EFFECTS OF SUBSTITUTING COTTONSEED MEAL PROTEIN WITH *Prosopis juliflora* SEED MEAL ON NILE TILAPIA GROWTH

BY

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DECLARATION

Declaration by the Candidate

I declare that this thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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DEDICATION

I solely dedicate this thesis to the Almighty God, who has seen me through it all. Glory and honour be to Him forever and ever.

ABSTRACT

By the year 2020 there will be need for an additional 37 million tonnes of farmed fish per year, globally. However, limited availability and high cost of high quality feed-stuffs hinders aquaculture development in Sub-Saharan Africa. Therefore, replacing highly demanded and costly ingredients, such as cottonseed meal (CSM), with cheaper, nutritious and locally available ingredients, like *Prosopis juliflora* seed meal (PJSM), is inevitable. This study evaluated the effects of substituting CSM protein with PJSM in fish diets on growth of *Oreochromis niloticus.* PJSM was ensiled for 28 days, using 5% formic acid. Ensiled and non-ensiled PJSM was used to replace CSM protein in a control diet of 250 g kg^{-1} protein, at 25, 50, 75 and 100%. Nine treatment diets were tested in triplicate. The growth performance experiment was conducted for 87 days in a completely randomized design, using 27 aquaria. Each aquarium was stocked with 30 frys of *O. niloticus,* weighing between 0.32-0.35 g. Fish were fed twice daily, 10 am and 4 pm, at a feeding rate of 10% live body weight in the first month and 5% in subsequent months. Length and weight were measured fortnightly. The bivariate analysis of weight and length was used to estimate growth parameters including maximum growth rate, weight at Critical Standing Crop (CSC), maximum length and maximum weight for each experiment. Analysis of Covariance (ANCOVA) was used to compare performance of the test diets. Ensiling led to a significant (p<0.001) reduction of crude protein in PJSM. Flavonoids, phenols and saponins were destroyed upon ensiling PJSM whereas alkaloids and tannins were not eliminated. Copper, Iron, Zinc, Nitrogen, Phosphorous and potassium were significantly ($p<0.05$) reduced in ensiled PJSM. Inclusion of non-ensiled PJSM at 50%, led to a significant (p<0.001) improvement in the growth of *O. niloticus,* compared to control diet. There was no significant difference on growth performance of *O. niloticus* upon replacement of CSM with ensiled PJSM, at 0, 25, 50 and 75%, except at 100%, where growth performance of the fish was significantly (p=0.0102) reduced. Dissolved oxygen, pH and temperature did not influence growth of fish for all the diets. Survival for fish fed diets containing ensiled PJSM ranged between 81.11-85.56% compared to 72.22-81.10% for fish fed diets containing non-ensiled PJSM. Thus, non-ensiled PJSM can be used to substitute CSM up to 50 % whereas ensiled PJSM can be used to replace CSM up to 75% in *O. niloticus* diets.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANCOVA	Analysis of Co-Variance
ANFs	Antinutritional factors
AOAC	Association of Analytical Chemists
AWA	Animal Welfare Association
CC	Carrying Capacity
CF	Crude Fibre
CP	Crude Protein
CSC	Critical Standing Crop
CSM	Cottonseed Meal
EAA	Essential Amino acids
ESP	Economic Stimulus Programme
FAO	Food and Agricultural Organisation
FCR	Feed Conversion Ratio
FWSM	Freshwater Shrimp Meal
Grate	Growth rate
HCL	Hydrochloric Acid
ISSP	Invasive Species Specialist Group
KEFRI	Kenya Forestry Research Institute
KMFRI	Kenya Marine and Fisheries Research Institute
NACOSTI	National Council of Science, Technology and Innovation
NCPF	Non-conventional Feed stuff
NFE	Nitrogen Free extracts
PJSM	*Prosopis juliflora* Seed Meal
SD	Standard deviation
USAID	United States Aid and International Development
VBGF	Von Bertalanffy Growth Function
WB	Wheat Bran

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CHAPTER ONE

INTRODUCTION

1.1 Background

About 100 million tons of fish are eaten worldwide each year and they provide fish protein to two and a half billion people, constituting at least 20 percent of the global animal protein. In developing countries, fish products contribute over 50% of people's dietary protein intake (Bhosale, Bhilave & Nadaf, 2010). Unfortunately, the natural water bodies can no longer sustain the rising demand for fish protein, due to pollution, reduction in water levels, among other factors (Obiero, Raburu, Okeyo-Owuor & Raburu, 2012). Furthermore, overfishing has greatly contributed to the critical drop of wild populations of fish and marine organisms.

It is therefore apparent that aquaculture is the most outstanding option that can provide for the rising fish food demand (Munguti, Kim & Ogello, 2014; Mission, 2014). Aquaculture is among the fastest growing food producing sectors. By 2012, aquaculture provided nearly half of all fish for human food, a record which is likely to increase to 62 percent by 2030 (FAO, 2014). The combined effect of development in aquaculture worldwide and the expansion in human global population led to the increase of average annual per capita from 0.7 kg in 1970 to 7.8 kg in 2008 (FAO, 2009). It has been predicted that by the year 2020, there will be urgent need for an additional 37 million tons of farmed fish per year to match fish protein demand globally (Packard, 2009).

However, limited availability and high cost of quality fish feeds is a major obstacle to the development of sustainable aquaculture in developing countries. For example, in East Africa like many other Sub-Saharan countries, aquaculture is under-developed and contributes less than 1% to global aquaculture production. In Kenya, although fish farming started in the early 20th century as a sport fishery (Vernon & Someren, 1960), its development has been stunted until 2009, when the Government of Kenya, through the Economic Stimulus Program (ESP), pumped in Kshs 6 billion for fish pond construction, provision of seed, feed and pond liners (Charo-Karisa & Gichuri, 2010). Despite the immense investment, little has been realised concerning sufficient and quality fish feed production.

The projected increase in global demand for farmed fish by the year 2020 and the anticipated increase in aquaculture production in Kenya, demands for more quality fish feeds (Packard, 2009; Nyonje, Charo-Karisa, Macharia & Mbugua, 2011). Feed is the most expensive operating cost, accounting for about 70% in intensive aquaculture (Thompson, Muzinic, Engler, & Webster, 2005). Fish farmers in sub-Saharan Africa, still use cereal brans, kitchen leftovers and green leaves as fish feed, because of poverty and lack of cheap, sufficient and quality fish feeds in the local markets (Madalla, 2008). Use of such feedstuffs may lead to deficiency of vital nutrients such as protein. Inadequate supply of vital ingredients denies fish the opportunity to realise their full potential. Protein is important for reproduction, growth and maintenance. Quality fish feeds provide protein that has the required quantities of the essential amino acids for the target fish species. The 10 indispensable amino acids are methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine (Santiago & Lovell, 1988). Protein requirements are usually lower for herbivorous and omnivorous fish, compared to carnivorous fish and those reared in intensive systems. In younger fish, protein requirements are higher as opposed to older and larger fish. Protein requirements as well, can be determined by the rearing environment, water temperature, water quality, genetic composition and feeding rates.

In complete fish feeds, protein is critical and the most costly nutrient, accounting for over 50% total feed cost. Fishmeal has traditionally been used as a source of fish feed protein, because of its richness in essential amino acids (Ogunji, 2004). Use of plant protein ingredients in fish feeds is a nutritional strategy to reduce the cost of fish meal (Bhosale *et al*., 2010). The conventional alternative plant proteins include cottonseed meal (Mbahinzireki, Dabrowski, Lee, El-Saidy, & Wisner, 2001), groundnut, sunflower, rapeseed (Jackson, Capper.& Matty, 1982), among others. These conventional plant ingredients are highly targeted because of their high protein content. Non-conventional ingredients such as aquatic macrophytes, acacia, *P. juliflora,* among others, are potential fish feed ingredients that have not been commonly used. Their limited use is because either they are not well understood, in order for them to be commercialized, or they have substances such as antinutrients that may be poisonous (Abowei & Ekubo, 2011). Most unconventional ingredients have no competition for

human consumption; they are cheap, available and have a high nutrional quality that can compare with conventional ingredients (Abowei & Ekubo, 2011).

1.2 Problem statement

Despite the availability of non-conventional fish feed ingredients, about a third of wild-caught fish are converted into fishmeal. Use of fish as an ingredient in fish feeds competes with man for food. Nevertheless, aquaculture has over dependently relied on fishmeal for many years. Fishmeal is costly, scarce and in great competition from other livestock industries. Alternatives to fish meal have relied on conventional plant ingredients like cottonseed meal, groundnut, sunflower, soya beans, and rapeseed, among others. Like fishmeal, conventional plant ingredients preferred for fish feeds, are also used for livestock feed and for human consumption, hence becoming more costly and scarce (Adewolu. &. Adamson, 2011). In addition, some of these conventional ingredients are limiting in some essential amino acids. Cottonseed meal, for instance, is limiting in lysine and methionine (Agbo, Madalla & Jauncey, 2011), in regard to *O. niloticus* amino acid requirements (Santiago & Lovell, 1988). Further, the cultivation of the oil seed ingredient is not affordable among the poor farmers, because of the high cost of inputs and the ultimate conflicting food security interests (Francis, Makkar & Becker, 2002).

Although Ingredients of plant-origin, classified as non-conventional feedstuffs (NCPF), can be used as alternatives to the costly and highly demanded conventional plant ingredients, most of them have unknown antinutritional factors that may decrease the bioavailability of nutrients (Makokha, Oniang'o, Njoroge, & Kinyanjui, 2002; Umeta, West & Fufa, 2005) and lead to malnutrition (Makokha *et al*., 2002; Alemu, 2009). For example, the presence of total free phenolics, tannins, phytic acid, trypsin inhibitor activity and lectins has been reported in *P. juliflora* seeds (Kathirvel & Kumudha, 2011).

1.3. Justification

A search for non-conventional, locally available, cost-effective and highly nutritious ingredients; which are not in competition for human, livestock and industrial uses, is

necessary. Ingredients such as acacia and *P.juliflora* seed*s* have been found suitable for incorporation in fish diets. *P. juliflora,* locally called '*Mathenge',* is widespread, cheap and easily available. The pods of this valuable multi-purpose plant have successfully been included in *O. niloticus* diets. In addition, its seeds, which have high levels of crude protein, have successfully been included in diets of other fish species, such as *Labeo rohita* (Bhatt, Chotiya & Shah, 2010). Unlike Cottonseed meal (CSM), *P. juliflora* seed meal (PJSM) is rich in lysine but has not been exploited as an ingredient for *O. niloticus* diets, to complement the lysine deficient CSM. Despite the presence of anti-nutrional factors reported in *P. juliflora* seeds, the anti- nutrients can be reduced or completely removed by use of efficient processes such as ensiling. Ensiling by use of formic acid is ideal for removing antinutrients and prolonging shelf life, however, no research has been done to investigate the effect of including ensiled PJSM in *O. niloticus* diets.

The present study intends to demonstrate the efficacy of reducing/destroying antinutrients in PJSM, by formic acid ensiling. Further, the study seeks to show the potential of substituting the scarce, costly and highly demanded CSM with PJSM in *O. niloticus* diets. This ultimately aims at promoting the production of cheap, sufficient and quality fish feeds for enhanced aquaculture production in Kenya.

1.4. Research objectives

1.4.1 Overall objective

To assess the potential of substituting cottonseed meal (CSM) with *P. juliflora* seed meal (PJSM) in production of *O. niloticus*.

1.4.2 Specific objectives

i. To determine the nutrient levels and presence of antinutrients in ensiled and non-ensiled PJSM.

ii. To determine the growth performance of *O. niloticus* fed on dietary formulations containing ensiled and non- ensiled PJSM.

iii. To find out the appropriate replacement levels of CSM with PJSM in dietary formulations, for growth of *O. niloticus.*

iv. To determine temperature, dissolved oxygen and pH levels in experimental tanks of *O niloticus* fed on diets containing ensiled and non-ensiled PJSM.

1.5 Hypotheses

This study was guided by the following hypotheses:

HA_1. There are significantly higher levels of nutrients in non- ensiled PJSM compared to ensiled PJSM.

HA_2. The level of anti-nutrients in non-ensiled PJSM is higher than the level in ensiled PJSM.

HA_3. There is significant difference on growth performance of *O. niloticus* fed on dietary formulations containing 0, 25, 50, 75 and 100% levels of ensiled and non-ensiled PJSM.

HA_4. Temperature, DO and pH, did not influence growth performance of *O. niloticus* in the various treatment tanks.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nile Tilapia culture and nutrition

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), belong to the family Cichlidae. The Cichlidae family is a large group of tropical freshwater fish with bilateral symmetry, regular vertical stripes throughout depth of caudal fin and are maternal mouth brooders. They are native to Africa, and especially in the coastal rivers of Israel and the Nile Basin. They are also indigenous to the Middle East (Froese & Pauly, 2015). Tilapia is regarded as one of the earliest cultured species, which can be traced to the early Egyptian times as far as 4000 years ago.

Nile tilapia is a preferred species in aquaculture because it feeds low on the food chain, feeds on a wide range of feeds, tolerates relatively poor water quality, has low susceptibility to diseases, responds well to handling, has high quality flesh and can breed in captivity all year round (Thomas & Michael, 1999). Nile tilapia can thrive in water temperature range of 12 – 35^0 C, a pH range of 6.5–8.5 and minimum dissolved oxygen levels above 3.0 mg/ l (Hussain, 2004). Nile tilapia is most preferred fish food because of high quality fillet. According to the Holy Scripture, the sixth chapter of the gospel of John and other gospels, tilapia is recognized as the fish that Jesus used along with the five loaves of bread to feed over 5000 people, among His miracles, Suresh's study (as cited in Madalla, 2008).

Due to flexibility in feeding, *O.niloticus* are also classified as omnivores (Jauncey, 1998; Beveridge & Baird, 2000). Different authors have outlined the ranges of protein requirements for *O. niloticus* as 18-50% (Craig and Helfrich, 2002); 25-55% (Jauncey, 1998); 30-50% (Jauncey and Ross, 1982). The protein and essential amino acid (EAA) requirements for the different sizes of *O. niloticus* are also quite variable (Santiago & Lovell, 1988). The fingerlings of *O. niloticus* require higher levels of dietary protein as opposed to larger fish (Jauncey & Ross, 1982).

