EFFECT OF *Glycine max*, *Rastrineobola argentea* AND *Caridina nilotica* MEALS ON WATER QUALITY, GROWTH PERFORMANCE AND SURVIVAL OF *Oreochromis niloticus* FRY

BY

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DECLARATION

I, the undersigned, certify that this thesis is my original work and has not been submitted for an award of a degree in any other university or institution.

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DEDICATION

I dedicate this thesis to the Almighty God and to my beloved family members.

ABSTRACT

Nile tilapia (*Oreochromis niloticus*), one of the main stay of Lake Victoria fisheries, was introduced in the 1950s and 1960s to boost capture fisheries. However, its production has declined dramatically over the years due to over exploitation and its consumption preference by the ever increasing Kenyan population. The decline of capture fisheries has made aquaculture production be considered as the future solution to the declining of capture fisheries and the availability of fish globally. Production of *O. niloticus* is still low in developing countries because of inadequate quality fingerlings and unavailability of good quality fish feeds due to high cost and scarcity of fishmeal raw materials. Hence, an eight-week experimental study was undertaken to determine the effect of soya bean meal (SBM) with reference to common fish feed ingredients like, Rastrineobola *argentea* meal (RAM), Freshwater shrimp (*Caridina nilotica*) meal (CNM) and commercial fish meal (CFM, control diet on water quality, growth performance and survival of *O. nilotica* fry. The study was done at the National Aquaculture Research Development and Training Centre (NARDTC) in Sagana, Kirinyaga County. Each tank was randomly stocked with 50 fry (mean wt. of 0.4 ± 0.01g) in triplicate glass aquaria tanks measuring 67.5 cm by 34.4 cm by 32.0 cm and fed on diets comprising RAM, SBM, CNM or CFM. Feeding was performed manually at 3% wet body weights twice daily in equal rations at 10. 00 hrs. and 16.00 hrs. except during the sampling days. Essential water quality parameters [temperature, dissolved oxygen (DO) and pH] were monitored daily at 9.00 hrs. and 15.00 hrs. The fry was randomly sampled for determination of specific growth rate (SGR) and feed conversion ratio (FCR) on a weekly basis between 7.30 hrs. to 9.00 hrs. Survival was determined on daily basis for eight weeks. Using Graph Pad prism software, data were analyzed using one-way analysis of variance to compare SBM and each of the three diets. Data revealed that there was no significant difference in water quality parameters between the experimental groups ($p > 0.05$) and the parameters were within the acceptable range, temperature (25-30^0 C), dissolved Oxygen (>3mg/¯L) and pH (6.5-9.0). The weights of fingerlings fed on SBM was higher than those fed on RAM or CNM ($p > 0.05$). However, those fed on CFM was considerably higher than those fed on SBM, but not significantly different. Specific growth was not significantly different ($p > 0.05$). The percent SGR in weight per day of fry fed on SBM was considerably higher than those fed on either RAM or CNM ($p > 0.05$). The differences in the FCR of fry fed on the three experimental diets were statistically significant ($p < 0.05$). The length gains over the 8-week study period of fry fed on SBM was considerably higher than those fed on RAM or CNM ($p < 0.05$) but not CFM. The percent SGR in length per day of fry fed on SBM was considerably higher than those fed on either RAM or CNM, but not CFM ($p < 0.05$). The survival of fry fed on SBM was significantly higher than those fed on RAM and CNM ($p < 0.05$). The SBM is not only considerably better than RAM and CNM but is also comparable to CFM in terms of its effects on water quality, growth performance and survival of *O. niloticus* fry. The result gave a distinctive evidence on the viability and sustainability of using soya bean meal as a complete diet for fry to fry in regard to fish farming. Therefore, from the study results, it is possible to completely replace fish meal with SBM in the diet of ON in aquaculture. Farmers could adopt the use of BM as replacement for expensive commercial feeds and very competitive fish ingredients like *R. argentea* or *C. nilotica*- in raising *O. niloticus* fry to fry. Further research is necessary to evaluate the full potential of SBM on grow-out *O. niloticus*.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BWG	Body weight gain
Ca	Calcium
Cl	Chlorine
CNM	*Caridina nilotica* meal
CP	Crude protein
EFA	Essential fatty acids
ESPFFEPP	Economic Stimulus Project Fish Farming Enterprise Productivity Program
FAO	Food Agricultural Organization
FCR	Feed conversion ratio
$\mathbf{H_2SO_4}$	Sulphuric Acid
K	Potassium
MC	Moisture content
MT	Metric tonne
Na	Sodium
NaOH	Sodium hydroxide
NARDC	National Aquaculture Research Development and Training Centre
$\mathbf{NH_3}$	Ammonia (gas)
$\mathbf{NH_4}$	Ammonium ions
RAM	*Rasterineobola argentea* meal
SBM	Soya bean meal
SGR	Specific growth rate

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

In aquaculture, feed accounts for over 50 percent of the production cost. Although considerable variation exists, cereal grains are the usual sources of carbohydrates in most of the aqua feeds and these cannot be economically supplemented with other sources (FAO, 2016). Fishmeal is the single most important source of protein in fish feed. The increased cost of energy (due primarily to soaring petroleum prices), El Niño effects, and increasing demand have resulted in a global increase in fishmeal price. The world price for fish feed ingredients averagely increased by 20-92 percent during the period between June 2007 and June 2008 (FAO, 2009). The increasing price of feed ingredients, in particular, fishmeal, fish oil and cereal, and increasing manufacturing and transportation costs have, therefore, had a compound effect on global production and the price of aqua feeds (FAO, 2009).

Aquaculture has not been immune to this global phenomenon, and specifically smallholders and rural farmers who are particularly susceptible to this global change. Therefore, the fallout may further contribute to their poverty and vulnerability, which could eventually induce small-scale producers to change businesses and/or may result in loss of livelihood.Freshwater aquaculture in Kenya started in 1920's and became popular in 1960's. However, it stagnated until 2003 when the production rose from 1000 metric tons (MT) to 4000 MT (FAO, 2016). Between the years 2006 and 2009, aquaculture production remained below 4895 MT until 2010 when 12,153 MT was realized (FAO, 2016). The government's nationwide Economic Stimulus Project - Fish Farming Enterprise Productivity Program (ESPFFEPP), which for the first time, received substantial funding triggered a rapid growth in the sector (Musa et al., 2012), and supported fish farmers by subsidizing fry, feed and pond construction. Despite the gains in growth following the ESP-FFEPP, aquaculture production in Kenya reduced from 24,096 MT in 2014 to 18,656 MT in 2015 and further to 14, 952 MT in 2016 (Opiyo et al., 2018). According to State Department of Fisheries (SDF), this decline is associated largely with low quality and quantity of fish farm inputs (SDF, 2016). Thus, there is urgent need to improve the production of freshwater aquaculture to bridge the deficit by, addressing the most pressing challenges on the unavailability of efficient and inexpensive farm-made feeds for different stages of fish development as was reported by Munguti et al., (2012).

Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1757) is a worldwide important species in aquaculture because of its fast growth, firm and tasty flesh, resistance against harsh conditions and ease production of fry under captivity (de Graaf et al., 1999; Gómez-Márquez et al., 2003). In the wild, *O. niloticus* starts to reproduce at a total length of 20–30 cm (150–250 g) (Gwahaba, 1973). However, under captivity *O. niloticus* reaches sexual maturity at a relatively smaller size of 8–13 cm (Suresh & Bhujel, 2012). Its suitability in aquaculture revolves around its ability to tolerate a wide range of environmental conditions as well as high plasticity in its food habits (Shoko et al., 2016). The fish also accepts a wide range of foods including those at lowest trophic level and the detrital food chain (Offem et al., 2009). *O. niloticus* represents 75% of the total fish produced from aquaculture in Kenya. In Kenya, the main challenge faced by the aquaculture industry is lack of quality seeds and feeds (Shitote et al., 2013; Munguti et al., 2014). Further, the survival rates in most hatcheries in Kenya are low (Opiyo et al., 2018), hence unable to meet the demand for *O. niloticus* fry by the farmers. Thus, there is need to develop quality feeds that would in turn lead to quality *O. niloticus* seeds for optimal production.

The major constraints faced by fish farmers in the feed sector in aquaculture production in Kenya, include; availability of required ingredients of desired quality and consistency nearer to the aquaculture farm site, appropriate processing techniques, easy accessibility to commercial feeds (Orina et al., 2018). One of the most pressing challenges in aquaculture in Kenya is the unavailability of efficient and inexpensive farm-made feeds for different stages of fish development (Munguti et al., 2012).

The quality of feed refers to the nutritional as well as the physical characteristics of the feed that allows it to be consumed and digested by the fish. Feed should be palatable to the fish with a good taste, smell and feel, and the contents of protein must be high, ideally in the range of 38-44% of crude protein for omnivorous and herbivorous fishes. In fish farming, nutrition is critical because feed represents 50% of the production costs (FAO, 2014). Feeding of fish in an aquaculture facility aims at producing the maximum weight of marketable fish within the shortest time possible and at a low cost. The feed to be used should be able to supply the necessary energy for movement and provide nutrients for body maintenance and growth. The contents of *O. niloticus* meal should be 28-45% protein, 0-90% fat, 1-46% fiber, and 1-13% ash (Cromwell, 2012; Kashindye, 2013; Phani, 2013). Compared to nursing of early *Clarias* species fry that require live or frozen zooplankton, nauplii or decasulated Artemia eggs, require a more precise and conscientious care as it uses artificially formulated diets. Therefore, deficiency of any nutrient, if severe enough, can adversely affect fish health either directly, by impairing metabolic functions, or indirectly, by making fish more susceptible to opportunistic disease-causing agents and may result into fry or fry mortality (Kashindiye et al. 2013).

Protein as one of the essential nutrients in fish growth, has been described as the most expensive and important components of fish feed and its major source is fishmeal. This is due to its high protein content, well balanced essential amino acid and fatty acid profiles, and also because of the high correlation between body Indispensable Amino Acid (IAA)and the IAA requirement pattern (Mambrini and Kaushik,1995). However, fishmeal is implicated in the prohibitive cost of sustainable aquafeed production because of its price increase, dwindling supply and competition from other users. This has necessitated increased research into alternative protein sources for fish (Ayoola, 2011).

Fish feeds are usually formulated from a combination of various ingredients to give a desirable crude protein (CP) level. In Kenya, most fish feeds contain *R. argentea* and/or *C. nilotica* as the major component(s). The silver cyprinid (Rastrineobola argentea) also known as the Lake Victoria sardine or Omena in Kenya, Mukene in Uganda and Dagaa in Tanzania, is a species of pelagic, freshwater ray-finned fish in the carp family, Cyprinidae. It ranks second to Nile perch among fish catches from Lake Victoria and it is the only endemic fish species which has remained abundant in Lake Victoria since the introduction of Nile perch, (*Lates niloticus* L), and *O. niloticus* (Kayenda et al., 2011; Wandera,1988). It is importantly used as food by the riparian communities notably in the areas of nutritional values, food security, incomes and foreign exchange earnings for the region (LVFO, Regional synthesis report, 2016). It has high crude protein content (>58.8%), rich in iron, zinc and calcium. However, it lacks critical components such as vitamins and omega-3 fatty acid, hence may not prove suitable as an independent O. *niloticus* diet (Musiba et al., 2014). Despite the high nutritive value, only up to 30% of the harvested is used for human consumption leaving the bulk (70%) for production of animal feeds (Kabahenda et al., 2011). In 2013, Society International Development reported that 42% of the 24 million children under the age of 5 years in East African Community had a stunted growth due to malnutrition and yet the region's production of *R. argentea* can sustainably cub the malnutrition problem.

Rastrineobola argentea's catch rate has presently declined due to its high demand as a result of overfishing and use of illegal gears during fishing expeditions in the lake by fishermen. It is overexploited for various uses hence its population has dwindled and efforts should be directed towards conservation of this species. Therefore, using it in fish meal may not be ecologically viable and sustainable for large scale fish feed production (Ogello et al., 2014).

