SUITABILITY OF REDWORMS, (*Eisenia foetida*) AS PROTEIN INGREDIENT OF FISH FEEDS FOR NILE TILAPIA AND AFRICAN CATFISH IN GREENHOUSE AND OPEN POND AQUACULTURE SYSTEMS

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has neither, wholly or in part, been submitted or presented for examination in any institution.

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Recommendation

This thesis has been submitted with our approval as University's Supervisors.

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DEDICATION

I dedicate this work to my beloved wife Anne Wamaitha Karanja, my son Asher Amuyunzu Akidiva, my parents Alfred Amuyunzu & Esther M'mboga Amuyunzu and my siblings: Margaret Kaveza, Lucy Vusaka, Holace Amuyunzu, Nelly Ang'adia, Tyson Musihiru and Branice Mudoto for their inspiration and moral support.

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ABSTRACT

This study assessed the suitability of redworms as a protein ingredient in fish feeds. Furthermore, the study evaluated the use of greenhouse technology to promote aquaculture of warm water fish species in high altitude areas characterized by low temperatures below optimum range of 25 to 33˚C. The study was conducted at the Agro-Science Park's Fish Farm in Egerton University from January to June 2017. A total of 240 catfish and 240 monosex male tilapia fingerlings were stocked in 30 hapa nets in greenhouse and 30 hapa nets in open pond at a rate of 8 fingerlings per net. Redworms were cultured for use in feed formulation. Two isonitrogenous diets containing 35% and 40% were formulated for tilapia and catfish respectively using redworms and fish meal as animal protein ingredients. Five redworm experimental diets containing 0:100%, 25:75%, 50:50%, 75:25% and 100:0% redworm and fish meal were formulated and fed to fish twice daily at 3% body weight. Selected physico-chemical parameters; temperature, conductivity, dissolved oxygen and pH were monitored daily while nutrients; nitrites, nitrates, ammonia, soluble reactive phosphorus and total phosphorus were analyzed monthly. Fish were sampled every fortnight to determine the Absolute Growth, Specific Growth, Food Conversion Ratio, Length Weight Relationship, and condition factor. There was no significant difference in the mean protein content of redworm meal 62.29±1.13 and fish meal 57.71±0.77 (t-test, df=1, $p > 0.05$). Similarly, there was no significant difference in mean lipid content of redworm (6.34±0.48) and fish meal (5.51±0.19). Temperature varied significantly in the two pond systems (t-test, $p<0.05$). There were significant differences in the Specific Growth rate, Absolute growth and mean weight gain of Nile tilapia and African catfish cultured in greenhouse and open pond systems (t-test, df=1, $p < 0.05$). Fish cultured in greenhouse pond had higher mean weight gain than those in the open pond system. However, there were no significant differences in the specific growth and Absolute growth rates of fish fed on the 5 redworm experimental diets (One Way ANOVA, df = 4, $p = 0.071$). Fish fed on diet containing 50% redworm diet gained highest weight. Nile tilapia and African catfish cultured in greenhouse pond had condition factors of 2.15 and 3.62 respectively while values of 0.87 and 0.88 in the open pond indicating isometric and allometric growth in greenhouse and open ponds, respectively. Calculated Food conversion ratio indicated that both experimental diets were efficiently utilized in greenhouse pond compared to the open pond. Therefore, this study recommends the use of redworm meal as an alternative protein ingredient and use of greenhouses in improving the growth rate of warm water fish species in aquaculture.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acids
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
CP	Crude Protein
CRD	Completely randomized design
Df	Degree of freedom
EC	Electrical Conductivity
FCR	Food conversion ratio
FM	Fish meal
GDA	Global Development Agenda
HUFA	Highly unsaturated fatty acids
K	Condition factor
KMFRI	Kenya Marine & Fisheries Research Institute
LWR	Length weight relationship
N	Normality
NNS	Non-Native Species
M	Molarity
P	Probability
PUFA	Polyunsaturated fatty acids
SD	Standard Deviation
SDGs	Sustainable Development Goals
Sq. M	Square meter
SRP	Soluble Reactive Phosphorus
TAN	Total Ammonia Nitrogen
TP	Total Phosphorus
UOM	Unit of measurement
UV	Ultra-Violet
WB	Wheat bran
WM	Worm meal

CHAPTER ONE

INTRODUCTION

1.1 Background information

The dwindling productivity of capture fisheries worldwide has increased the gap between fish supply and demand for the growing human population. The decline in capture fisheries production is mainly due to increased environmental pollution and overexploitation of available fish stocks (Cowx & Ogutu, 2019). Increase in fish production from aquaculture may reverse this trend and ensure continued supply of fish and fish products globally (Ababouch & Fipi, 2015; Aloo *et al.*, 2017).

In aquaculture, fish feeds account for 50-60% of total production costs (Prabu *et al.*, 2017). Most fish feeds are formulated from agro-based by-products like rice bran, wheat bran, groundnut peels, slaughterhouse waste, and distillers waste, together with a protein source (Kamble e*t al.*, 2018). Although both animal and plant proteins are used in formulation of fish feeds, animal-based proteins are more useful in promoting fish growth than plant proteins. This is due to their high-quality protein content, high palatability, digestibility, and balanced amino acid (AA) profiles (Hertrampf & Piedad-Pascual, 2012). However, most of the animal protein ingredients used in fish feeds are relatively expensive and geographically unavailable to most fish farmers (Kassahun *et al.*, 2012; Munguti *et al.*, 2006).

In Kenya, the main source of proteins used in fish feeds formulation is fish meal (FM). The supply of FM is however influenced by seasons of the year and competition from other sectors such as its use by humans for food and as livestock feed. These together with transportation costs from the source (lakes) to the farmers increase the production costs, making FM very expensive (Jabir *et al.*, 2014). To promote aquaculture in Kenya, a paradigm shift towards the use of readily available and cheap sources of protein in fish feed formulation is necessary (Oyoo *et al.*, 2012). There is a need for an alternative protein source to reduce over-reliance on FM protein in fish feeds given that its supply is declining rendering it unreliable and expensive in fish diets (Munguti *et al.*, 2014).

The aim of this study was to determine the suitability of redworms, *Eisenia foetida* as an alternative protein ingredient of fish feed production for culture of Nile tilapia and African catfish. Redworms (*E. foetida*) are viewed as a potentially excellent alternative protein ingredient for use in fish diets due to their high amount of protein, essential amino acids, fats, vitamins and minerals. Although there is scarce information in Kenya on the use of *E. foetida* worms in fish feeds production, proximate analysis shows that dried *E. foetida* have a protein content of 50-60%, 7-10% fats, 8-20% carbohydrates and mineral content of 2-3% (Satchell, 2012). The earthworms have low feeding costs, breed easily in artificial culture environments and have high reproduction rate. They can easily be cultured by farmers to form a reliable source of protein ingredient in fish feeds (Martinkosky *et al.*, 2017).

In aquaculture, growth and development of a given fish species is influenced by several physiological and environmental factors such as sex, feeding regime, stocking density and temperature. Temperature has been identified as a critical environmental factor influencing the growth rate of fish (Rountrey *et al.*, 2014). It directly influences the behaviour and metabolic activities of fish such as feeding and digestion thereby improving their growth rate (Mizanur *et al.*, 2014). Different fish species grow optimally at different temperature ranges and significant deviations of temperature beyond the desired range will negatively affect the growth rate of fish (Musa *et al.*, 2012). Optimum water temperature in the culture system increases metabolic rates, resulting in increased food demand by fish and faster growth (Craig *et al.*, 2017). Fish growth rate can, however, be greatly affected by factors such as food conversion ratio (FCR) and condition factor, all of which may confound the relationship between temperature and growth rate (Sarkar *et al.*, 2013).

Cold regions of Kenya, mostly highlands covering areas such as Nyandarua, Njoro, Kericho among others, have low temperatures which hinder the growth of warm water fish species including tilapia and catfish. In such regions, the large water temperature variation between day and night is very large which negatively influences fish growth rate. The large temperature variation results to stunted growth of fish. Stunted growth delays harvests, which discourages most farmers from practicing aquaculture despite proper feeding and water quality management. To promote aquaculture in such areas conventional measures that regulate temperature are needed. Therefore, the other aim of this study was to determine the suitability of greenhouse technology in regulating temperatures in aquaculture production systems in cold regions of Kenya.

1.2 Statement of the problem

In aquaculture, fish feeds account for 50-60% of the total production costs in the semi-intensive and intensive farming systems. In Kenya, formulated fish feeds have been used to enhance fish growth and development during culture. Among the ingredients used in fish feeds formulation, protein is the most important factor influencing fish growth. Fishmeal (FM) is heavily relied upon as a source of animal protein in formulation of fish feeds. However, challenges in its availability increases the overall cost of fish feeds. In addition, competition for its use from the livestock sector as feed has contributed to its unavailability. Increasing costs of fishmeal and adulteration of fish feeds by inclusion of low-level proteins by merchants have become very common problems which negatively affect the growth rate of cultured fish, consequently reducing profits to farmers. To promote aquaculture in Kenya, cheap, affordable and locally available protein ingredients are needed for formulation of fish feeds. Alternative sources to fishmeal with high protein content, amino acid profiles and palatability are needed.

Furthermore, water temperature is among the key physical environmental factors affecting physiology of fish through effects on overall metabolism and energy balances. Temperature determines how much energy fish obtains (through regulation of feeding behaviour and food intake), how much of that energy is acquired (through digestion and absorption) and how much of it can be allocated to key processes such as locomotion and growth. Warm water fish species such Nile tilapia and African catfish usually has a temperature range of 25 to 32 °C. for which physiological processes are optimized, and any deviation from these optimal temperatures results to dramatic effects on the overall health of fish and their survival. Culture of warm water fish species in cold regions of Kenya has been a challenge due to low temperatures below optimum level which results in stunted growth.

Based on the above background, the aim of this study was to assess the suitability of redworms, *Eisenia foetida*, as a protein ingredient of fish feeds formulation. Furthermore, the study also evaluated the potential use of greenhouse technology as a conventional method for temperature regulation in aquaculture in the high-altitude areas of the country such as Njoro which are characterized by low temperatures below the recommended optimum range of 25-32˚C.

1.3 Objectives

1.3.1 General objective

To assess the suitability of redworms (*Eisenia foetida*) as an alternative protein ingredient in fish feeds production for Nile tilapia (O*reochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in greenhouse and open-pond systems.

1.3.2 Specific objectives

i. To compare the water physico-chemical characteristics in the greenhouse pond and open pond systems.
ii. To compare the proximate compositions of redworms meal and fishmeal.

iii. To compare the growth performances, length-weight relationships and condition factors of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems fed on feeds formulated using redworm against fishmeal protein ingredients.

iv. To determine the food conversion ratios (FCR) of the formulated feeds fed to Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems.

1.4 Hypotheses

i. There are no significant differences in the water physico-chemical characteristics in the greenhouse pond and open pond systems.

ii. There are no significant differences in the proximate composition of redworms meal and fishmeal.

iii. There are no significant differences in the growth rates, length-weight relationships and the condition factors of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in the greenhouse and open pond fed on fish feeds formulated using redworm and fishmeal.

iv. There are no significant differences in food conversion ratios (FCR) of the formulated feeds fed to Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems.

1.5 Justification

Food security and poverty reduction are high priority thematic areas of the Global Development Agenda which call for multiple policies, economic and social actions to address consumer demand, access, supply and nutrition. In the United Nations’ Sustainable Development Goals (SDGs) document, which has been adopted by Kenya, goals 1, 2 and 3 outline strategies to end poverty and hunger, achieve food security and improve nutrition respectively to ensure healthy lives by the year 2030. Aquaculture addresses all these themes. Lack of quality protein fish feeds and low temperature in cold regions affect aquaculture, reduces fish supply, and increases fish demand in Kenya. Fishmeal, the highly preferred protein ingredient for formulation of fish feeds is unavailable to most fish farmers due to relatively high costs and competition from other sectors such as livestock which utilize it as a protein component in their feeds. Furthermore, the seasonality in its production reduces the supply of fishmeal in the market, leading to its unstable high prices. Temperature on the other hand, is directly linked to growth of fish. Where temperature range is below the species optimum range, stunted growth of fish is recorded. A cheap and locally available protein-based ingredient and a means for regulating temperature for fishponds are therefore needed to improve aquaculture production. This study aimed to investigate the suitability of redworms as an alternative source of protein ingredient in fish feeds. Redworms are rich in protein and minerals. They are easily cultured and have faster reproduction rates. In addition, their juveniles reach maturity stage in two months hence promising as an alternative to fishmeal protein in fish feeds. Further, this study also aimed to assess the use of greenhouse technology in regulating pond water temperature in cold regions of Kenya for improved aquaculture production of warm water fish species such as Nile tilapia and African Catfish.

1.6 Definition of terms

Absolute growth	The actual increase in size of fish per unit time under known or specific conditions.
Allometric growth	The increase in size of various organs or parts of an organism at different rates.
Ammonia toxicity	Unsuitable growth environment of fish which occurs when total ammonia in the pond is converted into un-ionized forms beyond the concentration of 2.0mg/L..
Cold area	In aquaculture, cold areas are regions where water temperature range falls below 18˚C and can result to stunted growth of warm water fish species like Nile tilapia and African catfish.
Condition factor	A measurement of the general health condition of fish as calculated by the ratio of body weight to body length; it is used to compare growth conditions of fish and is an indicator of environmental quality and fish wellbeing.
Diel variation	Changes occurring in a period of 24 hours.
Food conversion ratio	It is a rate measuring the efficiency with which the body of fish converts feed into the desired output.
Greenhouse effect	Warming those results when solar radiation is trapped by the atmosphere; caused by atmospheric gases that allow sunshine to pass through but absorb heat that is radiated back from the warmed surface of the earth.
Isometric growth	Growth that occurs at the same rate for all parts of an organism so that its shape is consistent throughout development.
Non-native species	Refers to a species introduced in an area through human action outside the species' natural past or present distribution.
Specific growth rate	A quantitative measure of increase in fish weight against time.
Stunted growth	Refers to impaired growth rate of fish development resulting from the nutritional and environmental imbalances.
Vermiculture	The science of culturing earthworms which convert organic waste into manure.

CHAPTER TWO
LITERATURE REVIEW

2.1 Fish feeds status in Kenya

Aquaculture sector has evolved over the past 2 decades increasing its potential in improving food and nutritional security thus playing a big role to Kenyan national food system (Obiero *et al.,* 2019). This has largely been attributed by major transformations which have impacted fish feeds, aquaculture management practices, and production technologies. These transformations have resulted to increased aquaculture production among the majority of small scale fish pond producers who account for the bulk of aquaculture production in the country (Ogello *et al.,* 2021).

According to Munguti *et al.* (2021), fish feeds quality and affordability remains key in safeguarding the profitability and viability of any fish farming enterprises. Fish feeds account for 50-60% of the total production costs in semi-intensive aquaculture aquaculture systems. Farmers are adopting appropriate feed management strategies to maximize on the returns. In Kenya, availability of quality feeds at affordable prices remains a major challenge calling for majority of small-scale farmers to adopt on-farm feed formulation strategies geared towards increasing fish production while maximizing on profits (Bundi *et al.,* 2018; Muteti, 2017).

In Kenya, onfarm feed formulation involves the use of locally available agro-based products of both plants such as wheatbran, ricebran, sunflower cake and maize bran and animal origin such as fish meal, blood meal, shrimp meal and bone meal. On farm feed formulation is however still faced with challenges of limited knowledge on proximate composition of the used ingredients to meet the nutritional requirements: proteins, lipids and energy levels for optimun growth performance of fish. The sector is also challenged by the seasonality and unavailability of fish meal (commonly used protein ingredient) making it relatively expensive as decereibed by Munguti *et al.* (2006).

2.2 Vermiculture

Vermiculture is the science of culturing earthworms for purposes of converting organic matter into manure. They degrade organic materials into finer particulate matter rich in nutrients, with considerable commercial potential as plant growth media or of soil amendment. Earthworms are therefore important in conditioning soil as well as managing decomposing organic wastes on farms. Furthermore, they are used in agricultural production of foliar feed and organic pesticides in crop production to enhance plant growth and control pests respectively (Garg &

Sharma, 2018). Other benefits of vermicomposting include providing an excellent source of protein in livestock feed production (Khan *et al.*, 2016). Culturing earthworms has advantages of producing useful protein ingredients which can replace animal protein sources in fish feeds as well as being used as a direct live feed for ornamental aquarium fishes and as bait for sport fishing (Mohanta, *et al.*, 2016; Musyoka *et al.*, 2019).

2.2.1 Vermiculture candidates

There are about 1800 known species of earthworms worldwide. Culturing earthworms has been successful in many parts of the world (Ibrahim *et al.*, 2016). Widely cultured earthworm species globally are Californian redworms (*Eisenia foetida*) whose main role has been to decompose manure as a way of managing organic matter (Garg & Sharma, 2017). The redworms (*E. foetida*) are mainly preferred in vermicomposting since they have high concentration of digestive enzymes that influence the decomposition of organic matter (Santamaría *et al.*, 2001). The earthworms, African night crawler (*Eudrilus eugeniae*), commonly cultured in West African countries and used as bait by anglers for fishing. Earthworm *Lumbricus rebellus* easily adapts to new culture environments hence can be used in vermiculture (Ibrahim *et al.*, 2016). Other types of worms widely used in vermiculture include *Perionyx excavatus* and *Lumbricus terrestris* (Jameson & Venkataramarujam, 2002). The natural habitats of these worms are soils loaded with organic matter, making their production cheaper since they feed on organic matter (Chakrabarty *et al*., 2011).

