunflower (*Helianthus annuus*) seed cake meal as sources of protein in practical diets of Nile tilapia (*Oreochromis niloticus L.)* in Tanzania

1.4 Specific Objectives

i). To evaluate effects of dietary inclusion of cotton (*Gossypium spp*) seedcake meal, soybean (*Glycine max*) meal and sunflower (*Helianthus annuus*) seedcake meal on fish growth and feed utilization of Nile tilapia (*Oreochromis niloticus L.*).

ii). To assess cost effectiveness of inclusion of cotton (*Gossypium spp*) seedcake meal, soybean meal (*Glycine max*) and sunflower (*Helianthus annuus*) seedcake meal in diets for Nile tilapia (*Oreochromis niloticus L*.).

1.5 Hypothesis

$H_{1:}$ There is no significant difference among tested diets on fish growth and feed utilization of Nile tilapia (*Oreochromis niloticus L.).*

H_2: There is no significant difference in cost-effectiveness of tested diets for Nile tilapia (*Oreochromis niloticus L).*

CHAPTER TWO

2.0 LITERATURE REVIEW

Tilapia is the third most important cultured fish in term of production after salmon and carp (FAO, 2010). They have acceptance by consumers worldwide and its annual rate of production is almost twice as big as the overall aquaculture production rates (FAO, 2011). Global annual production of tilapia species in 2007 was about 2.2 million tonnes and reaches about 2.5 million tonnes in 2010 (FAO, 2014). Tilapias are being cultured in all types of production systems in both fresh water and saltwater in tropical, subtropical and temperate climates (El Sayed, 2006)

Aquaculture in Tanzania began in 1950s century with pond culture of tilapia species native to the region, including Nile tilapia (*Oreochromis niloticus),* Mozambique tilapia (*Orechromis mossambicus* and zanzibar tilapia (*Oreochromis urolepis hornorum*) (Rice *et al*., 2006). Currently Nile tilapia (*Oreochromis niloticus*) is the most cultured species producing due to its good culture attributes such as fast growth, ease of reproducing, low susceptibility to diseases, tolerance to poor water quality, good taste, and wide market acceptability (FAO 2011). However, to attain fast growth profitably, the fish have to be fed on good quality and cost-effective feed (Aanyu, 2012). Nile tilapia (*Oreochromis niloticus*) is primarily herbivorous, though occasionally omnivorous (FAO, 2010). The fish is an efficient converter of waste foodstuff and capable of utilizing artificial supplemental feed (FAO, 2010). They have laterally compressed deep body, long mouth and a pharyngeal bone which was fused into single tooth for grinding feed (FAO, 2010).

Tilapia obtain nutrients from natural aquatic organism and other artificial diets such as plant and animal product and animal by product which is intended to support natural feed

(El-Sayed, 2006). Generally, fish need high protein diet because 65% of the protein ingested is excreted as ammonia through gills and solid wastes (Craig *et al*., 2002). Tilapias can efficiently utilize 35 to 40 % digestible carbohydrate (FAO, 2012). Minerals and vitamins are important for tilapia necessary for optimum growth and health especial intensive culture systems where limited natural foods are available (Hu *et al.*, 2006). Vitamins and minerals could be absorbed from cultured water together with those available in the feed for provision of maximum growth and health (Hu *et al.*, 2006).

2.2 Alternative Protein Sources

Fish meal is considered to be the most desirable animal protein source in aquaculture feeds due to its high protein content, balanced amino acid profile, high digestibility and palatability (Tacon and Metian, 2008). In 2010, fish accounted for 16.7 percent of the global population intake of animal protein and 6.5 percent of all protein consumed (FAO, 2014). World per capital fish supply reached 20 kg in 2014, due to vigorous growth in aquaculture and improved fisheries management (FAO, 2016).

There have been many studies focused on finding out alternative replacements to fish meal in fish diets which have high potential as fish feed (Tacon and Metian, 2008). Some of these fish meal replacers are soybean meal, (Lende *et al*., 2015; Dadger *et al*., 2009; Khan *et al*., 2013), cottonseed cake meal (Agbo *et al.*, 2011; Bazili, 2015), sunflower seedcake meal (El-Saidy *et al*., 2002; Mbahizireki *et al*., 2001), blood meal (Venou *et al.*, 2006) and shrimp meal (De Silva and De Silva, 1985). Some of alternatives sources of protein to fish meal are reviewed below:

2.3.1 Soybean meal.

Soybean feed production took off in the mid-1970s and then accelerated in the early 1990s due to growing demand (Steinfeld *et al*., 2006). Soybean meal is a by-product obtained after extraction of soybean oil (Hong *et al.*, 2004). Soybean meal is one of the greatest stimulating replacements to fish meal for fish diets due to high protein content, acceptable amino acid profile, fairly sensible price and stable supply (Storebbaken *et al*., 2000). The meal is usually classified into two main categories, the dehulled seeds which have higher protein (49 to 50% of protein + oil with 3% crude fiber) and hulled seeds have lower protein content (44 to 46% protein + oil with 6 to 7% crude fibre) (McDonald *et al*., 2002). In addition to having high protein content has good amino acid balance with high amounts of Lysine, Tryptophan, Threonine and Isoleucine that are often lacking from most cereal grains (Hardy, 2006). Soybean meal is restricted by the occurrence of ant-nutrition factors such as saponins, phytic acids, trypsin inhibitors, lectins, indigestible carbohydrates and allergens (Nengas *et al*., 1996). Trypsin inhibitor induce pancreatic enlargement, increase trypsin secretion and therefore lower nitrogen retention, growth and feed conversion efficiency (Van Eys *et al*., 2004). Luckily enough trypsin inhibitors are heat labile and are destroyed through heating during feed processing (McDonald *et al*., 2002).