Soya beans are species of legume most widely consumed as food worldwide. The nutritional suitability of soya beans includes vitamins (K- riboflavin, folate, B6- thiamin, and C), organic compounds (iron, manganese. Potassium, Phosphorus, Magnesium, zinc, selenium and calcium), and significant amount of dietary fiber, a very large amount of protein, and antioxidant (Jour et al., 2010). Soya bean is locally and readily available and it is the best plant protein source that is favoured as a supplement in diets of most farmed animals, especially *Oreochromis niloticus* species (Shiau et al., 1989; Ogello et al., 2014). Soya bean meal contains CP level comparable to CP levels of standard fish feeds (40 - 45% CP) in Kenya (Munguti et al., 2014).

Rastrineobola argentea are shiny silvery looking small pelagic fish which constitutes one of the main fish species in Lake Victoria. Its nutritional suitability includes high crude protein value, crude dietary fiber, good level of crude fat/lipid and ash. The high protein intake helps fish in rapid growth and healthy life (Kang'ombe, 2011). *Rastrineobola. argentea* has been used traditional in fish feeds (Olukayode & Emmanuel, 2012; Ogello et al., 2014), but due to its high demand and need for its conservation it may not be considered as a major source of proteins in commercial feeds, and therefore the potential of SBM in aquaculture need to be fully investigated as an alternative source of protein. Thus, there is need to evaluate the effect of SBM on growth performance and survival of *O. niloticus* fry in order to produce quality viable fingerlings. The use of SBM could reduce the cost of raising high quality *O. niloticus* fry, which is a major challenge in aquaculture industry.

Caridina nilotica is a species of freshwater shrimp in the family of Atyidae. *C. nilotica* in the present food web, is an important food source for the fish stocks and a major prey of the Nile perch in Lake Victoria (Bundi, 2015). This has increased predation pressure on its fishery. Therefore, making its future availability doomed if allowed to be used for aquaculture fish feed production. *C. nilotica* is seasonally harvested in Lake Victoria hence is not always available for feed manufacturers. Therefore, a fish feed ingredient that would supply the requisite nutrients and is readily available is required.

Commercial fish feeds are an important part of modern commercial aquaculture. They provide the balanced nutrition needed by farmed fish to grow to their full potential. Fish feeds since 1979 has had fishmeal and fish oil as the key components for farmed fish. Other ingredients like vegetable proteins, cereal grains, vitamins and minerals are usually combines in smaller proportions. Initially the fishmeal and fish oil came from processing of fish from the wild catch that were not suitable for human consumption. The type of fish species used could not attract a higher price in the market, thus referred to as reduction fisheries (IFFO, 2019). Since there is increase in demand for fish by the consumers around the world due to the ever growing population, increased income and greater awareness of fish as part of a healthy diet, a drive in research and development towards the replacement of fishmeal and fish oil by plant based protein is paramount. This would allow fish to continue providing the important health benefits for consumers, especially the low income earners within the communities.

Soya bean meal costs significantly less than most animal meals, including fish meal (Ayoola, 2011). In Kenya, market price of a two-kilogram of *Rastrineobola argentea* costs about USD. 3, depending on the season, *Caridina nilotica* costs USD 2 per two-kilogram tin, while Soya bean costs USD. 1.5 per two-kilogram tin. Moreover, the soya bean's quantity is more than that of *R. argentea* and *C. nilotica* at the same weight. While the cost of commercial fish feed especially Aller aqua (mostly used by fish farmers) ranges between USD. 1.80 to 2.0 per kilogram. Therefore, reducing feed cost is critical to improving efficiency and maintaining sustainability in aquaculture operations in Kenya.

Water is a critical factor in the life of all aquatic species. In aquaculture, any characteristic of water that affects the survival, reproduction, growth, or management of fish in any way is a water quality variable (Boyd, 2003). Fish performs its physiological activities in the water medium, hence, the overall performance of any aquaculture system is partly determined by its water quality (Alam & Al-Hafedh, 2006). Poor water quality stresses adversely affect fish growth with consequently low production and product quality (Iwama et al., 2000). Fish farmers are obliged to manage the water quality so as to provide a relatively stress-free environment that meets the physical, chemical and biological standards for the fishes' normal health and growth performance (Isyiagi et al., 2009). Thus, it is imperative to assess the effect of any fish feed formulation on the water quality parameter. However, the effect of soya bean meal, *R. argentae* meal, and *C. nilotica* meal during growth of fry to fingerlings in hatcheries on water quality parameters has not been fully investigated.

The growth performance and survival of fry raised on soya bean meal or soya bean-formulated diet has been previously evaluated. For instance, several studies reported the growth performance of *O. niloticus* raised on soya bean (Saijadi & Carter, 2004; Vielma et al., 2004; Tacon, 2008;

Nangas et al., 2008; Phani, 2013; Olaifa *et al*., 2010; Craig, 2012).However, the previous reports only evaluated soya bean as supplement hence, may not fully inform on its performance on *O. niloticus* growth. Despite soya bean having comparable crude protein levels to the standard feeds in Kenya (Munguti et al., 2014), the effect of SBM on growth performance and survival of *O. niloticus* fry remains largely unknown. The use of SBM could reduce the cost of raising high quality *O. niloticus* fry which is currently the major bottleneck in aquaculture industry.

Therefore, in the present study, the effect of SBM with reference to common fish feed ingredients [*R. argentea* meal (RAM) and *C. niloticus* meal (CNM)] and commercial fish meal (CFM) on water quality, growth performance (weight, length, feed conversion ratio and specific growth rate) and survival of *O. niloticus* fry was evaluated

1.2 Statement of the Problem

Aquaculture has been identified as sector that can be used to fight against poverty and malnutrition in developing countries. One of the big four agenda of the Kenyan government is food security where aquaculture has been considered a driving force. However, the major problems derailing aquaculture production are, unavailability of quality inputs in terms of seedlings and feeds besides credit, inadequate infrastructure, technology, poor extension support and ineffective or nonexistent policies as well as ensuring an investment climate conducive to industrial development in the sector. Quality seedlings and feeds as some of the most important in-puts, remain a big challenge. Fry survival depends on feeds, however the currently used feeds must be formulated and heavily rely on fishmeal's which are expensive, ecologically unsustainable and in most cases lack vital nutritive components. A plant based protein source readily available as an alternative to fish based meals is therefore required. Soya beam meal has the potential to provide the required nutritional needs of fry. However, the plant is administered to fish as a formulation and therefore its potential as an independent meal for fish fry growth and survival has not been investigated.

This may reduce the high dependency on use of fishmeal (*R. argentea*) whose production has already declined dramatically over the years due to over exploitation as fishmeal raw materials, competition for human consumption, animal feed and chicken feed production. Soya bean meal is a potential replacement of commercial feed since its crude protein levels is comparable to that of standard *O. niloticus* feeds used in Kenya. However, the effect of SBM on the water quality parameters, besides growth performance and survival of *O. niloticus* fry has not been fully evaluated. Thus, the present study evaluated the suitability of SBM in *O. niloticus* fry culture. This could provide quality feeds which in turn results in quality seeds hence optimal production to bridge the fish demand deficit.

1.3 Objectives of the Study

1.3.1 General Objective

To determine the effect of SBM, RAM, CNMon water quality, growth performance and survival of *O. niloticus* fry.

1.3.2 Specific Objectives

1. To determine growth performance of *O. niloticus* fry fed on SBM, RAM, CNM and CFM (control diet).
2. To determine survival of *O. niloticus* fry fed on SBM, RAM and CNM and CFM (control diet).
3. To determine the effect of feeding *O. niloticus* fry on SBM, RAM, CNM and CFM (control diet) on the water quality parameters.

1.3.3 Null Hypotheses

1. There is no significant difference in growth performance of *O. niloticus* fry fed on SBM, RAM and CNM and CFM (control diet).
2. There is no significant difference in survival rate of *O. niloticus* fry fed on SBM, RAM and CNM and CFM (control diet).
3. There is no significant difference on water quality on *O. niloticus* fry fed on SBM, RAM, CNM and CFM (control diet).

1.4 Justification of the Study

The aquaculture production in Kenya has dropped drastically since 2014 and with the average per capita annual fish consumption of 5Kg/person/year, falling below the FAO requirements of 20Kg/person/year. This is because aquaculture production has numerous challenges related to the fry. First, there is inadequate supply of fry to meet the demand by fish farmers. Secondly, quality *O. niloticus* fry feeds are largely unavailable or expensive (Munguti et al., 2014; Opiyo et al., 2018). Thus, there is need to develop a cost effective and readily available feeds that would guarantee production of high quality *O. niloticus* fry. In view of the above, the study explored the suitability of various meal diets for raising *O. niloticus* fry. Soya bean is recognized as a high-quality, and very digestible feed ingredient that is favored for addition to the diet of most farm animals, especially fish and shrimp. soya bean meal has; 45-53% protein, 5-39% fiber, 7.01% ash, 20% fats/lipids, 35% carbohydrates, vitamins (K- riboflavin, folate, B6- thiamin, and C), organic compounds (iron, manganese. Potassium, Phosphorus, Magnesium, zinc, selenium and calcium), and significant amount of dietary fiber, and antioxidant (FAO, 2012). Soya bean is also one of the world's best non-fish sources of essential omega-3 fatty acids, healthy proteins and unsaturated fats. In addition, soya bean has been widely used as a supplement in the formulation during production of livestock and human/baby foods (FAO, 2016). It is equally important to note that, deficiency of any nutrient, if severe enough, can adversely affect fish health either directly, by impairing metabolic functions, or indirectly, by making fish more susceptible to opportunistic disease-causing agents. Considering that the cost of soya bean is significantly less than most animal meals, including fish meal, its usage could reduce feed cost hence improving efficiency and maintaining sustainability in aquaculture operations. Therefore, replacement of fish ingredients with locally available plant material such as soya bean meal which is highly digestible and less expensive is imperative and could improve *O. niloticus* production.

1.5 Significance of the Study

First, an increased growth performance and survival of fry fed on soy bean compared to commercial feed could inform policy makers on the use of legume**s**in *O. niloticus* fingerling production and reducing over-reliance on fishmeal. Secondly, the adoption of soya bean as the main fingerling diet could lead to large-scale production of soya bean crop seeds by the farmers for *O. niloticus* fry resulting into improved incomes and livelihoods by the farmers. Third, the observed adoption of soya bean into fish meal may advance knowledge and understanding of aquaculture science on the use of seed crops in raising and developing *O. niloticus* fry.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Aquaculture

Aquaculture in Kenya comprises of freshwater and mariculture. Mariculture involves the farming ofmarine fish species such as, finfish (Milk fish) (Chanos chanos) and Grey mullets (*Mugil cephalus*); Shellfish (Mud crabs) (*Scylla serrata*), Oysters (*Saccosteria cucullata*), shrimp (*Penaeus monodon*) and Seaweeds (mainly *Kappaphycus alvarezii*) (Munguti et al., 2014). Mariculture is underdeveloped mainly due to accessibility problems, conflicts over land ownership, and lack of clear policies (KMFRI, 2017). Current mariculture production data indicates that there are over 100 MT of seaweeds, milkfish, shrimps, and mud crabs produced in small scale (Munguti et al., 2014) This is lower than the production of freshwater aquaculture which is currently at 14,852 MT (KMFRI, 2017).

Fresh water aquaculture involves cold and warm water culture. Cold water culture involves Rainbow trout (*Oncorhynchus mykiss*) in the Mount Kenya region while warm water fishes comprises of *O. niloticus* constituting 75%, African catfish (*Clarias gariepinus*), and other species comprising 25% (Mbugua, 2008). There have been efforts to culture some indigenous fish, like the African carp (*Labeo victorianus*), Ngege (*O. esculentus* and Victoria tilapia (*O. variabilis*) (Maithya et al., 2017; Orina et al., 2018). However, culture of these indigenous species have remained on experimental basis and are not widely adopted by farmers due to low survival and poor yields (Orina et al., 2018).