The redworms, *E. foetida* (figure 1) which are commonly known as the "compost worms", "manure worms", "California redworms", or "red wigglers" are extremely tough and adaptable worms, which are indigenous to most parts of the world and can be cultured easily on farms. Their faster reproduction rates make them suitable for culturing on farms as alternative protein ingredients for fish feeds (Musyoka *et al.*, 2019). Tacon *et al.* (1983) pioneered the research to establish the possibility of partially substituting fishmeal with redworms (*E. foetida*). Other innovative uses of *E. foetida* include production of foliar feeds for plant growth, production of organic pesticides to control insects in gardens and as a source of protein ingredient in poultry and fish feeds (Gunya, 2016; Musyoka *et al.*, 2019).

2.2.2 Conditions for Culturing of redworms

Key environmental factors that affect growth and reproduction of redworms include sufficient oxygen, temperature, light, moisture, pH, food and bedding materials. According to Sherman (2003), redworms survive in a pH range of 6.8 to 9.0. Adequate moisture should be maintained to help redworms breathe through their skin. Beds need to sustain a moisture range of 60% to 85% and temperature range of 12˚C to 26˚C for successful reproduction.

Feeding redworms is relatively cheap as they consume animal manure, compost, food scraps, shredded or chopped cardboard or paper, or almost any decaying organic matter or waste product. Horse, rabbit, swine and dairy manures are excellent food items (Jensen *et al.*, 2011). Use of wet poultry droppings is however discouraged since it contains high loads of nitrogen which can result in buildup of ammonia which is harmful to earthworms.

The culturing of redworms is easier at a farm level since both indoor bins and outdoor compost beds are used. Indoor methods are advantageous to the farmer as they can be used as a strategy of managing kitchen wastes. This method involves use of bins, wooden boxes or jerrycans of desired sizes whereby, decomposed manure is used as a starter medium onto which kitchen organic wastes are added on top. Earthworms are introduced to the bin after one or two weeks of setting up and watering of the bin (Sherman, 2003).

Figure 1: Redworms *Eisenia foetida, (Savigny 1826)* cultured for feed formulation.

On the other hand, outdoor methods of culturing *E. foetida* involve use of manure which acts as a bedding and food source for worms. Compost beds are common methods used in outdoor cultures. According to Fadaee (2012), the type of bedding materials used in vermiculture is important in determining the survival and growth of redworms. The beddings used should have a high absorbency of water to maintain moist conditions necessary for skin breathing activities in the worms. It should also have a sufficient air movement, to supply oxygen. Furthermore, the bedding materials should be of low protein materials. High protein levels result in rapid degradation and rise in temperature when decomposing hence detrimental to worms. These create inhospitable, often fatal conditions for the worms.

2.2.3 Reproduction of redworms, *Eisenia foetida*

Redworms, *E. foetida* breed fast. They are hermaphroditic but the eggs and sperms of each earthworm are located separately to prevent self-fertilization. Most species reproduce by cross-fertilization. They are either semi-continuous or continuous breeders, producing ova at most times of the year. However, most species produce cocoons parthenogenetically when food supply and environmental conditions are unsuitable for breeding.

Mature eggs and sperms are deposited in a cocoon produced by the clitellum, a swollen, saddle-shaped structure near the worm's head. Within the cocoon, the sperm cells fertilize the eggs, and then the cocoon slips off the worm into the soil. The incubation period of the cocoon is about four weeks after which they hatch into young ones which reach maturity in two months under optimum environmental conditions (Fadaee, 2012).

2.2.4 Use of redworms in fish feeds

According to Munguti *et al.* (2006), farmers practicing semi-intensive aquaculture systems aim to increase fish production through use of supplementary feeds. In Kenya, most of the semi-intensive farms, use on-farm formulated feed supplements since over-the counter feeds are relatively expensive to most farmers and in most cases majority of the ingredients are scarce, influenced by seasonality hence causing their prices to increase.

According to Ritcher *et al.* (2003), it is necessary to seek for a cost-effective feed replacement to supply dietary protein from locally produced inexpensive materials to avoid high feed costs. The redworms, *E. foetida* are among the alternative sources of protein being promoted by researchers in aquaculture globally to formulate cost effective fish diet (Jameson & Venkataramanujam, 2002). According to Kasye (2016), redworms have been documented as a viable ingredient in the formulation of poultry feeds. However, little information has been documented on its use in fish feeds (Mohanta *et al.,* 2016).

Owing to their high reproduction rates, low feeding costs, ease of breeding in captivity and their high nutritive value, redworms constitute an important protein source for fish feeds (Musyoka *et al.,* 2019). In fisheries, redworms are widely used as baits especially in sport fishing and as food in ornamental fish farming. Earthworms have also been used as live feeds in commercial fish farming and as a starter feed for juvenile fish especially carnivorous species such as catfish (Zakaria *et al.*, 2013).

Several feeding trials have been performed to test the suitability of earthworms as an alternative protein source in fish feeds (Ambasta & Kumari, 2013). According to Guerrero (2009), use of *P. excavatus* worms resulted to higher growth of *Oreochromis niloticus* when fed on fish feeds that contained 15% earthworm meal than fish fed on 25% fish meal. The earthworms used in this study were sun dried before being ground to a meal and incorporated into the fish feeds. According to a study on culturing *Heteroclarius* fingerlings conducted by Olele (2011), best growth rates in terms of mean weight gain (6.77g) specific growth rate (0.86), and protein conversion ratio (0.6) were obtained when *P. excavatus* worms replaced fishmeal at 50% level. Verma (2015), showed that when the earthworm *Hyperiodrilus euryaulos* meal was used to feed Nile tilapia broodstock, the prostaglandins hormones they contained induced gonad maturation in finfish. Its inclusion in fish feeds therefore had a positive impact on growth and fecundity of Nile tilapia broodstock.

2.2.5 Risk of Non-Native Species in Research

Non-Native Species (NNS), also known as alien species refer to species introduced through human action outside the species' natural, past or present distribution. Their use in research has caused considerable concern calling for NNS strategy in research (Carrie & Karin, 2011). This is because NNS causes both economic (Ricciardi *et al.*, 2000) and environmental (Mack *et al.*, 2000) impacts, including competition for resources with local species, reduced biodiversity, alteration of ecosystem properties such as nutrient cycling and hydrology, and increased environmental disturbances in the introduced areas (Carrie & Karin, 2011) as well as other unforeseen impacts (Secretariat, 2008).

NNS in most cases are introduced intentionally as pets, garden or aquarium flora and fauna, for recreational activities such as fishing or research as bio-controls. Currently, most research focuses on the negative impacts of NNS, although there is acknowledgement by some scientists that some NNS, including invasive ones can make a positive contribution both economically and environmentally (Carrie & Karin, 2011).

According to Zakaria *et al.* (2013), *E. foetida* are cultured in most parts of the world for vermicomposting purposes and as protein supplements especially in the poultry sector. In Kenya, there is no clear documentation on the introduction of redworms. Currently, *E. foetida* are used for composting manure, as fish and poultry feeds and for commercial purposes

(Mohanta *et al.*, 2016). So far, no negative economic and environmental impacts have been reported regarding the culture and use of *E. foetida*. Despite all these uses, there is no information on their nutrient content to inform their use as a protein ingredient in livestock, chicken and fish feeds. The current research aims to carry out proximate analysis of redworms and to test their suitability as an alternative protein ingredient in fish feed formulation.

2.3 Proximate analysis of fish feed ingredients

Proximate analysis of fish feed ingredients should be first carried out before fish feed is formulated (Stanton & Wagner, 2012). The main aim of proximate analysis is to establish the nutritional content of the ingredients to be used in fish feed formulation (Craig & Helfrich, 2002). The analysis considers the levels of crude protein (CP), crude fibre, lipids (fats), moisture content and ash present in the feed ingredients. Once the proximate composition of feed ingredients is established, it is easy to formulate a complete diet which is nutritionally balanced for fish growth and development (Bolorunduro, 2002).

2.4 Formulation of fish feeds

The primary objective of fish feed formulation is to balance the cost and protein ingredients to provide fish species with an acceptable feed which can be easily utilized (Goddard *et al.*, 2008). The desired feed should provide all the nutrients required by fish at different life stages as well as being present at the proper levels to yield optimum production at the minimum cost possible. According to Halver and Hardy (2002), feed intake by fish depends on the physical and chemical properties of the feed which includes the flavour, odour, texture, colour and stability of the feed in water.

The methods used in formulation of fish feeds include those that use algebraic equations and Pearson's square method (Halver & Hardy, 2002). Algebraic equations involve the use of equations to balance the ingredients. The method is commonly used in industries, but it is complicated to farmers. The Pearson square method in fish feed formulation is simple enough for on-farm feed formulations by farmers using locally available ingredients (Bhuiyan *et al.*, 2016).

2.5 Fish diet ingredients

The nutritional requirements of fish do not vary greatly within species (Lall & Dumas, 2015). Fish require energy and nutrients for growth, reproduction, and health. Fish nutritional requirements remain similar whether naturally or artificially fed. They require proteins, lipids, carbohydrates, minerals, vitamins and growth-promoting factors, which may come either from the surrounding aquatic environment or from the supplemented formulated feeds (Hasan, 2000).

2.5.1 Dietary protein

Protein is the basic component of animal tissues and is, therefore, an essential nutrient for both maintenance and growth (Mihalca *et al.,* 2010). Proteins are a source of amino acids, enzymes, hormones, and serve structural functions as well as a source of energy (Aladetohun & Sogbesan, 2013). Amino acids are broken down during digestion and used to produce new tissues and repair damaged ones. The ten essential amino acids (methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine) are important for fish growth and must be included in feeds. The protein level in each feed should be at optimum and biologically available to facilitate the growth of fish (Mihalca *et al.*, 2010).

Carnivorous fishes require a feed that has 50 percent of animal proteins while herbivores or omnivores require nearly 30 percent of animal protein in their feeds. Nile tilapia and African catfish for instance have an estimated gross protein requirement of 30% and 40% respectively (Hasan, 2000; Hels *et al.*, 2003). However, protein utilization by fish is affected by several factors which include water temperature, oxygen levels, presence of toxins in the culture environment, fish body size and stocking density (Hardy, 2010).

Protein requirement is usually high at the initial feeding stages of juvenile fish and decreases as the fish size increases. Juvenile fish require between 40 and 60% proteins in their feeds (Kaushik & Seiliez, 2010). The protein requirement for juvenile tilapia at hatching is estimated to be about 50% but decreases to about 30% as the fish increase in weight to 250g (Ali *et al.,* 2016). Commonly used animal protein ingredients include fish meal, freshwater shrimps and blood meal (Hardy & Tacon, 2002). These animal proteins can be mixed with plant-based protein ingredients such as cotton seed cake, sunflower cakes and defatted soybean meal when formulating fish feeds (Maina *et al.,* 2002).

2.5.2 Lipids

Lipids are excellent sources of energy compared to carbohydrates and proteins. Fish require an estimated lipid content in the range of 10–20% of the total dry weight of feed or between 4 and 15% of fat in the feed but this depends on water temperature since it influences the melting point of fats for digestion to occur (Sargent *et al.,* 2002). The basic constituents of lipids are fatty acids which can either be saturated or unsaturated. Lipids are sometimes associated with proteins (lipoproteins), minerals (phospholipids) and fats (glycerides). Lipids also supply essential nutrients which cannot be synthesized by the fish such as vitamins and trace elements. In broodstock nutrition, lipids are considered to be important in determining the quality of fingerlings, spawning and egg quality of many fish species. Lipids deficiency in broodstock feeds negatively affect fecundity, rate of fertilization and hatching rate of eggs (Izquierdo *et al.,* 2001).

2.5.3 Carbohydrates

Carbohydrates comprise organic compounds that are either aldehyde or ketone derivatives of polyhydric alcohols containing carbon, hydrogen, and oxygen. They are primarily used as energy sources and are important for their binding properties in floating pellets due to presence of starch (Başçınar *et al.,* 2007). The ability to utilize carbohydrates differs among trophic level of fish species with fish species at lower trophic level being more efficient users than those at high trophic levels. For instance, herbivorous fishes such as carps, are better than carnivores at digesting carbohydrates because they produce amylase enzymes in their digestive systems while Carnivorous fish such as catfish do not digest carbohydrates very well and therefore, they only need about 10 percent carbohydrates in their feeds (Khalifa *et al.,* 2017).

2.5.4 Vitamins

Vitamins are organic compounds required in fish feeds for normal growth, reproduction, and health (Lu *et al.,* 2007). They function in a variety of chemical reactions in the body. According to Matos *et al.* (2017), natural feeds contain balanced vitamin profiles essential to fish. On-farm formulated feeds however lack balanced profile of vitamins therefore the need for vitamin supplements. Furthermore, according to Lu *et al.* (2007), the body system of fish cannot synthesize vitamins at all or can only synthesize insufficient quantities for normal development, growth and maintenance hence they must be supplied in the diet.

Vitamins are classified according to their solubility in water and in fat. The water-soluble vitamins include thiamine, riboflavin, pyrodixine, folic acid, pantothenic acid, nicotinic acid, vitamin B_{12}, and vitamin C. These vitamins are required in small amounts but play a very important role in fish metabolism (Russell, 2004). For instance, vitamin C is an antioxidant and immune modulator. Fat soluble vitamins include vitamins A, D, E and K and are required in large amounts in fish feeds.

2.5.5 Minerals

Minerals are inorganic elements necessary in fish diet for normal body functions such as reproduction, movement and metabolism (Bhujel, 2001). Based on the quantity required in fish diet, minerals are divided into two groups known as macro-minerals and micro-minerals (Louis & Steven, 2002). The common macro-minerals include magnesium, sodium, chloride, potassium, calcium and phosphorous. These minerals regulate osmotic balance and aid in normal skeletal development of fish. The common micro-minerals are copper, iron, manganese, chromium, iodine, zinc and selenium. According to Boyd *et al.* (2008), micro-minerals play a vital role in the functioning of enzymes, hormones and other metabolic functions.

2.6 Length-weight relationship and Fish condition factor (K)

2.6.1 Length-weight relationship (LWR)

The length-weight relationship (LWR) is a useful biological tool in fishery assessment. It helps in predicting the weight of a given fish from the length required in the calculation of biomass (Keyombe *et al.,* 2015). The LWR is applicable in fish ecological sampling programmes, where it is usually easier to measure the length as compared to weight (Froese, 2006). The LWR of a particular species allows the inter-conversion of these parameters. Furthermore, one can carry out morphometric comparisons between species and populations using the LWR (King, 2007). The relationship between the length (L) and weight (W) of a fish is usually expressed by the equation $W = aL^b$, where a is the intercept and b is the allometry coefficient (Nehemia *et al.,* 2012). Values of the exponent b usually provide information on fish growth and is useful in calculation of fish condition factor (De Souza Braga & Gomiero, 2005; Mortuza & Pervin 2008). In ecological surveys, when $b = 3$, the increase in weight is isometric. When the value of b is other than 3, weight increase is termed as allometric (positive if $b > 3$, negative if $b < 3$). Growth conditions associated with no change in body shape as the fish grows is termed as isometric growth. This is a useful tool that provides important information concerning the structure and function of fish populations (Richter *et al.,* 2000; Riedel *et al.,* 2007).

2.6.2 Fish condition factor (K)

Sekitar *et al.* (2015) describes fish condition factor as a key indicator to denote the well-being of fish in relation to their growing environment hence important in assessment of fish stocks. Measurements of condition factor are generally intended to act as indicators of tissue energy reserves, with the expectation that, a fish in relatively good condition should demonstrate higher growth rates, greater reproductive potential and higher survival rate (Froese, 2006). In aquaculture, condition factor is used in monitoring the degree of feeding activity of a species to determine whether it is making good use of its feeds (Ambrósio & Lizama, 2002).

In ecological surveys, fish condition factor (K) is calculated by the equation $K = (Wx100)/L^b$ (De Souza Braga & Gomiero, 2005) whereby L is the total length of fish (cm), W is the weight of fish and b is the weight at unit length. The K-value is influenced by several factors including type of feed, sex, water quality, season, and stress. When, K- values are greater than one (1) it implies fish are grown in well-conditioned environment (Sharawy *et al.*, 2017).

2.7 Influence of water quality on fish growth

Water quality is among the most important limiting factors in aquaculture, which is often, less understood and therefore overlooked by most farmers until it affects fish production (Boyd, 2008; Keremah *et al.*, 2014). Water quality is influenced by factors such as temperature, pH, dissolved oxygen, conductivity, hardness, turbidity, alkalinity, ammonia and carbon (IV) dioxide (Ehiagbonare & Ogunrinde, 2010).