2.2 Nutritional requirements, fish feed formulation and production

Development of high quality and cost effective feeds requires acquaintance with the target fish species' nutritional needs. The nutritional requirements vary with species and size (Craig & Helfrich, 2002), and can only be met by preparation of balanced dietary formulations and application of appropriate feeding practices (Gatlin, 2010). A balanced diet for fish requires protein, lipids, digestible carbohydrates and fibre, mineral and vitamin supplements. In order to carry out proper diet formulation, it is imperative that each batch of feedstuff meant for fish diet preparation, be subjected to proximate analysis. Evaluation of feedstuff proximate composition is done by chemical analysis to estimate six parameters including crude protein, ether extracts (lipids or fats), Nitrogen Free Extracts (NFE), crude fibre, ash and moisture content (Nakyewa, 2013). Further, suspected antinutritional factors, which are common in feed ingredients, requires evaluation as well (Jauncey & Ross, 1982). In feed formulation, the price of ingredients, the ultimate cost of feed, ingredient availability, anti-nutritional factors and palatability of a formulated diet are some of the essential factors that require attention (Azevedo, Cho, & Bereau, 1998).

2.2.1 Nutrient groups

Carbohydrates, proteins and lipids are the nutrients the body metabolizes to give energy for physical activities and physiological processes. Fish that are herbivorous and omnivorous have lower nutritional dietary protein compared to carnivorous fish species. The carnivores excrete ammonia through gills with limited energy requirements. Carbohydrates, proteins and lipids have gross energy values of 4.15, 5.65 and 9.45 kilocalories per gram (k cal/g), respectively. Not all gross energy is digested and absorbed for metabolism. Therefore, digestible energy (DE) of feed is a percentage of gross energy. There is no specific dietary carbohydrate requirement for fish. However, dietary formulations for carnivorous fish usually contain below 20% soluble carbohydrate whereas those of omnivorous fish species contain between 25-45% soluble carbohydrates (Gatlin, 2010).

Proteins comprise carbon (50%), nitrogen (16%), oxygen (21.5%), and hydrogen (6.5%) (Craig & Helfrich, 2002). The proteins that support different body functions

include enzymes, hormones and immunoglobins. Protein has amino acids that are unique for different species of fish. Omnivorous and herbivorous fish species require between 25 to 35% crude protein (Gatlin, 2010). There are about two hundred amino acids in nature and only twenty of them are common. Among the twenty, ten are essential and cannot be synthesized by fish. The essential amino acids include: Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Tryptophan, and must be provided in fish diets (Santiago & Lovell, 1988; Craig, & Helfrich, 2002; Gatlin, 2010). The body of fish can synthesize the ten common non-essential amino acids, and some are available in dietary proteins.

Essential amino acids should be provided in balanced levels and required amounts only in order to provide desired growth and good health. Any excess in dietary protein is economically and environmentally disadvantageous (Lovell & Lovell, 1988; Gatlin, 2010). Amino acids imbalance can cause reduced growth performance in fish through amino antagonism or toxicity (Lovell & Lovell, 1988). This occurs when some amino acids are supplied beyond their required levels and consequently causing an increase in the requirement for other structurally similar amino acids. On the other hand, dietary excesses of some amino acids may cause direct toxicity in fish, and this may not be corrected by additions of other structurally similar amino acids (Lovell & Lovell, 1988).

Lipids are high energy nutrients for fish (Craig, & Helfrich, 2002; Gatlin, 2010) and can be used for protein sparing. There are three categories of fatty acids: saturated fatty acids (do not have double bonds), polyunsaturated fatty acids (have greater than two double bonds) and highly unsaturated fatty acids (have greater than four double bonds). Lipids supply essential fatty acids that assist in transporting fatty soluble acids. About 15% of fish diets comprise lipids. Higher levels of lipids are used in fish diets to reduce the high cost of diets, by sparing protein. However, higher levels of fat deposits at the liver can decrease health and market quality of fish. Fish require fatty acids of omega 3 and 6 families. Freshwater fish require an 18 carbon n-3 fatty acid, linolenic acid (18:3-n-3). Freshwater fish cannot synthesize such fatty acid and therefore must be supplied in the diets (Craig, & Helfrich, 2002). Diets deficient in essential fatty acids leads to reduced weight gain.

Micronutrients comprises of inorganic elements that fish require for tissue formation, metabolic functions and osmoregulation. They are classified into two groups, macro-minerals and micro-minerals based on the quantities required in the diet and that stored in the body. Macro-minerals include: Phosphorous, magnesium, calcium, chloride, sulphur, potassium and sodium. Phosphorous is considered a critical macro-mineral in fish diets because it is limiting in water. It is a constituent of bones and scales. Phosphorous deficiency leads to impaired growth, reduced feed efficiency, reduced tissue mineralization and impaired skeletal formation in juvenile fish (Lall, 2002). Sodium, chloride and potassium are abundant in water and diets. They are electrolytes for acid-base balance and osmoregulation in the fish body (Lall, 2002). Magnesium is abundant in most feedstuffs and is important in homeostasis and cellular respiration.

Micro-minerals are also referred to as trace minerals and they include: Cobalt, chromium, copper, iodine, iron, manganese, selenium and zinc. Due to low levels of copper, Iron, Manganese, selenium and zinc in diets, they may lead to reduced bioavailability of other dietary nutrients. Thus, they should be supplemented in fish diets (Gatlin, 2010).

Vitamins are organic compounds required in relatively small quantities for structural and metabolic functions. There are two groups of vitamins based on their solubility. Fat-soluble vitamins which include: vitamin A (retinol), vitamin D (cholecalciferol), vitamin E (alpha-tocopherol) and vitamin K. Fat soluble vitamins are metabolized and deposited in association with body lipids. Fish can live for long periods without the fat-soluble vitamins and any signs of their deficiency (Gatlin, 2010).

Water-soluble vitamins include: vitamin C (ascorbic acid), vitamin $B_{12,}$ folic acid, choline, biotin, inositol, niacin, thiamine, riboflavin, pantothenic acid and pyridoxine. Enough quantities of this group of vitamin is not stored in the body of fish. As a result, deficiency signs occur within a short period, especially in young and rapidly growing fish. Functions of these water soluble vitamins and the amounts needed by various fish species has been outlined by Halver (2002).

2.2.2 Fish feed formulation and production

Fish feed formulation and preparation refer to the practice of blending available feed-stuff to form dietary mixtures that meet specified requirements for a fish species (Jauncey & Ross, 1982; Bhosale *et al*., 2010). However, fish feed formulation should strike a balance between quantitative nutrient requirements and other considerations such as cost, digestibility and performance efficiency (Munguti, Liti, Waidbacher, Straif & Zollitsch, 2006). Feed formulation basically blends ingredients into a form that can be fed to fish (Gatlin, 2010). Feed formulation involves grinding feed ingredients into desired particle size, mixing them, moistening and heating them under pressure to attain the desired product form. Mostly, compression pelleting is used in making sinking pellets. Cooking and extrusion produce pellets that either sink or float. Diets for small fish are produced by different methods such as micro binding, micro coating and microencapsulation procedures. The processing procedures and diet forms of a given fish species, depend on nutritional requirements of the species and the type of culture system. Therefore, all steps in feed production should be guided by well-established quality control to ensure the production of quality feeds. The guidelines are prepared to ensure production of feeds that can meet the target species' needs in terms of nutrients and physical characteristics, and in turn enhance distribution in the preferred culture system (Gatlin, 2010).

2.3 Fish feed ingredients

2.3.1 Wheat bran

Wheat bran is a by-product of common wheat (*Triticum aestivum* L.) used for animal feeds (Heuzé, Tran, Bastianelli, Hassoun & Lebas, 2015). It involves a small starchy endosperm of the wheat kernel, the cuticle, pericarp and seed coat. Good quality wheat bran should be in form of large dry flakes and have a fair coating of flour. It is used to lighten heavy dietary mixtures (Gohl, 1982). Wheat bran is recommendable for livestock and other animals. High fibre content limits the use of wheat bran in herbivorous and omnivorous fish. A rate of 2-5% of extruded wheat bran has been recommended for use in diets. Wheat bran nutrient digestibility in Nile tilapia was found to be highest for amino acids, 78-87%, and lowest for energy, 37-39% (Heuzé *et al*., 2015). In a

study that evaluated growth performance of Nile tilapia fed on three different cereal brans, growth was highest for tilapia fed on maize bran, followed by 1.5% body weight, obtained with wheat bran. Nevertheless, wheat bran was more profitable. The lowest growth was observed on tilapia fed on rice bran (Liti, Mugo, Munguti & Waidbacher, 2006).

2.3.2 Fishmeal

Animal proteins, though expensive, are superior nutritionally because of their amino acid profiles. Fishmeal has proven to be palatable, highly digestible and rich in essential amino acids, fatty acids, energy and minerals (Ogunji, 2004). It is top among the highly rated protein sources for fish. Others include: soybean meal, shrimp meal, blood meal, cottonseed cake and palm kernel cake, respectively (Onuoha, 2014). A good growth performance of fish fed on fish meal based diets has been reported by several authors (Wee, Kerdchuen & Edwards, 1986; Onuoha, 2014).

Recent statistics reveal that 36% of the total global fisheries catches is converted into fishmeal and oil for poultry, pigs and fish feeds (Jacquet, Hocevar, Lai, Majluf, Pelletier & Pitcher, 2010).The steady growth in aquaculture yields has resulted to doubled demand for fishmeal, the most favoured proteinous ingredient for fish diets. Inspite of the high demand, the supply has become unstable due to over fishing, pollution, climate change and bad weather (Naylor *et al*., 2001). The increase in fishmeal consumption and decline in wild water fish catches, calls for alternative ingredients to replace fishmeal (Ogello, Munguti, Sakakura & Hagiwara, 2014), which is the most expensive protein ingredient in fish diets, so far (Bhosale *et al*., 2010).

2.3.3 Cottonseed meal (CSM)

Cotton (*Gossypium* spp.) is important globally because of its fibre and oil seed. However, it is majorly cultivated for fibre. Cotton is commonly cultivated in India, China and USA, and thus, there are two genetically different groups of cottonseed, the Asiatic and American varieties. Cottonseed meal/cake is produced in a process whereby cotton fibre is separated and the seed is recovered for oil extraction. Cottonseed oil can be extracted using mechanical method, direct solvent extraction and pre-press solvent extraction, which results in 97% oil extraction (Heuzé *et al*., 2015). After extraction of

the oil, cottonseed cake is obtained as a by-product, which can be ground to form a meal. The protein content and nutrient levels in cottonseed meal/cake varies with the type of processing technique (Jauncey and Ross ,1982; Heuzé *et al*., 2015) and also the variety of cotton (Jauncey and Ross, 1982). The processing of cottonseed produces about 26% hull, 9 % linters, 16% oil and 42% meal (Padley, 1994). CSM is commonly used as a feed ingredient in domesticated animal feeds, because of its high protein content. The amino acid profile for CSM has been evaluated and reported by several authors (Gohl, 1982; Ojewola, Ukachukwu & Okulonye, 2006; Munguti, Waidbacher, Liti, Straif & Zollitsch, 2009; Heuzé *et al*., 2015).

As a proteinous feed ingredient, CSM is commonly used to supply protein for ruminants. The global supply of CSM was about 14.7 million tons in 2009 of which 10.9 million tons were used to feed livestock (Heuzé *et al*., 2015). The leading producers of CSM in the world include China, India, Pakistan, Brazil and USA, accounting globally for about 80% production (FAO, 2012).

Cottonseed protein is of good quality despite deficiencies in methionine, cystine and lysine (Jauncey and Ross, 1982). It also has low levels of calcium (Gohl, 1982). Further, the major impediment in the usage of CSM is the presence of gossypol. Gossypol is a polyphenolic yellow pigment found in most varieties of CSM (Jauncey and Ross, 1982; Ojewola *et al*., 2006). CSM contains between 0.03 to 0.2% of gossypol (Jauncey and Ross, 1982; Jones's study (as cited in Ojewola *et al.,* 2006). Gossypol is toxic to monogastric (non-ruminants) animals as well as young and reproductive ruminants (Heuzé *et al*., 2015). Gossypol inhibits enzymes pepsin and trypsin in the digestive system and therefore affects the process of protein digestion, reduces appetite and causes constipation. Gossypol also renders lysine unavailable to fish (Jauncey and Ross, 1982).

Several studies involving different fish species have been conducted to determine the inclusion level and suitability of CSM in dietary formulations for fish. Agbo, Madalla & Jauncey (2011) replaced fishmeal protein with CSM at 0, 25, 50 and 75%. The findings revealed that replacements at 25 and 50% did not adversely affect the growth of *O. niloticus.* However, replacement of fishmeal at 75% level significantly reduced fish growth, an observation, which was attributed to the low levels of lysine, methionine

and threonine. In another study, CSM protein replaced 67% of fish meal protein, growth retardation and poor feed utilization in *O. niloticus* were reported (Fagbenro and Davies, 2000). The poor growth and feed utilization were also attributed to low levels of lysine, methionine and threonine and high levels of gossypol and trypsin inhibitors. The tolerable levels of gossypol and trypsin for tilapia are reported to be 1.6 g kg^{-1} and 5 g kg^{-1}, respectively (Francis, Makkar & Becker, 2001). Nevertheless, Dixon (1981) and Jackson *et al*. (1982) recommend that CSM can be used even at 100% levels depending on the available lysine, gossypol and the oil extraction method used.