From the nutritional point of view, fish is a composite of very high nutritional quality, it is rich in most vitamins, proteins, minerals, fats and essential amino-acid and a very valuable nutritious part of human diet. This idea has been justified by some biological experiments that have proved its nutritional equivalency to those of meat, milk, and eggs (FAO, 1995a). This has properly placed fish in an especially important category of food, in which its unavailability for human consumption may lead to serious malnutrition.

The average per capita annual fish consumption in 2010 was 5 kg $person^{-1}$ $year^{-1}$ which is below the FAO recommended average of 20 kg $person^{-1}$ $year^{-1}$ and the contribution of fish to overall animal protein intake in Kenya is still very low (5.7%) (FAO, 2016). Freshwater fish consumption in 2014 was estimated at 195,206 tonnes. To meet the gap between fish production locally and the increasing demand for food fish, Kenya imports about 5900 MT annually from other countries such as China, India, Pakistan, Japan, Korea and Uganda (SDF, 2014). The bulk of imports in 2013 were frozen *O. niloticus* (14%) originating from China. Total fish imports reached 5853 MT in 2014, whilst those of *O. niloticus* increased from 14% (2013) to 30.8% (2014) (SDF, 2014).

The Kenyan government nationwide Economic Stimulus Project - Fish Farming Enterprise Productivity Program (ESPFFEPP), which for the first time, received substantial funding triggered a rapid growth in the sector (Musa et al., 2012), and supported fish farmers by subsidizing fry, feed and pond construction. During the ESP-FFEPP, earthen ponds were constructed in most parts of the country after mapping areas which were suitable for aquaculture. The ESP-FFEPP was implemented within the 2009 and 2010 financial year, leading to an increase in fish pond area from 220 ha in 2008 to 468 ha in 2009 and a total gross land for aquaculture from 728 ha (2008) to 825 ha (2009) (Opiyo et al., 2018). Despite the gains in growth following the ESP-FFEPP, aquaculture production in Kenya reduced from 24,096 MT in 2014 to 18,656 MT in 2015 and further to 14, 952 MT in 2016 (Figure 2.1) (Opiyo et al., 2018). The establishment of county governments and subsequent removal of aquaculture from the functions of the national government to county governments also led to a reduction in aquaculture activities in several counties in Kenya which lacked support programs for fish farming (SDF, 2016).

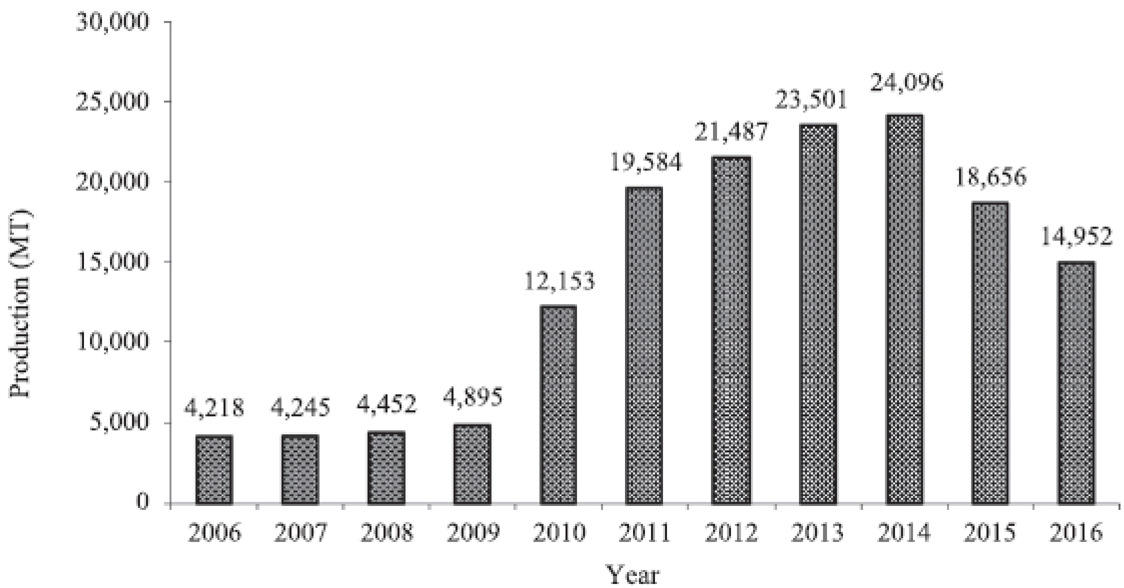

Figure 2.1. Aquaculture production in Kenya. The trends in aquaculture production over a ten-year period (Adapted from Opiyo et al., 2018).

2.2 Physical and Ecological Aspects of *Oreochromis niloticus*

Oreochromis niloticus is silver in colour with olive/grey/black body bars. It often flushes red during the breeding season (Picker & Griffiths, 2011). *O. niloticus* grows to attain a maximum length of 62 cm and 3.65 kg (FAO, 2012). The average total length of *O. niloticus* is 20 cm (Bwanika *et al.* 2004). It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation (Picker & Griffiths, 2011). *O. niloticus* is an omnivore, hence feeds on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films and even other fish and fish eggs (FAO, 2012). *O. niloticus* feed either via suspension filtering or surface grazing, trapping plankton in a plankton rich bolus using mucus excreted from their gills

(Fryer & Iles 1972). *Oreochromis niloticus* exhibit trophic plasticity according to the environment and the other species they coexist with (Bwanika et al.,2007).

Oreochromis niloticus can live longer than ten years. Food availability and water temperature are the limiting factors to growth for *O. niloticus* (Kapetsky & Nath, 1997). Optimal growth is achieved at 28-36°C and reduces with decreasing temperature (FAO, 2012). Their ability to vary their diet may also result in variation in growth (Bwanika et al.,2007). In aquaculture ponds, *O. niloticus* can reach sexual maturity at the age of 5-6 months (FAO, 2012).

2.3 Aquaculture Potential of *Oreochromis niloticus*

Oreochromis niloticus is a worldwide important species in aquaculture because of its fast growth, firm and tasty flesh, resistance against harsh conditions and ease production of fry under captivity (de Graaf et al., 1999; Gómez-Márquez et al., 2003). In the wild, *O. niloticus* begins to reproduce at a total length of 20–30 cm (150–250 g) (Gwahaba, 1973). However, under captivity *O. niloticus* reaches sexual maturity at a relatively smaller size of 8–13 cm (Suresh & Bhujel, 2012). Its suitability in aquaculture revolves around its ability to tolerate a wide range of environmental conditions as well as high plasticity in its food habits (Shoko et al., 2016). The fish also accepts a wide range of foods including those at lowest trophic level and the detrital food chain (Offem et al., 2009).

2.4 Fish Nutrition and Feed Ingredient

Nutrition is vital in fish farming because it addresses the interaction of nutrients and other food substances in relation to maintenance, growth, reproduction, health and disease of an organism. Fish require high protein diet because it promotes their optimal growth and health, but as much as 65% of the protein may be lost to the environment (Munguti et al. 2014). Mostly in form of nitrogen is excreted as ammonia (NH_3) by the gills of fish, while only 10% is lost as solid wastes. Lipids typically comprise about 15% of fish diets, supply essential fatty acids (EFA) and serve as transporters for fat-soluble vitamins. Up to about 20% of dietary carbohydrates can be used by fish vitamins being organic compounds are necessary in the diet for normal fish growth and health (Lim & Webster, 2006).

Soy beans or soya beans (Glycine max) are a type of legume native to eastern Asia. They are a species of legume most widely consumed as food and its products are readily available in form of soy flour, soy protein, tofu, soy milk, soy sauce, and soybean oil (Food Data Central, 2019). The nutritional suitability of soya beans includes vitamins (K- riboflavin, folate, B6- thiamin, and C), organic compounds (iron, manganese. Potassium, Phosphorus, Magnesium, zinc, selenium and calcium), and significant amount of dietary fiber, a very large amount of protein, and antioxidant (Kang'ombe, 2011). Soybeans contain phytonutrients that are linked to various health benefits and a rich source of various plant compounds, including isoflavones, saponins, and phytic acid. Isoflavones in particular mimic estrogen (McMichael-Philips et al., 1989) and responsible for many of soybeans' health effects including Cancer in women-breast and men – prostrate (De Mejia et al., 2003). Soya bean meal appears as a promising substitute since it is an affordable high quality source of protein. However, the presence of anti-nutritional factors like trypsin inhibitors, lectins, glycinin, ß-conglycinin, saponins, phytates, and oligosaccharides do negatively affects the growth and the general health of fish, thus limiting its inclusion as fish food (Castro, Maria, 2014). To reduce anti-nutritional factors that degrade protein ability and decrease flow of dietary amino acids to the intestine, soya bean meal is commonly heat or chemically treated. According to Graham, and Vance,2003, proper heat treatment is critical to achieve optimum quality of soya bean; undercooked soya bean, retains anti-nutritive factors, while overcooked soya bean results in damaged amino acids, particularly Lysine, resulting in reduced biological availability. Heat treated SBM increases the total flow of essential amino acids to the intestine and also supports higher milk yield during processing in the factory (Castro, Maria, 2014).

Rastrineobola. argentea are shiny silvery looking small pelagic fish which constitutes one of the main fish species in Lake Victoria. Its nutritional suitability includes high crude protein value, crude dietary fiber, good level of crude fat/lipid and ash (*R. argentea* has 47.9% to 58.8% CP, 5.0% to 7.5% CF, 1.5% to 18.2% ash)(Mustapha et al., 2014; Phani, 2013; Ambitsi et al., 2007)**.** A part from the mentioned nutritive values of *R. argentea*, some of the health benefits include; the availability of calcium as a nutrient that is essential for formation of strong bones and teeth. Calcium is also known to be important for normal cell functioning, muscle contraction, message transmission through nerves and release of hormones. The high protein intake helps fish in rapid growth and healthy life. *Rastrineobola argentea*is used in animal feeds because of its high nutritional quality for fish growth and also the most popular food around Lake Victoria and highly targeted by fishermen due to its market driven demand (Munguti et al. 2014).

*Caridina nilotica*is a species of freshwater shrimp in the family of Atyidae**.** Its nutritional suitability includes 33.0% to 63.5% CP, 1.8% to 5.0% CF and 2.5% to 22.8% ash (Mustapha et al., 2014; Phani, 2013; Ambitsi et al., 2007)**.** *Caridina nilotica* in the present food web, is an important prey item food source for many fish species and a major prey of the Nile perch and *R. argentea* in Lake Victoria, the second largest fresh water lake in the world (Bundi, 2015; Budeba and Cowx, 2007; Matsuishi et al.,2006; Balirwa et al., 2003). It is also being used as bait in haplochromine hand-line fisheries (Budeba, 2007). The sustainability of the fisheries of Lake Victoria depends on among other things, abundance and availability of *C. nilotica*. Its decline in the aquatic ecosystem may deprive other fish species that solely depend on it as their food, a chance of growing. This is one of the reasons of choosing it as an important treatment diets in the study.

Commercial fish feeds in Kenya, usually contain 24–30% crude protein for *O. niloticus* (Liti et al., 2006). These feeds are too expensive for some farmers such that, most farmers use locally formulated mixed feeds (Charo-Karisa et al., 2013). The feeds are made by mixing dried freshwater shrimp (*C. nilotica*), with rice bran or maize bran with *R. argentea*) meal (Munguti et al., 2014). This practice does not lead to formulation of balanced diets required by the fish leading to poor growth and nutritional deficiencies (Opiyo et al., 2018).Micro-minerals (trace minerals) are required in small amounts as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. In order for fish to grow fast and healthy, it needs to be fed with feeds which contain all essential nutrients, however getting quality fish feeds has always been a challenge to African nations since commercial feeds are unaffordable to their small-scale fish farmers. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health, but a gap steal lingers on the scientifically proven plant protein based fish feeds (Lim & Webster, 2006; Craig, 2012; Kashindye et al.,2013).