Soybean meal can be included at different levels in fish diets depending on fish species, stage of growth and dietary protein levels. EI Sayed (2006) noted that Soybean meal has about 75% inclusion level in Nile tilapia fingerling diet, without methionine supplementing. Similarly, (Lin *et al.*, 2011) indicated that protein from Soybean meal could substitute less than 75% of fishmeal protein without influencing the growth performance of juvenile tilapia. Goda *et al*. (2007) suggested that, both soybean meal (SBM) and full fat soybean meal (FFSB) supplemented with DL-methionine and L-lysine can completely replace dietary fish meal on growth and feed utilization of Nile tilapia *Oreochromis niloticus* and *Tilapia galilae* fingerlings. Al-Kenawy *et al*. (2008) demonstrate that soybean diet (SBM25) could replace the fish meal in diets for Nile tilapia fingerlings without negative effects on growth, with high net economic returns.

2.3.2 Cotton seedcake meal

Cotton seedcake meal is a by-product obtained after extracting oil from cotton seeds (Oil World, 2011). Its value on protein levels depends on plant characteristics and efficiency of oil extraction (FAO, 2012). Cotton seedcake meal is less digestible than soybean meal in many fish species, including rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*O. niloticus*) and channel catfish (*Ictalurus punctatus*) (Chen *et al*., 2002; Morales *et al*., 1999; Smith *et al* .,1995; Li *et al*., 2006).

Inclusion levels of cotton seedcake meal tilapia diet vary depending on processing method and dietary level. According to Bazili (2015) 20% cotton seed meal could be safe and useful for Nile tilapia *O. niloticus L.* fingerlings production. Similarly, Yue *et al*. (2008) pointed that up to 33.76% cotton seed meal could be used to replace up to 60% of soy bean meal in diets for juvenile hybrid tilapia. El-Saidy *et al.* (2004) reported that 41.25% inclusion level of cotton seed meal could replace 75% of soy bean meal in Nile tilapia fingerlings. In addition, mechanical extracted cotton seed meal could replace 100% of soy bean meal in Rainbow trout juveniles (Lee *et al*., 2002). Likewise, Agbo *et al*. (2011) indicated that cotton seed meal could replace at least 50% of fish meal protein in the diet of *O. niloticus* without adversely affecting growth and feed utilization.

Problem limiting use of cotton seed meal as a fish feed is its gossypol content (Hertrampf *et al*., 2000). However, when cotton seeds are extracted, gossypol glands are broken, releasing the gossypol in the cake. The effects of processing (pressure, heating, solvent, duration) on the free gossypol content are variable (Zahid *et al*., 2003). Heat processing decreases gossypol content thus improving the nutritional value (Mena *et al*., 2001: Mena *et al*., 2004: Nunes *et al*., 2010).

2.3.3 Sunflower seedcake meal

Sunflower seedcake meal is a by-product after extraction of oil from sunflower seeds (FAO, 2011). In terms of production word wide, it is the fourth most important oil meal after soybean meal, rapeseed meal and cotton seedcake meal (FAO, 2011). Sunflower seedcake meal can be made from whole or decorticated seeds, and can be mechanically and/or solvent-extracted (Manguti *et al*., 2012). The quality of sunflower seedcake meal depends on the plant characteristics and on the processing methods i.e. dehulling, mechanical and/or solvent extraction (Maina *et al*., 2002). Sunflower seed meal is one of the major protein meals used for fish feeding, its protein content ranges from 29 to 33% dry matter content for non-dehulled meals and 35 to 39% DM for dehulled meals (Olvera-Novoa *et al*., 2002). The fibre content is directly linked to the presence of hulls: crude fibre ranges from 27 to 31% DM for non-dehulled meals and from 20 to 26% for dehulled and partially dehulled (Maina *et al*., 2002). Solvent-extracted sunflower seed meals contain about 2 to 3% dry matter of residual oil, but mechanically-extracted meals may contain up to 30% oil depending on the efficiency of pressing (Maina *et al*., 2002). One particularly interesting trait of sunflower seed meal is the absence of any intrinsic anti-nutritional factors and hence it does not require any processing before being used in feed formulation (Olvera-Novoa *et al*., 2002).