Water quality in fish ponds directly affects feed utilization efficiency (Alabaster & Lloyd, 2013), as well as fish growth rates, fish health and survival Deterioration in water quality can result in fish being stressed and vulnerable to diseases which can result to slow growth rates and in severe cases of fish kills (Ojwala *et al.*, 2020; Pucher *et al.*, 2015).

2.7.1 The influence of temperature on fish growth in aquaculture

Growth of fish is influenced by factors such as temperature, sex, feed quality and stocking density. According to Musa *et al.* (2012), temperature is one of the important environmental factors influencing fish growth. Temperature positively affects the physiological and metabolic activities which influence the growth rate of fish (Rountrey *et al.*, 2014). Studies by Turker (2009) indicate that with increasing temperature, digestion rate in fish increases upto a certain level then it remains constant. The growth rate in fish decreases with decreasing temperature beyond optimal range for different fish species. This decreased growth rates delay harvesting of fish (Mizanur *et al.*, 2014). In aquaculture it has been observed that optimum water temperature increases metabolic rates, resulting in increased food demand and faster growth (Craig *et al.*, 2017).

Every fish species has an optimum range of temperature in which growth performance is maximum (Rountrey *et al.*, 2014; Sarkar *et al.*, 2013). Nile tilapia, for instance, has an optimum temperature range of 25 to 33˚C with 28˚C being the most suitable temperature for culture. Within the optimum temperature range, the growth rate of fish is usually at optimum due to the enhanced enzymatic response (Elliott & Hurley, 2003). Catfish on the other hand performs best in temperature range of 25 to 34˚C but can tolerate temperature as low as 16˚C below which the growth performance deteriorates (Payne *et al.,* 2016).

In the Kenyan high-altitude areas, of Rift valley region such as Mau, Kericho, Njoro, among other areas, long rains occur during the months of April to August. These are the coldest months in which variability in diel temperatures is high dropping upto 12˚C. The low temperatures create a big challenge to aquaculture practitioners as they cause stunted growth of farmed fish especially Nile tilapia (Musa *et al.,* 2012). In such areas, most farmers rely on open pond systems for fish production. However, temperature variations during the day and night are very wide in these ponds. At daytime, water is heated up by solar energy upto 24˚C on average while at night temperatures drop to as low as 11˚C affecting the physiological functioning of fish (Musa *et al.,* 2012). According to Payne *et al.* 2016, low temperatures below optimum level affects growth of Nile tilapia through reduced metabolism rates which results in slow intake of food. Furthermore, under low temperatures below the optimum level, fish will utilize more energy to regulate its internal body temperature hence stunted growth (Elliott & Hurley, 2003). This therefore calls for conventional methods of practicing aquaculture in cold areas such of Njoro within the Rift valley and other highland areas in Kenya. The use of greenhouse technology is known to raise and control temperatures throughout the production system of crops. These occurs through *'the greenhouse effect'* whereby solar radiation is trapped by the geomembrane of the greenhouse and protected from escaping thus absorbed and retained within the internal environment. It is this phenomenon that makes greenhouses suitable for aquaculture. The trapped solar radiation is absorbed by water in the fish culture system. In doing so, it prevents drastic temperature drop of water thus favouring fish growth (Fuller, 2007). This technology can as well be adopted in aquaculture (Becker & Frei, 2005; Jain, 2007; Li *et al.,* 2009).

2.7.2 The effect of water pH and carbon (IV) oxide levels on water quality

Water pH correlates negatively with carbon dioxide available in water (Olalekan *et al., 2015*). The pH in fish ponds varies throughout the day due to respiration and photosynthesis within the water column but it should be maintained between 6.5 to 9.0 (Boyd & Zhou, 2015). The main source of carbon (iv) oxide is respiration. At night, phytoplankton and fish respire by consuming oxygen resulting to production of carbon (iv) oxide within the water column. Released carbon (iv) oxide reacts with water to form carbonic acid which lowers the fish pond water pH. This consequently reduces the capacity of fish's blood to carry oxygen due to the lowered blood pH at the gills resulting to fish stress which negatively affects their growth rate.

The stocking density and primary productivity are two main important aspects to consider when controlling the effects of water pH in aquaculture. Usually, the growth of fish is limited in water with a pH less than 6.5, and fish reproduction ceases and sometimes mortalities occur in fingerlings of most fish at a pH less than 5.0. In tilapia and catfish aquaculture, fish death is almost certain at a pH of less than 4.0 or greater than 11.0 (Bhatnagar & Devi, 2013).

2.7.3 Ammonia toxicity

Ammonia is the primary nitrogenous waste product of fish. According to Farrelly *et al.* (2015), the main source of ammonia in fish ponds is the breakdown of proteins in fish feeds. Fish digest protein and excrete ammonia through their gills and in their droppings. The amount of ammonia excreted by fish will positively correlate with the amount of protein in the feeds given to the fish. Ammonia also gets into fish ponds through bacterial decomposition of organic matter including the unutilized feeds, dead aquatic plants such as algae and other types of organic matter which find their way into the fish ponds and exists in ionized and un-ionized forms which are non-toxic and toxic forms, respectively (Akinshola & Mustapha, 2016).

The total ammonia nitrogen (TAN) in fish ponds is composed of toxic (un-ionized) ammonia (NH_3) and non-toxic (ionized) ammonia (NH^+_4). Under normal circumstances, a fraction of the TAN exists as toxic (un-ionized) ammonia, but a balance exists between it and the non-toxic (ionized) ammonia Ammonia toxicity in fish ponds occurs when the total ammonia mostly exists in un-ionized form (NH_3) and is dependent on pH and temperature (Boyd, 2008).

The proportion of TAN in the toxic form increases as the temperature and pH of the water increase. According to KS *et al.* (2016), for every pH increase of one unit, the amount of toxic (un-ionized) ammonia increases about 10 times. Ammonia toxicity is more pronounced in fish ponds which are heavily stocked beyond their carrying capacity. In most cases, toxic levels of un-ionized ammonia for short-term exposure lies in the range of 0.06 to 0.1mg/L, and sub lethal effects may occur at level beyond 0.1mg/L. Management of ammonium in aquaculture should therefore be considered at a concentration of <0.1mg/L for fishpond production. This can be achieved through reduced feeding rates, liming and water exchange (Bhatnagar & Raparia, 2016).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

The study was conducted at the Agro-Science Park fish farm in Egerton University (figure 2). The university has a geographical reference of S 0˚22'11.0", E 35˚55'58.0" within the Kenyan Rift valley in Njoro, Nakuru County. It is situated at an altitude of 1,800 m above sea level with a temperature range of 17˚C to 22˚C but the minimum temperature can drop to 11˚C during cold months of April to August characterized by long rains. Average annual rainfall received in the area is 1,200±100 mm.

Agro-science park fish farm was operationalized in 2014 as an initiative of the Division of Research and Extension to promote aquaculture research at the University. The farm produces Nile tilapia and African catfish fingerlings, fish feeds and provides aquaculture extension services to farmers within Nakuru County. Apart from rearing Nile tilapia and African catfish the farm has plans to start ornamental fish farming in the near future. The farm's main source of water is River Njoro which is a second order stream originating from the Mau Forest catchment. The farm also receives water from a rain fed dam/reservoir whose water passes through the treatment plant before use within the university.

3.2 Culture of redworms

The culturing of redworms, *E. foetida* was carried out at the Agro-science park fish farm within Egerton University from January to September 2016 before harvesting them for fish feed formulation and thereafter feeding of fish. The redworms were cultured in black plastic containers within a sheltered house structure (shade) to ensure easy management and control of their movement (figure 3 and 4). Dried grass was mixed with goat and chicken dung to prepare indoor compost beds. The beds were watered daily to facilitate decomposition and to maintain a suitable moisture condition. After two weeks of setting up the beds, mature pure breed worms from Kiambu Organic Farm were introduced into the beds. A large black 0.3mm polythene paper was used to cover the top of the bed to maintain temperature and prevent direct light from reaching the worms since they are photophobic (Jameson & Venkataramanujam, 2002). After every three weeks, dried grass was added to the bed and mixed with previously decomposed organic matter for feeding the worms. Harvesting of the earthworms started in the third month. At the end of study period, the redworm bed materials were destroyed to prevent the worms getting into the surrounding environment. This was achieved through incineration to ensure that no redworms remained in the local area (Davis, 2006; Sagoff, 2005). After the incineration process, the soils from the bed were examined for presence of redworms. No redworms were observed.

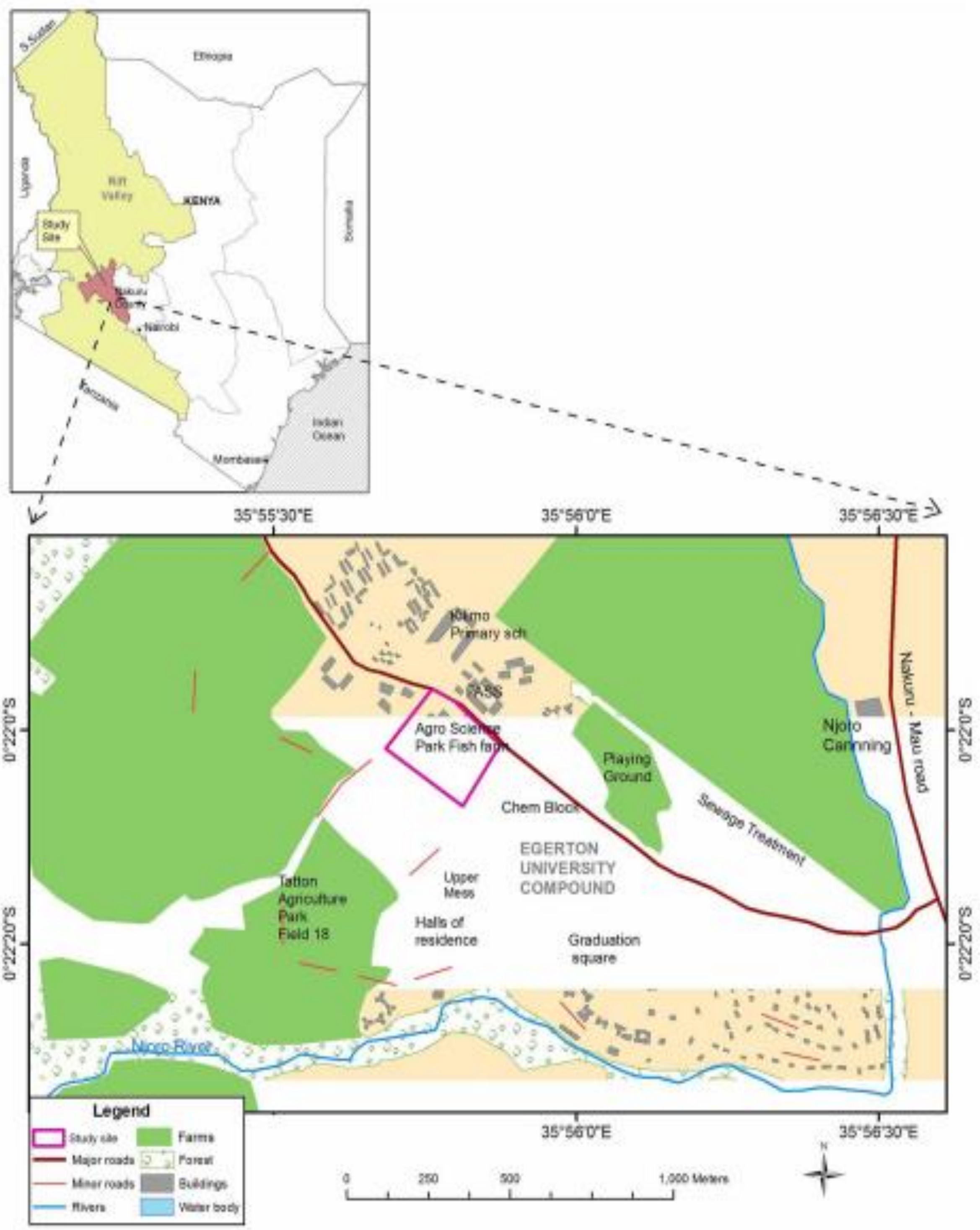

Figure 2*:* A map of Egerton University showing the site of ponds where the study was conducted (*Redrawn from Q-GIS)*.

Figure 3: The shade structure where redworms, *E. foetida* were cultured for use in the feeding trial.

Figure 4: Containers with decomposing organic matter used for culturing the redworms, *E. foetida*.

3.3 Harvesting and preparation of redworms for fish feed formulation

Harvesting of the worms was done manually by hand picking as recommended by Jameson and Venkataramanujam (2002). The bedding materials containing the earthworms were dug using a garden rake to expose the worms which were handpicked and placed into plastic containers. Processing of worms for fish feed formulation was done by thoroughly rinsing the worms in clean water followed by 30 minutes waiting period for the worms to evacuate undigested matter from their guts. Final rinsing of the worms in clean water was done followed by oven-drying at 80˚C for 3 hours and grinded using a mortar and pestle for proximate analysis and feed formulation.

3.4 Proximate analysis of feed ingredients

Proximate analysis was carried out to determine the chemical composition of the redworms and other feed ingredients used in fish feed formulation. The animal-based protein feed ingredients used in fish feed formulation included redworms and fish meal. These were used alongside other plant-based ingredients such as sunflower, Soya meal and wheat bran. The analysis comprised of chemical tests to determine the moisture, crude protein, lipid, crude fibre and ash content.

3.4.1 Determination of Dry Matter (DM) and moisture content in the feed ingredients

Dry Matter (DM) and moisture content were determined using the drying method described by the Association of Official Analytical Chemists, AOAC (2012). A cleaned crucible was dried for one hour in an oven previously heated to 105˚C and later allowed to cool in a desiccator. The crucible was weighed (W1) and approximately 2g of the ground feed sample added. The crucible containing the feed sample was oven dried for 2 hours at 105˚C followed by cooling in a desiccator. The sample was reweighed to determine the final weight (W2). The DM and moisture content in the sample were calculated according to equations 1 and 2 (AOAC, 2012)

$$\%DM = \frac{(W2-W1)}{Wf} \times 100 \qquad (1)$$

Where, W_1 is the weight of empty dish (g), W_2 is the weight of dish and feed sample after drying (g) and W_f is the weight of the feed used in grams.

$$\%\,Moisture = 100 - \%\,DM \qquad (2)$$

3.4.2 Determination of Crude Protein (CP) content in the feed ingredients

Determination of crude protein was carried out using Semi-micro Kjeldahl method (Balthrop *et al.*, 2011). Approximately 0.5g of ground feed ingredient sample (W), was weighed in digestion tubes. The samples were digested using Kjeldahl digester for 3 hours at an average temperature of 420 ± 20˚C and allowed to cool for 10–20 minutes. Cooled samples were distilled using 50ml of 40% NaOH. The distillate containing ammonia was trapped in 4% boric acid. A mixed indicator was then added and titrated against 0.02N HCL to the endpoint (indicated by colour change from colorless to pink). The percentage of CP in the sample was calculated using equation 3 as described by Balthrop *et al.* (2011);

$$\% \, CP = \frac{\frac{a \times b}{1000} \times 14 \times 100 \times 6.25}{c} \quad (3)$$

Where: a is the amount in ml of HCL used, b is the Normality of the HCL used for titration, c is the weight of analyzed feed sample, 14 is a constant indicating the molecular weight of nitrogen and 6.25 is the Conversion factor from nitrogen content into crude protein content.

3.4.3 Determination of Ash Content in dried feed ingredients

Ash content in the dried feed samples was determined by the incineration method described by Balthrop *et al.* (2011). The incineration crucibles were pre-dried, cooled and weighed (W1). Approximately 2g of the ground feed powder was added into the crucibles. The contents were incinerated for 3 hours in a muffle furnace at a temperature of 550 ˚C. The crucibles containing the residue were cooled in a desiccator and re-weighed (W2). The percentage ash content was calculated using the equation 4 given by Balthrop *et al.* (2011).

$$\% \, Ash = \frac{W2 - W1}{Wf} \times 100 \quad (4)$$

Where: W_f is the weight of the feed used (g), W_1 is the weight of empty dish (g) and W_2 is the weight of dish and residue after incineration (g).

3.4.4 Determination of Crude Fats in feed ingredients

The Soxhlet method was used to determine crude fats according to procedures described by AOAC (2012). Approximately 2g of ground feed sample was weighed into an extraction thimble and covered with a fat-free cotton wool. The extraction thimble was transferred into an extractor. Dried conical flask was weighed (W1) and 95ml of petroleum ether added. The extractor was then connected to the conical flask before heating started. Crude fats in the sample were extracted for 6 hours.

The solvent was distilled until the flask was nearly free from the solvent. The flask was left in a fume hood overnight to evaporate all the solvent. The flask with residue was dried for 1.5 hours, cooled and re-weighed (W2).

The percentage Crude Fat was calculated using equation 5 (AOAC, 2012).

$$\% \, crude \, fat = \frac{W2 - W1}{\mathrm{Wf}} \times 100 \quad (5)$$

Where: Wf is the weight of the feed used (g), W1 is the weight of flask (g), and W2 is the weight of flask and fat residue (g).