Fish seed are sourced from hatcheries which are either owned by the government or private farmers. Between the years 2010 to 2016, the government owned National Aquaculture Research and Development Training Centre (NARDTC), Sagana supplied 30.3% of fry and fry while private hatcheries contributed 69.7% (Nyonje et al., 2018). The total demand for both African catfish and tilapia fry across Kenya was estimated at 100 million $yr-^{1}$ in 2010 (Musa et al., 2012). The common methods used in Kenya for fingerling production are open ponds, tanks and hapas in ponds. Fry are collected from the spawning units at 0.03–0.05 g and stocked into nursery units for rearing to the fingerling stage (5 g) before they are stocked into grow out facilities. Currently, there are a total of 127 authenticated hatcheries in Kenya with a capacity to produce 96 million fry annually (Nyonje et al., 2018).

Early juvenile fish (0.02-10.0 g) require a diet higher in protein (45-50%), lipids (10%), vitamins (trace elements) and minerals (trace elements) and lower in carbohydrates (8% crude fiber) than sub-adult fish (10-25 g) which require protein (30-35%), more energy from lipids (10-15%) and carbohydrates (10%) for metabolism. Adult fish (>25.0 g) require even less dietary protein (28-30%) for growth and can utilize even higher levels of carbohydrates (maximum of 40%) as a source of energy. The contents of an ideal fish meal are; protein-60-77%, fat 10-15%, fiber 1.46%, and ash 13-9%, and that of soya bean meal are; protein 45-53%, Fat 10-25%, Fiber 5.39%, and ash 1-7.01% (FAO, 2015; Kashindye, 2013; Phani, 2013; Cromwell, 2012; Nyandat et al., 2007).

Studies have reported the growth performance of *O. niloticus* raised on various diets. Workagegn et al. (2014) reported the growth performance and feed utilization efficiency of juvenile Nile tilapia, *O. niloticus* L. fed different types of diets formulated from six experimental diets containing soybean, bone meal and groundnut as basal feed ingredients which accounted as 60% of the total amount of ingredients. The highest growth performance in terms of final body weight, weight gain and specific growth rate, and feed utilization efficiency were observed on the fish fed diet control diet followed by the fish fed diet with 40% wheat and rice, while the fish fed diet with 40% coffee husk had the lowest. The survival rates were similar regardless of the dietary content. However, the study evaluated the performance of soy bean as a supplement, hence may not inform on the full potential of SBM in *O. niloticus* fry culture.

In another study, the growth performance and survival rate of *O. niloticus* subjected to different feeding frequencies were evaluated in cage culture. Juveniles were fed a diet containing 30% CP composed from Niger seed (*Guizotia abyssinica*) cake (20%), mill sweeping (16%), meat and bone meal (28%),wheat bran (32%) and wheat flour (4%)] at 3% of their body weight. The mean specific growth rates (SGR), feed conversion ratio (FCR), mean weight gain, mean daily gain and condition factor significantly varied with the frequency of feeding (Alemayehu & Getahun, 2017). Nevertheless, the study only focused on the effect of feeding frequency and not on different meals and only focused on byproducts as ingredients.

2.5 The use of Soya Bean as Fish Feed

Dry soybeans comprise of34% to 53%, proteins, 35% carbohydrate, 20% fat, about 5% ash, and is high in dietary fiber. Soya bean is considered to be a source of complete protein (i.e. contains significant amounts of all the essential amino acids) and as such a candidate for addition to the diet of most farmed animals and fishes (Mustapha et al., 2014; Phani, 2013; Ambitsi et al., 2007).

According to Cromwell, (2012), soybean is also one of the world's best non-fish sources of essential omega-3 fatty acids, healthy proteins and unsaturated fats. Soy proteins are fed to farmed fish and shellfish to support their growth and healthy development because soybeans are the best non-fish sources of essential omega-3 fatty acids, healthy proteins and unsaturated fats. Furthermore, Kapinga et al.(2013), observed that soya bean meal when fed fish do provide a consistent quality, supply of nutrients, the most common source of plant protein used in compound aquaculture feed, and the most prominent protein ingredient substitute for fishmeal in aquaculture feeds.

A comparative study of tilapia feeds by Mlaponi et al. (2013) based on animal blood protein (25% crude protein), soya bean (25% crude protein), and a market based commercial feed) also indicated a better growth performance for the soya bean diet selective to the others, therefore supported this assertion. As a substitute to fish meal in tilapia diets, SBM has been used together with croquette seed meal (Kashindye et al., 2013), Azolla Africana meal, Cassia fistula meal (Adebayo et al., 2004), alfalfa meal (Krichen, 2007), tomato by-product meal, ulva meal, cotton seed meal and waste date meal (Azaza et al., 2006). However, little is known on its use as fish meal.

According to Roman-Eguia et al. (2013) in Asia, many fish feed studies and fish breeders have ascertained that without supplemental feeding, the resultant outcome is always invariably least number of fish and lowest body weights. Supplemental feeds so far have improved the production of cultured fish, but in ideal situation, producers use fish as protein source which has resulted in decline of the particular species used.

2.6 Effect of Environmental Conditions on the Growth and Survival of Farmed Fish

Water is a critical factor in the life of all aquatic species. In aquaculture, any characteristic of water that affects the survival, reproduction, growth, or management of fish or other aquatic creatures in any way is a water quality variable (Boyd, 2003). In all culture systems, fish performs its physiological activities such as breathing, excretion of wastes, feeding, maintaining salt balance and reproduction in the water medium. The overall performance of any aquaculture system is partly determined by its water quality (Alam & Al-Hafedh, 2006). Poor water quality stresses and adversely affects fish growth with consequently low production, profit and product quality (Iwama et al., 2000). Production is reduced when the water contains contaminants that can impair development, growth, reproduction or even cause mortality to the cultured species

(Shoko et al., 2014). Monitoring water quality is therefore an important procedure in aquaculture as a way of keeping in check the levels of the various parameters and adopting various intervention measures in situations where values are in levels that are detrimental to the production of the cultured organisms. The highest survival rates have been reported in fish fed on quality artificial diets compared to those that rely on natural feeds within their environment (Sanker et al., 2017). However, little is known on fish fed on meals.

2.6.1 Temperature

Temperature range of 25-30°C as ideal for the production of tilapia in the tropics. The values outside this range impede biochemical reactions in fish, thus affecting their growth as well as quality of their meat or muscles. This is because temperature has a direct effect on enzymatic activity, also controls the rate of response to toxic chemicals. For example, it accelerates the conversion of ionized ammonia to non-ionized form which is more toxic. Temperatures above 30°C are known to encourage stress induced disease and mortality which in turn lowers survival (Yaro et al., 2005).

2.6.2 Dissolved Oxygen

Dissolved oxygen (DO) refers to the level of free, non-compound oxygen present in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a body of water. In limnology, DO is an essential factor to the fish growth second to water itself so much that when the level is too high or too low can harm aquatic life and affect water quality. Low DO is usually the first water quality constraint to fish growth in intensively managed ponds (Shanmugaarasu et al., 2016). Commonly cultured species of tilapia and the catfish usually survive routine dawn DO concentrations of less than 0.5 mg/L, levels considerably below the tolerance levels for other cultured fish species (Shoko et al., 2015; Shanmugaarasu et al., 2016) survival in water with low DO is due, in part, to their ability to extract DO from the film of water at the water –air interface when DO is below 0.1 mg/L. In aquarium tanks, the provision of aerators generates sufficient oxygen and helps in maintaining relatively high DO levels. Furthermore, fish tend to raise DO content level because they stir up the water and increase the contact between water and air. The activities of fish can also make the distribution of oxygen more uniform (Shoko et al., 2015; Shanmugaarasu et al., 2016).

2.6.3 pH

Shanmugaarasu et al. (2016) reported diurnal pH range of 6.5 - 9.0 as ideal for optimum production of fish in the tropics. In a study, Yaro et al. (2004) reported that prohibition of recycling of nutrients in ponds at values below 5.5 reduces fish appetite. Hossain et al. (2017) found that pH values >8.5 encourages nitrogen loss through ammonia volatilization, a process primarily controlled by photosynthetic aquatic biomass.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

An eight-week study on the effect of SBM, RAM and CNM for *O. niloticus* fry was carried out between August 2015 to October, 2015 at the National Aquaculture Research Development and Training Centre, Sagana (NARDTC) (Latitude of 0°40'10.25"S and longitude of 37°12'22.01"E). The Centre is located in Kirinyaga County, approximately 104 Km Northeast of Nairobi City, at an altitude of 1231 meters above mean sea level (Appendix 1). The farm is supplied with water from River Ragati by gravity all-year round. The water is diverted through a 1 m wide and 0.5 m deep canal. The average speed of the feeder canal is 63.5 cm/second.

3.2 Experimental Design

All the raw materials (soya been seeds, dried *Rastrineobola argentea* and dried *Caridina nilotica*) used for diet preparations were purchased from the local market (Gikomba) in Nairobi, Kenya, and processed into flour form at NARDTC, except for Commercial feed meal (Aller aqua-from Germany). The test trial feeds were not formulated because the interest was to find out the ability (efficacy) of *O. niloticus* fry to feed and survive on soya bean meal, and SBM's effect on water quality during growth period.

Three day old *Oreochromis niloticus* fry were sourced from brooders' ponds within NARDTC and stocked into acclimatization tank measuring 1 m x 0.5 m x 0.5 m for 14 days while being fed on a commercially formulated fish feed. No mortality was experienced on the fry during acclimatization period. Fifty uniform-sized (0.4 ± 0.01 g) healthy fry were randomly distributed, in triplicate, to glass aquarium experimental (treatments) tanks measuring 67.5 cm, 34.4 cm and 32.0 cm. There were three experimental groups i.e.T1 (SBM-43.6% CP), T2 (RAM- 62.8% CP), T3 (CNM- 38.4% CP), and control T4 (CFM- 28% CP), commercial control diet).The fry was starved for 24 hours to eliminate variation in weight due to residual food in the gut, prepare the gastro-intestinal tract for the experimental diets and increase the appetite of the fry. Each of the feed was administered twice daily to the fry at 10.00 hrs. and 16.00 hrs after working out their feed rations, and was done manually at 3% of fish's wet body weights for 8 weeks. Each tank had an inlet and outlet connected to a water drainage system. The pumping unit consisted of two water pumps and an aerator which ran alternatively for twenty-four hours to ensure continuous water flow.

3.3 Processing of Raw Materials and Preparation of Test Diets

Soya bean seeds were sorted, roasted to remove anti-nutritional inhibitors, cooled and milled into flour. The flour was sieved to remove larger particles that could hinder the palatability of the feed to fry. The sieved soya bean flour was further dried in an oven at 105°C for 6 hours and allowed to cool.

Dried *R. argentea* and *C. nilotica* fish meals from Lake Victoria, were sorted out to remove all the unwanted materials and further sun dried before subjecting to proximate content analysis. The two were separately milled using the normal flour miller and sieved to remove large particles. The sieved *R. argentea* and *C. nilotica* flour was further dried in an oven at 105°C for 6 hours and allowed to cool. The prepared experimental feeds were packaged and stored in a cool dry place. No formulation of the prepared feeds took place as it was not part of the objectives of the study.

3.4 Water Quality Measurements

Water quality parameters, which included temperature, pH, and dissolved oxygen (DO) were measured daily in situ using a multi-purpose model Hi-9024 microcomputer (Hanna Instruments

Ltd., Chicago, and IL., USA. Water samples for unionized ammonia and nitrites were collected in

small bottles from experimental tanks on sampling days (weekly basis) (I have not seen results for these nutrients anywhere in the document) . Sample filtered using 0.45 µm membrane filters after sub-sampling 100 ml, preserved using concentrated H_2SO_4 and stored in polyethylene bottles under refrigeration at about 4°C for further analyses.

3.5 Proximate Content Analyses of Fry Diets

In this study, the potential of raising *O. niloticus* fry on SBM was evaluated in reference to RAM, CNM and CFM. Prior to the evaluation in terms of effect on water quality, growth performance and survival, the proximate contents of the feeds such as crude protein, lipid, moisture and ash were analysed as described by AOAC, 1995 method and reported in table 3.1. The analysis was applied to the test trial feeds as a control to check whether they meet the specifications or requirements established by WHO and FAO standards.