Sunflower seedcake meal is often used to replace soybean meal since it is free of trypsin inhibitors and has higher vitamin content than soybean meal (Hertrampf *et al.*, 2000). It has been reported to replace 10 to 25% of fish meal in the diets of Nile tilapia

Oreochromis niloticus L. (El-Saidy *et al*., 2003), and redbreast tilapia fingerlings *Tilapia rendalli* (Olvera-Novoa *et al*., 2002). It has been recommended that decorticated sunflower seedcake meal could replace much higher proportion of fish meal (Maina *et al*., 2002; Maina *et al*., 2003)

2.3.4 Blood meal (BM)

Blood meal (BM) is a powder made from fresh and clean blood which is readily available in abattoirs (Aladetohun and Sogbesan, 2013; Venou *et al*., 2006). Blood meal has high crude protein content ranging from 42 to 87.6% and is an excellent source of Lysine (Venou *et al*., 2006). Blood has different method of processing to obtain blood meal which includes are cooking and fermenting dried blood meal (Kingori *et al*., 1998). Fermented blood meal combination with molasses is more acceptable to fish because is palatable, digestible and safe ingredient used in fish feed formulation than cooked dried blood meal (Samaddar *et al*., 2015). It has been acceptable replacement for other protein sources in animals as well as fish production diets. However, it is not powerfully utilized by tilapia due to low digestibility and poor essential amino acid profile (El-Sayed, 1999).

Ogunji and Wirth (2001) in their study on alternative protein sources as substitute for fish meal in the diet of juvenile Nile tilapia observed that a proper combination of about six percent of blood meal with soybeans, , groundnut cake and wheat bran could provide the 42 to 45% protein needed by the fish. McDonald *et al*. (2010) suggested that appropiate inclusion levels of fermented blood meal may improve growth and feed utilization of tilapia by boosting dietary Lysine levels of the respective diets.

2.3.5 Freshwater shrimps

Caridina nilotica are common tropical freshwater shrimps growing to about 30 mm, are reported to be as protein source in fish feeds (De Silva and De Silva, 1985). They can easily be collected from the wild or can be cultured easily in high density in small ponds (Jauncey and Ross, 1982). Freshwater shrimps have a high potential inclusion in tilapia feeds because it is not used as human food (Munguti *et al*., 2012). Freshwater shrimp's meal is a good source of protein range from 60 to 65% CP (Jauncey and Ross, 1982). In fish diets it can replace about 50% to 100% fish meal without changing fish performances (El-Sayed, 1998). 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*.

2.4 Conclusion

It is concluded from the literature review that Nile tilapia is primarily herbivorous, though occasionally omnivorous. It is an efficient converter of waste foodstuff and can be able to utilize artificial feed. However, its production in Tanzania is limited by poor quality feeds, high costs and limited supply of fish meal which is primarily used as source of protein in tilapia diets. Alternative protein sources from plants and animal by products such as soybean meal (SBM), cotton seed cake meal (CSC), sunflower seed cake meal (SFSC), blood meal (BM) and freshwater shrimp meal are nutritious and readily available ingredients in most parts of Tanzania. Unavailability of certain amino acids such as Methionine, Lysine, Cystine, Threonine and Isoleucine can be avoided through addition of vitamins/mineral mix during feed formulations. However, in fish ponds such vitamins can be obtained through pond fertilization. The presence of ant-nutritional factors that inhibit digestion of protein in fish could be rendered harmless through heat treatment during fish feed processing.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the experiment

The study was conducted at Sokoine University of Agriculture (SUA) in the Department of Animal, Aquaculture and Range Sciences (DAARS). The University is located between 6^o to 7^o S and 37^o to 38^o E which is 3 km from Morogoro municipality at the base of Uluguru Mountains. The annual rainfall amount ranges between 600 to1000 mm per annum and the temperature ranges 26 to 35.5 ^oC from October to January and 20 to 27 ^oC from April to August.

3.2 Feed Ingredients and their Processing

Soybean, cotton seed cake, sunflower seed cake, freshwater shrimps and fish (*Restrineobola argentea*) locally known as "Dagaa" from Lake Victoria were purchased from livestock feed shops in Morogoro Municipality. Soybeans were sorted manually to remove impurities, soaked for 24 hours in clean tap water under the ratio of seed to water of 1:3. Thereafter, the seeds were boiled for about 45 minutes to eliminate the enzymes commonly known as ant-nutritional inhibitor that inhibit digestion of most leguminous plant protein, then washing using clean tap water. The beans were spread on polythene sheets for sun drying and then soybean were milled into soybean meal. Cottonseed cake, sunflower seed cake, *Restrineobola argentea* and freshwater shrimps were sorted,b oven dried at 60 ^oC for 12 hours, milled to obtained different meal ready for incorporation.

Fresh animal blood was purchased from abattoir in Morogoro Municipality. The fresh blood was weighed and 20% of molasses was added and thoroughly mixed by stirring with a wooden stick, there after it was left to stand at a room temperature for at least 14 days, the mixture being stirred at least once per day, the fermented blood meal was then sun dried for 3 to 5 days to produce fermented dried blood meal. The aim of fermenting blood was to improve digestibility and palatability of blood meal.