3.4.5 Determination of Crude Fibre content in the feed ingredients

Crude fibre was determined using the filtration method as described by Balthrop *et al.* (2011). Approximately 2g of finely ground feed sample was weighed in a filtration unit for pre-treatment using petroleum ether. The content was digested in 150ml of 0.15N sulphuric acid by boiling for 30 minutes, then cooled, filtered and washed with 10ml acetone. The residue was then boiled in 150ml of 0.23M potassium hydroxide for 30 minutes, cooled, filtered and washed with 10ml acetone. The washed residue was then transferred onto a crucible, oven dried for 4 hours at 105˚C and re-weighed after cooling in a desiccator (W1). The residue was then incinerated at 550˚C for two hours in a muffle furnace, cooled and crucible weighed again (W2). The percentage crude fibre was calculated using equation 6 (Balthrop *et al.*, 2011).

$$\% \, crude \, fibre = \frac{(W2 - W1)}{Wf} \times 100 \quad (6)$$

Where: Wf is the weight of the feed used (g), W1 is the weight crucible and residue after drying (g), and W2 is the weight crucible and residue after incineration (g).

3.5 Fish feed formulation using Pearson Square method

Fish feed formulation was carried out using the Pearson Square Method (Barrows & Hardy, 2002). Fish feeds with 35 and 40% crude protein (CP) for tilapia and catfish respectively were formulated using redworms and fish meal as animal protein ingredients. Sunflower and soya meal were used as plant protein ingredients while wheat bran was used as an energy source in the feed. The formulated feeds contained 0, 25, 50, 75 and 100% redworm powder.

Ingredients used in fish feeds formulation were placed on the Pearson square as shown in figure 5. The desired CP of 35% and 40% was inserted at the center of the square at ago. The protein percentage (values) in the ingredients were placed in the left corners of the square. The levels of protein of each foodstuff were subtracted from the desired CP of the feed being formulated. The differences were placed on the right corners of the square diagonally opposite the feedstuff considering the absolute values. The difference in percentage protein content represented the proportion of overall proteins used in feed formulation. The percentage difference between the protein ingredients used and the desired CP of the feed being formulated represented the proportion of energy source ingredients required during formulation.

Calculations for the protein and energy to be used in formulation were done using equations 7 and 8 (Barrows & Hardy, 2002).

$$Protein \ = \frac{(Y-Z)}{(Y-Z)+(X-Z)} \times 100\% \quad (7)$$

$$Energy \ = \frac{(X-Z)}{(Y-Z)+(X-Z)} \times 100\% \quad (8)$$

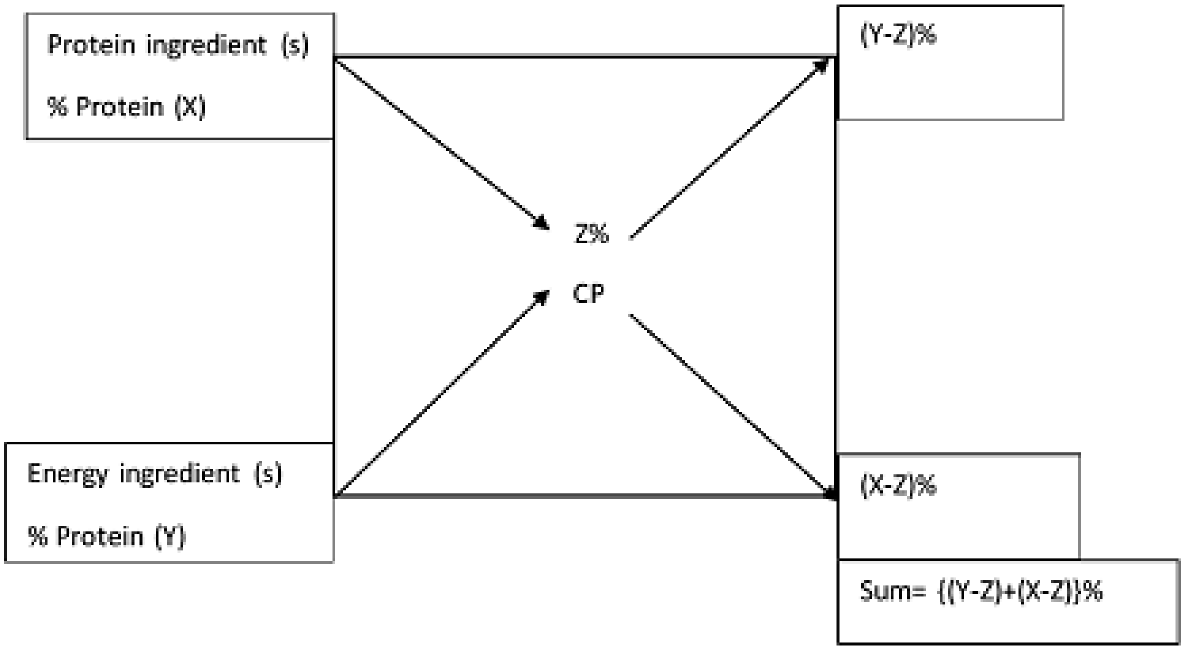

Figure 5: Use of Pearson's Square method in formulation of fish feeds using one protein ingredient.

In cases where both fish meal and redworms were used as protein ingredients and wheat bran and rice bran used as energy sources in formulation, Pearson's square method was used as described by Barrows and Hardy (2002). The protein levels and their mixing ratio were first determined.

For instance, suppose a feed of 35% CP was to be prepared using redworms, fish meal, wheat bran and rice bran whose protein content are 55%, 60%, 13% and 11% were to be used in formulation. The two protein ingredients were mixed in a ratio of 1:2 (redworms: fish meal) and 1:1 (wheat bran and rice bran).

Using the Pearson's square method, the desired crude protein (CP) level of feed (35%) was placed in the middle of the square. The two ingredients were separated into two groups and the protein level of each group calculated as per the specified proportion. Equation 9 and 10 show the averages of the feed ingredients calculated from the specified ingredients.

Protein sources: Redworms = 1×55=55%

Fishmeal = 2×60=120%

$$Average = \frac{(55+120)}{3} = 58.33\% \qquad (9)$$

Energy sources: wheat bran =1×13=13%

Rice bran =1×11=11%

$$Average = \frac{(13+11)}{2} = 12\% \qquad (10)$$

The two protein ingredients and energy sources, along with their calculated protein contents were then placed in each corner at the left-hand side of the square as shown in figure 6. The percentage of individual feed ingredient used in formulation was then calculated as described by Barrows and Hardy (2002) in equations 11, 12, 13 and 14. Diagonal subtraction was carried out to find the proportion of protein and energy ingredients to be included in the feed (23% and 23.33% respectively).

Protein source (23%) = Redworm meal $= \frac{1}{3}\times 49.64\% = 16.55\%$ (11)

Fish meal $= \frac{2}{3}\times 49.64\% = 33.09\%$ (12)

Energy source (23%) = Wheat bran $= \frac{1}{2}\times 50.36\% = 25.18\%$ (13)

Rice bran $= \frac{1}{2}\times 50.36\% = 25.18\%$ (14)

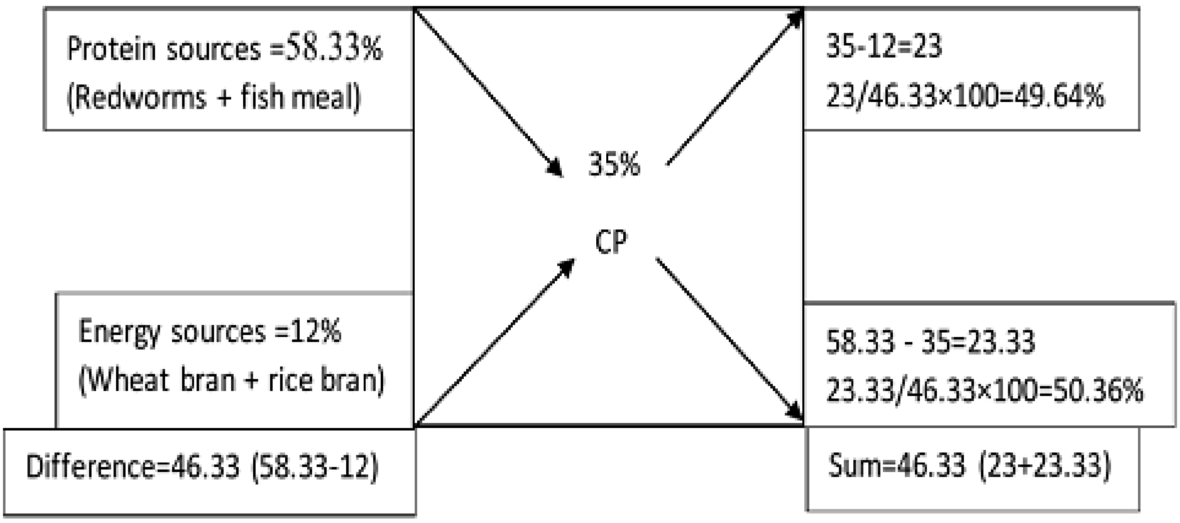

Figure 6: Use of Pearson's Square method in formulation of fish feeds using more than one protein ingredient.

3.6 Fish growth experimental design

A complete randomized design (CRD) with five treatments of redworms composition at the level of 0%, 25%, 50%, 75% and 100% CP replicated three times was set in an open and greenhouse pond as shown in figure 9.

Figure 7: Experimental set-up for fish feeding studies inside the greenhouse pond.

Figure 8: Experimental set-up for fish feeding studies in the open pond.

Thirty hapa nets each measuring (2m ×1m ×1m) were set in each pond, with a total of 60 hapa nets each stocked with 8 fingerlings (4 fingerlings per meter square). A total of 240 catfish and 240 monosex male tilapias were stocked in hapa nets under the two environments for a period of six months (December 2016 to June 2017) as shown in figure 7 and 8.

Isonitrogenous fish diet of 35% and 40% CP was formulated using fishmeal and redworm meal for Nile tilapia and African catfish respectively. Redworm inclusion in formulated feeds was done at four levels; 25%, 50%, 75% and 100% with 0% inclusion used as a control diet.
The control diet was formulated to contain fish meal as the only source of protein (0% redworms). Formulated rations were administered twice a day at 3% body weight (BW).

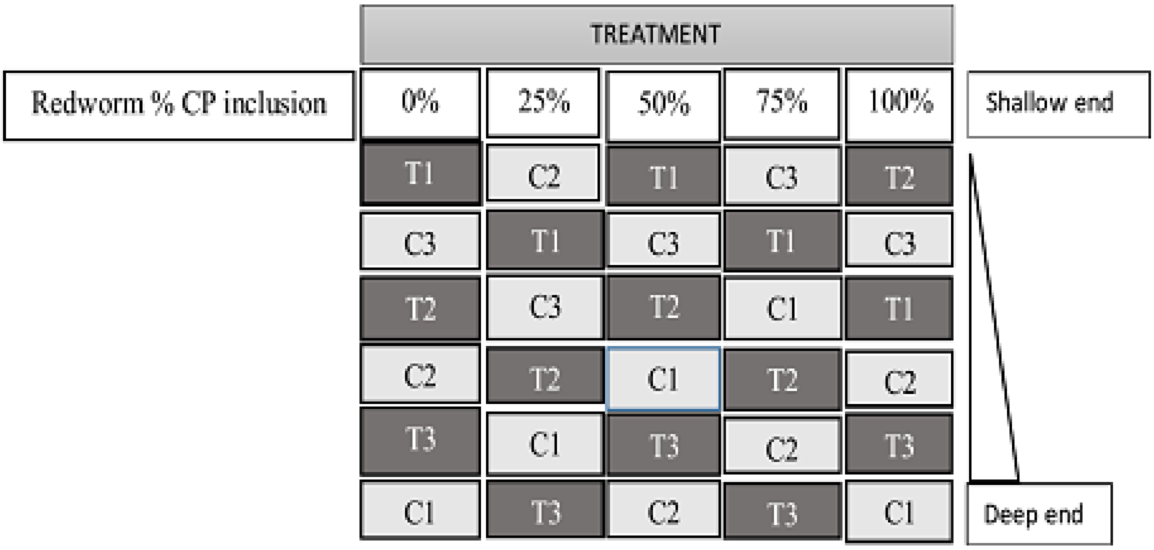

Figure 9: Experimental set-up in the pond culture of Nile tilapia and African catfish under different feeding regimes. T-tilapia, C-catfish, CP-crude protein, 1, 2 & 3 are experimental replicates.

3.6.1 Determination of fish growth performance

Sampling for fish growth parameters was done every fortnight and growth performance indicators which include absolute growth (AG) and specific growth rate (SGR) of fish calculated based on the equations 15 and 16 (Agbo *et al.*, 2011).

a. **Absolute Growth (AG)**

$$Absolute\ growth\ = \frac{Wf - Wi}{T} \tag{15}$$

Where: *Wi* is initial weight, *Wf* is the final weight and *T* is the time in days.

b. **Specific Growth Rate (SGR)**

$$Specific\ growth\ rate = \frac{In(Wf) - In(Wi)}{T} \times 100\% \tag{16}$$

Where: *In is the natural log, Wi* is initial weight, *Wf* is the final weight and *T* is the time in days.

3.6.2 Determination of Feed Conversion Ratio (FCR)

The FCR is a ratio of the weight gained by fish after consuming a known amount of feed over a given period. This was calculated using equation 17 from Agbo *et al.* (2011).

$$FCR = \frac{Amount\ of\ feed\ fed\ (kgs)}{weight\ gained\ by\ fish\ (kgs)} \quad (17)$$

3.6.3 Determination of length-weight (L-W) relationship

Fish Length-weight relationship was determined using equation 18 from Bolger and Connolly (1989).

$$W = aL^b \quad (18)$$

Where: W is the weight of fish (g), L is the total length of fish (cm), a is the rate of change of weight with length and b is the weight at unit length.

3.6.4 Determination of fish Condition factor (k)

The condition factor (K) of the cultured fish was determined using equation 19 from De Souza Braga and Gomiero (2005):

$$K = \frac{100W}{L^b} \quad (19)$$

Where: K is the condition factor, W is the weight of fish (g), L is the total length of fish (cm), b is the weight at unit length.

3.7 Water sampling and measurement of physico-chemical parameters

The physico-chemical parameters of fishponds including temperature, conductivity, dissolved oxygen and pH were measured *in situ* daily at 0800hrs using 'HACH' (HQ40d) multi probe meter at three selected points (deep end, shallow end and middle of pond) randomly. After every month, a diel sampling of all the physico-chemical parameters was done at an interval of two hours.

Water samples for nutrient analysis of Ammonia nitrogen-NH_4-N, Nitrate Nitrogen-NO_3-N, Nitrite Nitrogen-NO_2-N, Soluble Reactive Phosphorus-SRP and Total Phosphorus-TP were collected after every four weeks over the sampling period in triplicates in the two pond systems using 500ml acid-washed plastic bottles. The samples were transported in a cool box to the Limnology laboratory in Biological Sciences department of Egerton University for analysis immediately on arrival. Nutrients (Nitrogen and phosphorus) concentrations were determined calorimetrically following conversion from sample absorbance values using known standard curves.

3.8 Nutrients analysis

3.8.1 Determination of nitrogen in water sample

The analyzed Nitrogen components in the water samples included Ammonia-nitrogen (NH_4-N), Nitrite nitrogen (NO_2-N) and Nitrate-nitrogen (NO_3-N). Ammonium-nitrogen (NH_4-N) was determined by phenol-hypochlorite method (APHA, 2004). In this method, 2.5ml sodium salicylate solution and 2.5ml of hypochloride solution were added to 25ml of the filtered water samples from the fish ponds. The samples were then incubated in the dark for 90 minutes and thereafter, their absorbance read at a wavelength of 665nm using GENESYS 10 UV scanning spectrophotometer. The absorbance was used to calculate NH_4-N concentration using the equation derived from the standard calibration curve.

Nitrite-nitrogen (NO_2-N) was determined using the sulphanilamide method (APHA, 2004). 1ml of sulphanilamide solution was added to 20ml of filtered water sample and left to settle for 8 minutes. 1ml of N-Naphthyl-(1)-ethylendiaminedihydrochloride solution was then added and left to settle for another 10 minutes. The absorbance was read at a wavelength of 540nm. The concentration was calculated using the equation derived from the nitrite standard calibration curve.

Nitrate-nitrogen (NO_3-N) was determined by sodium-salicylate method (APHA, 2004). 1ml of sodium salicylate solution was added to 20ml of water sample and left to evaporate at 80 ºC to complete dryness. Resulting residue was then dissolved using 1ml H_2SO_4, followed by addition of 40ml distilled water and thereafter 7ml potassium-sodium hydroxide tartarate solution. The absorbance was read at a wavelength of 420 nm and concentration calculated using the equation derived from the Nitrate standard calibration curve.