Table 3.1. Proximate content (%) of fish diets used

Parameters	SBM	RAM	CNM	CFM
Moisture content	11.2	12.1	6.94	12.2
Ash	11.5	13.6	10.4	11.6
Fat/lipid	10.5	8.8	5.11	8.5
Crude protein	43.6	62.8	38.4	28

Note:

RAM: *R. argentea* meal

CNM: *C. nilotica* meal

SBM: Soya bean meal

CFM: Commercial feed meal.

Daily feed supplied was recorded and few remnants collected two hours before the start of feeding. The amount of food to be provided being adjusted following weekly sampling for the determination of gain in weight and length per treatment which lasted for eight weeks (56 days). Each tank was completely drained and thoroughly scrubbed on the day of sampling.

Measurement and analysis? This sentence is hanging

3.6 Sampling Design and Data Collection

At stocking, fifty uniform-sized (0.4 ± 0.01 g) healthy O. *niloticus* fry were randomly distributed, in triplicate, to glass aquarium experimental (treatments) tanks measuring 67.5 cm, 34.4 cm and 32.0 cm after taking their weights and lengths. The tanks for test treatment diets were labelled as; T1 (SBM-43.6% CP), T2 (RAM- 62.8% CP), T3 (CNM- 38.4% CP), and T4 (CFM- 28% CP), commercial control diet).*Oreochromis niloticus* fry for each treatment diet were weighed and length measured using an analytical balance (Shimadzu Analytical Balance AUW320series) and a ruler after placing each fry on a glass petri dish. This was done to determine the initial weight (W_{t1}) and total length ((Ln_{t1}) respectively, and recorded. The recorded weights and lengths of fry were calculated to determine their feeding rations**.** Each week between 7.30 hours to 9.00 hours, all the survived fish (fry) from each tank were individually weighed (W_{t2})and their lengths(Ln_{t2}) measured for calculation and recorded on weekly basis using the same procedure during stocking and marked as the sampling day. The procedure was concurrently done weekly at the same time until the end of the experimental period. The procedure was repeatedly done on weekly basis so as to determine the size and length at that time and immediately returned to their various cleaned and marked aquaria tanks. Growth and feed efficiency were monitored in terms of the final weight, weight gain (expressed as the percent of initial body weight at the end of the experiment), specific growth rate (SGR) (In final body weight – In initial body weight/time, expressed as % per day),and feed conversion ratio (FCR, or feed fed / wet weight gain). All the calculations were done based on each feed test treatment.

The water quality in the aquarium tanks i.e. temperature (˚C), pH, and DO (mg/L) were recorded daily at 8.00 hours and 15.00 hours before administering feeds. The survival rates were based on the number of deaths recorded on daily basis from each tank and was calculated per test treatment.

3.7 Growth Parameters for *O. niloticus* fry

The following formulae were used to calculate variables in this study:

$$\text{Length gain} = \text{Average final total length} - \text{Average initial total length}$$

Weight gain = Average final weight – Average initial weight

% SGR in weight = In (final weight in grams) – In (initial weight in grams) X 100/t (in days)

SGR in length = In (LT(t+i)/LT (t)

Where: LT = Total length of fish

LT (t+1) = next length at age (t+1)

t = Initial length at age t

$$\text{FCR} = \frac{\text{Total feed given (g)}}{\text{Body weight gain (g)}}$$

$$\%\ \text{Survival rate} = \frac{\text{Total number survived}}{\text{Total number stocked}} \times 100$$

3.8. Statistical Analyses

All data generated during the study were stored in Microsoft Office Excel. Averages were calculated using Microsoft Office Excel. Statistical analysis and graphs were performed using Graph Pad Prism software version 5.03 (Graph Pad Software Inc., California, USA). Distribution of data generated was verified using D'Agostino and Pearson omnibus K2 test. Data were presented as mean ± standard error of mean (SEM). Statistical comparisons in the water quality parameters for treatments groups were performed using one-way analysis of variance (ANOVA) with Dunnett's post-test where there were significance differences. Further, statistical differences in the growth parameters of fry fed on different diets were determined by one-way ANOVA. Similarly, statistical differences in the survivals of fry fed on different diets were determined by one-way ANOVA. A difference with a $p< 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1. Growth Performance of *O. niloticus* fry fed on RAM, CNM, and SBM or CFM

4.1.1 Weights

The weights of *O. niloticus* fry fed on CFM was not significantly different from those fed on SBM, RAM or CNM (Figure 4.1A; One-way ANOVA, $p = 0.2231$). Furthermore, there was no significant difference among and between the treatment groups (SBM, RAM and CNM) (Figure 4.1B; $p = 0.2537$). The weights of fry fed on CFM was considerably higher than those fed on each of the three diets. Further, fry fed on SBM exhibited considerably higher weight followed by RAM then CNM.

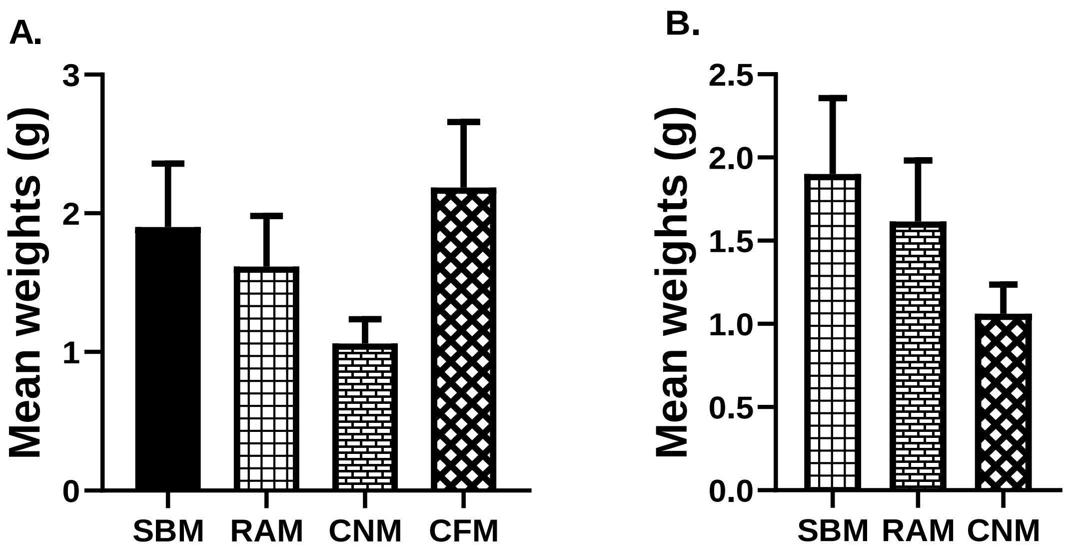

Figure 4.1. Weights of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The mean weights of CFM versus each of the other diets (4.1**A**), and among and between treatment diets (4.1**B**) are presented. The experiment was performed in triplicates. The bars depict mean ± SEM. There was no statistical difference as determined by one way-ANOVA (p > 0.05).

Further, weights gain by *O. niloticus* fry fed on CFM were not significantly different from those fed on SBM, RAM or CNM (Figure 4.2A; One-way ANOVA, p = 0.1871). Similarly, there was no significant difference among and between the treatment groups (SBM, RAM and CNM) (Figure 4.2B; p = 0.2500). The weight gains by fry fed on CFM was considerably higher than those fed on each of the three diets. Further, fry fed on SBM exhibited considerably higher weight followed by RAM then CNM.

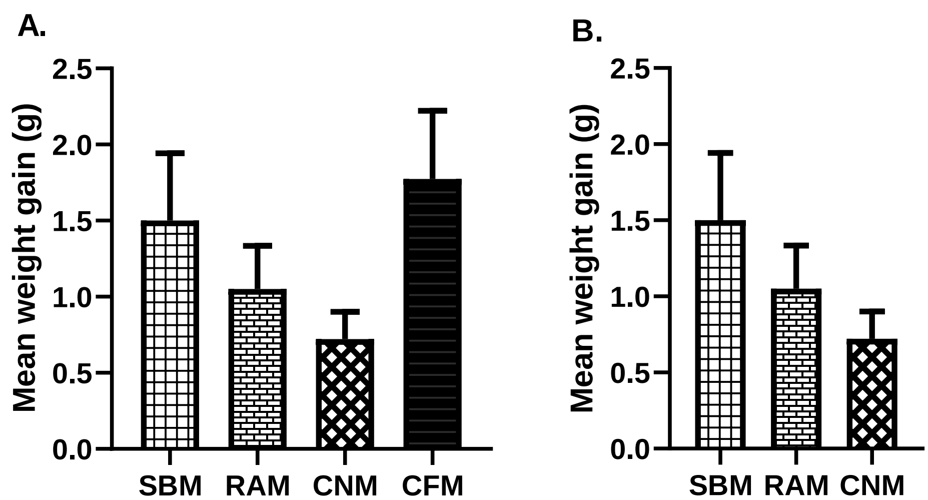

Figure 4.2. Comparison of weight gains of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The mean weight gains of CFM versus each of the other diets (Figure 4.2A), and among and between treatment diets (Figure

4.2B) are presented. The experiment was performed in triplicates. The bars depict mean ± SEM. There was no statistical difference as determined by one way-ANOVA ($p > 0.05$).

4.1.2 Specific Growth Ratein Weight

In the present study, the percent SGR in weight per day was significantly different among fry fed on the four diets (one-way ANOVA, $p = 0.0118$). Dunnett's post-test revealed that the percent SGR in weight per day of fry fed on CFM was significantly higher than those fed on CNM (Figure 4.3A; $p = 0.0056$). However, there was no significant difference in percent SGR in weight per day among and between the treatment groups (Figure 4.3B, one-way ANOVA, $p = 0.0705$). These results indicated that SGR in weight per day of fry fed on CFM was significantly higher than those fed on CNM and considerably higher than those fed on RAM and SBM. Notably, fry fed on SBM had slightly higher SGR in weight followed by RAM then CNM.

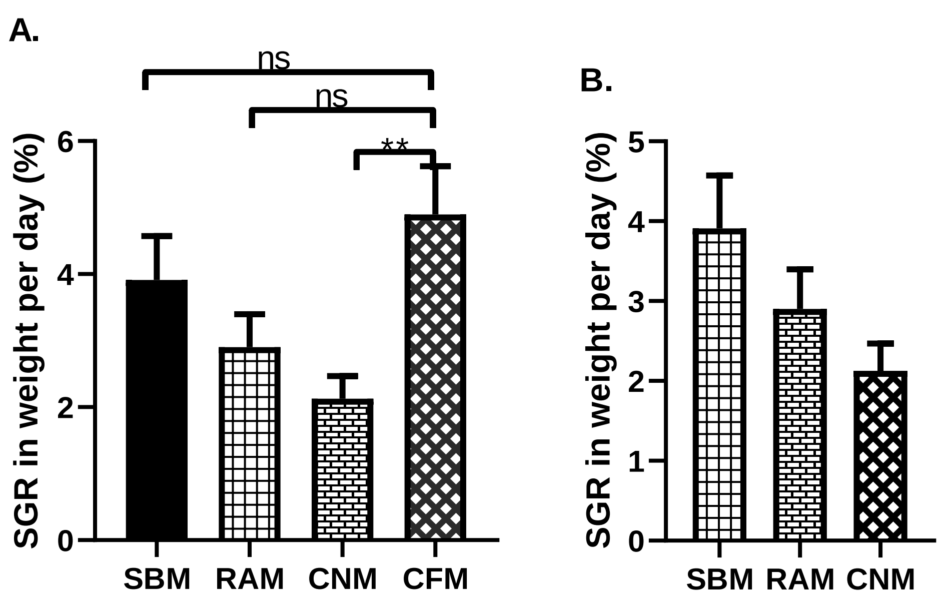

Figure 2.3. Comparison of SGR in weight of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The percent SGR in weight per day of CFM versus each of the other diets (Figure 4.3A), and among and between treatment diets (Figure 4.3B) are presented. The experiment was performed in triplicates. The bars represent mean ± SEM. Statistical comparison between CFM and each of the treatment group was determined using one way-ANOVA with Dunnett's post-test (ns, $p > 0.05$; **, $p < 0.05$).