3.3 Determination of Chemical Composition of Feed Ingredients and Formulated Diet

Chemical composition of the ingredients and diets were determined through standard methods (AOAC, 2005). Dry matter (DM) content was determined by drying feed ingredients and diets to constant weight gain in an oven (E 115, WTB binder 7200, Germany) for 24 hours at 105 ^{0}C. Crude protein (CP) was determined using the Kjeldahl method where a sample with a known weight was digested (Digestion system 12 1009 Digester, Tecator, Sweeden), distilled (2200 Kjeltic Auto Distillation, Foss Tecator, Sweden) and titrated (Digitrate, Tecator, Sweden) to determine amount of nitrogen which was then multiplied by a factor of 6.25 to get crude protein content of the sample.

Ether extract (EE) was determined by using Soxtec extraction machine (Soxtec system HT 1043 Extraction unit, Tecator and Sweeden) using petroleum ether at 40 to 60 ^{0}C boiling range. Crude fiber (CF) was determined by 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 diets in a muffle furnace (N31R, Nabertherm, West Germany) at 550 ^{0} C for 4 hrs. Nitrogen free extract (NFE) was determined by subtracting the sum of moisture, crude protein, ether extract, crude fiber and ash from 100. Gross energy (Kcal) (1kj = 4.186) as adopted from NRC, 1993.

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

Where:

GE = Gross Energy,

CP = Crude Protein,

EE = Ether Extract,

Kcal = Kilocalories,

NFE = Nitrogen Free Extract.

3.4 Diet Preparation and Formulation

Five (5) isonitrogenous (30g 100g-1), isolipidic (10g 100g-1) and isoenergetic (18kJg-1) diets were formulated by using computer program and prepared by 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 a 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 25%(SBM25), CSM diets contained CSM at 20%(CSM20) and SFSM diets contained SFSM at 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 (8–14%) 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 (Table 1).

Table 1: Ingredients levels (% dry matter basis) of experimental diets.

			Diets		
Ingredients (%)	**Control**	**25%(SBM25)**	**20%20CSM**	**20%20SFSM**	**BLEND**
FM	22	5	5	5	5
SBM	30	25	0	0	5
CSM	0	0	20	0	10
SFSM	0	0	0	20	10
MM	35	39	42	35	36
SHRM	0	8	10	12	10
SFO	3	4	5	4	4
BM	0	9	8	14	10
WM	8	8	8	8	8
**VIT/MIN	2	2	2	2	2
Total	100	100	100	100	100
CP (30 g 100 g-1)	30	30	30	30	30
*Cost(TSHS/kg)	2216	1641	1346	1266	1346

FM = Fishmeal, SBM = Soybean meal, CSM=Cotton seedcake meal, SFSM=Sunflower seedcake 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.
*****USD 1= TSHS 1600 (Accessed 20 March, 2015).
******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.

During compounding of diets, the relevant proportions of dry ingredients were weighed using a sensitive digital weighing balance (Mettler PM 11, Mettler Instrument LTD, Switzerland) and then thoroughly mixed using hand in a clean plastic container. The mixture was pelleted using meat grinder, sundried on polythene sheets (Plate 1), the resulting pellets were dried for 48 hrs at (60 to 70 ^{o}C) and frozen packed in airtight containers prior to use.

Plate 1: Pelleted feed spreaded on polythene sheets for sun drying. Source: Beata (2016)

3.5 Experiment Procedure

Tilapia fingerlings (*O. niloticus)* were obtained from fish ponds belonging to the Department of Animal, Aquaculture and Range science. The fingerlings were bulk weighed using a digital weighing balance (Mettler PM 11, Mettler Instrument LTD, Switzerland). Tilapia fingerlings were acclimatized to experimental conditions for one week prior to the experiment while fed on control diet. Fifteen round concrete tanks with diameter of 2 meters and depth of 1meter (Plate 2) were stocked with the fingerlings at density of eight fingerlings per tank, making a total of 120 fingerlings for the whole experiment.

Plate 2: Round concrete tank at Magadu fish farming. Source: Beata (2016).

Experimental diets were randomly assigned to the tanks in triplicates. Each diet was fed to satiation (but not exceeding 5% of body weight per day) twice per day at 09:30 a.m. and 16: 30 pm; Water quality were monitored by regular change the water after two days to ensure that water quality remained well within limits recommended for Nile tilapia culture, the experiment lasted for 174 days from May to November, 2015. Fingerlings in each treatment were weighed once a month to determine monthly weight gain and adjust feed ration.

3.6 Data Collection

Fish weights and feed intake were recorded on monthly basis to determine growth, feed utilization and cost effectiveness indices as follows:

3.6.1 Growth

Growth parameters were determined in terms of weight gain (WG) in grams, average daily gain (ADG) in g $fish^{-1}day^{-1}$ and specific growth rate (SGR) in % day^{-1}.