3.8.2 Determination of phosphorus in water samples

Soluble reactive phosphorus (SRP) was analysed using the ascorbic acid method (APHA, 2004). The prepared reagents of ammonium molybdate solution (A), sulphuric acid (B), ascorbic acid (C) and potassium antimonyltartrate solution (D) were mixed in a ratio of A: B: C: D=2:5:2:1. The resulting mixed solution was added to the filtered water sample at a ratio of 1:10 and the absorbance read at 885nm wavelength using a GENESYS 10UV scanning spectrophotometer after 15 minutes of reaction. The concentration was determined from known concentrations of SRP standard solutions (APHA, 2004).

Total phosphorus (TP) was determined using ascorbic acid method, whereby 20g of unfiltered water sample was digested in persulphate reagent to reduce particulate phosphorus present in the sample into soluble reactive phosphorus-SRP form. After the persulphate digestion, evaporated water was replaced, and TP analyzed as SRP using ascorbic acid method. The concentration of TP was determined from the equation derived from the standard calibration curve of known TP standard solutions (APHA, 2004).

3.9 Data management and analysis

MS Excel (2013) was used to store data and carry out descriptive data analysis. Collected data was tested for normality and homogeneity of variance using the Shapiro-Wilk test and $\log_{10}(x+1)$ transformed. SPSS data package (version 21) was used for descriptive and inferential data analysis. In all the statistical analyses, 5% level of significance was used.

For objective 1, standard statistical computation was used in the evaluation of proximate composition of redworms meal against fishmeal was done. Student t-test was used to compare the nutrients (crude fibre, crude fats, ash content, dry matter and crude protein content) in the two feed ingredients after proximate analysis.

For objective 2, Second-order polynomial regression analysis was used to compare the growth performance of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) in open pond and greenhouse pond. One-way ANOVA was used to compare the growth performance of fish fed on feeds formulated using redworm against fishmeal protein ingredients for the five experimental diets.

For objective 3, Regression analysis of log-weight against log-length of each fish species determination of the length-weight relationship and condition factor of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems.

For objective 4, One-way ANOVA was used in determination of the food conversion ratio (FCR) of the formulated feeds fed to Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems.

CHAPTER FOUR

RESULTS

4.1 Water quality in culture systems

4.1.1 Diel variations of physico-chemical parameters

4.1.1.1 Temperature

The diel temperature in the two pond environments were significantly different ($t_{0.05}$, df=93, $P < 0.05$) with greenhouse recording higher mean temperature compared to the open pond (figure 10). Greenhouse pond recorded a temperature range of 22.3-27.5˚C and a mean diel temperature of 24.6±1.9˚C with a peak of 27.5˚C recorded at 1400hrs while lowest temperature of 22.3˚C being recorded at 0800hrs. In the open pond, mean temperature of 18.5±3.5˚C was recorded with highest temperature of 23.5˚C at 1600hrs while the lowest temperature recorded was 13.8˚C at 0600hrs.

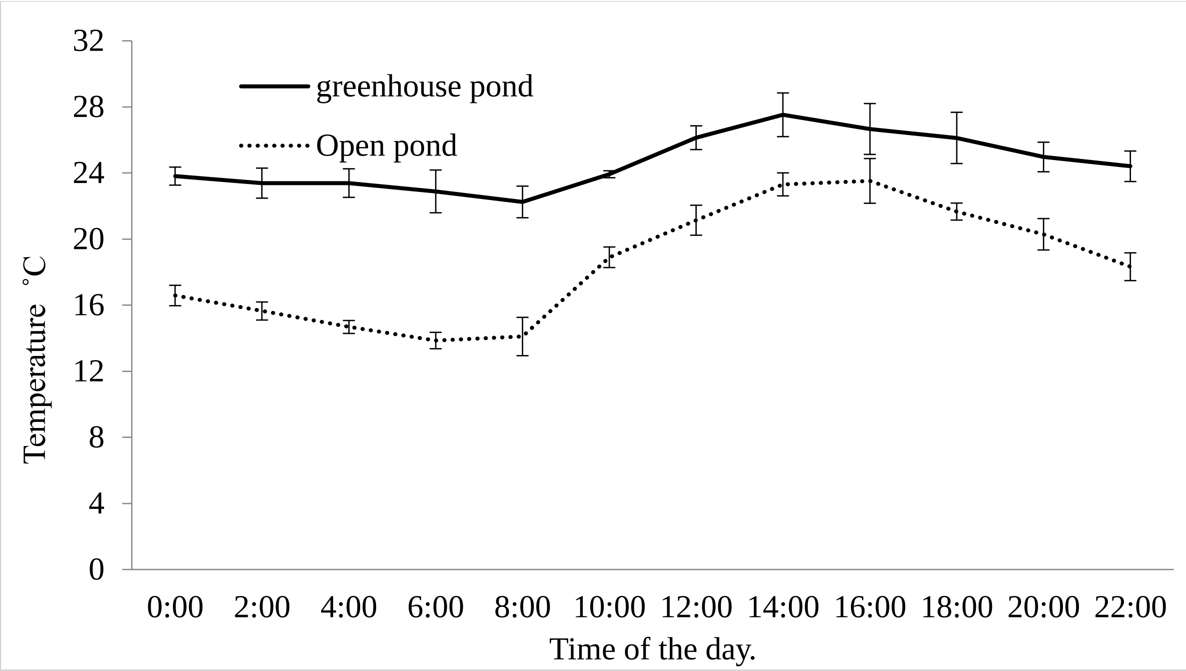

Figure 10: Diel temperature variation over time in the greenhouse and Open Pond systems (Error bars represents standard deviation of the data set).

4.1.1.2 Dissolved Oxygen

The diel pattern of dissolved oxygen concentration recorded in the two ponds did not vary significantly ($t_{0.05}$, df=93, $P > 0.05$). However, the mean diel oxygen concentration was higher in the open pond than in the greenhouse pond (figure 11). In the open pond, mean diel oxygen concentration of 5.48±1.37mg/L was recorded while the greenhouse pond had a diel oxygen concentration of 4.38±1.36mg/L. In the greenhouse pond, the highest oxygen concentration of 7.0mg/L was recorded at 1400hrs while the lowest value recorded was 2.87mg/L at 0600hrs.

In the open pond, the highest oxygen recorded was 10.04mg/L at 1400hrs while the lowest value was 2.96mg/L at 0800hrs.

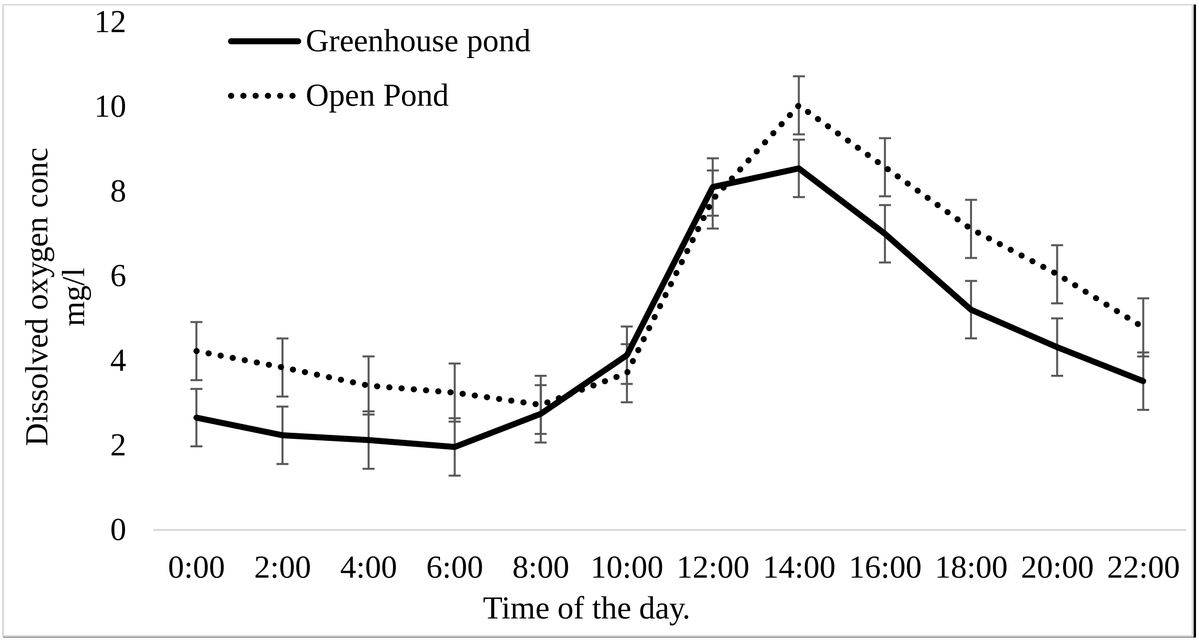

Figure 11: Diel oxygen variation over time in the greenhouse and Open Pond systems (Error bars represents standard deviation of the data set).

4.1.1.3 pH

The diel pH values recorded in the two pond systems did not vary significantly ($t_{0.05}$, df=93, $P > 0.05$) as shown in Figure 12.

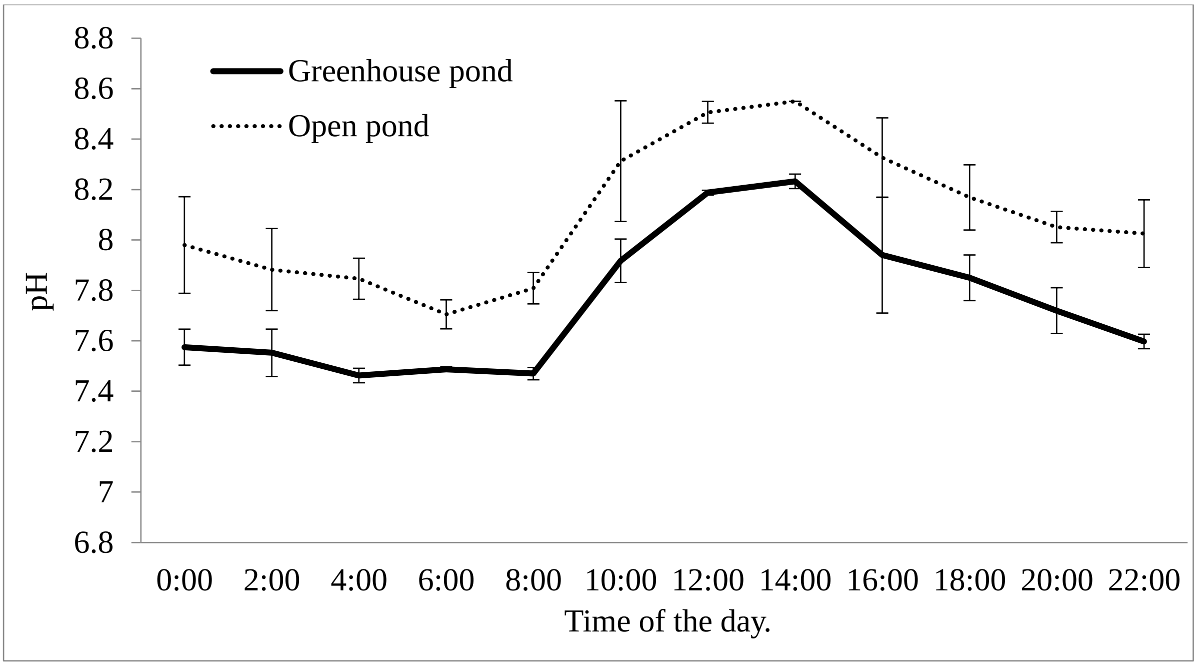

Figure 12: Diel pH variation over time in the greenhouse and Open Pond systems. (Error bars represents standard deviation of the data set).

In the greenhouse pond, diel pH values ranged from 7.44 to 8.27 which were slightly lower than those recorded in the open pond whose diel range was 7.63 to 8.55. On the diel cycle, the highest pH values of 8.55 and 8.23 were recorded at around 1400hrs in the greenhouse and open pond respectively. The pH in the two pond systems rose between 0400hrs and 0600hrs. During this time, pH values of 7.75 and 7.46 were recorded in the greenhouse and open pond respectively.

4.1.1.4 Electrical conductivity

The diel electrical conductivity in the two ponds did not vary significantly ($t_{0.05}$, df=93, $P > 0.05$). In the greenhouse pond, a mean diel electrical conductivity of 578.07±21.02 µs/cm, was recorded. This was higher than the open pond's mean diel electrical conductivity value of 500.15±39.81µs/cm (figure 13).

The highest mean conductivity of 628.02±14.02µs/cm in the greenhouse pond was recorded at 1600hrs but dropped to 522.38±8.02µs/cm at 0600hrs. In the open pond, the highest mean conductivity of 524.22±17.02µs/cm was recorded at 1600hrs, and dropped to 444.95±12.02µs/cm at 0400hrs.

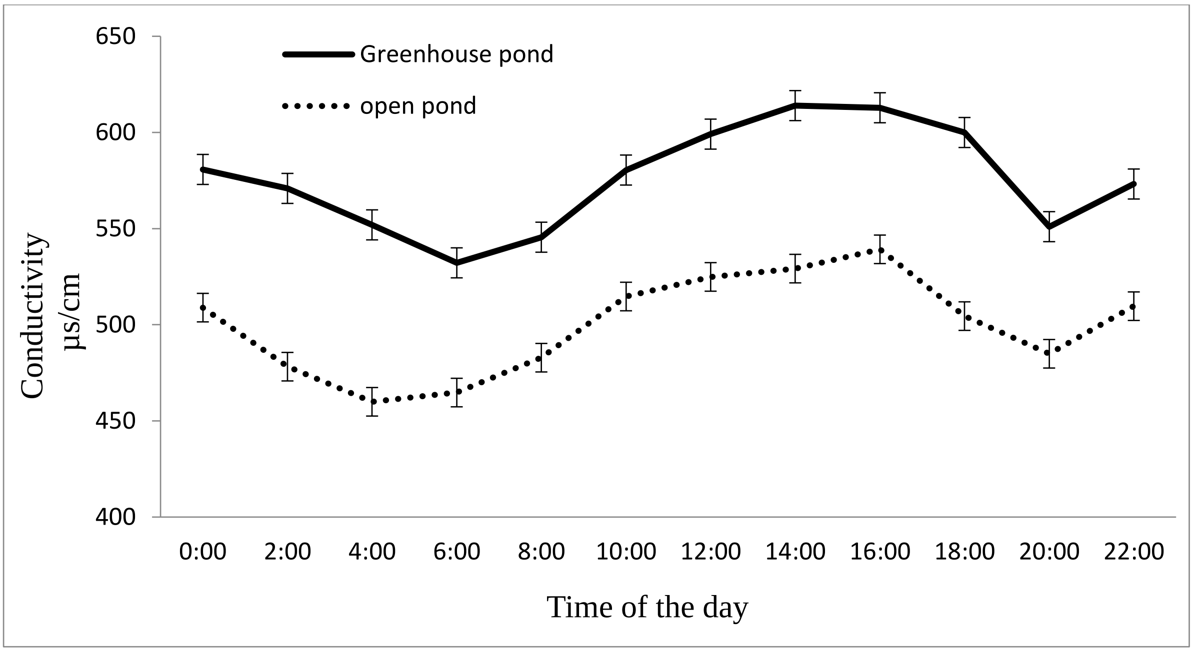

Figure 13: Diel electrical conductivity variation over time in the greenhouse and Open Pond systems (Error bars represents standard deviation of the data set).

4.1.2 Mean daily variations of physico-chemical parameters

A summary of results for the measured physico-chemical parameters obtained from the two pond environments are shown in table 1. Throughout the study period, there was a significant difference in the mean daily temperatures recorded in the two ponds ($t_{0.05}$, df=41, $P < 0.05$) with greenhouse pond recording higher temperature values compared to open pond. Greenhouse pond had a mean temperature of 23.6±1.7˚C with maximum temperature of 27.1˚C and minimum of 20.9˚C while in the open pond system, maximum temperature of 24.4˚C and minimum of 15.6˚C with mean daily value of 18.7±2.1˚C were recorded.

There was no significant difference in the mean oxygen concentration recorded ($t_{0.05}$, df=41, $P > 0.05$) in the two pond systems during the study period. However, mean oxygen concentration values recorded in the open pond was higher than that of the greenhouse pond. In the greenhouse pond, the maximum and minimum oxygen concentration were 8.34mg/l and 0.38mg/L respectively with a mean of 3.25±1.04mg/L. Open Pond environment recorded maximum and minimum oxygen concentration of 9.3mg/L and 1.13mg/L respectively with a mean oxygen concentration of 3.57±2.31mg/L.

The values of pH in this study were not significantly different between the two pond systems ($t_{0.05}$, df=41, $P > 0.05$). In the greenhouse pond, the recorded pH ranged from 7.45 to 8.22 while in the open pond, pH values were from 7.76 to 8.45. Electrical conductivity (EC) values recorded from the two pond systems had no significant difference, with greenhouse pond recording higher EC compared to open pond ($t_{0.05}$, df=41, $P > 0.05$). The greenhouse pond had a mean conductivity value of 578.07±21.02µs/cm while open pond recorded a mean conductivity value of 500.15±39.81µs/cm.

Table 1: A summary of physico-chemical parameters in greenhouse pond and open pond systems during the study period (Mean ± SD, n =42).