4.1.3. Feed Conversion Ratio

The FCR of *O. niloticus* fry fed on CFM were not statistically different from those fed on SBM, RAM or CNM (Figure 4.4A; One-way ANOVA, $p = 0.9443$). Moreover, no significant difference was observed in FCR among and between the treatment groups (SBM, RAM and CNM) (Figure 4.4B; $p = 0.9879$). The FCR of fry fed on CFM was slightly higher than those fed on each of the three diets. Further, fry fed on RAM had slightly higher FCR followed closely by SBM then CNM.

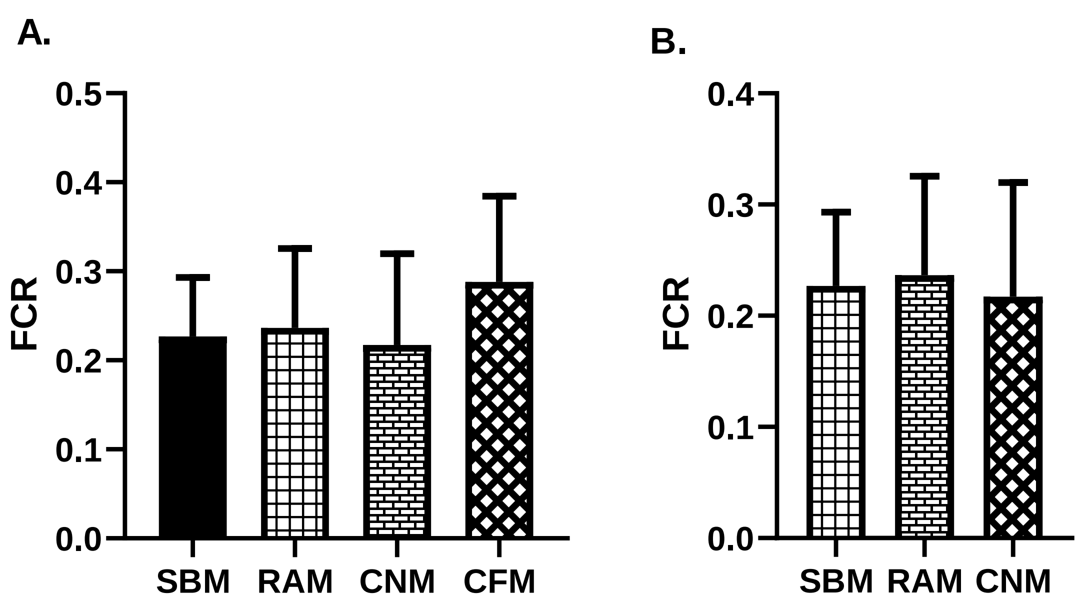

Figure 4.4. Feed conversion ratio of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The mean FCR of CFM versus each of the other diets (Figure 4.4A), and among and between treatment diets (Figure 4.4B) are shown. The experiment was performed in triplicates. The bars showed mean ± SEM. There was no statistical difference as determined by one way-ANOVA ($p > 0.05$).

4.1.4. Lengths

The lengths of *O. niloticus* fry fed on CFM was not significantly different from those fed on SBM, RAM or CNM (Figure 4.5A; One-way ANOVA, $p = 0.1749$). Furthermore, there was no significant difference among and between the treatment groups (SBM, RAM and CNM) (Figure 4.5B; $p = 0.2616$). The lengths of fry fed on CFM was considerably higher than those fed on each of the three diets. Further, fry fed on SBM had considerably higher length followed by RAM then CNM.

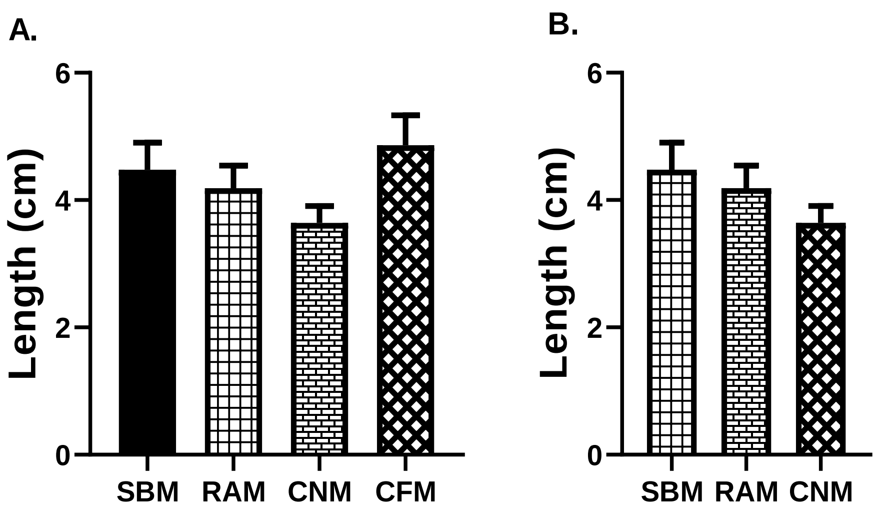

Figure 4.5. Lengths of *O. niloticus* fry fed different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The mean lengths of CFM versus each of the other diets (Figure 4.5A), and among and between treatment diets (Figure 4.5B) are presented. The experiment was performed in triplicates. The bars depict mean ± SEM. There was no statistical difference as determined by one way-ANOVA ($p > 0.05$).

In addition, length gain by *O. niloticus* fry fed on CFM was not significantly different from those fed on SBM, RAM or CNM (Figure 4.6A; One-way ANOVA, $p = 0.1738$). Furthermore, there was no significant difference among and between the treatment groups (SBM, RAM and CNM) (Figure 4.6B; $p = 0.2596$). The length gains by fry fed on CFM was considerably higher than those fed on each of the three diets. Further, fry fed on SBM had considerably higher length gain followed by RAM and CNM.

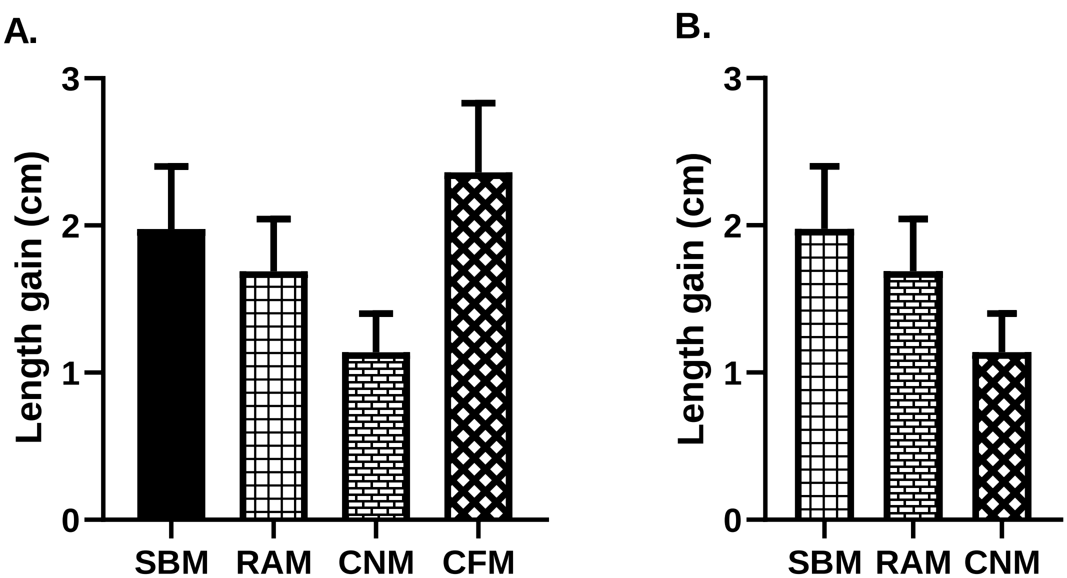

Figure 4.6. Length gains by *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The mean length gains by fry fed on CFM versus each of the other diets (Figure 4.6A), and among and between treatment diets (Figure 4.6B) are shown. The experiment was performed in triplicates. The bars depict mean ± SEM. There was no statistical difference as determined by one way-ANOVA ($p > 0.05$).

4.1.5 Specific Growth Rate in Length

The percent SGR in length per day was significantly different among fry fed on the four diets (one-way ANOVA, $p = 0.0234$). Dunnett's post-test revealed that the percent SGR in length per day of fry fed on CFM was significantly higher than those fed on CNM (Figure 4.7A; $p = 0.0084$). On the contrary, there was no significant difference in percent SGR in length per day among and between the treatment groups (Figure 4.7B, one-way ANOVA, $p = 0.0598$). These results showed that SGR in length per day of fry fed on CFM was significantly higher than those fed on SBM. The fry fed on SBM had slightly higher length followed by RAM then CNM as the trial test diets.

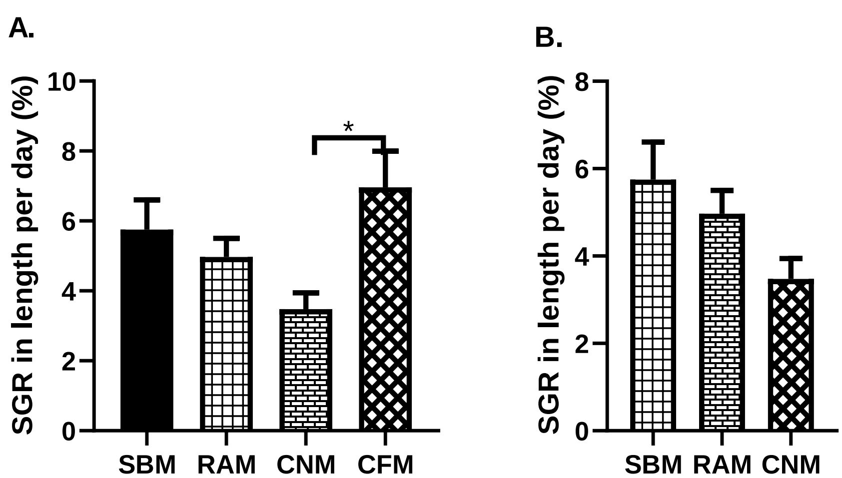

Figure 4.7 SGR in length of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The percent SGR in length per day of CFM versus each of the other diets (Figure 4.7A), and among and between treatment diets (Figure 4.7B) are presented. The experiment was performed in triplicates. The bars represent mean ± SEM. Statistical comparison between CFM and each of the treatment group was determined using one way-ANOVA with Dunnett's post-test (ns, $p > 0.05$; *, $p < 0.05$).

4.2 Survival of *O. niloticus*fry fed on RAM, CNM, and SBM or CFM

In the present study, the survival of *O. niloticus* fry fed on the four diets was evaluated weekly for 8 weeks. The percent SGR in weight per day was significantly different among fry fed on the four diets (Figure 4.8A; one-way ANOVA, $p< 0.0001$). Dunnett's post-test revealed that the percent SGR in percent survival of fry fed on CFM was significantly higher than those fed on SBM ($p< 0.0001$), RAM ($p< 0.0001$) or CNM ($p< 0.0001$). Similarly, However, there was a significant difference in percent survival of fry fed on the treatment diets (Figure 4.8B; one-way ANOVA, $p= 0.0066$). Tukey's post-test revealed that the percent survival of fry fed on CNM was significantly lower than for those fed on SBM ($p = 0.0109$) and RAM ($p= 0.0189$). These results indicated that fry survived better in SBM and best in CFM but poorest in CNM.