3.6.1.1 Weight gain (WG)

WG was calculated by difference between the final weights (FNWT) measured at the end of 180 days and the initial weight (INWT) measured at the start of the experiment;

$$\boldsymbol{Weight\ gain\ (WG) = FNWT - INWT}$$

3.6.1.2 Average daily gain (ADG)

ADG was determined by dividing weight gain in g fish $^{-1}$ by duration of experiment in number of days:

$$ADG = \frac{final\ weight\ gain - initial\ weight\ gain}{number\ of\ days}$$

3.6.1.3 Specific growth rate (SGR) (% day $^{-1}$)

SGR is percentage of increase in body weight per animal per day was computed as follows:

$$\boldsymbol{SGR(\%days^{-1}) = \frac{[(In\ final\ weight - In\ initial\ weight)]}{(number\ of\ days)}}$$

3.6.2 Feed utilization

Daily feed intake (DFI), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER) ware used to determine feed utilization as follows;

3.6.2.1 Daily feed intake (g fish $^{-1}$ day $^{-1}$)

Is the amount of feed consumed in a specified time period.

$$DFI = \frac{Total\ Feed\ Intake\ (g)}{Number\ of\ Days}$$

3.6.2.2 Apparent feed conversion ratio (AFCR)

AFCR was calculated as the grams of feed provided to fish to produce one gram of whole fish.

$$AFCR = \frac{feed\ intake\ (gram\ dry\ weight)}{live\ weight\ gain\ (g)}$$

3.6.2.3 Protein efficiency ratio (PER)

PER was calculated per tank from weight gain and the amount of protein offered.

$$PER = \frac{Live\ weight\ gain\ (g)}{crude\ protein\ intake\ (g)}$$

3.6.2.4. Survival rate (SR)

SR in percentage was calculated by per number of fish at the end and number of fish stocked at the beginning

$$SR(\%) = \frac{Number\ of\ fish\ at\ the\ end}{Number\ of\ fish\ at\ the\ beginning} \times 100$$

3.6.3 Cost Effectiveness

Cost effectiveness of experimental diets was determined through multiplication of price (Tanzania shillings) of each diet per kg by its apparent feed conversion ratio.

$$\text{CE} = \text{diet price per kg x apparent feed convention ratio}$$

Cost of the diets was calculated using prevailing prices for the feed ingredients in Tanzania as follows; fishmeal, TSH 4000.00 per kg, soybean, TSH 2000.00 per kg, cotton seedcake meal, TSH 1000.00 per kg and sunflower seedcake meal, TSH 600.00 per kg (1USD=TZS 1600.00).

3.10 Data Analysis

A completely randomized design (CRD) was used in assigning dietary treatments to culture units. The main statistical hypothesis tested was “there is no significant difference between treatment means”. One way analysis of variance (ANOVA) was used to determine differences between treatment means which were judged significant at $P<0.05$. Post-hoc analysis was done where significant differences existed between treatments means using Tukey's Honest Significant Difference Test (Steele and Torrie, 1980). Analyses were performed using SPSS software version 16 (SPSS Inc.). Before analysis data were tested for normality and homogeneity of variance using Levene's test and transformed in case of non–conformity. The model used to test the effect of the diets on these parameters was

$$y_{ijk} = \mu + D_i + \varepsilon_{ijk}$$

Where:

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

μ = Overall mean,

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

ε_{ijk} = Random error.

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Composition of Ingredients

Results of chemical composition of ingredients are shown in Table 2. Blood meal had the highest crude protein while the lowest crude protein was found in sunflower seed cake. Soybean meal had the highest crude lipid content and blood meal was relatively low. In terms of crude fibre content, sunflower seed cake meal had the highest crude fibre content and fish meal had the lowest. Ash content was highest in freshwater shrimp meal and lowest in soybean meal. Highest nitrogen free extract content had observed in Freshwater shrimp meal and fish meal had the lowest. Soy bean meal had highest gross energy content and least in Sunflower seed cake meal.

Table 2 Chemical composition and gross energy of the individual ingredients used in the study (as % DM)

Items	BM	FM	SBM	FSM	CSC	SFSC
Moisture	4.7	4.8	4.1	4.2	4.0	5.0
Crude Protein	82.9	65.5	49.7	47.6	45.0	26.0
Crude Lipid	1.9	12.8	28.3	3.1	10.2	11.5
Crude Fibre	1.3	1.0	12.4	6.5	18.0	34.8
Ash	5.2	9.6	1.1	10.2	2.7	2.1
NFE	4.0	6.3	4.4	28.4	20.1	20.6
GE(Kcal/g)	501.9	516.1	565.4	414.5	432.7	339.9

BM = Blood meal, FSM = Freshwater shrimp meal, FM = Fish meal, CSM = Cotton seed meal, SBM = Soybean meal, SFSM = Sunflower seed meal, NFE = Nitrogen free extract, % = Percentage, DM = Dry matter, GE = Gross energy, g = gram, Kcal = Kilocalories.