	Type of Pond							
	Greenhouse Pond				Open Pond			
	Temp ˚C	Oxy mg/l	pH	Cond µs/cm	Temp ˚C	Oxy mg/l	pH	Cond µs/cm
Mean	23.56	3.57		578.07	18.72	3.25		500.15
±SD	±1.74	±2.34		±21.02	±2.09	±2.31		±39.81
Min	20.90	0.38	7.45	528	15.60	1.13	7.76	430
Max	27.13	8.34	8.22	628	24.40	9.31	8.45	521
Range	6.23	7.96	1.77	100	8.80	8.18	2.10	91

4.1.3 Correlation analysis of physico-chemical parameters

Pearson Correlation analysis was carried out on the physico-chemical data to see the nature of their relationship. The results are presented in table 2. In both ponds, it was observed that there were positive linear relationships between the temperature, oxygen, pH and conductivity.

Table 2: Correlation matrix between physico-chemical parameters in the greenhouse pond and open pond systems.

Pond type	Parameters	Temperature	Oxygen	pH	Conductivity
Greenhouse	Temperature	1			
	Oxygen	0.802**	1		
	pH	0.811**	0.904**	1	
	Conductivity	0.730**	0.532**	0.574**	1
Open pond	Temperature	1			
	Oxygen	0.906**	1		
	pH	0.823**	0.775**	1	
	Conductivity	0.686**	0.591**	0.740**	1

**Correlation is significant at the 0.01 level (2-tailed).

4.1.4 Concentration of nutrients in the two pond systems

Results of the analyzed nutrients (Ammonium, Nitrites, Nitrates, Soluble reactive Phosphorus and Total Phosphorus) in the two pond systems are presented in table 3. The Nitrogen components (Ammonium, Nitrites and Nitrates) varied in the two pond systems. Ammonium concentrations in the two ponds were significantly different ($t_{0.05}$, df=17, $P < 0.05$), with higher mean value of 0.42±0.03mg/L recorded in the greenhouse pond compared to 0.21±0.02mg/L in the open pond system.

Nitrite-Nitrogen concentrations from the two pond systems had no significant difference ($t_{0.05}$, df=17, $P > 0.05$). Nitrite concentration in greenhouse pond was lower with a mean value of 0.02±0.01mg/L compared to the mean of 0.03±0.01mg/L in the open pond. Similarly, Nitrates from the two ponds were significantly different ($t_{0.05}$, df=17, $P < 0.05$), with lower mean value recorded in the open pond than in the greenhouse pond. Nitrate had mean concentration of 0.03±0.01mg/L and 0.05±0.01mg/L in the greenhouse and open pond system respectively.

There was no significant variation in soluble reactive phosphorus in the two pond systems ($t_{0.05}$, df=17, $P > 0.05$). The SRP concentrations in the greenhouse pond were higher than in the open pond. The SRP had a mean concentration of 0.13±0.02mg/L and 0.12±0.02mg/L in the greenhouse and open ponds respectively. Similarly, the concentration of TP in the two pond systems did not vary significantly ($t_{0.05}$, df=17, P > 0.05). The recorded mean TP concentrations were 0.61±0.05mg/L and 0.49±0.03mg/L in the greenhouse and open ponds respectively.

Table 3: Nutrient concentrations in greenhouse pond and open pond systems during the study period (Mean ± SD, n =18).

	Pond Type	
Parameter	**Greenhouse**	**Open**
Ammonium (mg/L)	$0.42±0.03^{a}$	$0.21±0.02^{b}$
Nitrite (mg/L)	$0.02±0.01^{a}$	$0.03±0.01^{a}$
Nitrate (mg/L)	$0.03±0.01^{a}$	$0.05±0.01^{b}$
SRP (mg/L)	$0.13±0.02^{a}$	$0.12±0.02^{a}$
TP (mg/L)	$0.61±0.05^{a}$	$0.49±0.03^{a}$

Values in the same raw with different superscripts are significantly different.

4.2 Proximate composition of Feed ingredients

The proximate composition results of crude protein, crude lipids, moisture, ash and crude fibre expressed in terms of percentages are shown in table 4.

Table 4: Results of proximate composition for experimental feed ingredients. Values indicate % means ± standard deviation.

Feed	**Proximate composition (%)**				
ingredient	Protein	Lipids	Moisture	Ash	Fibre
Fishmeal	$57.71±0.77^{a}$	$5.51±0.19^{a}$	$6.07±0.06^{a}$	$9.25±0.23^{a}$	$5.15±0.08^{b}$
Redworms	$62.29±1.13^{a*}$	$6.34±0.48^{a*}$	$7.97±0.06^{a*}$	$12.23±0.12^{a*}$	$3.02±0.80^{c}$
Sunflower	$40.13±0.40^{b}$	$2.74±0.05^{c}$	$3.95±0.10^{b}$	$8.00±0.19^{b}$	$15.47±0.37^{a*}$
Soya meal	$36.13±0.50^{b}$	$4.71±0.36^{b}$	$3.24±0.53^{b}$	$8.70±0.20^{b}$	$6.68±0.05^{b}$
Wheat bran	$12.54±0.48^{c}$	$2.11±0.06^{c}$	$2.63±0.02^{b}$	$1.73±0.22^{c}$	$13.07±0.13^{a}$

Values with different superscripts in the same column are significantly different P<0.05), the highest value in column is indicated by *.

The crude protein content obtained varied significantly from one feed ingredient to another (One Way ANOVA, df = 4, $P < 0.05$). Animal based protein ingredients recorded the highest crude protein content compared to plant ingredients. Redworm meal had the highest percentage mean protein level of 62.29% followed by fishmeal with a mean crude protein of 57.71%. Among the analyzed plant ingredients, sunflower had the highest crude protein mean of 40.13% followed by soya-bean meal with mean crude protein of 36.13% while wheat bran recorded the lowest mean crude protein of 12.54%.

Proximate results of extracted crude lipids in the five feed ingredients were significantly different (One Way ANOVA, df = 4, $P < 0.05$). Animal-based ingredients contained higher lipid content compared to plant-based ingredients. Redworm meal had a mean lipid content of 6.34% followed by fishmeal with mean lipid content of 5.51%. Lipids in plant-based ingredients were highest in soya bean meal which had 4.71% followed by sunflower with 2.74% while wheat bran had the lowest content of 2.11%.

The moisture content among the analyzed feed ingredients was significantly different (One-way ANOVA, df = 4, $P < 0.05$). Animal based ingredients had higher levels of moisture compared to plant ingredients. Redworm meal recorded the highest mean moisture content of 7.97% followed by fishmeal with mean moisture content of 6.07%. Sunflower, soya bean meal and wheat bran recorded mean moisture contents of 3.95%, 3.24% and 2.63% respectively.

There was a significant difference in mean ash content among the analyzed feed ingredients (One Way ANOVA, df = 4, $P < 0.05$). Higher levels of ash were found in animal-based feed ingredients than plant-based ones. Redworm meal had a mean ash content of 12.23% followed by fishmeal with a mean of 9.25%. Wheat bran had the lowest mean ash content of 1.73% followed by sunflower and soya bean meal with mean ash content of 8.0% and 8.7% respectively.

The proximate composition of crude fibre in the five feed ingredients varied significantly (One Way ANOVA, df = 4, $P < 0.05$). Results indicated that plant-based ingredients had the higher fibre content than animal-based feed ingredients. Among the plant-based ingredients, sunflower, wheat bran and soya bean meal had mean crude fibre content of 15.47%, 13.07% and 6.68% respectively. These values were higher compared to those of fish meal and redworms which contained 5.15% and 3.02% respectively.

4.3 Growth Performance of Nile tilapia and African catfish

4.3.1 Growth performance indices Mean Weight Gain (MWG), Absolute Growth (AG) and Specific Growth Rate (SG)

The fish growth parameters of mean weight gain (MWG), absolute growth (AG) and specific growth (SG) rates of Nile tilapia and African catfish in the two pond systems are summarized in tables 5 and 6 respectively. Growth rates of both fish species cultured in the greenhouse pond were significantly higher than those in the open pond (t-test, df=1, $p < 0.05$).

There was no significant difference in the growth rates of Nile tilapia fed on diets containing different redworm rations (Figure 14). The growth rates of Nile tilapia fed on redworm rations of 0%, 25%, 50%, 75% and 100% showed no significant difference in the greenhouse pond (One Way ANOVA, df = 4, $p = 0.778$) and in the open pond (One Way ANOVA, df = 4, $P > 0.05$). however, the mean weight gain of Nile tilapia fed on 50% redworm inclusion ration recorded the highest mean weight gain of 251.78±6.33g in the greenhouse pond and 60.77±7.08g in the open pond environment.

Nile tilapia fed on control diet in the greenhouse and open ponds had a mean weight gain of 245.35±2.97g and 56.85±10.04g respectively while Nile tilapias fed on 100% redworm inclusion in the greenhouse and open pond had mean values of 244.43±5.76g and 55.93±6.01g respectively. African catfish cultured in the greenhouse pond recorded significantly higher growth than those in the open pond (t-test, df=1, $p < 0.05$). Growth rate assessment of African catfish cultured in the greenhouse pond fed on rations (0%, 25%, 50%, 75% and 100% redworm inclusion) showed no significant difference (One Way ANOVA, df = 4, $P > 0.05$). Similarly, there was no significant difference in the growth rates obtained for catfish fed on the five rations in the open pond (One Way ANOVA, df = 4, $P > 0.05$).

Evaluation of the five redworms rations performance revealed that 50% redworm inclusion feed produced the highest mean weight gain for catfish cultured in the greenhouse and open ponds (figure 15). Catfish in the greenhouse and Open Pond recorded a mean weight of 435.33±10.05g and 111.35±13.4g respectively. The performance in terms of final weight of catfish fed 0% redworms (control diet) and 100% redworms had less variation. The mean weight gain of catfish fed on the control diet (0% redworms) in the greenhouse pond and open pond was 414.22±10.43g and 107.86±17.10g respectively while when fed on 100% redworms diet a mean weight of 414.62±7.07g and 103.17±17.07g were achieved for the catfish raised in the greenhouse and open pond.

Table 5: Mean (±SD) values of weight gain, Absolute growth and Specific growth rate of Nile tilapia fed on experimental redworm rations in the two pond systems.

	Treatments (redworm inclusion)									
Growth	**0% (control diet)**		**25%**		**50%**		**75%**		**100%**	
Parameters	**Ghs P**	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**
Initial length (cm)	4.87± 0.40	5.08± 0.27	4.93± 0.14	4.91± 0.27	4.94± 0.29	4.93± 0.14	5.08± 0.27	4.94 ±0.29	4.91± 0.27	4.87± 0.40
Final length (cm)	24.29± 0.47	18.63± 1.19	24.01± 0.50	17.57± 0.21	24.12± 0.78	18.71± 0.93	23.82± 0.54	17.45 ±0.55	23.82± 0.64	18.41± 0.68
Initial weight (g)	2.02± 0.31	2.04± 0.24	2.01± 0.12	1.99± 0.24	2.02± 0.34	2.06± 0.12	2.04± 0.24	2.02 ±0.34	1.99± 0.24	2.02± 0.31
Final weight (g)	247.37± 3.17	58.89± 10.91	243.33± 3.62	50.07± 6.55	**253.80± 7.69****	**62.83± 8.37****	242.80± 7.67	49.57 ±4.48	246.42± 7.06	57.95± 6.48
WG (g)	245.35± 2.97	56.85± 10.04	241.32± 2.77	48.08± 6.01	**251.78± 6.33****	**60.77± 7.08****	240.76± 7.06	47.55± 4.07	244.43± 5.76	55.93± 6.01
AG (g)	1.40± 0.02	0.32± 0.06	1.38± 0.02	0.27± 0.04	**1.44± 0.04****	**0.35± 0.05****	1.38± 0.01	0.27± 0.03	1.37± 0.04	0.32± 0.04
SGR	1.16± 0.03	0.83± 0.07	1.12± 0.04	0.80± 0.03	**1.20± 0.02****	**0.85± 0.03****	1.13± 0.04	0.79± 0.04	1.16± 0.03	0.83± 0.04

WG, AG, SGR, Ghs P and O P = Mean Weight gain, Absolute growth, Specific Growth Rate, Greenhouse pond and Open pond, respectively

The highest Value is indicated by superscript ** and bolded.

Table 6: Mean (±SD) values of weight gain, Absolute growth and Specific growth rate of African catfish fed on experimental redworm rations in the two pond systems.

	Treatments (redworm inclusion)									
Growth Parameters	**0% (control diet)**		**25%**		**50%**		**75%**		**100%**	
	Ghs P	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**	**Ghs P**	**O P**
Initial length (cm)	6.00± 0.46	5.89± 0.19	6.17± 0.22	5.93± 0.25	6.36± 0.26	5.97± 0.21	6.02± 0.28	5.85± 0.39	6.29± 0.39	5.98± 0.29
Final length (cm)	37.31± 0.73	34.57± 1.83	36.61± 1.18	33.81± 1.63	39.43± 0.74	34.78± 1.57	37.25± 1.59	33.25± 1.99	38.13± 0.56	33.77± 1.79
Initial weight (g)	1.49± 0.21	1.44± 0.04	1.49± 0.08	1.47± 0.09	1.53± 0.15	1.47± 0.09	1.47± 0.09	1.46± 0.09	1.53± 0.20	1.47± 0.07
Final weight (g)	416.22± 10.43	107.86± 17.10	398.26± 6.24	103.24± 14.33	**436.86± 12.65****	**112.82± 14.72****	397.22±3 6.42	102.25± 16.72	416.15± 7.91	108.04±1 7.77
WG (g)	414.73± 8.12	106.42± 12.02	396.77± 5.02	101.77± 12.03	**435.33± 10.05****	**111.35± 13.04****	377.75±2 4.98	100.79± 14.08	414.62± 7.07	103.17± 17.01
AG (g)	2.35± 0.06	0.61± 0.10	2.27± 0.04	0.58± 0.08	**2.49± 0.07****	**0.64± 0.08****	2.26± 0.06	0.57± 0.09	2.37± 0.05	0.59± 0.09
SGR	1.40± 0.03	1.07± 0.08	1.38± 0.01	1.05± 0.05	**1.41± 0.11****	**1.08± 0.17****	1.39± 0.02	1.05± 0.04	1.39± 0.09	1.06± 0.06

WG, AG, SGR, Ghs P and O P = Mean Weight gain, Absolute growth, Specific Growth Rate, Greenhouse Pond and Open pond, respectively

The highest Value is indicated by superscript ** and bolded.

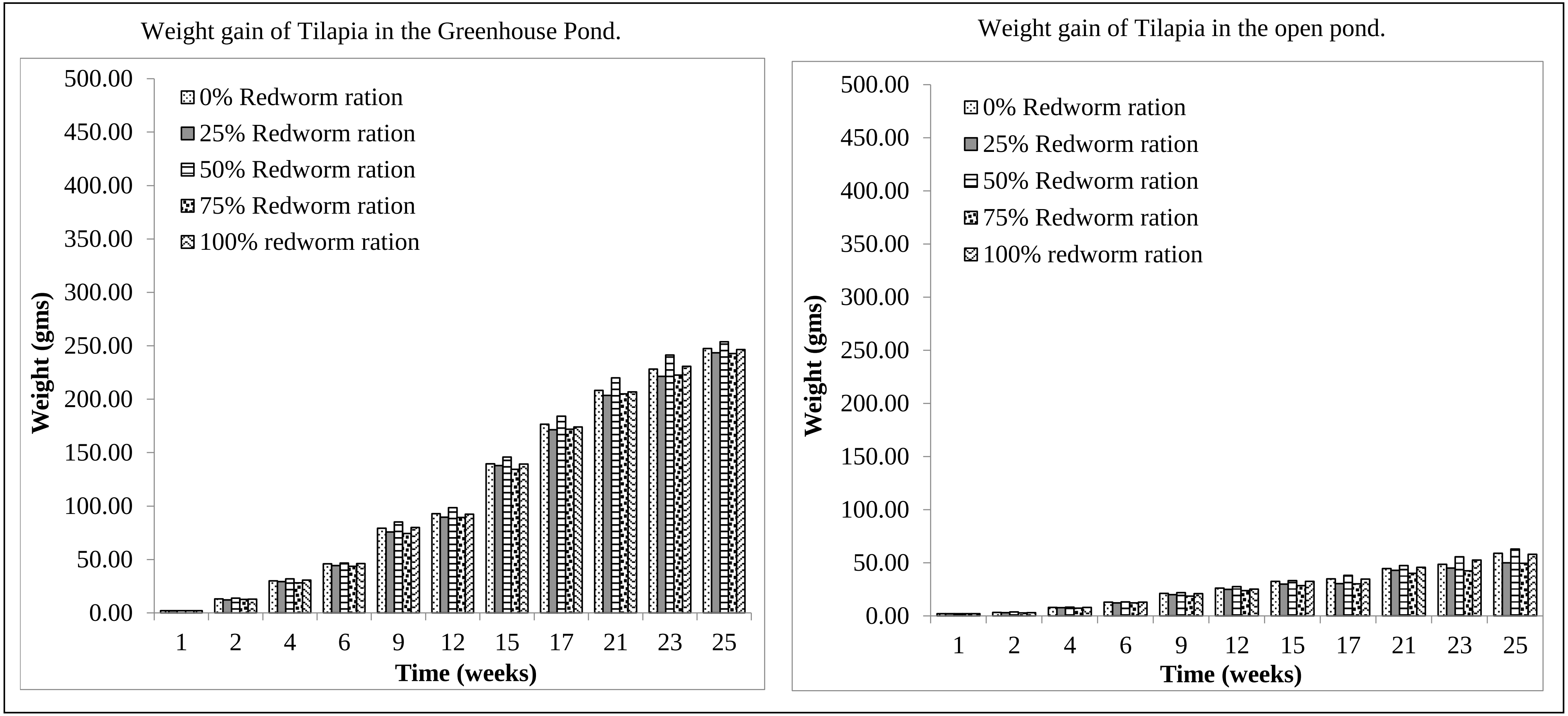

Figure 14: The relationship of weight gain against time of Nile tilapia (*Oreochromis niloticus*) cultured in greenhouse and open pond.