2.3.4 *Prosopis juliflora*

The plant *P. juliflora* (Sw.) DC, is a shrub in the Fabaceae family and subfamily Mimosoideae, which is commonly referred to as Mathenge in Kenya and mesquite (in other places). It is indigenous to Mexico, Caribbean, Central, Northern and Southern America (Heuzé, Tran, Boval & Renaudeau, 2015). It is considered an invasive weed in Asia, Australia and Africa, while in other places. *P. juliflora* is xerophytic and can thrive under a wide range of soil types and moisture conditions (Orwa, Mutua, Kindt, Jamnadass, & Simons, 2009).

There are also reports that mathenge drains water out of the soil and competes effectively with grasses, particularly in dry areas; hence becoming a nuisance weed (Orwa *et al*., 2009; World Agroforestry Centre, 2012). *P juliflora* is a tropical species mostly invading arid and semi-arid areas. The fast adaptiveness of the species enables it outcompete the native plant species, leading to invasions, which are detrimental to the environment and the livelihoods of the inhabitants of the affected areas (Walter, 2011).

In Africa, *P. juliflora* was introduced in 25 countries and is possibly present in every arid or semi-arid country in Africa. According to Pasiecznik & Felker (2001), earliest introductions to Africa may have occurred in Senegal, South Africa, and Egypt in the early to late 19th century. The exact origins of *P. juliflora* in East Africa are unclear but it is speculated that it may have been dispersed by livestock from Sudan, South Africa or by traders from India or South Africa (Walter, 2011). Africa countries like Sudan, Ethiopia and Kenya have suffered the consequences of *Prosopis* infestations in

recent years. The plant has invaded large areas of irrigated farmlands, degraded land, watercourses, floodplains and even blocking bends of highways (Walter, 2011).

Documentation about introductions of *P. juliflora* in Kenya show that in 1973, seeds sourced from Brazil and Hawaii were planted for the rehabilitation of quarries near Mombasa (Pasiecznik & Felker, 2001). In Early 1980s, the same varieties were introduced into the semi-arid districts of Baringo, Tana River and Turkana. This was done with the aim of providing fuel products, rehabilitation and preservation of the indigenous vegetation from overexploitation (Pasiecznik & Felker, 2001).

In Baringo, Kenya, *P. juliflora* has caused severe problems ranging from reduction of pasture for livestock, by depressing the growth and survival of indigenous vegetation; increasing malaria incidences, due to increased populations of *Prosopis* bushes; reduced land for cultivation, reduced farming opportunities; disfiguration of livestock gums and tooth decay, which result in deterioration of livestock health and sometimes death. The weed has thorns that are harmful to both human and livestock, among other problems (Mwangi and Swallow 2005). As a result, there are divergent views among Baringo residents, whereby majority advocate for complete eradication of *P. juliflora* whereas minority support its commercial exploitation (Miranda *et al*., 2009).

P. juliflora has several uses and thus is a valuable multi-purpose tree. Some of the key uses include use as: posts, fuel wood, poles, sawn timber, charcoal and pods for fodder. In addition, *P. juliflora* gives other numerous products including chemical extracts from the wood or pods, honey from the flowers, medicines, exudate gums, fibres, tannins and leaf compost (Miranda *et al*., 2009; Orwa *et al*., 2009). The tree is also planted for soil conservation (Orwa *et al*., 2009).

The seeds of *Prosopis* are reported to be trapped between the gums and teeth of livestock causing swelling of the gums (Miranda *et al*., 2009). Majority of effects of *P.juliflora* products on livestock are attributed to antinutrients, which are toxic to animals (Miranda *et al*., 2009). Presence of antinutritional factors has limited the use of *P.juliflora* seeds as a feed ingredient (Pugalenthi, Vadivel, Gurumoorthi, & Janardhanan, 2004). Total free phenolics and tannins constitute the major anti-nutrients, which are in high concentrations in *P. juliflora* seeds (Kathirvel and Kumudha, 2011).

Despite the antinutrients, the pods have successfully been included in *O. niloticus* diets (Mabrouk, Hilmi & Abdullah, 2008). The seeds of *P. juliflora* have been evaluated by different authors (Sawal, Ratan.& Yadav, 2004; Choge, Pasiecznik, Harvey, Wright, Awan, & Harris, 2007; Kathirvel & Kumudha, 2011) for potential use in fish feeds. *P. juliflora* seeds contain varying nutritional contents including up to 30-40 % protein and much less fibre (3-7 %) than that of the pods (11-35%) (Sawal *et al*., 2004; Choge *et al*., 2007). Kathirvel and Kumudha (2011) reported ranges of various nutrients in *P. juliflora* seeds as follows: crude protein, 26.69 - 29.84%; crude lipid, 11.89 -13.75%; total crude fibre, 8.78 - 9.89%; ash 3.99 - 4.95% and carbohydrates, 42.45 - 46.37%. Further reports revealed that *Prosopis* seeds are rich in potassium (K), Calcium (Ca), magnesium (Mg) and Phosphorous (P) (Kathirvel and Kumudha, 2011).

Based on the proximate composition of *P. juliflora* pods and seeds, several growth performance experiments have been conducted with different animals including fish. Such experiments have revealed that replacement of fishmeal with *P. juliflora* seed meal by 50%, resulted to depressed growth in *L. rohita* fingerlings even after soaking or autoclaving the seeds. However, inclusion at less than 30% seed meal promoted reasonable growth performance of *L. rohita* fingerlings (Bhatt, *et al*., 2010). Thus, the present study endeavoured to evaluate the performance of ensiled and non-ensiled PJSM products on *O. niloticus.*

2.4 Antinutritional factors (ANFs)

According to Aganga and Tshwenyane (2003), the anti-nutritive factors (ANFs) are substances present in normal metabolism of natural feedstuffs by animal species, which affect feed utilization. These substances are not intrinsic to the characteristics of the plant in question, but to the animal type. Thus, the effects are animal specific, for example, trypsin inhibitors do not adversely affect ruminants, because most of the antinutritional factors are destroyed in the rumen, whereas they are not destroyed in monogastric animals (Kumar, 1992). Plants have many compounds, which, based on their nature and concentration may have valuable or harmful effects on the consumer. The ANFs are commonly referred to as “allelochemicals”. The ANFs can reduce animal productivity and cause toxicity when animals consume feeds rich in the allelochemicals, Jurgen’s study (as cited in Njidda & Ikhimioya, 2012). According to

Harborne (1989) ANFs in plants is nature's way of securing stored nutrients from destruction by consumers and protecting the structure and integrity of reproductive elements of the plant.

Use of plant protein materials like legume seeds, a variety of oilseed cake, leaf meals, and root tubers, as ingredients for fish feed, is restricted by the presence of antinutrients. There are four categories of ANFs: Some ANFs affect protein utilisation and digestion (protease inhibitors, (tannins, saponins and lectins) and others reduce mineral solubility and utilisation (Phytates, phytic acid, gossypol pigments, oxalates-oxalic acid and glucosinolates). The third category inactivates or cause increased requirement of certain viatamin (anti vitamin A, D, E and K and anti vitamin B, thiamine, nicotinic acid, pyridoxine, cyanobalamin) and lastly the miscellaneous substances (mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents and phyto-estrogens) (Raj ,1987; Francis *et al*., 2001). ANFs can also be classified into two groups, based on response to heat: protease inhibitors, phytates, lectins, goitrogens and anti-vitamins are heat labile whereas saponins, non-starch polysaccharides, antigenic proteins, estrogens and some phenolic compounds are heat resistant, Rumsey *et al's* study (as cited in Francis *et al*., 2001).

2.5 Ensiling

Improving the protein quality of plant ingredients requires suitable processing. However, some processing techniques are inefficient and do not reduce or eliminate antinutritional substances in the feedstuffs. According to Cruz, Kijora, Wedler, Danier & Schulz (2011), ensiling is one of the processes that have the capacity to improve the quality of feeds significantly and prolong their shelf life as well.

Acid ensiling is a process where the target material is chopped/ground, placed in a non-metallic container, preferably glass or plastic. Thereafter, the material is soaked in distilled water (10 % by volume) and mixed with an acid solution, stirring severally until liquefaction occurs. The liquid or semi-liquid formed is called silage. Grinding or mincing the feed is crucial for the distribution of enzymes within the target material. In acid silages, enough acidity is ensured to create ideal environment (pH 4-4.5) for

enzymatic action as well as inhibition of bacterial and fungal growth. The amount of acid is dependent on the kind of feedstuff being ensiled and the nature of the acid.

According to Martens, Tiemann, Bindelle, Peters & Lascano (2013), ensiling is an appropriate method for treating grains. Oetterer (2002), confirms that formic acid is ideal for preparation of chemical silage. The silage made using formic acid has a pH that can be used directly by fish without the need to neutralize. When organic acids, such as formic and propionic acid are used in ensiling, no pH adjustment is necessary at end of the process as in the case when mineral acids, like sulphuric and hydrochloric acids are used. Ensiling causes liquefication of feedstuffs, which can either be stored in liquid state or dried to produce a meal (Moreda & Sullivan, 1997). Silage can be made using either plant or animal ingredients, in separately or combined forms. Silages have been successfully made using organic acids or mixtures of organic and mineral acids (Moreda & Sullivan, 1997). However, Green, Wiseman & Cole (1983) and Oetterer (2002), advocated for use of organic acids because preservation is achieved at a higher pH at which neutralization of the silage is not required before use. The duration of ensiling process is also dependent on the nature of the acid, for example, ensiling with lactic acid takes about 60 days whereas it takes about 30 days with formic acid to complete the process (Cussen, Merry, Willians & Tweed, 1995).

Ensiling leads to reduction of both nutrients and antinutrients in plant based ingredients. Earlier studies reveal significant reduction of nutrients such as protein and crude fibre upon ensiling with lactic acid (Jones, 1975). The reduction in crude fibre was attributed to partial acid hydrolysis of hemicelluloses (Jones, 1975), while that of protein was associated with proteolysis which in turn was dependent on the plant species (Cussen *et. al.,* 1995). Significant reduction of trypsin, phytates, soluble tannins and oxalates, in raw aquatic plants, has been reported upon ensiling by fermentation (Cruz *et al*., 2011).

Several studies have reported different forms of ensiling processes and materials, and the suitability for their use in fish feeds. For example, El-Sayed, Mansour & Ezzat (2003) reported that ensiling by use of bacterial fermentation is essential for water hyacinth (*Eichhornia crassipes*), at inclusion levels of 20% or more into Nile tilapia (*O. niloticus*) diets. In another study, *Lemna* leaf meal was reported to be better util-

ized by *L. rohita* diets (El-Sayed *et al*., 2003) when fermented than raw. Cruz *et al*. (2011) recommended that bacterial fermentation is one of the novel ways to improve nutritional quality of plant ingredients, and especially macrophytes. Ensiling enhances their suitability for inclusion in fish diets by destruction of the antinutritional factors.

2.6. Fish growth

Fish growth is the change of body mass with time. Growth can be anabolic (increasing body mass) or catabolic (decreasing body mass). Fish growth can be influenced by one or by a combination of quantity and quality of feed, water temperature, age, health, species and genetics. Traditionally, growth in aquaculture experiments consider only yield at the end of the experiment and disregard data collected during intermediate samplings. As a result, a multivariate model, which is an expansion of Gulland and Holt Plot is necessary in capturing growth information based on intermediate sampling data (Hopkins K.D, M.L. Hopkins & D. Pauly, 1988). The method uses multiple regression equations to evaluate the effect of environmental variables on growth. The basic assumption being that growth rate of fish in terms of length decreases linearly as the fish grow larger.

Absolute growth rates are used to evaluate treatment effects in aquaculture yield trials. However, they can only be applied for groups of fish with same starting size, under identical environmental conditions and equal periods of time (Hopkins *et al*., 1988). Conversely, instantaneous growth rates and growth functions like the Von Bertalanffy growth function (VBGF) are not restricted to similar, initial size, or period of time. Changes in weight and length can easily be measured using balances and rulers, as appropriate. Therefore, they are often used to assess whole body fish growth. A relationship between weight (W) and length (L) of fish can be expressed as adapted from Pauly (1993).

$W = \mathbf{a} \times L^{b}$

a and b are constants, a is the multiplicative factor whereas exponent b should be close to 3 (2.5-3.5).When b is 3 weight increase proceeds proportionately as the cube of length (isometric). However, when b # 3 weight increases disproportionately with L^3

(Allometric). Allometric growth can either be positive (b>3) or negative (b<3) (Jobling, 2002).

Feed Conversion Ratio (FCR) is the amount of feed required to grow one kilogram of fish. When FCR is low, it implies that the feed quality is high and at the same time an indicator of feed utilization efficiency of formulated feeds. Specific Growth Rate (SGR) is used to assess the production of fish in a given duration of time. Ln (weight at harvest - weight at stocking) / production period * 100. When fish are small SGR can be greater than 3 while fish over 1 kg have average values of 1. Smaller fish can eat a larger percentage of their body weight per day (Mosig & Fallu, 2004). Asymptotic Length ($L\infty$) also referred as L infinity, is a parameter of the von Bertalanffy Growth Function (VBGF), showing the average maximum length the fish of a given population is likely to reach if left to grow for a considerably long period (Froese, Palomares & Pauly, 2000). Asymptotic weight ($W\infty$) is a parameter of the VBGF, that show the maximum mean weight the fish of a given population is expected to reach if they were left to grow for a substantially long period (Froese *et al*., 2000).

Survival rate is the number of fish alive after a specified time interval. The number of living fish is divided by the original number of fish after the defined period (Ricker, 1975).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted at University of Eldoret (Figure 3.1). The University of Eldoret is situated along Eldoret-Ziwa road, 9 km north of Eldoret town, in Uasin Gishu County, Kenya. Eldoret has a tropical monsoon climate with annual precipitation average of about 1103 mm. The town is located at an altitude of around 2073 m above sea level. The average temperature is 16.6 °C whereas the average minimum temperature is 9 °C and occurs in the months of January, April, June, July, August, September and December. The average maximum temperature is 26 °C, and is reported in February and March. The experimental fish were acquired from University of Eldoret fish farm whereas the laboratory analysis work was conducted at the Fisheries laboratory I, II, Chemistry laboratory II, III, and Biotechnology laboratory. Ensiling of the test ingredient and the growth performance experiment for *O.niloticus* were conducted at the Zoology laboratory, University of Eldoret.