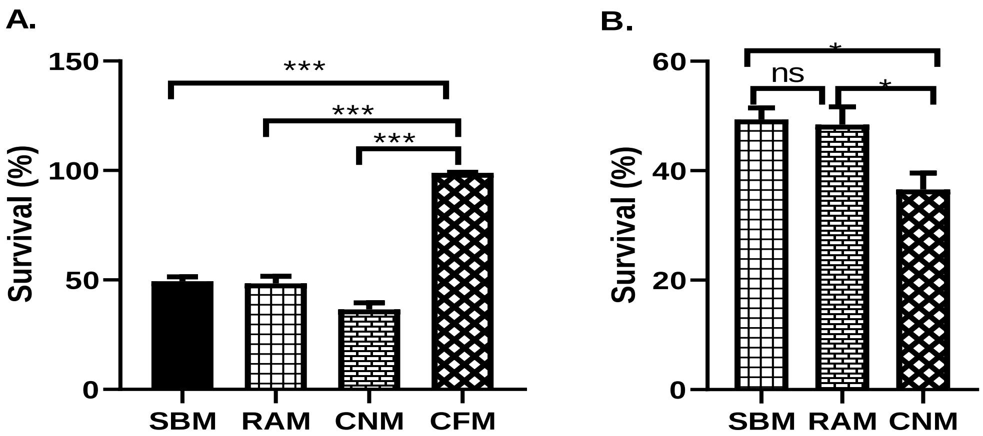

Figure 4.8. Survival rate of *O. niloticus* fry fed on different diets.

The fry was fed on SBM, RAM, CNM or CFM for 8 weeks. The percent survival of fry fed on CFM versus each of the other diets (Figure 4.8A), and among and between treatment diets (Figure 4.8B) are shown. The experiment was performed in triplicates. The bars show mean ± SEM. Statistical comparison was performed using one way-ANOVA with Dunnett's or Tukey's post-test (ns, $p > 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

4.3 Effect of Different Diets on water Quality Parameters

Water quality is a key determinant of the growth performance of any fish species and can contribute to inter-tank variations in growth performance. To this end, the physico-chemical parameters of water, such as dissolved oxygen, temperature, and pH, in each treatment tank was measured to determine if the diets had any effect on water quality.

4.3.1 Water Temperature

There was no significant difference in the water temperatures of the aquaria supplied with the four feeds (One-way ANOVA, $p = 0.8516$). The temperature of aquarium tank supplied with

SBM was slightly higher than that supplied with RAM, CNM or CFM. However, the differences were not significantly different ($p > 0.05$) (Table 4.1).

4.3.2. Dissolved Oxygen

In the present study, no significant difference was observed in the DO of the aquaria supplied with the four feeds (One-way ANOVA, $p = 0.2802$). Although, the DO in aquarium tank supplied with SBM was slightly lower than that supplied with RAM, CNM or CFM, the differences were not significantly different ($p > 0.05$) (Table 4.1).

4.3.3. pH

There was a significant difference in the pH of the aquaria supplied with the four feeds (One-way ANOVA, $p = 0.0152$). The pH of water in aquarium tank supplied with SBM was significantly lower than that supplied with RAM ($p < 0.05$). However, the pH of aquaria supplied with SBM was slightly lower than that supplied with CNM or CFM ($p > 0.05$) (Table 4.1).

Table 4.1. Effect of various diets on water quality

Parameters	SBM	RAM	CNM	CFM
Temperature (°C)	25.82 ± 0.37^{a}	24.94 ± 0.82^{a}	25.71 ± 0.96^{a}	25.55 ± 0.79^{a}
DO (mg/L)	6.01 ± 0.32^{a}	6.02 ± 0.32^{a}	6.37 ± 0.19^{a}	6.79 ± 0.36^{a}
Ph	8.52 ± 0.11^{a}	8.83 ± 0.15^{a}	8.90 ± 0.14^{b}	8.58 ± 0.15^{a}

Note:

Four feeds SBM, RAM, CNM or CFM were applied on aquarium tanks in triplicate for 8 weeks. The temperature, DO and pH of water in the aquarium tanks were measured weekly. The values represent mean ± SEM. Statistical comparison was performed using one-way ANOVA with Dunnett's post-test. Different letters per row depict statistical significance between SBM and any of the other diets ($p < 0.05$).

CHAPTER FIVE

DISCUSSION

5.1 Growth Performance of *O. niloticus* Fry Fed on RAM, CNM and SBM or CFM

Dietary protein is normally considered to be of crucial importance in fish nutrition and feeding, therefore quality and sufficient supply of dietary protein is required for rapid growth (Jauncey and Rose, 1982; Lovell,1989). In the present study, the dietary protein level and other digestible food ingredients (carbohydrates, Minerals, Omega -3 fatty acids and Vitamins) in SBM were adequate to optimize both weight gain and the feeding efficiency in Nile tilapia, *O. niloticus fry*(Figures 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7).

In the present sy, *O. niloticus* fry fed on SBM exhibited comparatively higher growth performance (weights, lengths, SGR and FCR) than RAM and CNM, of which was only comparable to CFM. The outcome indicated that full replacement of fishmeal with soya bean meal did not compromise growth (Figures 4.1, 4.2, and 4.3).

Overall Specific Growth Rate ranged between 1.8% (0.1692 g/day) and 3.8% (1.9 g/day). The present findings agree with several previous reports, which showed that soya bean contributes to good *O. niloticus* growth performance (Saijadi & Carter, 2004; Vielma et al., 2004; Tacon, 2008; Phani, 2013). In contrast, the present findings are not consistent with reports by Olaifa et al., (2010) and Craig & Helfrich, (2012) who used formulated diets and registered stunted growth. The discrepancy between the present and two previous reports could be related to differences in their diet composition and to different rearing conditions. The previous reports only evaluated soya bean as supplement, hence, may not fully inform on the potential of SBM on *O. niloticus* growth performance. The soy bean's nutritional balance as reported by Mlaponi et al., (2015), accounts for 34% to 53%, proteins, 35% carbohydrate, 20% fat about 5% ash, and high in dietary fibre on dry matter basis could have given it a higher chance of maintaining fry's growth increment throughout the study period. This is comparable to the current study on the proximate content analysis performed on the test trial diets prior to commencement of the experiment (SBM had 43.6 % crude protein, 10.5% fat/lipids and 11.5% Ash (Table: 1).The soya bean performance during the experimental period could also be attributed to its high digestibility and increased bioavailability of protein and minerals in the plant as reported by Debnath et al. (2005); Yoo et al. (2005). In the current study, no significant differences ($p>0.05$) were observed in the growth parameters for the three experimental diets administered to the *O. niloticus* fry (Figures 4.1 and 4.2), Weights of *O. niloticus* fry fed on different diets (figure 3), Comparison of weight gains of *O. niloticus* fry fed on different diets (figure 4), Comparison of specific growth rates (SGR) in weights of *O. niloticus* fry fed on different diets, (figure 5) and Feed conversion ratio of *O. niloticus* fry fed on different diets. Data on the body composition of fish allows assessment of the efficiency of transfer of nutrients from feed to fish and helps in predicting the overall nutritional status (Ali et al., 2000). In most cases retention of energy and deposition of new tissue results in an increase in the weight of an animal, and the weight of young fish is usually a reliable indicator of adequacy of the nutritional and management regimes (Kefi, et al., 2013).

In the current study, data on fry's body composition indicated no significant differences ($P>0.05$) among treatments. As reported by Kefi, et al., (2013), an increase in the body protein of fish at the end of any experiment shows that fish's growth is as a result of protein synthesis and tissue production and not only to fish weight but also to lipid deposition of which soya bean is an obvious candidate. The outcome of the study is in agreement with Ayoola's (2010) report, that the protein quality of the feed fed the fish determines whether the feed material accepted by fish is balanced or not. In the current study, the proximate analysis of the test trial diets for experiments revealed that the highest crude fat content was obtained in SBM and this could be due to the availability of sufficient energy in the diet. This assertion is also supported by Abdelghany (2003), who reported that diets containing soya bean meal for *O. niloticus* increased body crude protein.

The present findings demonstrated SBM acceptance amongst the *O. niloticus* fry compared to RAM and CNM. While the low growth in fish fed diet with RAM and CNM, could be attributed to lack of carbohydrate, and vitamin components in the two diets. The outcome suggests that SBM promotes and subsequently leads to increased growth and high yield of *O. niloticus* fry. Thus, SBM could be utilized for raising *O. niloticus* fry to fry stage in hatcheries as an alternative to the costly commercial fish feeds.

5.2 Survival Rate of *O. niloticus* Fry Fed on RAM, CNM and SBM or CFM

One of the important factors in the hatchery venture is the survival rates of fry. An efficient hatchery system should provide conditions that minimize fry mortalities as much as possible. In view of this, the present study evaluated the effect of SBM on fry survival. The survival rate over the eight-week period of *O. niloticus* fry fed on CFM was nearly constant compared to fry fed on SBM and RAM throughout the experimental period. While fry fed on CNM were disadvantage (Figure: 4.8). This could have been attributed to the fact that there was direct continuity in the consumption and utilization of the same feed from the acclimation period to the beginning of experiment. For the fish fed on SBM or RAM, the survival dropped between weeks one to three then remained nearly constant with stunted growth on RAM for the rest of the period. This could have been attributed to lack of essential nutrients or balanced diet in RAM. Further, the results presented demonstrated that the fry survival was dependent on the diet fed with SBM than RAM and CNM diets, as it exhibited higher survival rates (Figure: 4.8). The present findings are consistent with previous findings in which soya bean diet and fishmeal diets were shown to have almost similar survival rates of Juvenile Red Snapper, *Lutjanus campechanus* (Davis et al. 2007). However, the previous report only focused on solvent-extracted soya bean supplements and do not inform on the full potential of SBM in raising *O. niloticus* fry. The drop in survival in the first three weeks could be attributed to the poor uptake of the newly introduced diets to fish fry. It is not clear why the survival rates of fry fed on CNM continued to drop from week one to the seventh week of the study period. A possible explanation for this observation is that CNM could be lacking some essential nutrients required for *O. niloticus* fry survival. From results, CFM exhibited survival rates higher than SBM. A potential explanation for this could be related to the unknown value of amino-acid profile and low mineral intake in soya bean, that limited its efficient utilizationby *O. niloticus*during the experimental period. However, with mineral supplementation, SBM fed fry could result in higher survival rates of *O. niloticus* fry in the hatcheries (El Sayed 1999). Probably a study should be conducted to see the survival rates if the SBM is supplemented with minerals and a known value of amino-acid.

5.3 Water Quality Parameters

Monitoring water quality parameters in fish culture systems is critical as the variables influence fish physiological processes. Water quality is important in fish culture and ensures optimal growth and survival. Thus, in this study, water quality was monitored throughout the experimental period. The ranges from results of water quality parameters were within recommended levels for warm water fish growth and survival (pH-6.5-8.5, Temperature 20-30^0 C and DO >5mg/L—FAO,1993).

The fish diets used did not adversely affect the physico chemical parameters used (Mean range of; dissolved oxygen (DO) 6.01 – 6.79 mg/l, temperature 24.94 – 25.82 °C, pH 8.52 – 8.90).

Feeding rate is known to affect the level of DO (Edea et al., 2018). The high level of DO in this study indicated that the optimal feeding rate was adopted. Feed wastes may lead to water deterioration, thus bringing significant changes in ecosystem structure and functioning (Da, 2012).

The results of the study showed that in order to achieve satisfactory *O. niloticus* fry growth, SBM can comfortably be used as it kept the conditions of all the monitored key water quality indicators well within acceptable ranges (Table 4.1) for fish of the same size.

The physico-chemical parameters obtained in this study were within the standards required for the growth of freshwater fish i.e. a temperature between 17°C and 32°C (Hecht et al., 1988); pH between 6.5-9.0 (Hepher & Pruginin, 1981); DO content ≥ 3 mg/L (Viveen et al., 1985). The present findings demonstrated that SBM is suitable for *O. niloticus* fingerling production since it does not adversely affect the quality of water (temperature-24.94 ± 0.82, DO- 6.79 ± 0.36 and pH- 8.83±0.15) Table: 4.1.