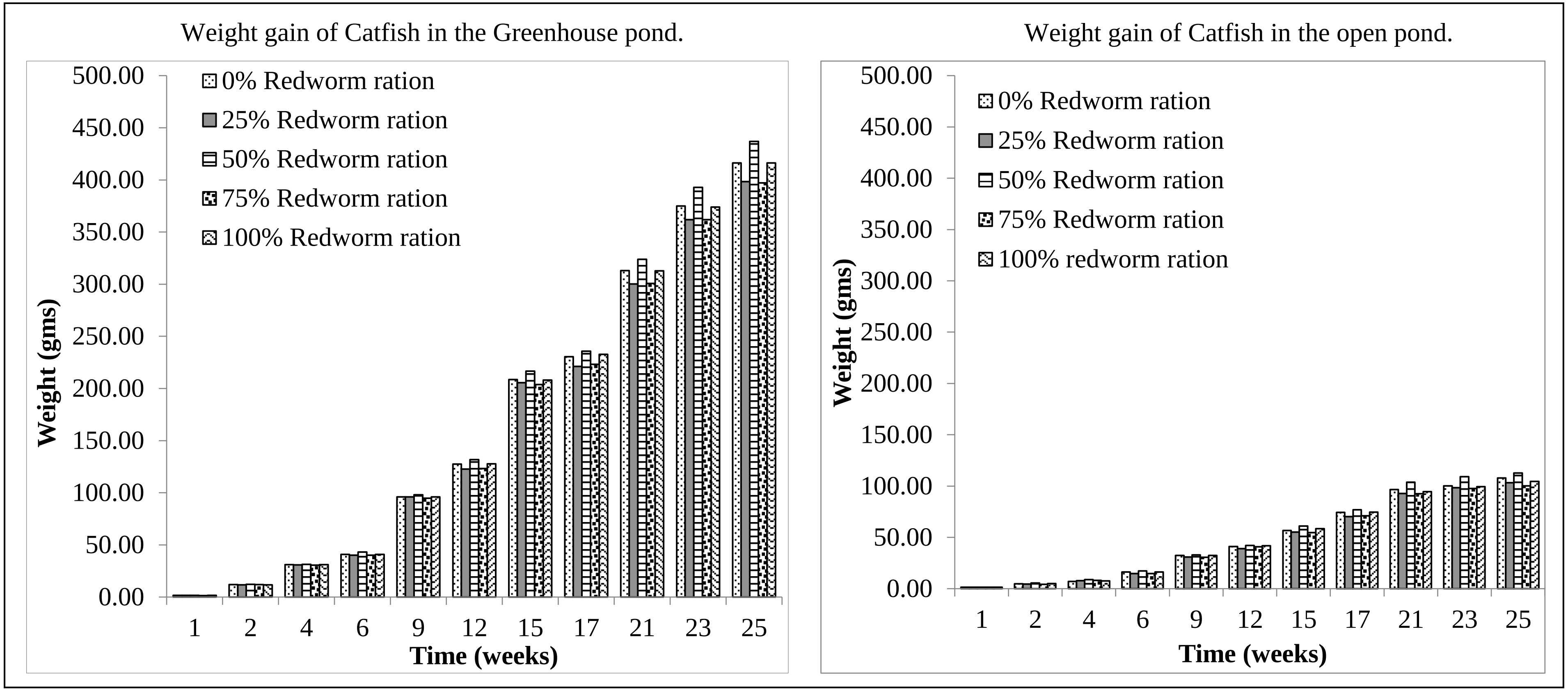

Figure 15; The relationship of weight gain against time of African Catfish (*Clarias gariepinus*) cultured in greenhouse pond and open pond systems.

4.3.2 Absolute growth (AG)

The absolute growth (AG) values of Nile tilapia and African catfish are presented in tables 5 and 6. Nile tilapia from greenhouse and open pond had absolute growth rates in the range of 1.39 to 1.48 and 0.30 to 0.40 respectively when fed on the five redworm experimental diets (0%, 25%, 50%, 75% and 100%), which were statistically not significant (t-test, df=1, $p > 0.05$). On the other hand, African catfish recorded AG values in the range of 2.31 to 2.56 and 0.66 to 0.72 in the greenhouse and open pond, respectively. These values were also statistically not significant (t-test, df=1, $p > 0.05$).

4.3.3 Specific growth rate (SGR)

The specific growth rate of Nile tilapia cultured in the greenhouse pond were statistically different from those in the open pond (t-test, df=1, $p < 0.05$), with the values ranging from 1.16 to 1.22 and 0.83 to 0.88 respectively when fed on the five redworm experimental diets (table 5). There was no significant difference in the SGR of Nile tilapia fed on the five redworm experimental diets (0%, 25%, 50%, 75% and 100%) in the greenhouse pond (One Way ANOVA, df = 4, $P > 0.05$) and in the open pond (One Way ANOVA, df = 4, $P > 0.05$). However, comparison of five redworm experimental diets showed that 50% redworm inclusion diet had the highest mean specific growth rate of 1.20±0.02 and 0.85±0.03 in greenhouse and open pond, respectively.

The SGR of African catfish cultured in the greenhouse and open ponds were significantly different (t-test, df =1, $p < 0.05$), with values in the range of 1.39 to 1.52 and 1.09 to 1.26 respectively (table 6). Evaluation of the five redworm experimental diets on growth performance of African catfish showed no significant difference in the SGR of African catfish in greenhouse (One Way ANOVA, df = 4, $P > 0.05$) and those cultured in the open pond (One Way ANOVA, df = 4, $P > 0.05$). In the two pond systems, feed ration containing 50% redworm inclusion gave the highest mean specific growth rate value (1.41±0.11 for greenhouse pond and 1.08±0.17 for open pond) when compared to other rations.

4.3.4 Length-Weight Relationship (LWR) and Fish Condition Factor (K)

4.3.4.1 Length-Weight Relationship (LWR)

Nile tilapia cultured in the greenhouse pond recorded an isometric growth with an allometry coefficient represented by b-values of 3.15, 3.14, 3.13, 3.15 and 3.16 when fed on diets containing 0%, 25%, 50%, 75% and 100% redworm ration respectively (table 7). The b-values varied insignificantly from one another (One Way ANOVA, df = 4, $P > 0.05$). In contrast, Nile tilapia cultured in the open pond had recorded a negative allometric growth represented by b-values of 2.29, 2.29, 2.29, 2.34 and 2.29 when fed on feed containing 0%, 25%,50%,75% and 100% redworms respectively. These values were not significantly different (One Way ANOVA, df = 4, P > 0.05). Comparison of the b-values of Nile tilapia cultured in the greenhouse pond and open pond indicated significant differences (t-test, df =1, P > 0.05).

African catfish cultured in the greenhouse pond recorded an isometric growth represented by b-values of 3.13, 3.13, 3.08, 3.12 and 3.11 when fed on diets containing 0%, 25%, 50%, 75% and 100% redworm meal respectively. On the other hand, African catfish cultured in the open pond recorded a negative allometric growth represented by b-values of 2.41, 2.43, 2.40, 2.43 and 2.41 when fed on diets containing 0%, 25%, 50%, 75% and 100% redworm meal respectively. These values had no significant difference (One Way ANOVA, df = 4, P > 0.05). However, calculated mean b-values of African catfish in the greenhouse pond and open pond had a significant difference (t-test, df =1, p < 0.05).

4.3.4.2 The Fish Condition Factor (K)

Nile tilapia cultured in the greenhouse pond, recorded condition factor (K) values of 2.10, 2.11, 2.21, 2.23 and 2.06 respectively whereas in the open pond they recorded K-values of 0.87, 0.88*, 0.90, 0.87 and 0.87 when fed with diets containing 0%, 25%, 50%, 75% and 100% redworm meal. Evaluation of the effect of diets on K-factor in individual pond environment revealed that there were no significant differences (One Way ANOVA, df = 4, P > 0.05). On the other hand, evaluation of the effect of diets on the fish condition factor between the two pond systems revealed that the Nile tilapia cultured in the greenhouse had K-values being significantly higher (t-test, p < 0.05) compared to those in the open pond (table 8).

The calculated K-values of African catfish cultured in the greenhouse pond were 3.67, 3.48, 3.81, 3.45 and 3.66 when fed on redworm ration, 0%, 25%, 50%, 75% and 100%, respectively, results which were not significantly different from one another (One Way ANOVA, df = 4, P > 0.05). On the other hand, in the open pond, the calculated K values for African catfish were 0.88, 0.86, 0.90, 0.86 and 0.88 when fed on 0%, 25%, 50%, 75% and 100%, redworm ration respectively. These results showed no significant difference in the K-values of African catfish when fed on the five experimental diets, (One Way ANOVA, df = 4, P > 0.05). However, when the K-value data for the African catfish cultured in the greenhouse and open pond systems were compared, there was a significant difference (t-test, P > 0.05) with higher K-values in greenhouse pond (table 7).

Table 7: Regression equations and condition factor of Nile tilapia and African catfish in open and greenhouse pond systems.

Pond type	Fish species	Diet Ration	Regression equation ($W = aL^b$)	a intercept	b exponent	r^2	K	Growth description
Greenhouse pond	Nile Tilapia	$R_{0\%}$	$W=1.95L^{3.15}$	1.96[b]	3.15[b]	0.98	2.10[a]	Isometric
		$R_{25\%}$	$W=1.94L^{3.14}$	1.94[b]	3.14[b]	0.99	2.11[a]	Isometric
		$R_{50\%}$	$W=1.92L^{3.13}$	1.92[b]	3.13[b]	0.99	2.21[a]	Isometric
		$R_{75\%}$	$W=1.99L^{3.15}$	1.99[b]	3.15[b]	0.99	2.23[a]	Isometric
		$R_{100\%}$	$W=1.95L^{3.16}$	1.95[b]	3.16[b]	0.99	2.06[a]	Isometric
	African Catfish	$R_{0\%}$	$W=2.56L^{3.13}$	2.56[b]	3.13[b]	0.98	3.67[a]	Isometric
		$R_{25\%}$	$W=2.55L^{3.13}$	2.55[b]	3.13[b]	0.98	3.45[a]	Isometric
		$R_{50\%}$	$W=2.49L^{3.08}$	2.49[b]	3.08[b]	0.98	3.81[a]	Isometric
		$R_{75\%}$	$W=2.52L^{3.12}$	2.52[b]	3.12[b]	0.98	3.45[a]	Isometric
		$R_{100\%}$	$W=2.56L^{3.11}$	2.56[b]	3.11[b]	0.98	3.66[a]	Isometric
Open pond	Nile Tilapia	$R_{0\%}$	$W=1.34L^{2.29}$	1.34[a]	2.29[a]	0.96	0.87[b]	Allometric
		$R_{25\%}$	$W=1.34L^{2.29}$	1.34[a]	2.29[a]	0.95	0.88[b]	Allometric
		$R_{50\%}$	$W=1.32L^{2.29}$	1.32[a]	2.29[a]	0.96	0.90[b]	Allometric
		$R_{75\%}$	$W=1.33L^{2.34}$	1.33[a]	2.34[a]	0.95	0.87[b]	Allometric
		$R_{100\%}$	$W=1.35L^{2.29}$	1.35[a]	2.29[a]	0.96	0.88[b]	Allometric
	African Catfish	$R_{0\%}$	$W=1.39L^{2.41}$	1.39[a]	2.41[a]	0.96	0.88[b]	Allometric
		$R_{25\%}$	$W=1.41L^{2.43}$	1.41[a]	2.43[a]	0.96	0.86[b]	Allometric
		$R_{50\%}$	$W=1.40L^{2.40}$	1.40[a]	2.40[a]	0.96	0.90[b]	Allometric

	$R_{75\%}$	$W=1.41L^{2.43}$	1.41^{a}	2.43^{a}	0.96	0.86^{b}	Allometric
	$R_{100\%}$	$W=1.44L^{2.41}$	1.44^{a}	2.41^{a}	0.96	0.88^{b}	Allometric

Where W = Weight (g), a Intercept = Gradient, b intercept = Slope, L = Standard length (cm), r = Correlation coefficient. Columns with different superscript are significantly different.

4.4 Food Conversion Ratio (FCR)

The results of the amount of feed given and the fish weight gain by the end of the experimental study are summarized in table 8.

Table 8: Food conversion ratio of experimental diets fed to Nile tilapia and African catfish in the greenhouse and open pond systems.

Pond type	**Fish type**	**Redworm feed ration** (%)	**Total Feed (kgs) fed daily**	**Fish weight gain (Kgs)**	**Feed Conversion Ratio (FCR)**
Greenhouse	Nile tilapia	0	2.98	1.62	1.84[a]
		25	2.83	1.55	1.83[a]
		50	3.06	1.69	1.81[a]
		75	2.80	1.50	1.86[a]
		100	2.91	1.57	1.85[a]
	African catfish	0	11.33	6.24	1.81[a]
		25	10.85	5.97	1.82[a]
		50	11.71	6.55	1.78[a]
		75	10.82	5.96	1.82[a]
		100	11.34	6.24	1.82[a]
Open	Nile tilapia	0	1.90	0.88	2.25[b]
		25	1.74	0.75	2.32[b]
		50	2.03	0.94	2.25[b]
		75	1.70	0.74	2.28[b]
		100	2.00	0.87	2.30[b]
	African catfish	0	8.20	3.71	2.21[b]
		25	7.96	3.65	2.18[b]
		50	8.41	3.85	2.18[b]
		75	7.93	3.64	2.18[b]
		100	8.20	3.70	2.22[b]

Superscripts a & b indicate significance level in the feed conversation ration. Those with a similar superscript are not significant different.

A total of 120.7 Kgs of formulated fish feed was used during the feeding trial with 9.37Kgs and 40.7Kgs of feed used on Nile tilapia and African catfish respectively in the open pond, while 14.58Kgs and 56.05Kgs of feed was used in the greenhouse pond to feed Nile tilapia and African catfish, respectively.

The FCR were calculated based on weight gain and the average amount of fish feed fed daily to the fish in the two systems. The five redworm experimental diets fed to Nile tilapia in the open pond had FCR values that ranged from 2.25 to 2.32 and were significantly higher than 1.81 to 1.86 value range in the greenhouse pond (t-test, df =1, $P < 0.05$). On the other hand, the FCR of the redworm experimental diets given to African catfish in the open pond were in the range of 2.18 to 2.22, being significantly higher than the greenhouse pond values which ranged from 1.78 to 1.82 (t-test, df =1, $p < 0.05$).

CHAPTER FIVE

DISCUSSION

5.1 Water quality parameters in greenhouse and open pond environments

5.1.1 Temperature

During the study period, greenhouse pond recorded temperature mean of 23.56±1.74˚C with 27.13˚C and 20.90˚C being highest and lowest temperature recorded respectively. These temperatures were higher compared to open pond whose water temperature had mean of 18.72±2.09˚C with 21.42˚C and 21.42˚C highest and lowest temperatures recorded. The daily mean temperature was higher in the greenhouse pond than in the open pond. Diel monitoring indicated that temperature dropped by 5.3˚C and 9.7˚C in the greenhouse pond and open pond respectively. Furthermore, mean temperatures recorded at night were higher in the greenhouse than in open pond. This is evidence that the greenhouse pond was more cushioned against the drastic temperature drop than the open pond.

The higher temperature values recorded in the greenhouse pond could be due to the '*greenhouse effect'*. During sunshine, total solar radiation received by the greenhouse geo-membrane cover is partly reflected, absorbed and transmitted inside the greenhouse through walls and roofs. A large portion of this transmitted radiation is absorbed by water and hence utilized in raising water temperature (Mohapatra *et al.*, 2007; Tiwari, 2003). Findings of this study were in line to Ghosal *et al.* (2005) whose study concluded that water temperature in greenhouses can increase by 3˚C to 9˚C daily. On the other hand, Khemakorn (2015) recommended the use of greenhouses in aquaculture as they can raise water temperature by upto 5˚C daily. The results of temperature changes obtained in the open pond are consistent with the findings of Ogello *et al.* (2017) studying the growth rate of Nile tilapia in the open earthen ponds. Findings of this study were similar the findings of Solomon *et al.* (2013) whose results indicated a temperature range of 24 to 28˚C in fish ponds for raising Nile tilapia.