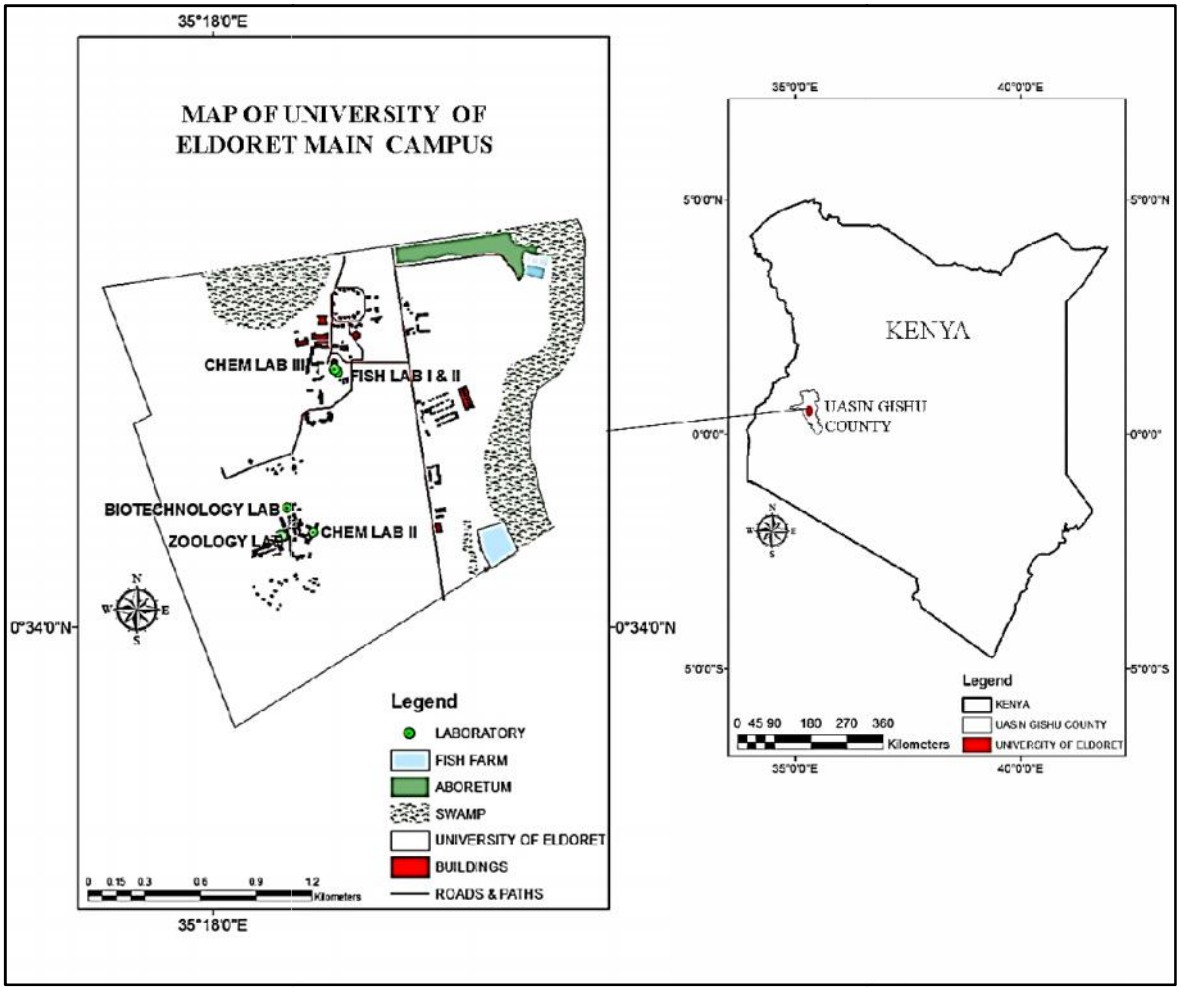


Figure 3.1 Map showing fish farm and laboratories where chemical analysis and growth performance experiments were conducted (Source: Author, 2016)

3.2. Preparation of ingredients

Selection of the test ingredient, *Prosopis juliflora* seed meal (PJSM), was based on its crude protein level, availability and its non-use for human consumption (Kassahun, Waidbacher, & Zollitsch 2012). Samples of *P. juliflora* seeds were collected in December 2013. Ripe and dry *P. juliflora* pods were harvested from *P. juliflora* trees (Figure 3.2) at Kampi ya Samaki town, Marigat sub-county, Baringo County. The Lakeshore town lies towards the southern shore of Lake Baringo. The area has a semi-arid climate with two rainy seasons and an annual rainfall of about 600 mm. The long rains occur between the months of April to August whereas short rains occur in Octo-

ber/November (Odada, Onyando & Obudho, 2006). Temperature ranges from 26^0 C to 39^0 C. The soils are clay and clay loam (Hickley *et al.*, 2004).

After harvesting, *P juliflora* pods (Figure 3.2) were packed in clean gunny bags and transported to University of Eldoret. Thereafter, the samples were cleaned to remove dirt and sun dried for two days. To release seeds, dry *P. juliflora* pods were cut longitudinally using a pair of scissors and the seeds packed in clean polythene bags. The seeds were dried in an oven at 70^oC for 6 hours and then ground using an electric grinder (Disk Mill Model FFC-23, Made in China). PJSM was packed in sample bottles and kept in a clean and dry place in the laboratory for ensiling, chemical analysis and preparation of experimental diets for *O. niloticus*.

Wheat bran and cottonseed meal were purchased from animal feed stores in Eldoret. Fresh water shrimp was bought from the Eldoret Municipal market. Wheat bran, FWSM and CSM were sorted separately and dried in an oven at 70^oC for 6 hours, and thereafter ground into meal using an electric grinder (Disk Mill Model FFC-23, Made in China). The meals were well packed and stored in a cool and dry place.

3.3 Ensiling of *P. juliflora* seed meal

About 1500 g of PJSM was mixed with 300 ml of water in a 2- litre glass bottle. About 90 ml of formic acid (equivalent of 5% volume of mixture) was added gradually with constant stirring to adjust the pH to 4 -4.2 (Santhosh, Sini & Mathew, 2007). The container was tightly sealed immediately after stirring. The mixture was kept for 28 days, after which the entire sample turned into semi-solid product. The resulting silage was placed in a plastic tray and then exposed in the sun to reduce the moisture level. After partial drying, the ensiled material was broken into small granules using a mortar and pestle and then left for further drying in the sun. The dry silage was ground into a meal using an electric grinder. Ensiled PJSM was kept in a tightly sealed 2 kg plastic container for further use in the preparation of dietary formulations and chemical analysis.

a (Source: https://en.wikipedia.org.) b (Source: https://www.sagebud.com)

C (Source: http:// www.pinterest.com) d (Source: http://www.jdgseeds.com)

Figure 3.2 *Prosopis juliflora* plant: a) flowers, b) ripe pods, c) harvested pods, d) extracted seeds.

3.4 Proximate analysis of feed ingredients

Proximate analysis involved determination of moisture, crude protein, crude lipid, crude fibre, ash and nitrogen-free extracts. The analysis was done for feed ingredients according to standard methods (AOAC, 1984).

3.4.1 Moisture content

Moisture content was determined by drying triplicate samples of 2 g in pre-weighed porcelain dishes, to constant weight in an oven at 105°C. The samples were then cooled in a desiccator and weighed. The average loss in weight was recorded as the moisture content of the samples according to Olvera-Novoa, *et al*., 1994), as follows:

$$\text{Moisture content (\%)} = 100\left\{\frac{(B - A) - (C - A)}{B - A}\right\}$$

Where:

A = weight of clean, pre dried crucible (g)
B = Crucible weight + weight of wet sample (g)
C = Crucible weight + weight of dry sample (g)

3.4.2 Crude protein

Crude protein was determined using the Kjeldahl method (Thiex, *et al*., 2002). This procedure involved the digestion of samples in concentrated sulphuric acid followed by steam distillation of ammonia into boric acid and then titration of the blue-green boric acid-ammonia complex with 0.01 M hydrochloric acid. Samples of 0.4 g were placed in digestion tubes in triplicates and 15 ml of concentrated sulphuric acid were added to each tube. One Kjedahl digestion tablet was added to each sample. A set of three digestion tubes was treated similarly but without samples to act as a control. The digestion tubes with their contents were placed in a digestion block. The samples were digested in a fume cupboard for 3 hours after which the digests were transferred into volumetric flasks. After topping up each flask to 100 ml, the digests were then ready for the distillation process

About 10 ml of the diluted digest material were pipetted into the distilling tube then 8 ml of 40% Na0H was added. The tube with the contents was mounted into the distilling unit. The mixture was heated with steam to distil ammonia into 25 ml of 4 % boric acid + 3 drops of Mixed indicator (0.1% methyl red and 0.2% bromocresol green in alcohol). The distillation process took between 15-20 minutes, with colour change of the distillate from pink to blue. Thereafter, the collected distillate was titrated back to the pink end point using 0.01 N HCl. The volume of the acid used in titrating each sample was recorded. Nitrogen content and crude protein of the sample were thus calculated according to Thiex *et al*. (2002) as follows:

$$\text{Nitrogen in sample (\%)} = 100\left\{\frac{\text{A x B x 0.014}}{\text{C (g)}}\right\}$$

Where:

A = Hydrochloric acid used in titration (ml)
B = Normality of standard acid
C = Weight of sample (g)

Crude protein (%) = nitrogen in sample × 6.25

3.4.3 Determination of ash content

Triplicate samples of 2 g of samples were placed in pre-weighed porcelain dishes, were charred on a hotplate for 1 hour and thereafter transferred and ashed in a muffle furnace at 600^0C for 6 hours. The samples were transferred to a desiccator and allowed to cool to room temperature before weighing them. The percentage ash was determined according to Olvera-Novoa *et al*. (1994), as follows:

$$\text{Ash content (\%)} = 100\left\{\frac{\text{A} - \text{B}}{\text{C}}\right\}$$

Where:

A = weight of crucible with ashed sample (g)
B = weight of empty crucible (g)
C = weight of sample (g)

3.4.4 Crude lipid determination

Crude lipids were determined using Soxhlet extraction method. Clean round bottomed flasks were dried in an oven, at 105 ^{0}C for about 1 hour and then placed in a desiccator to cool to room temperature. Each flask was weighed soon as it was removed from the desiccator and weight recorded. About 2 g of each sample were weighed in triplicates and placed in labelled thimbles and covered with cotton wool. The thimbles carrying samples were placed into the Soxhlet extractors. The flasks filled half way with petroleum ether and fixed to the extractors, were placed on mantle heaters. The extractors were connected to a cooling unit with a condenser and left to run for 6 hours. Thereafter, the thimbles with samples were removed and the solvent in the extractors was recovered. The flasks were dried in air and then oven dried for about 30 minutes at 105^0C. The flasks containing oil were put into a desiccator and allowed to cool to

room temperature. The flasks and the extracted oil were weighed and percentage ether extract was estimated according to Olvera-Novoa *et al*. (1994), as follows:

$$\text{Crude Lipid content (\%)} = 100\left\{\frac{B - A}{C}\right\}$$

Where:

A = weight of clean dry flask (g)
B = weight of flask with fat (g)
C = weight of sample used (g)

3.4.5 Crude fibre determination

Evaluation of crude fibre involved ashing of the portion remaining after digesting samples with solutions of sulphuric acid and sodium hydroxide. Dried fat-free triplicate samples, each weighing 2 g, were placed into a 600 ml beaker into which 200 ml of 0.255 N hot sulphuric acid was added. Thereafter, the mixture was boiled gently for 30 minutes; distilled water was used to keep the volume at 200 ml and to clear particles attached to the sides of the beaker. The digested mixture was centrifuged at a wavelength of 3000 nm and the supernatant decanted off. The residue was rinsed thrice with boiling water. The residue was then transferred back to the 600 ml beaker and 200 ml 0.313 N hot sodium hydroxide solution added, the mixture was boiled gently for 30 minutes. As for the acid digestion, distilled water was used to keep the volume at 200 ml and to clear particles attached to the sides of the beaker. After 30 minutes the contents were filtered through porous crucible and washed with boiling water, then 1% hydrochloric acid and then again with boiling water. The residue was washed twice with alcohol and dried in an oven for 2 hours at 105 °C. Thereafter the digest was cooled and weighed. The pre- weighed sample residues were placed in pre-weighed crucibles, dried on a hotplate, reweighed, and thereafter ashed in a furnace at 600 °C for 4 hours. Crude fibre was determined according to Olvera-Novoa *et al*. (1994), as follows:

$$\text{Crude fi} \quad \text{content (\%)} = 100\left\{\frac{A - B}{C}\right\}$$

Where:

A = weight of crucible with dry residue (g)
B = weight of crucible with ashed residue (g)
C = weight of sample (g)

3.4.6 Determination of Nitrogen Free Extracts (NFE)

The Nitrogen-free content was calculated by difference method according to Olvera-Novoa *et al*. (1994), as follows:

Nitrogen-free extract (%) = 100 - (A + B + C + D + E)

Where:

A =Moisture content (%)
B = crude protein content (%)
C = crude lipid content (%)
D = crude fibre content (%)
E = ash content (%)

3.5 Mineral analysis

Mineral analysis was done by digesting samples using a digestion mixture in a digestion block at about 350^0C for 3 hours. The digest materials were subjected to nitrogen, phosphorous, sodium, potassium, copper and zinc tests.

3.5.1 Preparation of digestion mixture

Selenium powder, 0.21 g, was added to 7.0 g of lithium sulphate then to 175 ml of 30% hydrogen peroxide and mixed well. About 210 ml of concentrated sulphuric acid was added while cooling in an ice-bath. This was then stored at 4^0C for stability purposes (Okalebo, Gathua & Woomer, 2002).