Among the monitored water quality indicators, water temperature has a major influence on the amount of food consumed by a fish (Jobling, 1998)). Barrows and Hardy (2001) concluded that the best temperature for rapid efficient growth is that at which appetite is high and maintenance requirements/or the energy cost of living are low. The scenario remained the same during the eight (8) week study period as feed left overs were not eminently found.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

6.1 Summary

In summary, the present study showed that *O. niloticus* fry fed on SBM exhibited good growth performance just like the commercial feed and considerably higher growth performance than the two trial diets (RAM and CNM). Further, the findings of the present study showed that survival of *O. niloticus* fry fed on SBM was higher than those fed on the other two trial diets (RAM and CNM). The study further, demonstrated that SBM does not affect the water quality parameters and that the parameters were within the accepted limits.

6.2 Conclusions and Implications

This is the first study reporting on the use of unformulated soya bean meal in *O. niloticus* fry production. Based on the results presented, the following conclusions can be drawn:

1. The *Oreochromis niloticus* fry fed on soya bean meal performed as well as those fed on commercial feed. The nutrient composition of both control and experimental diet (SBM) were within the recommended range for *O. niloticus* fry rearing. Thus, soya bean meal could potentially be utilized as the main *O. niloticus* fry diet.
2. The *Oreochromis niloticus* fry fed on soya bean meal had higher survival rates than those fed other trial diets (RAM and CNM). Thus, soya bean meal could be used to raise *O. niloticus* fry with minimal mortalities.
3. The water quality parameters in both experimental and control diets set ups remained constant and within the recommended optimal range throughout the experimental period. As the main trial test diet, SBM is suitable for *O. niloticus* fry culture since it effectively utilized with very minimal or insignificant adverse effect on water quality.

6.3 Recommendations

6.3.1 Recommendation for the Present Studies

1. It is recommended that soya bean meal should be adopted in hatcheries for raising *O. niloticus* fry to fingerling since registered better growth performance and survival rates.
2. We recommend replacement of animal based protein source,*R. argentea* and *C. nilotica* with a plant protein based source, Soya bean, as an austerity measure towards the conservation and preservation of the ecological function of the two animals that are already under threat.

6.3.2 Suggestions for Future Studies

1. The present study only obtained the proximate crude protein content of the diets. There is need to determine the levels of both essential and non-essential amino acids for promoting tissue growth and health of *O. niloticus* fry. Further, there is need for evaluation of Soya bean key components, such as carbohydrates, vitamins and omega-3 and an evaluation of the economics of its use in *O. niloticus* fry production as a substitute to animal protein-based feeds.
2. The present study was conducted in a laboratory-controlled set-up which has its own inherent limitations. Thus, there is need to evaluate the survival and growth performance of *O. niloticus* fry fed on different diets in outdoors or natural conditions. Further studies might be necessary to establish effect of SBM on grow out *O. niloticus*.
3. The present study evaluated the effects of SBM on a few water quality parameters. There is need for a comprehensive analysis on the effects of SBM on several water quality parameters such as nitrates and nitrite which are very paramount in water quality.

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APPENDICES

Appendix 1. Physical map of the study area

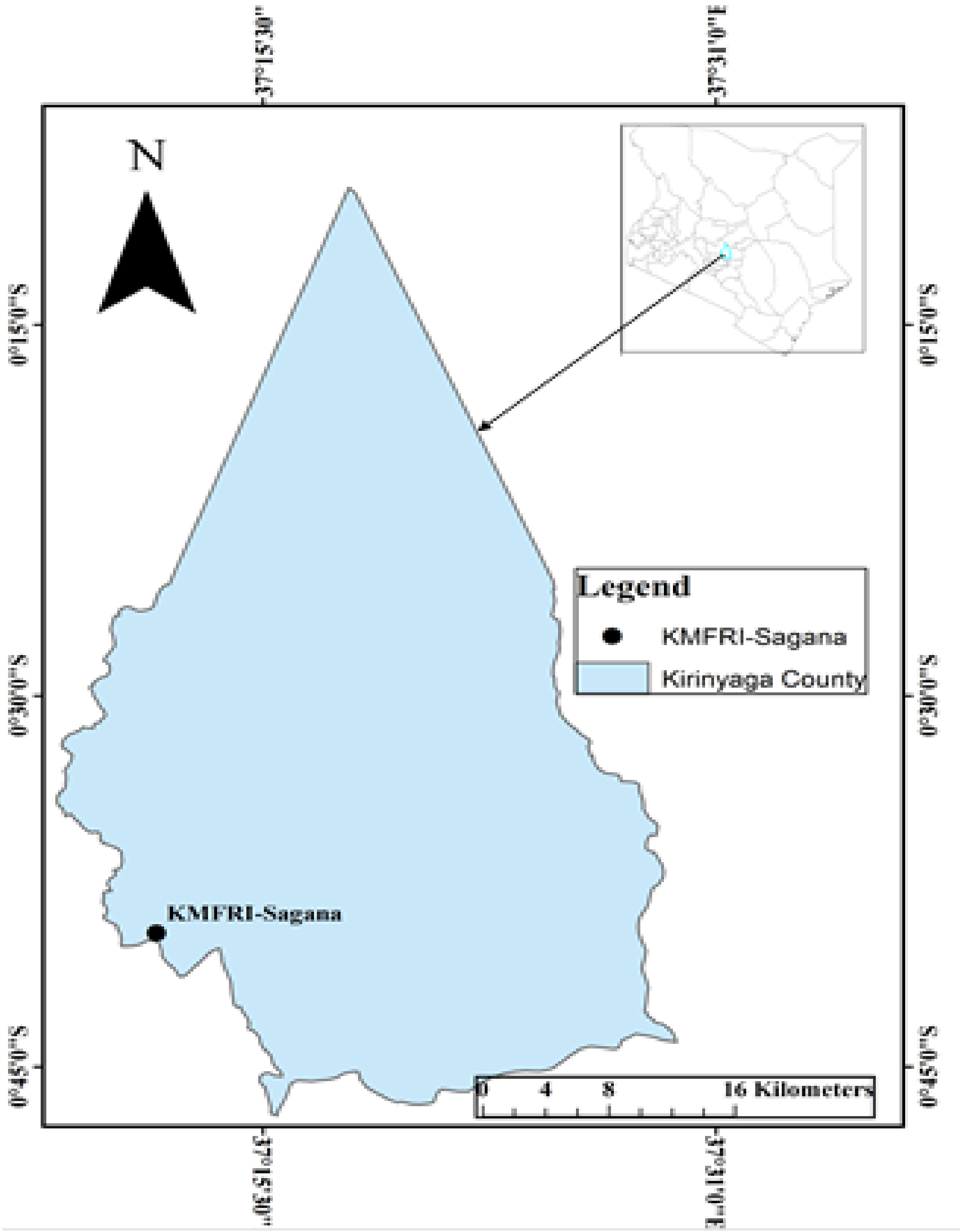
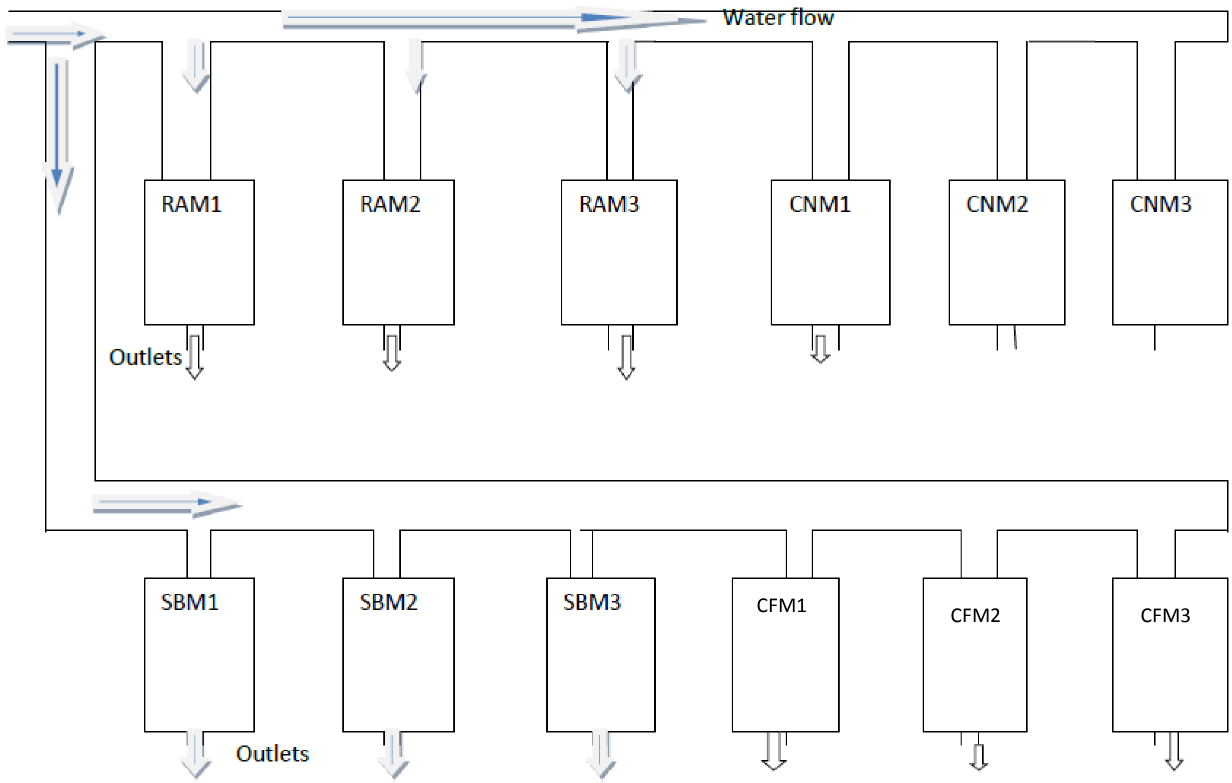


Figure 3. Map showing the location of NARDC in Sagana, Kirinyaga County, Kenya.

Appendix 2. Arrangement of experimental tanks



Key:

RAM1-3------------Rastrineobola argentea meal

CNM 1-3------------Caridina nilotica meal

SBM1-3-------------Soya bean meal

CFM 1-3---------------Commercial feed meal

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

FROM: Secretary - MUERC

DATE: 13th December, 2017

TO: Veronica Obiero Ombwa
PG/MSc/0040/2013
Department of Zoology
School of Biological and Physical Sciences
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00491/17

RE: Proposal Reference Number MSU/DRPI/MUERC/00491/17 **Comparative Study on the Efficacy of Soya Bean Meal, *Rastrineobola argentea* Meal and *Cardina Niloticus* Meal for Nile Tilapia (*Oreochromis Niloticus*) Trewavas Juveniles**

The Maseno University Ethics Review Committee (MUERC) is pleased to inform you that your proposal application was reviewed and discussed in the Committee meeting held on 23rd November, 2017.

In its review, the Committee noted the following **Minor Corrections** to be made before Ethics Clearance is granted:

i. There needs to be an improvement on the general presentation of the document i.e there are numerous formatting, typographical errors, font size inconsistencies etc.
ii. Describe in details the specified conditions under which the moisture content will be determined. Provide the source of the method used.
iii. Describe why the Biuret method of protein determination is preferred over other methods despite the fact that Biuret method targets peptide bonds and in that sense not specific for amino acids composition of proteins which the study seems to be interested in.
iv. For every method proposed to be used, indicate any modifications which will be done, if any. Attach the method (kit) in the appendix for more detailed description
v. State how you will minimize the potential biases and limitations for the study.
vi. Attach the CV for all the co-Investigators (supervisors)

The Committee granted the Investigators thirty (30) working days to make corrections and submit a final draft proposal to MUERC Secretariat for consideration and approval.

Please submit **one (1) copy of corrected draft proposal** and a **signed cover letter,** detailing the sections (page numbers and paragraphs) where corrections are made. Include your **proposal Ref Number** on the cover letter. All **changes** in the **revised proposal** should be written in **bold** text.

Thank you.

DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES
13 DEC 2017
MASENO UNIVERSITY

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