4.2 Chemical Composition of Diets

The chemical composition of diets is shown in Table 3. Results showed that moisture content was highest in soybean meal diet followed by blends diet, control diet, cotton seedcake meal diet (CSM20) and relatively lowest in sunflower seedcake meal diet (SFSM20). Crude lipid was similar in all test diets except control diet. Control diet had highest crude protein, crude lipid, Ash and gross energy compared to other diets. The highest fibre content was observed in sunflower seedcake meal diet follow by blend diet, soybean meal diet, cotton seed meal diet and lastly control diet.

Table 3: Chemical composition of experimental diets fed to Nile tilapia.

Diets	Control	SBM25	CSM20	SFSM20	BLEND
Moisture (%)	10.40	11.20	9.50	8.50	10.50
Crude protein (%)	34.20	32.30	29.50	30.50	30.90
Crude lipid (%)	11.00	10.10	10.00	10.10	10.00
Crude fibre (%)	3.00	4.22	3.81	6.20	4.40
Ash (%)	6.48	4.50	5.00	6.00	5.30
NFE (%)	34.92	37.68	41.69	38.70	38.90
GE(kcal g^{-1})	443.8	436.2	438.4	430.3	434.4

CSM = Cotton seedcake meal, SBM = Soybean meal, SFSM = Sunflower seedcake meal, NFE = Nitrogen free extract, GE = Gross energy, g = gram, Kcal = Kilocalories.

4.3 Effects of Different Diets on Growth Performance, Feed Utilization and Cost-effectiveness

Differences in body weight gain of fish fed the experimental diets became noticeable after the first month of the experiment as shown in Figure 1.

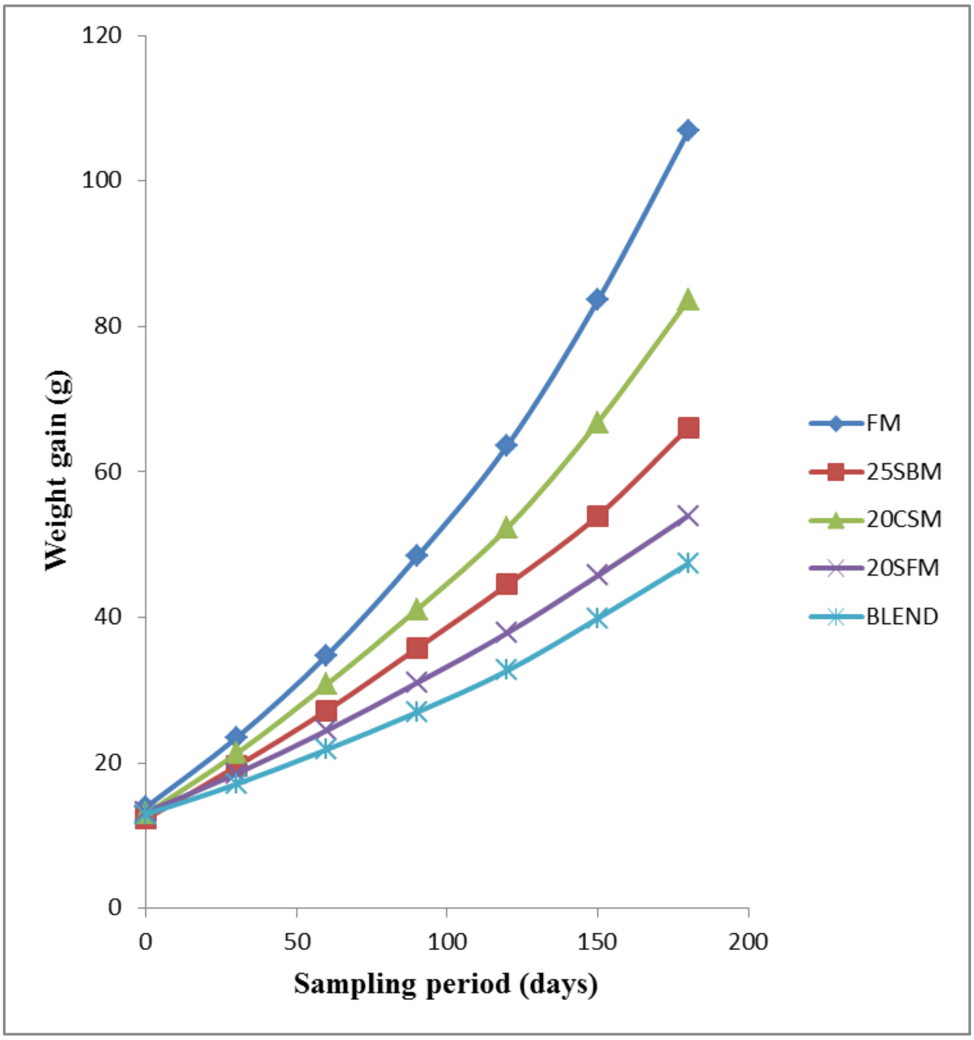

Figure 1: Growth trends of Nile tilapia (*O.niloticus*) fed on different experimental diets

Performance in terms of growth, feed utilization and cost effectiveness is shown in Table 4. There was no significance difference ($p<0.05$) in final weight of fish fed cotton seed cake diet (CSC20) and those fed diet FM and soybean meal diet (SBM25). Fish fed diet SFSM20 and BLEND had significantly lower final weights.