3.5.2 Digestion and analysis of samples

Digestion and preparation of samples for mineral analysis followed the procedures outlined by Okalebo *et al*. (2002). About 0.3 g of finely grounded samples were placed in triplicate, into dry and clean digestion tubes. Digestion mixture, 4.4 ml, was added

to each tube and to reagent blanks. The tubes were heated in a block digestion at 350^0 C for 3 hours, until the digest material became colourless. The tubes were removed from the digestion block and cooled to room temperature. Thereafter, 25 ml of distilled water was added to the digest material and mixed well. The contents were then transferred into 50 ml volumetric flasks where they were topped up to the mark and allowed to settle. The clean solutions were filtered and used for analysis of nitrogen, phosphorous, sodium, potassium, copper and zinc.

Total nitrogen and phosphorous were determined by colorimetric method whereas sodium and potassium were determined by flame photometry (Okalebo *et al*., 2002). Copper (Cu), Iron (Fe) and Zinc (Zn) were determined using atomic absorption spectrophotometer (AAS) (Okalebo *et al*., 2002).

3.6 Phytochemical analysis

3.6.1 Solvent extraction.

About 5 g of each sample, was separately dispersed in 50 ml of water, 70% ethanol, acetone and chloroform. The solutions were left to stand at room temperature for 24 hours and then filtered with whatman No. 1 filter paper. The product was used for phytochemical screening using testing procedures for alkaloids, flavonoids, phenols, saponins, oxalates and tannins (Ugochukwu & Arukwe Uche, 2013)**.**

3.6.2 Determination of antinutrients

Alkaloids were tested by taking a fraction of extract and treating it with 3 drops of Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of reddish brown precipitate (or colouration) (Ugochukwu & Arukwe Uche, 2013)**.**
In testing for flavonoids, 2 ml of extract were treated with a few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which turns colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids (Ugochukwu & Arukwe Uche, 2013)**.**

Phenols were tested by taking three drops of 5% weight/volume $FeCl_3$ and adding to 1 ml of the extract. A greenish precipitate indicated the presence of phenols, Awe and

Shodipo's study (as cited in Adeyemi and Olorunsanya, 2012). About 2 ml of sample extracts were each added separately to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins (Ugochukwu & Arukwe Uche, 2013).

Test for tannins also referred to Braymer's test, was effected by taking 1 ml of a freshly prepared 10% weight/volume ethanolic KOH (0.01 M) and adding to 1 ml of each sample. A dirty white precipitate indicated the presence of tannins (Sofowora's study as cited in Adeyemi and Olorunsanya, 2012). Test for Oxalates was effected by adding a few drops of glacial ethanoic acid to 3 ml of the extracts. A greenish black colouration was to indicate presence of oxalates (Ugochukwu & Arukwe Uche, 2013).

3.7. Diet formulation

Crude protein levels of ensiled and non-ensiled PJSM, Fresh water shrimp meal (FWSM), Cottonseed Meal (CSM) and Wheat bran (WB) were determined. The crude protein values obtained for ensiled and non-ensiled PJSM were used in the formulation of eight experimental diets, each having 25% crude protein. PJSM and CSM were the main variables and a source of dietary plant protein for *O. niloticus*. The other ingredients, FWSM and WB were not varied in diets. In all the diets, 12 g of animal protein, provided by FWSM, were kept constant. Percentage crude protein values for CSM and WB were subjected to a 2 x 2 square matrix method, which uses determinants (Saxena, Pathak & Kumar, 2013). The matrix gave output values which were used to derive mixing ratios for all the diets (Table 3.1). Diet formulation was effected in Microsoft Excel 2007.

The control diet was formulated using FWSM, CSM and WB and did not contain any PJSM (Table 3.1). Four diets were formulated whereby ensiled PJSM was included by replacing CSM protein at 25, 50, 75 and 100% levels (Table 3.1). The other four diets were formulated and non-ensiled PJSM was included by replacing CSM protein at 25, 50, 75, 100% levels (Table 3.1). Alpha cellulose was used in varying quantities as a filler to attain 100% or 100 g of each diet in the formulation process (Table 3.1).

3.8 Diet preparation

The feed preparation process involved weighing of feed ingredients in appropriate ratios to make 100 % of each diet (Table 3 1). The diets were made of varying levels of ensiled and non-ensiled PJSM. The different ingredients were then mixed thoroughly in a plastic hand-washing basin, making a homogenous mixture for each experimental diet. The dietary mixtures were moistened and pelleted using a meat mincer. The pellets were sun dried and stored in pet dishes at room temperature, for experimental feeding.

Table 3.1. Mixing ratios (g) for ingredients in diets; E=Ensiled, NE= Non-ensiled, PJSM= Prosopis juliflora Seed Meal, FWSM=Freshwater Shrimp Meal, CSM=Cottonseed Meal and WB=Wheat Bran.

	DIETS (% inclusion of PJSM)								
	0	**25**		**50**		**75**		**100**	
	Control	**E**	**NE**	**E**	**NE**	**E**	**NE**	**E**	**NE**
FWSM (g)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
CSM (g)	29.3	22.0	22.0	14.7	14.7	7.3	7.3	0.0	0.0
WB (g)	45.7	45.7	45.7	45.7	45.7	45.7	45.7	45.7	45.7
PJSM (g)	0.0	10.1	8.4	20.2	16.8	30.2	25.1	40.3	33.5
α-cellulose (g)	13.0	10.2	11.9	7.4	10.8	4.8	9.9	2.0	8.8
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

3.9 Source of experimental fish and fish handling

One thousand mixed sex of *O. niloticus* fry, weighing between 0.32-0.35 g, were acquired from the Department of Fisheries and Aquatic Science fishponds, University of Eldoret. Fish were handled according to the Animal Welfare Act (AWA) (Carroll & Santi, 2010). The AWA require that minimum standards of care and treatment be given to animals, such as those used in research. Some of the animals specified in AWA include: cattle, camels, buffalos, sheep, goats, pigs, fish, horse, mule, ass, dog and any other wild or domestic animal, kept in captivity or otherwise. Fish fry were transported in batches of 200 in 20 litre buckets, to the wet lab, in the department of Zoology, University of Eldoret. The one thousand fish were acclimatised to experi-

mental conditions for 2 weeks, in five, 80-litre, glass-holding tanks, each holding 200 fish. During the two weeks, the fish were fed on control diet (Table 3.1) to satiation. All dead fish were removed and discarded. After two weeks, the initial lengths and weight of 810 fish were measured using a measuring board and weighed using a digital balance (citizen scale, CY 204). The weighed fish were randomly stocked into 27 experimental tanks and fed on experimental diets for 3 months.

3.10 Fish holding facilities and maintenance

A total of 27 glass aquaria, each measuring 45 x 30 x 30 cm, were used for growth performance experiments. The experiment was conducted in the wet lab section within Zoology Laboratory I, in the Department of Biological Science, University of Eldoret. An aeration system (comprising of an air pump, air tubes and air stones) and temperature regulation thermostat were fixed to each of the tanks. Eight reservoir glass tanks of 80 litres capacity and a PVC tank of 200 litres capacity were placed above the aquaria stand to provide top up water. The reservoir tanks were filled with tap water using a 6 metre long horse pipe and then the water was left to settle for at least two days before it could be used to refill experimental tanks. The settling period for reservoir water, was meant to allow de-chlorination to take place. The experimental room was always kept closed and a room heater switched on to ensure warm reservoir water at room temperature. The inner surfaces of experimental tanks were wiped carefully using a hand towel after every two days and the dirty water siphoned to ¼-way using ½-inch horse pipe into a 20-litre bucket. The cleaned and drained experimental tanks were refilled to ¾ full by siphoning reservoir water using a six-metre long horse pipe.

3.11. Experimental design

A completely randomized design was used in evaluating the growth performance of *O. niloticus* fed on experimental diets containing ensiled (E) and non-ensiled (NE) PJSM. A control diet and eight treatment diets were applied each in three replicates into 27 aquaria, each stocked with 30 fish. The treatments were allocated randomly using random numbers, generated online (https://www.random.org/integers). The diets included, 0% (control), 25% E, 25% NE, 50% E, 50% NE, 75% E, 75% NE, 100% E, 100% NE (Figure 3.1).

3.12 Experimental fish feeding and measurements of length and weight

After acclimatization, fish were fed on experimental diets twice a day, for 87 days, from February 2014 to May 2014, at 10 am and 4 pm, at a feeding rate of 10% of total body weight for each aquarium tank in the first month and 5% in subsequent months. The rations were adjusted fortnightly, according to total fish weight for each of the experimental tanks. The fish were weighed and their lengths measured, fortnightly. Weight was measured using a digital balance (Citizen Scale, CY 204, Poland) to the nearest 2 decimal places while length was measured to the nearest 1 mm using a measuring board.

3.13 Water quality parameters

Water quality parameters, dissolved oxygen; temperature and pH, were monitored weekly to ensure that they were within tolerable levels for *O. niloticus* (Hussain, 2004). Temperature, Dissolved Oxygen and pH were measured insitu, using a Multi-parameter meter (Standard Sensor Module, Hand held Water quality Meter-model WQC-24).

3.14 Evaluation of growth performance, feed utilisation and survival

Growth performance was evaluated based on weight (W) and length (L) of fish, as follows:

$W = a \times L^b$; where:
W=Body weight of fish in grammes
L=Total length in centimetres
a=is the intercept of the regression line
b=is the slope of the regression line

a is the multiplicative factor whereas exponent b should be close to 3 (2.5-3.5) (Pauly, 1993).

Asymptotic length was determined as follows:

$L_\infty = (b/1-mk)$
Where:
L_∞ =Asymptotic Length
b=slope of reciprocal of Length against Ln of Weight
Ln=natural log.
m=slope
mk= Exponent of intercept

Asymptotic weight was determined as follows:

$W_\infty = a \times L_\infty^{b}$
Where:
W_∞= Asymptotic weight
a x L_∞^{b}
=(Exponent of the intercept of Ln Weight against Ln of Length) x power
(L_∞,b (slope of LnW against LnL).

Specific Growth Rate (SGR) (% day^{-1}) was determined as follows:

$$SGR = \frac{100x\{Ln(Wz) - Ln(Wi)\}}{t}$$

Where:
W_z=Final body weight
W_i = Initial body weight
t= time in days
Ln natural logarithm

Feed Utilisation was evaluated using final individual fish weight (g), final weight gain (g), average daily gain (g per fish per day) and Feed conversion ratio (FCR), according to Jones and De Silva (1998). The indices were calculated as follows:

Feed Intake (g $fish^{-1}$ day^{-1}) was determined as follows:

FI= FIf/t
Where:
FIf =Total feed intake per fish
t = number of days

Feed Conversion Ratio (FCR)

$$FCR = Ff/Wg$$

Where
Ff=Amount of feed fed (g)
Wg =Weight gain (g).

Percentage survival is an indicator of nutritional status of fish and should always be high.

$$\% \text{ Survival} = \{(Fi-Ff)/ Fi\} x\ 100$$

Where:
F_i =Initial number of fish
F_f = Final number of fish

3.15. Statistical analysis

Length and weight data were log transformed and analysed using simple regressions in Statgraphics trial version 16.1.11 (32-bit) and Microsoft excel 2007. Analysis of Co-variance (ANCOVA) was used to compare slopes of regression lines for length against weight. Multiple regression was used to determine the relationship between water quality parameters and the weights of *O. niloticus* fed on different diets. A t test was used to determine the differences of nutrient levels between ensiled and non-ensiled *Prosopis juliflora* seed meal (PJSM). All inferences were accepted at $\alpha = 0.05$.

CHAPTER FOUR

RESULTS

4.1 Nutrients and antinutrients analysis

4.1.1. Proximate composition for ingredients and diets

Proximate analysis results for ensiled and non-ensiled *P. juliflora* seed meal (PJSM), demonstrated that ensiling led to reduction of crude protein, crude fibre and ash content. Crude protein levels reduced significantly ($p<0.001$) by 16.75 %, crude fibre content levels reduced significantly ($p=0.011$) by 25.10 % and ash content levels also decreased significantly ($p=0.001$) by 27.77%. On the other hand, Crude lipid and moisture content levels did not increase significantly upon ensiling whereas Nitrogen Free Extracts (NFE) increased significantly ($p=0.013$) by 16.52% (Table 4.1).

Crude protein, crude lipid and ash content levels ranged from 13.85-58.63%, 5.06-6.10% and 1.99-3.93% respectively for Freshwater shrimp meal (FWSM), Cottonseed meal (CSM) and Wheat bran (WB) (Table 4.1). Crude protein for control diet and treatment diets ranged from 24.56- 25.28% (Table 4.2).

4.1.2 Mineral analysis for ensiled and non-ensiled PJSM

There was a reduction in copper (Cu), Iron (Fe), Zinc (Zn), Nitrogen (N), Potassium (K) and Phosphorous (P) in ensiled PJSM compared to non-ensiled PJSM (Table 4.3)

Table 4.1 Proximate composition for diet ingredients (mean ± SD, n=3); DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF=Crude Fibre, NfE=N-free Extracts, PJSM=*Prosopis juliflora* Seed Meal, FWSM=Freshwater Shrimp Meal, CSM= Cotton-seed Meal and WB=Wheat Bran.

Sample	DM	CP	EE	Ash	CF	NfE
Non-ensiled PJSM	88.2 ±0.60[a]	30.92 ±3.57[a]	10.00 ±0.58[a]	4.43 ±0.09[a]	7.73 ±0.39[a]	35.12 ±3.61[a]
Ensiled PJSM	87.8 ±0.29[a]	25.74 ±0.32[b]	11.00 ±0.58[a]	3.20 ±0.00[b]	5.79 ±0.20[b]	42.07 ±0.80[b]
FWSM	94.27±0.22	58.63±0.58	6.10±0.10	1.99±0.55	-	-
CSM	93.43±0.59	32.16±1.74	5.80±0.20	3.93±0.31	-	-
WB	95.09±0.18	13.85±0.67	5.06±0.14	3.84±0.33	-	-

Different superscripts within columns indicate significant difference at p<0.05

Table 4.2 Crude Protein (CP) for experimental diets, (mean ± SD, n=3); C=Control, E=Ensiled, NE=Non-ensiled and PJSM= *P. juliflora* Seed Meal.

	% PJSM inclusion in diets				
Diets	0	25	50	75	100
	C	NE	NE	NE	NE
CP	25.11 ±0.25[a]	25.08 ±0.13[a]	25.13 ±0.26[a]	25.09 ±0.25[a]	25.06 ±0.25[a]
	-	25.28 ±0.21[a]	25.22 ±0.61[a]	24.56 ±0.71[a]	24.91 ±0.33[a]

Similar superscripts within a column indicate the difference was not significant at p>0.05

Table 4.3 Mineral levels in PJSM, (mean ± SD, n=3); PJSM=*P. juliflora* Seed Meal, Cu=Copper, Fe=Iron, Zn=Zinc, N=Nitrogen, K=Potassium, Na=Sodium and P=Phosphorous.

	Cu mgl^{-1}	Fe mgl^{-1}	Zn mgl^{-1}	N mgl^{-1}	P mgl^{-1}	Na mgl^{-1}	K mgl^{-1}
Non ensiled PJSM	0.048 ±0.001[b]	1.509 ±0.001[b]	0.198 ±0.000[b]	87.407 ±0.000[b]	0.196 ±0.000[b]	0.564 ±0.000[a]	3.077 ±0.002[b]
Ensiled PJSM	0.039 ±0.000[a]	0.891 ±0.002[a]	0.049 ±0.017[a]	78.148 ±0.017[a]	0.178 ±0.00[a]	0.564 ±0.001[a]	2.115 ±0.000[a]

Different superscripts within columns indicate significant difference at $p<0.05$

4.1.3. Qualitative analysis of antinutrients in PJSM.

The presence of alkaloids, flavonoids, phenols, saponins and tannins was evident in non-ensiled PJSM, following the positive tests during the qualitative evaluation of non-ensiled PJSM extracts (Table 4.4). Negative tests observed for flavonoids, phenols and saponins, in ensiled PJSM extracts, is evidence of complete elimination of the antinutrients in ensiled PJSM. Positive tests were observed for alkaloids and tannins in ensiled PJSM extracts, an evidence of their presence in PJSM, even after ensiling (Table 4.4). Oxalates were not detected either in ensiled or non-ensiled PJSM (Table 4.4).

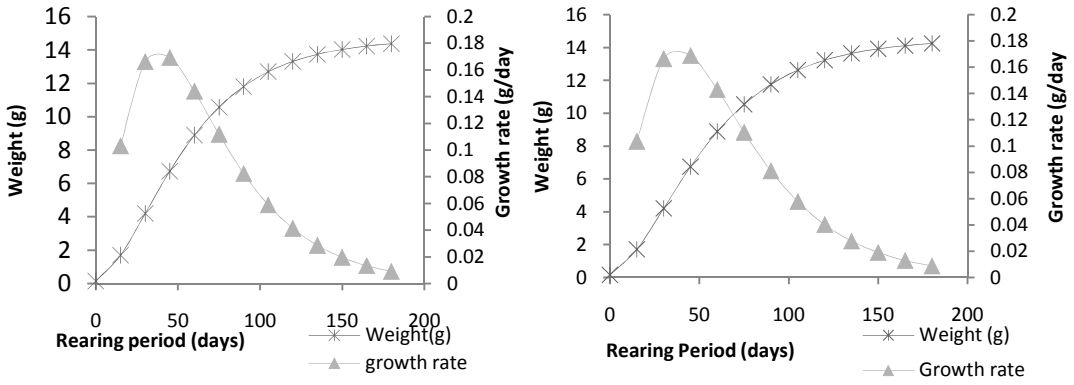
Table 4.4 Antinutrient status for ensiled and non-ensiled PJSM, extracted using different solvents.

	Non-ensiled PJSM				**Ensiled PJSM**			
	Solvents				Solvents			
ANFs	Water	70% ethanol	Ace-tone	Chloro-form	Water	70% ethanol	Ace-tone	Chloro-form
Alkaloids	+++	++	+	++	++	+	+	+
Flavon-oids	++	++	++	++	-	-	-	-
Oxalates	-	-	-	-	-	-	-	-
Phenols	+	+	-	+	-	-	-	-
Saponins	+++	++	-	++	-	-	-	-
Tannins	+++	++	-	-	+	-	-	+

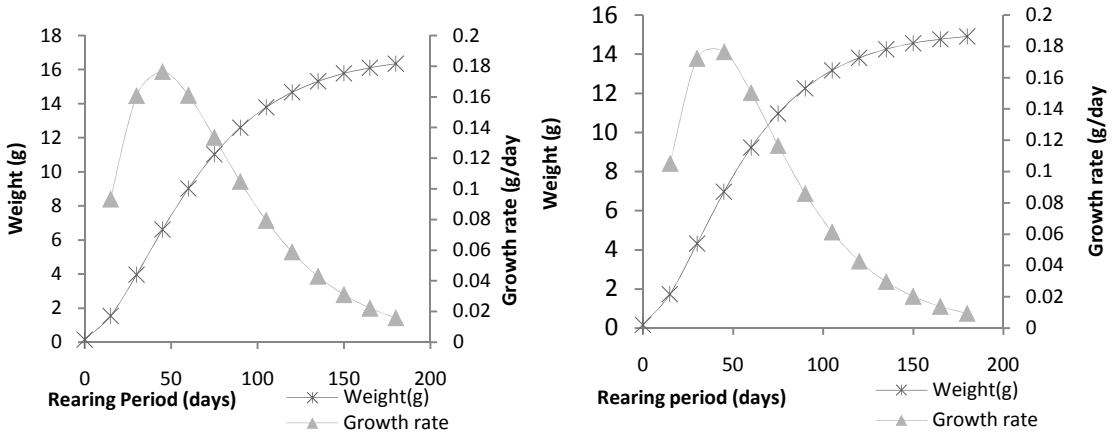
'+' Present '-' absent

4.2 Growth performance for *O. niloticus* fed diets containing ensiled and non-ensiled PJSM

For all dietary treatments, *O. niloticus* reached Critical Standing Crop (CSC) at day 45 (Figure 4.1 and 4.2). Beyond the 45th day, growth rate declined gradually for all dietary treatments. The maximum growth rates at CSC ranged between 0.169-0.174 g day^{-1} at weights ranging between 5.94-6.14 g for *O. niloticus* fed diets containing ensiled PJSM (Figures 4.1), whereas for fish fed diets containing non-ensiled PJSM, maximum growth rates at CSC ranged between 0.169-0.177 g day^{-1} at weights ranging from 6.31-6.97 g (Figures 4.2). The highest growth rate, 0.177 g day^{-1}, was obtained at 50% replacement of CSM with non-ensiled PJSM (Figure 4.1 c) while the lowest growth rate, 0.169 g day^{-1}, was obtained at 50% replacement of CSM with ensiled PJSM (Figure 4.2 e). Fish fed diets containing 50 % non-ensiled PJSM, attained the highest asymptotic weight, 16.92 g, (Figure 4.1 c) whereas fish fed diet containing 100% non-ensiled PJSM had the least asymptotic weight, 13.93 g (Figure 4.1 e). Asymptotic lengths ranged from 10.57 cm to 11.00 cm.



(a) 100% CSM inclusion, 0% non-ensiled PJSM **(b) 25% non-ensiled PJSM inclusion**



(c) 50% non-ensiled PJSM inclusion **(d) 75% non-ensiled PJSM inclusion**

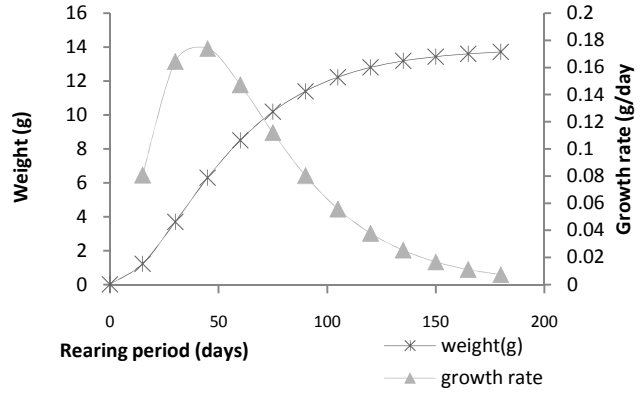
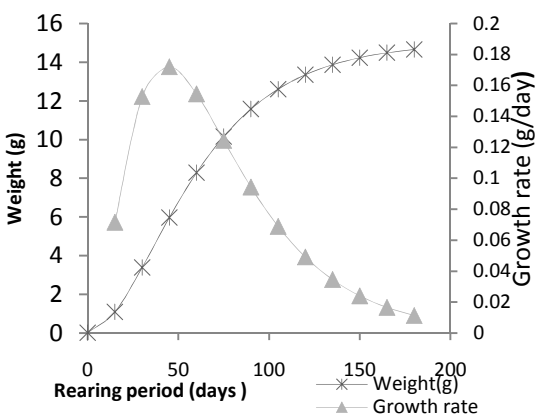
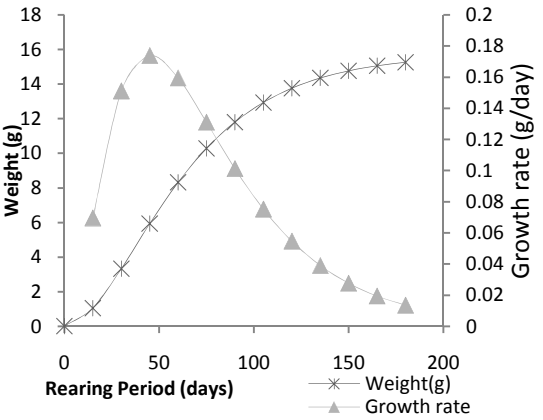


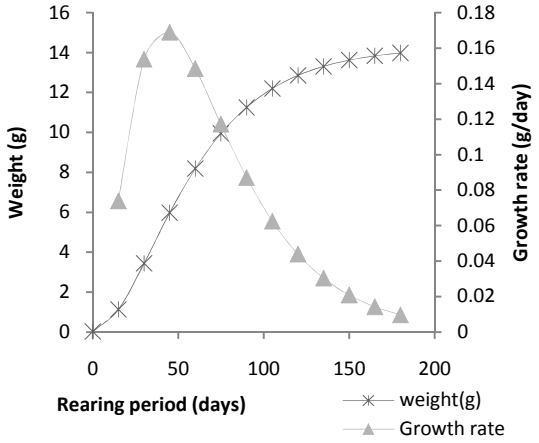
Figure 4.1 Growth rate (g day^{-1}) and weight (g) against time (days) for *O. niloticus* fed on diets containing non-ensiled PJSM at 0, 25, 50, 75 &100%.



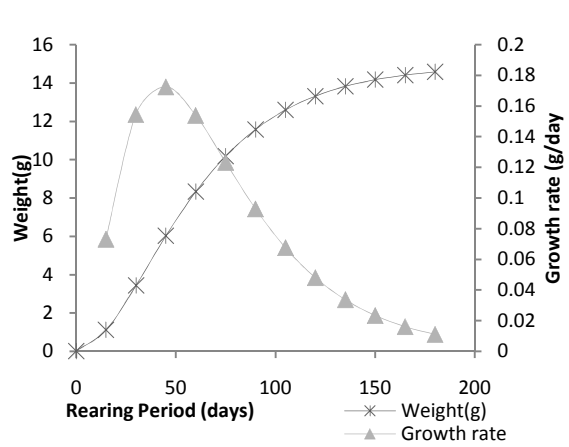
(a) 100% CSM inclusion, 0% ensiled PJSM



(b) 25% ensiled PJSM inclusion



(c) 50% ensiled PJSM inclusion



(d) 75% ensiled PJSM inclusion

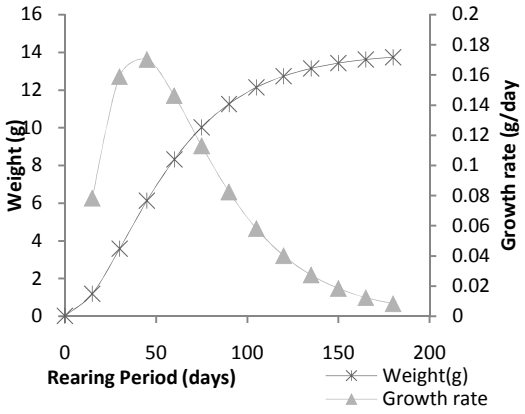


Figure 4.2 Growth rate (g day^{-1}) and weight (g) against time (days) for *O. niloticus* fed on diets containing ensiled PJSM at 0, 25, 50, 75 &100%.

Feed Intake (FI) and Specific Growth Rate (SGR) for *O. niloticus* decreased with increased PJSM inclusion in dietary formulations. On the other hand, Food Conversion Ratio (FCR) increased with increased inclusion of PJSM (Table 4.5).

Table 4.5 Specific Growth Rate (SGR), Feed Intake (FI) and Feed Conversion Ratio (FCR) for *O. niloticus* fed on control diet (0%) and diets containing ensiled (E) and non-ensiled (NE) PJSM, (mean ± SD, n=3); C=Control diet.

	DIETS (% Inclusion of PJSM)								
	0	25		50		75		100	
	C	E	NE	E	NE	E	NE	E	NE
SGR %	2.83	2.85	2.60	2.80	2.60	2.61	2.40	2.56	2.40
FI g/day	0.070 ±0.007	0.068 ±0.001	0.066 ±0.002	0.057 ±0.003	0.073 ±0.005	0.058 ±0.004	0.068 ±0.000	0.058 ±0.006	0.053 ±0.004
FCR	1.75	1.72	2.2	1.71	2.0	2.07	2.5	2.40	2.9

4.2.1. Growth performance at varying levels of replacement of CSM with ensiled and non-ensiled PJSM in *O. niloticus* diets.