Table 4: Growth performance, nutrient utilization and cost effectiveness of experimental diets (mean + SE, n=3) for 174 days

Parameter	FM	SBM25	CSM20	SFSM20	BLEND
Initial weight (g)	13.96±1.06^{a}	12.31± 1.78^{a}	13.04±2.26^{a}	13.23±1.59^{a}	12.95±0.28^{a}
Final weight (g)	111.48±2.24^{a}	87.69±10.03^{ab}	105.65±2.5^{a}	69.40±5.54^{b}	62.67±10.57^{b}
Weight gain(g)	92.48±3.69^{a}	53.73± 2.18^{ab}	70.56±7.34^{b}	40.74±1.45^{c}	34.45±3.50^{c}
Average daily gain (g /fish/day)	0.510±0.02^{a}	0.297±0.01^{ab}	0.390±0.04^{b}	0.227±0.01^{c}	0.193±0.02^{c}
Average feed intake (g /fish/day)	0.771±0.03^{a}	0.759±0.04^{ab}	0.859±0.07^{b}	0.609±0.04^{c}	0.567±0.04 ^{c}
Apparent feed conversion ratio	1.60±0.09^{a}	2.55±0.04^{bc}	2.21±0.06^{b}	2.71±0.09^{cd}	3.04±0.10^{d}
Apparent protein efficiency ratio	1.98±0.14^{a}	1.23±0.02^{c}	1.57±0.04^{b}	1.21±0.04^{c}	1.09±0.38^{c}
Specific growth rate (% /day)	2.58±0.00^{a}	2.24±0.06^{abc}	2.55±0.02^{ab}	2.30±0.04^{bc}	2.24±0.09^{c}
Survival rate (%)	87.50±0.00^{a}	66.66± 8.33 ^{a}	66.66±4.17 ^{a}	70.83±4.17 ^{a}	66.66±8.33 ^{a}
Cost effectiveness (TSHS./kg)	4547.6.0±52.00^{a}	3960.4±41.46^{b}	3189.8.5±105.44^{d}	3277.9±31.94^{d}	3590.0±21.89^{c}

^{a,b,c,d} Different superscripts in the same row indicate significant difference at $p < 0.0$

The control diet (FM) had no significance difference (p<0.05) in weight gain (WTGN) with fish fed soybean meal (SBM25) diet. Fish fed sunflower seedcake meal diet (SFSM20) and BLEND diet had significantly lower weight gain to other diets. Moreover fish fed cotton seed cake diet (CSM20) and FM diet had significant difference (p<0.05) on weight gain. Fish fed soybean meal diet (SBM25) and FM diet had no significance difference (P<0.05) on average daily gain (ADG). Fish fed sunflower seedcake meal (SFSM20) diet and BLEND had significantly lower average daily gain. There was no significance difference (P<0.05) in average daily gain of fish fed soybean meal diet (SBM25) diet and cotton seed cake diet (CSM20).

Fish fed sunflower seedcake meal (SFSM20) diet and BLEND had significantly lower average feed intake (AFI). There was no significance difference (P<0.05) in average feed intake of fish fed soybean meal diet (SBM25) diet and cotton seed cake diet (CSM20). Fish fed soybean meal diet (SBM25) and FM diet had no significance difference (p<0.05) on average feed intake. There was no significance difference (P<0.05) in apparent feed conversion ratio (AFCR) of fish fed soybean meal diet (SBM25) diet and cotton seed cake diet (CSM20). Fish fed sunflower seedcake meal (SFSM20) diet and BLEND had significantly lower apparent feed conversion ratio (AFCR). Fish fed FM diet had significantly lower feed conversion ratio (AFCR).

Fish fed soybean meal (SBM25) diet, sunflower seedcake meal (SFSM20) diet, BLEND had significantly lower apparent protein efficiency ratio (APER). There was significance difference (P<0.05) in apparent protein efficiency ratio (APER) of fish fed FM diet and cotton seed cake diet (CSM20). There was no significance difference (p<0.05) in specific growth rate (SGR) of fish fed cotton seed cake diet (CSC20) and those fed diet FM and soybean meal diet (SBM25). Fish fed diet SFSM20 and BLEND had significantly lower specific growth rate (SGR).

There was no significance difference ($p<0.05$) in survival rate (SR) of fish fed cotton seed cake diet (CSC20) and those fed diet FM, sunflower seedcake meal (SFSM20) diet, BLEND and soybean meal diet (SBM25) diet. There was no significance difference ($p<0.05$) in cost effectiveness of fish fed cotton seed cake diet (CSC20) and fish fed sunflower seedcake meal (SFSM20) diet. Fish fed BLEND diet had significantly lower in cost effectiveness. Fish fed soybean meal (SBM25) diet and FM diet was no significant difference ($p<0.05$) in cost effectiveness.