The results on the effects of including PJSM in *O. niloticus* diets, show that replacement of CSM with non-ensiled PJSM from 0 to 50 % led to increase in growth performance of *O. niloticus* followed by a decline in fish growth at levels beyond 50% replacement. There was low growth at inclusion of non-ensiled PJSM up to 25% whereas inclusion at 50%, led to a significant increase (p<0.001) in growth of *O. niloticus* relative to the control diet. Inclusion of non- ensiled PJSM at 75% and 100% levels, led to a gradual decline in growth (Figure 4.3).

There was no significant difference on growth performance of *O. niloticus* upon replacement of CSM with ensiled PJSM at 25, 50 and 75% compared with the control. Replacement of CSM with ensiled PJSM from 0%-25 % led to a slight increase in growth performance of *O. niloticus*, followed by a gradual decline in growth when CSM was replaced between 25-100 %. Inclusion of ensiled PJSM at 100% led to a significant (p=0.0102) reduction in *O. niloticus* growth compared to the control (Figure 4.4).

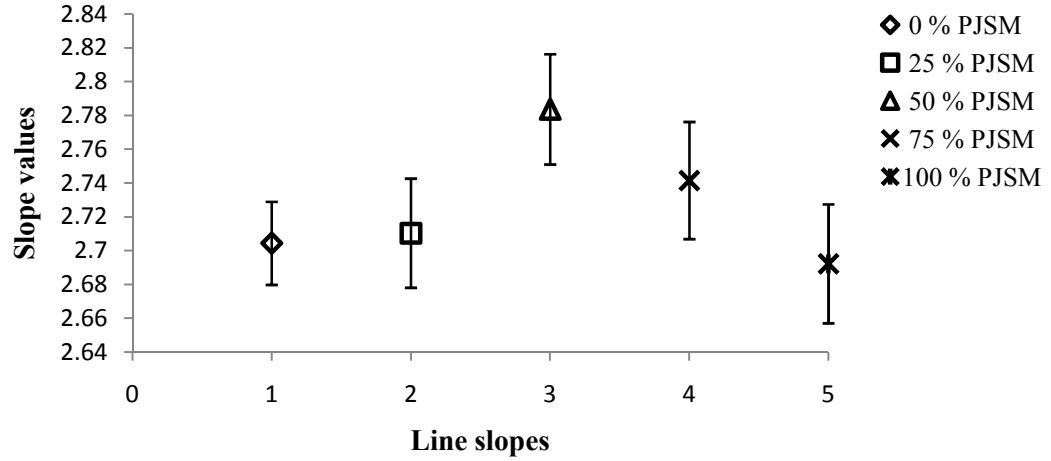


Figure 4.3 Deviations of treatment diet slopes from control diet slope for regression lines of lengths against weights for *O. niloticus* fed on control diet (0 %) and diets containing non-ensiled PJSM.

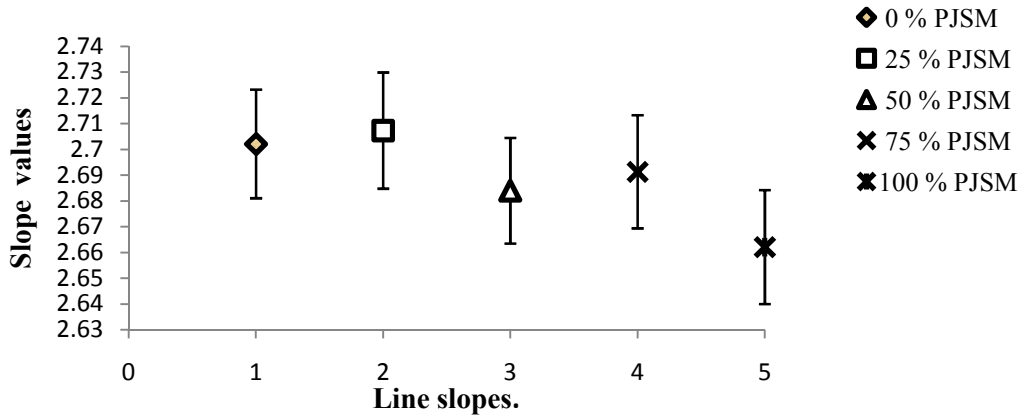


Figure 4.4 Deviations of treatment diet slopes from control diet slope for regression lines of lengths against weights for *O. niloticus* fed on control diet (0 %) and diets containing ensiled PJSM.

4.3. Survival

Survival rates for *O. niloticus* fed dietary formulations containing ensiled PJSM, ranged from 81.11%-85.56% whereas for fish fed dietary formulations having non-ensiled PJSM, it ranged from 72.22%-81.10% (Table 4.6).

Table 4.6 Survival (%) for *O. niloticus* fed on diets containing *P. juliflora* seed Meal (PJSM)

	DIET (% PJSM)				
Inclusion level of PJSM	**0**	**25**	**50**	**75**	**100**
Control diet	85.6	-	-	-	-
Ensiled PJSM	-	85.6	82.2	81.1	82.2
Non-ensiled PJSM	-	74.4	72.2	81.1	72.2

4.4 Water quality variables and growth of *O niloticus*

The water quality variables measured during the present study included: dissolved oxygen (DO), temperature and pH. The values for DO, temperature and pH ranged from 3.40-3.93 mg l^{-1}, 27.03-27.33^0 C, and 7.17-7.23, respectively (Table 4.7 and 4.8). The multiple regression analysis between weight and the water quality variables showed that there was no significant relationship (p=0.4036) between the growth of *O niloticus* fed diets containing non-ensiled PJSM and water quality. Similarly, for *O niloticus* fed diets containing ensiled PJSM, there was not a significant relationship (p=0.0795) between growth and the water quality variables.

Table 4.7 Water quality variables measured in experimental tanks for *O. niloticus* fed on control diet (0%) and diets containing ensiled PJSM, (mean ± SD, n=3).

% inclusion of ensiled PJSM.	**Temperature ^{o}C**	**Dissolved Oxygen (DO) (mgl^{-1})**	**pH**
0	27.23±0.21^a	3.42±0.26^a	7.21 ±0.47^a
25	27.20 ±0.26^a	3.90±0.35^a	7.20±0.25^a
50	27.23±0.31^a	3.93±0.42^a	7.21±0.47^a
75	27.22±042^a	3.43±0.23^a	7.19±0.65^a
100	27.23±0.49^a	3.42±0.32^a	7.23±0.49^a

Similar superscripts within a column indicate the difference was not significant at p>0.05

Table 4.8 Water quality variables measured in experimental tanks for *O. niloticus* fed on diets containing non-ensiled PJSM, (mean ± SD, n=3).

% Inclusion of non-ensiled PJSM.	Temperature^{o} C	Dissolved Oxy-gen(DO) (mg l^{-1})	pH
25	27.33±0.55^{a}	3.41±0.65^{a}	7.21±0.11^{a}
50	27.33±0.40^{a}	3.42±0.64^{a}	7.17 ±0.17^{a}
75	27.03±0.12^{a}	3.40±0.27^{a}	7.21±0.03^{a}
100	27.16±0.31^{a}	3.42±0.18^{a}	7.20±0.10^{a}

Similar superscripts within a column indicate the difference was not significant at p>0.05

CHAPTER FIVE

DISCUSSION

5.1. Effects of ensiling on the chemical composition of PJSM

This study set out to investigate the potential of formic acid ensiling in destruction of antinutrients in PJSM. In addition, the study sought to determine if ensiling had an effect on nutrient levels in PJSM. The findings demonstrated that ensiling of PJSM, significantly reduced crude protein, ash content and trace mineral elements whereas moisture content and crude lipids increased in ensiled PJSM. The decrease in crude protein content in ensiled products could be attributed to the acid hydrolysis of protein during the process of ensiling (FAO, 2007). This finding agrees with that of Ramasubburayan, Iyapparaj, Subhashini, Chandran & Immanuel (2013) who reported a drop of 10% in protein when fish was ensiled in 3% formic acid for 30 days. Geron *et al*. (2007) and Vidotti (2002) reported a decrease in crude protein when tilapia fillet residues were ensiled in a mixture of formic and sulphuric acid at a ratio of 1:1. Similarly, Lyimo, Mugula & Elias (1992) observed a decrease in crude protein and attributed the reduction to loss of sulphur amino acids. Most ensiled ingredients in the present study showed a significant increase in Nitrogen Free Extracts (NFE). On the contrary, there was a general reduction in crude fibre content in most of the ensiled ingredients. The decrease in crude fibre was probably due to hydrolysis into simple carbohydrates, which in turn may have led to increase of the NFE. The assumption agrees with the concept of acid hydrolysis causing the breakdown of non-reducing sugars to reducing sugars (Sadasivam, 1996). Although in the present study, formic acid ensiling led to increase in NFE, ensiling by fermentation is reported to have led to decrease in NFE, and this was attributed to the activities of the fermenting microbes (Larson, 2003).

Qualitative evaluation for alkaloids, flavonoids, oxalates, phenols, saponins and tannins led to either reduction or complete elimination of antinutrients in ensiled ingredients. The reduction and absence of antinutrients in ensiled ingredients could be attributed to acid hydrolysis during ensiling, which caused degradation of these substances.

5.2 Growth performance trends for *O. niloticus* fed diets containing ensiled and non-ensiled PJSM

This study sought to determine the growth performance of *O. niloticus* fed diets containing ensiled and non-ensiled PJSM by looking at growth, the critical standing crop (CSC), specific growth rates and maximum weights and lengths. In the present study, CSC was reached at the 45th day, for *O. niloticus,* despite the different dietary treatments. The point at which growth decline occurs for each individual is referred to CSC (Liti *et al*., 2006). According to Hepher (1978) CSC is an important concept that regulate the management of fish production and harvests in aquacultural systems. Several studies have indicated that CSC of tilapia in semi-intensive ponds must occur during the first month of culture at a weight between 30-40 g (Diana, Lin & Jaiyen, 1994; Diana, Lin & Yi, 1996). In the present study, growth performance experiments were conducted in aquaria where CSC was reached at the 45th day and at weights ranging between 5.94-6.97 g.

The maximum growth rates at CSC ranged between 0.169-0.174 g day^{-1} for *O. niloticus* fed diets containing ensiled PJSM whereas fish fed on diets containing non-ensiled PJSM had maximum growth ranging from 0.169-0.177 g day^{-1}. Okonji, Bayoko & Alufohai (2013) evaluated the effects of culture systems on *O. niloticus* growth and reported growth rate of 0.387 g day^{-1} for fish reared in an intensive system and fed on a commercial diet. Specific growth rates ranging between 3.05-3.94 were reported by Opiyo, Ngugi & Rasowo (2014) who conducted a study in aquarium tanks where *O. niloticus* fingerlings were fed on a commercial diet. The specific growth rates in the present study ranged between 2.36-2.85 g day^{-1}. Overall, the growth performance observed in the present study was relatively lower, compared to those reported earlier. The lower growth observed may be attributed to diets which may have been deficient in protein and nutrients, such as vitamin and minerals.

5.3 Growth performance of *O. niloticus,* at different inclusion levels of ensiled and non-ensiled PJSM in diets.

This study aimed at finding out the appropriate level at which PJSM could replace CSM to realize better growth of *O niloticus*. Findings of the present study revealed

that replacement of CSM protein with that of non-ensiled PJSM from 0% to 50 % led to improved growth performance of *O. niloticus,* followed by a decline in growth, at inclusion of PJSM beyond 50%. The significant increase in growth of *O. niloticus* at 50% inclusion of PJSM could be attributed to synergetic complementation of nutrients and dilution of anti-nutritional factors in the mixture of CSM and non-ensiled PJSM.

Growth of *O. niloticus* declined when PJSM was included beyond 50%. This result could be attributed to nutrient deficiencies and higher concentration of anti-nutritional factors in the dietary formulations. At 0 and 25% inclusion of PJSM, CSM dominated in supplying dietary protein whereas PJSM was the main source of protein at 75% and 100% inclusion of PJSM in diets. Protein supply from a single ingredient of plant origin cannot support good growth of fish due to deficiency in one or more of essential amino acids (Jauncey & Ross, 1982). Plant proteins are usually deficient in sulphur amino acids such as methionine and lysine and cannot support adequate growth of fish (Noreen & Salim, 2008; Soltan, Hanafy & Wafa, 2008)**.** Fish like other animals; require 10 essential amino acids , which should be presented in right proportions for protein synthesis (Santiago & Lovell, 1988). The essential amino acid requirements for *O. niloticus* (Santiago & Lovell, 1988) were not met fully by either those provided by CSM (Heuzé *et al*., 2015) or PJSM (Kathirvel & Kumudha, 2011). Essential amino acid levels of CSM indicate that three essential amino acids, lysine, methionine/cystine and threonine were limiting, compared to six, cystine/methionine, tyrosine/ phenylalanine, isoleucine, threonine, tryptophan and histidine, which were limiting in PJSM. It is therefore apparent that PJSM was an inferior ingredient compared to CSM, in terms of amino acids. One advantage PJSM has over CSM in regard with amino acids, is that lysine is in surplus relative to *O. niloticus* requirement. Therefore, it is possible that the growth increase between 0 and 50 % inclusion levels was due to supplementation of lysine. All essential amino acids should be supplied in the fish diets (Santiago & Lovell, 1988), in right proportions. Based on amino acid levels in PJSM (Kathirvel & Kumudha, 2011) and the essential amino acid requirements for *O. niloticus* (Santiago & Lovell, 1988), the limiting essential amino acids which may have greatly influenced the reduction of *O.niloticus* growth include phenylalanine, methionine, isoleucine, tryptophan, threonine and histidine, particularly, when PJSM was included beyond 50 % in diets. This deduction agrees with findings by Kathirvel &

Kumudha (2011) which show that *P. juliflora* seeds are deficient in cystine/methionine, tyrosine/phenylalanine and histidine.