Cotton seed cake meal and sunflower seed cake meal diets were significantly more cost-effective than the control, BLEND and soybean meal diets ($p<0.05$). CSM20 was the most cost-effective diet (TSH 1358.80) followed by SFSM20 (TSH 1269.70), BLEND (TSH 957.60) and least in SBM25 (TSH 587.20) while the highest cost to produce a kilogram of fish was observed in control FM diet. In general, cotton seedcake (CSM 20) diet with an inclusion level of 20% CSM had comparable performance to the control diet but more cost-effective compared to other experimental diets used in this study.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The present study was examined cotton seed cake meal, soybean meal and sunflower seed cake meal as sources of protein in practical diets of Nile tilapia (*O. niloticus L.)* Fish fed fishmeal control diet had higher performance than all other diets but comparable to soy bean meal diet (SBM25). Fish meal considered to be the most desirable animal protein source in aquaculture feeds due to its high protein content, balanced amino acid profile, high digestibility and palatability (Mohammad, 2008). Fish meal has been used as a feedstuff since the 19th century in Northern Europe and is now used worldwide (Tacon, 2008).

Soybean meal is a good plant protein source (Brown *et al*., 2008, Fagbenro *et al*., 2008). It has well-balanced amino acid with high amounts of Lysine, Tryptophan, Threonine and Isoleucine that were often lacking in cereal grains (Hardy, 2006). Soya bean meal has the best protein quality among plant protein feedstuffs used as alternative protein sources to fish meal in aqua feeds (Fagbenro *et al*., 2008). El Saidy and Gaber (2002) reported that soybean meal could be used as a single source of protein for *O. niloticus* without interfering growth performance when supplemented with methionine and lysine.

However, performance of fish fed diet containing cotton seed meal (CSM20) had comparable to those fed diet SBM25 but better performance than those fed diets containing sunflower seed meal (SFM20) and blend of seed cakes (BLEND). Good performance of cottonseed meal is attributed to its palatability (Li and Robinson, 2006).

Cottonseed meal is less digestible than soybean meal in many fish species, including rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*), (Chen *et al*., 2002; Morales *et al*., 1999; Smith *et al* 1995; Li *et al*., 2006). Cottonseed cake meal has high protein content (Soltan *et al*., 2011). The performance of CSM20 diet in the present study reaffirms earlier findings by Bazili (2015) reported that CSM can be included up to 25% in practical diets for Nile tilapia containing 5% FM without compromising feed cost and diet quality.

Apparent feed conversion ratio of Nile tilapia fed on CSM20 and SBM25 diets observed in the present study was significantly lower than other experimental diets except the control diet, the result indicating two diets are better feed for Nile tilapia culture. Similar finding was reported by El-Saidy *et al*. (2004). Likewise, similar finding were reported by Mbahinzireki *et al*. (2001); Agbo *et al*. (2011) and Bazili (2015), thus supporting that cotton seed meal can partially replace fish meal as a source of protein in formulated feeds for Nile tilapia. Similarly, Garcio-Abiado *et al*. (2004) reported that fish fed diets containing 25 to 50% CSM showed similar body weight gain and total lengths to the fish fed control diet at the completion of the 16 week trials. El-Sayed (1990), observed that dietary inclusion levels of 20 to 30% CSM could be safe and useful for Nile tilapia.

The poor performance of fish fed SFSM20 diet is most likely due to higher fiber content contained in sunflower seed meal (Olvera-Novoa et al., 2002). The fibre content is directly linked to the presence of hulls: crude fibre ranges from 27 to 31% DM for non-dehulled meals and from 20 to 26% for dehulled and partially dehulled (CETIOM, 2003). Sunflower meal from 0% to 36.5% resulted in lower DM intake and lower performance (Tacon *et al.*, 1984 cited by Hertrampf *et al*., 2000). The BLEND diet which had highest inclusion level of plant proteins led to lower fish performance in terms of growth and feed utilization. It is well established that high inclusion levels of plant proteins leads to poor growth (El-Sayed, 1999).

Generally, all test diets were more cost effective compared to control diet with Diet CSM20 being most cost effective. Similar results were observed by Fagbenro (1999) who recommended that inclusion of locally available feedstuffs especially agricultural by products reduce the cost of a complete tilapia feeds. Similar finding are previous reported by Bazili (2015). A higher inclusion level of fishmeal in the control diet makes the diet more expensive than other experimental diets. El-Saidy and Gaber (2003) stated that high inclusion of fish meal increases cost of feeds. Costs of compounded diets containing fishmeal as a primary protein source can be expected to rise due to prices fish meal (El-Saidy, 2006). The present result shows that reduction of fishmeal dietary inclusion to 5% has economic benefits for fish producers.

5.2 CONCLUSION AND RECOMMENDATION

Findings from this study showed that diets containing soybean meal (SBM25) and cotton seed cake (CSM20) performed better than diets containing sunflower seed cake (SFM20) and BLEND in terms of growth and feed utilization. Furthermore, diet CSM20 was most cost effective than all other diets. It is therefore recommended that diet CSM20 be tested under farmer managed conditions.

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