The increment in growth performance of *O. niloticus* at 25-50% inclusion of non-ensiled PJSM, suggest that CSM, as a single plant protein source, could not have supported better growth of *O. niloticus* without complementation of deficient lysine and threonine with those from PJSM. Nakyewa (2013) reported better growth of *O. niloticus*, which were fed on formulations rather than on single ingredients and attributed the improved growth to better amino acid complementation in formulations. The findings in the present study are in line with those of Agbo *et al*.(2011), who reported reduced growth performance of *O. niloticus* when fishmeal was replaced with 75 % of CSM. The authors attributed the negative growth effects to low levels of lysine, methionine and threonine in CSM. In another study involving *L. rohita*, growth was depressed when the fish were fed on diets where PJSM replaced 50% of fishmeal (Bhatt *et al*., 2010).

The increase in growth realized at 25% inclusions of PJSM was lower than that observed when PJSM was included between 50% levels. The observed lower improvement of growth of *O. niloticus* between 25% inclusion of PJSM, was probably due to the presence of trypsin inhibitors and gossypol given the high levels (75-100%) of CSM in the diets. Francis *et al*. (2001) reported the presence of phytic acid, phytoestrogens, gossypol, antivitamins and cyclopropenoic acid in CSM. Dietary protein can be reduced due to formation of less digestible phytate-protein complexes. Phytates have been reported to affect nutrient availability to fish including phosphorus and heavy metal cations through chelating process (Duffus & Duffus, 1991). The levels of gossypol in CSM range from 0.01% to 1.3%. and is reported to lower utilization of feed and depress growth in fish (Raj, 1987). Gossypol is also reported to cause deficiencies of some amino acids, such as methionine, when indigestible gossypol–protein complexes are formed. Francis *et al*. (2001) reported poor growth and feed utilization when *O. niloticus* was fed diets containing high levels of gossypol. It is most likely that the low response in fish growth observed at 25% PJSM and 0% inclusion of PJSM in diets may have partly been caused by interference of digestive enzymes by gossypol, because of the high levels of CSM.

At 75 and 100% inclusion of non-ensiled PJSM, a lower growth of *O. niloticus* was observed. The seeds of *P. juliflora* are not widely used as an ingredient in animal feeds due to the presence of antinutrients (Pugalenthi *et al*., 2004). Kathirvel & Kumudha (2011) reported the presence of total free phenolics, tannins, phytic acid, trypsin inhibitor activity and lectins in *P. juliflora* seeds whereby total free phenolics and tannins had the highest concentrations. In the present study, inclusion of non-ensiled PJSM at 75 and 100% levels led to poor growth of *O. niloticus,* possibly due to a high concentration of antinutrients in the respective diets. The inference that high concentration of antinutrients was due to high inclusion levels of PJSM in *O. niloticus* diets, concur with the findings of Madalla (2008), who reported higher levels of phenols, tannins and saponins when Moringa leaf meal was increased in Nile tilapia diets.

The findings of the present study and those of earlier studies suggest that CSM or PJSM, cannot be used individually as main suppliers of dietary protein in *O. niloticus* diets. This may be due to high concentrations of some antinutrients and nutrient deficiencies. When the two ingredients are combined and included in proper proportions, complementation of nutrients and dilution of anti-nutrients may occur. The complementation and dilution are likely to enhance palatability and availability of nutrients leading to improved growth of fish. The observed rejection of pellets when fish were fed diets containing CSM or PJSM as the main suppliers of protein could be associated with the presence of tannins and saponins. Saponins lower palatability due to their astringent taste (Agbo *et al*., 2011).

There was no significant difference on growth performance of *O. niloticus* upon replacement of Cotton Seed Meal (CSM) with ensiled PJSM at 25, 50 and 75%. Replacement of CSM with ensiled PJSM at 100%, led to significant decline in growth of *O. niloticus*. There was no difference in growth of *O. niloticus* fed on control diet and diets containing ensiled PJSM at 25, 50 and 75%. This could be attributed to enhanced nutrient availability and removal of toxins because of hydrolysis during the ensiling process. This view is supported by findings from a study by Cavalheiro, de Souza & Bora (2007), in which fishmeal substituted with shrimp waste silage at 33.3% and 66.6% led to similar growth performance. The significant reduction in *O. niloticus* growth, observed at 100% inclusion of ensiled PJSM, was possibly caused by hydroly-

sis resistant antinutritional factors. This finding presents the same opinion with the results of Soltan & Tharwat (2006) who reported significant reduction in growth, where fishmeal was replaced with an ensiled mixture of fish by-products and rice bran.

5.4 Survival rates of fish fed diets containing ensiled and non-ensiled PJSM

Survival of *O. niloticus* fed diets containing ensiled PJSM, was higher compared to fish fed diets having non-ensiled PJSM. The presence of antinutrients such as tannins, saponins, among others, in diets, may have had toxic effects (Kumar, 2003) to the fish fed diets containing non-ensiled PJSM, and this may have influenced the low survival rate.

5.5 Water quality variables and growth performance

The water quality variables in the present study were monitored to ensure that fish were reared within optimum required conditions for growth. The monitored variables ranged from 3.40-3.93 mg l^{-1}, for DO; 27.03-27.33^{0}C, for temperature and 7.17-7.23, for pH. For optimal growth, *O. niloticus* require DO above 3 mg l^{-1} (Ross, 2000); temperature between 22-29 ^{o}C (Sarig, 1969; Mires, 1995) and a pH range of 7-9 (Ross's study (as cited in Mjoun *et al.,* 2010). However, other studies have reported a relatively higher temperature range (30-32 ^{o}C) for tilapia optimal growth (Popma & Masser, 1999). In this study, DO, temperature and pH, remained within the optimal levels for culturing *O. niloticus*. Therefore, water quality conditions did not influence the growth performance trend, given that DO, Temperature and pH values did not differ significantly with the weights of fish for the different treatments.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Inclusion of non-ensiled PJSM in dietary formulations, at 25 and 50 % enhanced growth performance of *O. niloticus* compared to the control, whereas inclusion beyond 50 % led to growth decline. Inclusion of non-ensiled PJSM at 50% led to the highest growth performance in this study.

There was no difference in growth performance of *O. niloticus* fed on cotrol diet and diets containing ensiled PJSM at 25, 50 and 75%. This suggests that ensiling led to enhanced growth especially when PJSM was included in diets beyond 50%, due to reduction and destruction of antinutrients such as, flavonoids, alkaloids, saponins and tannins in PJSM. Thus, ensiling improved the inclusion level of PJSM from 50 to 75 % in diets of *O. niloticus.*

Ensiling led to reduction of crude protein, crude fibre, trace elements and elimination of anti-nutritional factors such as flavonoids, phenols and saponins. However, ensiling led to increase in Nitrogen free Extracts (NFEs) as a result of hydrolysis of crude fibre into simple carbohydrates. Dissolved Oxygen (DO), temperature and pH were within acceptable ranges for *O. niloticus* growth and did not influence growth performance, in the present study.

6.2 Recommendations

1. It is recommended that non-ensiled *P. juliflora* seed meal (PJSM) can replace cottonseed meal (CSM) up to 50%, whereas ensiled PJSM can replace CSM up to 75% in *O. niloticus* diets.
2. Though ensiling did not destroy all the antinutrients in PJSM, in this study, it is a simple and convenient way of processing fish feed ingredients.

6.2.1 Recommendation for further research

1. The dietary formulations used in this study, especially those containing up to 50% non-ensiled PJSM and up to 75% ensiled PJSM, should be tested in a semi-intensive system, such as hapa nets, to try the possibility of better growth performance of *O. niloticus*.
2. For better growth in aquarium tanks, the diets can be fortified with vitamin and mineral premixes.
3. Finally, ensiling is likely to give better results if it can be used along with other methods, such as soaking, boiling, autoclaving, roasting and drying for complete destruction of antinutrional factors in PJSM.

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APPENDICES

Appendix I: Slope values for fish fed on diets containing non-ensiled PJSM and probability values showing how individual slopes differ from the control slope.

ANCOVA				
Parameter	Estimate	Standard Error	T Statistic	P-Value
Contol LnL	2.707	0.012	216.984	0.000
LnL* Diet B (25%)	0.001	0.020	0.072	0.943
LnL* Diet C (50%)	0.073	0.021	3.517	0.000
LnL* Diet D (75%)	0.035	0.021	1.614	0.107
LnL*Diet E (100%)	-0.015	0.022	-0.690	0.490

Appendix II: Slope values for fish fed on diets containing ensiled PJSM and probability values showing how individual slopes differ from the control slope.

ANCOVA				
Parameter	Estimate	Standard Error	T Statistic	P-Value
LnL* Diet A (Control)	2.70210000	0.010765	251.01500	0
LnL* Diet B (25%)	0.00518726	0.015756	0.329223	0.742000
LnL* Diet C (50%)	-0.0181506	0.014998	-1.21017	0.226200
LnL* Diet D (75%)	-0.0107900	0.015545	-0.69412	0.487600
LnL*Diet E (100%)	-0.0400181	0.015582	-2.56816	0.010200

Appendix III: Multiple Regression, LnW, for *O. niloticus* fed diets containing non-ensiled PJSM, against DO, pH and Temperature

		Standard	*T*	
Parameter	*Estimate*	*Error*	*Statistic*	*P-Value*
LnW	-3.13326E-11	0.0237228	-1.32078E-9	1.0000
DO	0.00146021	0.0278281	0.0524726	0.9581
pH	-0.0334084	0.0283416	-1.178780	0.2385
Temp	0.04796500	0.0324196	1.479510	0.1390

Dependent variable: LnW; Independent variables: DO, pH and Temperature

Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	2.925	3	0.975	0.970	0.404
Residual	1773.080	1773	1.000		
Total (Corr.)	1776.000	1776			

Appendix IV: Multiple Regression, LnW, for *O. niloticus* fed diets containing ensiled PJSM, against DO, pH and Temperature

		Standard	*T*	
Parameter	*Estimate*	*Error*	*Statistic*	*P-Value*
CONSTANT	7.95724E-12	0.023697	3.3579E-10	1.0000
DO	0.0457944	0.0295312	1.55071	0.1210
PH	0.0483644	0.0266489	1.81487	0.0695
Temp	0.0272305	0.0302007	0.901651	0.3672

Dependent variable: LnW; Independent variables: DO, pH and Temperature

Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	6.770	3	2.260	2.260	0.076
Residual	1769.230	1773	0.990		
Total (Corr.)	1776.000	1776			

Appendix V: Essential amino acid (EAA) profiles for CSM, PJSM and the essential amino acid requirements for *O. niloticus*

Essential amino acid (EAA)	**EAA requirements for *O. niloticus***	**EAA profile for PJSM**	**EAA for CSM .**
Lysine	5.12	7.90	4.20
Arginine	4.20	8.70	11.1
Histidine	1.72	1.50	2.90
Valine	2.80	5.20	4.30
Leucine	3.39	8.60	5.90
Isoleucine	3.11	2.80	3.20
Threonine	3.75	2.30	3.30
Tryptophan	1.00	ND; 0.75 (in FAO, 1973)	1.10
Methionine/ Cystine	3.21	Trace; 2.48 (in FAO, 1973)	3.03
Phenylalanine with Tyrosine,	5.54	4.50	7.80
	Adapted from (Santiago and Lovell, 1988)	Adapted from- Kathirvel and Kumudha (2011)	Adapted from (Heuzé *et al*., 2015)

Appendix IV: Experimental photos

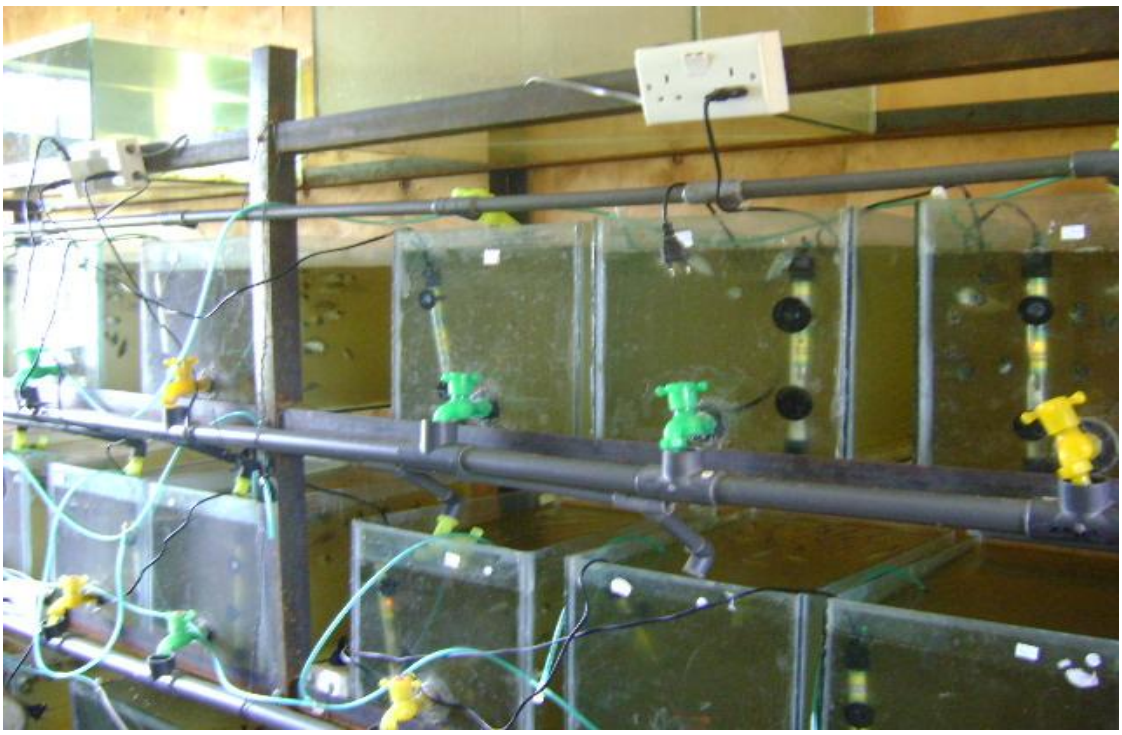

Plate 1: Growth performance experimental set up at Zoology Laboratory, University of Eldoret (Source: Author, 2016)

Plate 2: Back distillation during determination of crude protein, Fisheries Laboratory 1, University of Eldoret (Source: Author, 2016)