5.1.2 Dissolved Oxygen

The higher mean daily oxygen concentration recorded in the open pond could be due to the mechanical aeration by wind which was present in the open pond as opposed to greenhouse pond which had been shielded from wind effect. Mean oxygen concentration values recorded in the greenhouse are similar to the findings of Ani-Sabwa *et al.* (2012) while rearing catfish, *Clarias gariepinus* in a greenhouse pond. In a separate study, mean oxygen concentrations recorded by Eyo *et al.* (2013) were in the range of 3 to 5.7mg/L, similar to the results obtained in this study. Generally, mean oxygen concentrations recorded during the day were higher than those at night in both systems. The diurnal differences in oxygen concentration could be due to increased respiration activities by organisms within the water and absence of photosynthesis by plants within the systems at night (Cooper *et al.*, 2002).

According to Ehiagbonare and Ogunrinde (2010), main sources of oxygen in aquaculture ponds includes diffusion from air into the water column, mechanical aeration by wind or aeration systems, and photosynthesis by aquatic plants. In aquaculture ponds, dissolved oxygen concentrations will vary throughout the day, and are affected by several factors including water temperatures, fish stocking density, salinity, amount of aquatic vegetation and number of other aquatic animals present in the ponds (Boyd, 2015; Koç & Yilmaz, 2014). In this study, temperature, respiration activities and mechanical aeration due to 'wind effect' were the main factor affecting the changes in oxygen concentrations in the two pond environments.

5.1.3 pH variation in the ponds

In aquaculture, pH determines the solubility and availability of some chemical forms of various compounds in water, some of which can be toxic to fish. The survival and maximum growth of most freshwater fish species will occur in a pH range of 6.5 to 8.5 (Boyd & Zhou, 2015). Furthermore, Stress and low growth rates occur in fish ponds whose pH increases beyond 9, and above 11 fish death occurs. Death of fish when the pH is beyond 11 may be due to increased proportion of Total Ammonium Nitrogen (TAN) in the form of unionized ammonia (Toxic form of ammonia) in water whose concentration increase with increase in pH (Bhatnagar & Devi, 2013).

The pH in this study ranged from 7.76 to 8.45 and 7.45 to 8.22 in the greenhouse and open pond respectively, which are within the recommended range 6.5 to 8.5. However, the pH values recorded in the open pond were lower than those in the greenhouse pond although not significant. The difference observed resulted from Dilution effect of rainfall was more prevalent in the open pond as opposed to greenhouse pond which was covered (Peyami, 2016). The pH ranges in this study agree with those of Chakravartty and Mondal (2016) who obtained pH ranges of 7.58 to 8.02 and 7.85 to 8.85 in two earthen ponds for culturing Nile tilapia in Indian Sundarbans.

Diel monitoring of pH in this study revealed that, relatively higher pH values occurred during daytime than nighttime. The diurnal dynamics observed in pH could be explained by the combined effect of respiration and photosynthesis taking place in ponds at night and daytime respectively. During daytime, the rate of photosynthesis increases which removes carbon (IV) oxide from the system increasing pH. The reverse occurs at night when more carbon (IV) oxide is produced whose reaction with water produces a weak acid leading to a drop in pH levels (Giang *et al*., 2008; Thich, 2008; Ut *et al.,* 2016).

5.1.4 Electrical conductivity (EC) variation in the two ponds

The recorded mean daily Electrical Conductivity (EC) was higher in the greenhouse pond compared to the open pond. The differences in the observed EC could be due to dilution effect by rainfall in the open pond as opposed to greenhouse pond (Hayashi, 2004). The EC values obtained from both ponds in this study were within the acceptable range of 100-2,000 µs/cm recommended in aquaculture (Stone & Thomforde, 2004). The results of this study agree with those of Abdel-Satar and Geneid (2009) who obtained an EC range of 308 to 687µs/cm in earthen ponds in Egypt. Similar values were obtained by Ogello *et al.* (2017) working on *O. niloticus* in Kisii region of Kenya. Hussain *et al*. (2013) working on open ponds for culturing *Nile tilapia* in Egypt also recorded EC values of 232.76 to 330 (µS/cm), which were lower than the ones obtained in this study but within the recommended EC range.

Diel monitoring of EC in both ponds showed relatively higher values during the day than nighttime. According to Hayashi (2004), EC correlates positively with temperature. This is because increasing temperature affects EC through ionic concentration, as many salts tend to be more soluble with increasing temperatures. Warmer water during the day unlike cold water at night will dissolve more minerals and salts explaining the higher ionic concentration during the day (Oyem *et al.,* 2014).

5.1.5 Nutrient concentrations in greenhouse and open pond environments

5.1.5.1 Nitrogen: Ammonia, Nitrite and Nitrates

In fish ponds, ammonia exists in two forms as ammonium ions (NH_4^+) which is not toxic and the un-ionized ammonia (NH_3) which is toxic to fish. The presence of ammonia in fish ponds is normal since it results from fish metabolism, microbiological decay of organic matter and from excess feeds present in the water column. The NH_3 concentration in fish ponds positively correlates with the increasing water temperature and pH. The desirable level of toxic ammonia in fish ponds should not exceed 0.1mg/L (Santhosh & Singh, 2007).

The ammonium ions were within the recommended levels being less than 0.5mg/L in both earthen and greenhouse ponds. However, mean ammonium ion concentration in the greenhouse pond was higher compared to the open pond, probably due to relatively higher temperature present in the greenhouse pond. Higher temperatures favour denitrification process by the microbes which reduce $NO_2^-$and NO_3^- ions into NH_4^+. In a study by Ali (2013), the ammonium levels increased with increasing water temperatures. Findings of this study were similar to those obtained by Makori *et al*. (2017) who recorded NH_4^+ concentrations in the range of 0.02 to 0.05mg/L in earthen fish ponds within Busia County in Kenya.

Nitrite-N is the unstable form of nitrogen produced during the process of nitrification which transforms ammonium ions into nitrate by bacterial activity. Nitrite has been identified as a compound affecting fish production (Mook *et al.,* 2012). When nitrites exceeding 1mg/L get absorbed in the blood stream of fish, they bind to hemoglobin reducing its capacity to carry oxygen in the body. Nile tilapia requires nitrites concentration levels in the range of 0 to 1mg/L (Stone & Thomforde 2004). The mean nitrite levels of 0.02±0.01mg/L and 0.03±0.01mg/L recorded in the greenhouse and pond open respectively were within the acceptable limits not exceeding 1mg/L. The open pond recorded higher nitrite concentration than the greenhouse pond probably due to the oxidation of ammonium ions, yielding nitrite as an intermediate state (Thullen *et al.,* 2008). Furthermore, relatively higher oxygen levels in open pond could have favoured the oxidation of ammonium into nitrites (Mook *et al.,* 2012). Nitrite results in this study were in line with the findings of Ehiagbonare and Ogunrinde (2010) who recorded nitrite concentration ranging from 0.01 to 1.02mg/L in Nigerian open ponds.

The Nitrate-N is the harmless form of nitrogen resulting from autotrophic activities of Nitrobacter bacteria combining oxygen and nitrite during the process of nitrification. Kumar *et al.* (2017), recommends maximum levels of nitrates in aquaculture to be maintained below 4.00mg/L in order to prevent eutrophication. In this study, mean nitrate levels in open pond and greenhouse pond were 0.05±0.01mg/L and 0.03±0.01mg/L respectively, values which were within the acceptable limits not exceeding 4.00mg/L. The mean concentration of nitrates in the open pond was higher than in the greenhouse pond system probably due to more oxygen concentration in the open pond favouring the bacterial autotrophic activities and oxidation of nitrites. The mean nitrate concentrations obtained in this study are comparable to values reported by Ehiagbonare and Ogunrinde (2010) who obtained nitrate concentration range of 0.02 to 0.04mg/L in Nigerian open ponds.

5.1.5.2 Soluble Reactive Phosphorus and Total Phosphorus

Soluble reactive phosphorus (SRP) and total phosphorus (TP) are the most important forms of phosphorus nutrients which determine primary production in fish ponds. Cycling of phosphorus in pond systems is a dynamic and complex process which involves adsorption and precipitation reactions, interchange with sediments and uptake by aquatic biota (Toma, 2011). Possible sources of phosphorus in this study include incoming water and other autochthonous sources such as pond sediments. Greenhouse pond recorded slightly higher SRP and TP concentrations of 0.13±0.02mg/L and 0.61±0.05mg/L respectively. However, these values compared insignificantly to the open pond's SRP and TP concentrations of 0.12±0.02mg/L and 0.49±0.03mg/L respectively. The differences observed could possibly be resulting from the temperature differences observed in the two systems. The higher mean temperatures in the greenhouse pond increased the breakdown of organic matter into nutrients. The warmer temperatures accelerated the biological and chemical reduction processes in the greenhouse pond, releasing bound phosphate from sediments into water column (Bhatnagar & Devi, 2013).

The SRP concentrations obtained from both pond systems were much higher than the range of 0.02 to 0.06mg/L obtained by Toma (2011) in tilapia ponds in Kurdistan region of Iraq. The TP concentrations obtained from both pond systems were slightly lower than 0.51± 0.01mg/L reported by Elnady *et al.* (2010) in the study of effects of fertilization and low-quality feed on water quality and growth of Nile tilapia in ponds. Shahabuddin *et al.* (2012), studying the effect of rice straw mat on water quality parameters in earthen ponds obtained TP concentrations in the range of 0.27 to 0.60mg/L values which are comparable with the findings of this study.

5.2 Proximate Composition of Feed ingredients

In Kenya, majority of semi-intensive fish farming systems rely on Agro based by-products as ingredients of fish feeds. This is due to the fact that they are readily available and cheaper than commercial feeds. However, storage conditions, shelf-life and processing procedures of these ingredients interfere with their nutritional composition (Munguti *et al.*, 2012). It is therefore important to carry out proximate analysis on fish feed ingredients to ascertain their nutritional composition before using them in feed formulation. Animal protein is important in fish feeds due to its high protein quality and balanced amino acid profiles when compared to plant proteins (Nunes, 2014). The results in this study indicated that, animal-based ingredients had higher protein content compared to plant-based protein ingredients. Similar results were obtained by Musyoka *et al.* (2019) and Nalwang'a *et al.* (2009).

Proximate composition of feed ingredients indicated that redworms *E. foetida* had the highest protein content followed by fishmeal. Redworms' proximate analysis yielded a mean crude protein content of 62.29%. These results were compares to those of Gunya *et al.* (2016) who recorded crude protein content of 66.2%. Results also compares with findings by Vodounnou

(2015), who recorded a mean protein of 59.00%, and 61.05 reported by Ng, *et al.* (2001) working on the same redworm species. Fish meal recorded a mean protein content of 57.71% which was not significantly different from a mean of 54.34%, a value obtained by Al-Mahmud *et al.* (2012).

Analysis of sunflower, soya meal and wheat bran yielded a protein content of 40.13%, 36.13% and 12.54%, respectively, which did not differ much from those reported by Al-Mahmud *et al.* (2012) of 42%, 38.05% and 14% respectively. The observed differences could be explained by the differences in their sources/origin, storage conditions, shelf life and value alterations along the supply chain of the feed ingredients.

Animal-based ingredients had the highest crude lipid content when compared to the plant-based feed ingredients. These values are in line with findings reported by Tom (2002) with mean lipid content of 5.6%, 2.9%, 4.8% and 3% for fishmeal, sunflower, soya bean and wheat bran, respectively. Dynes (2003) working on *E. foetida* recorded a mean lipid content of 10%, a value that was higher than the one obtained in this study. Bhuiyan *et al.* (2016) analyzing *E. foetida* worms recorded a mean lipid content of 6.04% which is similar to the results obtained in this study. From this study, it can be concluded that redworms, *E. foetida* have higher protein and lipids content than fishmeal. The observed variations in proximate compositions from other studies might be associated with the differences in specific-ecology, food, seasons, life stages and reproductive states of the *E. foetida* as reported by Ntukuyoh *et al.* (2012).

Redworms also had higher ash content than fishmeal as documented in other studies. For instance, Vital *et al.* (2016), documented ash values of 8.8% for *E. foetida*, 9.02% for fishmeal, 3.1% for sunflower, 5.6% for soya bean and 2.0% for wheat bran. Kedar (2016) reported ash content of 12.7% in *E. foetida* and 15.3% in fishmeal. Allan *et al.* (2000) working on soya bean meal and wheat bran obtained an ash content of 8.0 and 1.8 respectively. El-Saidy and Gaber (2003) analyzing soya meal and sunflower obtained ash content of 6.0 and 7.6 respectively. Disparities observed in the results might have resulted from the harvesting and processing procedures where soya meal could have mixed with soil (Munguti *et al.*, 2021; Preston *et al.*, 2010).

Plant ingredients had higher fibre content than animal-based ingredients. Sunflower for instance, recorded the highest mean fibre content followed by wheat bran. Similar studies conducted by Vital *et al*. (2016) recorded fibre content of 18.7% in sunflower, 9.9% in wheatbran, 6.0% in soya bean, 4.2% in fishmeal and 1.6% in *E. foetida*. Kedar *et al.* (2016)

reported a fibre content of 7.8% and 5.8% in *E. foetida* and fishmeal respectively. Munguti *et al.* (2006) working on rice bran recorded a mean fibre content of 12.7% which concurs with the values obtained in sunflower and wheat bran in this study.

Proximate analysis for moisture content revealed that redworm meal had the highest moisture content followed by fishmeal while wheat bran had the lowest value. It is worthy to note that moisture levels obtained in all the feed ingredients used in this study did not exceed the recommended maximum level of 10% an indication that the ingredients were well dried.

All the proximate analysis values obtained in the feed ingredients were within the acceptable levels of fish feed ingredients. According to Kinyuru *et al.* (2015), fish feeds should maintain certain standards to qualify as aquaculture feed. The feed should have moisture content of less than 10%, a crude fat/lipids range of 5 to 15%, crude fibre content ranging from 5 to 20%, ash content of less than 10% and protein content ranging from 25 to 50%. Similar standards have been documented by Munguti *et al.* (2021) who recommends that fish feed should contain a protein range of 18 to 50%, lipids from 10 to 25%, carbohydrate 15 to 20%, ash < 8.5% and moisture content of <10% alongside minerals and vitamins.

5.3 Fish growth performance

The growth performances in terms of final weight and weight gain of both Nile tilapia and African catfish cultured in the greenhouse pond were significantly higher than those of the open pond. Evaluation of the growth performance of Nile tilapia fed on the five redworm rations in the greenhouse resulted into similar final weight gain which was not significantly different from one another. Furthermore, a similar case was recorded for African Catfish cultured and, in a greenhouse, and fed on similar five redworm rations. In the open pond, final weight gain of Nile tilapia when fed on the five redworm rations had results which had no significant difference. This pattern was also similar to that of African Catfish whose final weight gain when fed on the five redworm rations had no significant difference.

In both pond systems, despite there being no significant variations in the five redworm diet performance, there was an observable difference in terms of final weight gain. Nile tilapia and African catfish fed on 50% redworm inclusion recorded the highest weight gain compared to the other four redworm rations. Results of this study compares to that of Ahmed (2020) who, obtained the higher growth performance of fish when fed on 50% redworm inclusion diet.

It was also observed that in both ponds, Nile tilapia and African catfish fed on the control diet containing 0% redworms and 100% redworm ration had similar weight gain, an attribute which might be as a result of the proximate evaluations of fish meal and red worm meal whose crude lipid and protein content had no significant difference.

5.3.1 Absolute Growth (AG) and Specific Growth Rates (SGR)

The findings in this study indicated that the AG and SGR of fish cultured in the greenhouse pond were significantly higher than those in the open pond for both Nile tilapia and African catfish cultured. Higher AG range of 1.39-1.48 and SGR values 0.30-0.40 obtained in the greenhouse pond compared with findings of Santos *et al.* (2013) who obtained AG range of range 1.31-1.45 and SG range of 0.27-0.42. The observed difference could be explained by the relatively warmer water temperature experienced compared to open pond since the other selected water quality parameters (pH, conductivity and dissolved oxygen) were within the recommended levels for aquaculture.

In aquaculture, every fish species has an optimum range of temperature in which growth performance is optimal (Biro *et al.,* 2007; Oyugi *et a/.,* 2011). Warm water fish species for instance grow well in a temperature range of 24 to 32˚C. This is the temperature range which is suitable for most tropical freshwater fish (Ani-Sabwa *et al.,* 2012; Stickney 2000). Temperature has a direct effect on important factors like oxygen demand, food requirements and food conversion efficiency which influence fish growth (Houlihan *et al*., 2001; Islam *et al.,* 2006). At higher temperatures, demand for oxygen and food intake increase. Temperature consequently improves food conversion ratio resulting into faster growth rate of fish. Low temperatures in aquaculture results to reduced feed intake and poor feed conversion ratios hence low growth rate of fish (Khemakorn, 2015).

On the other hand, low values of AG and SGR of Nile tilapia and African catfish cultured in the open pond can be explained by low temperatures known to cause stress in fish hence interfering with growth (Temprasit *et al.,* 2015). A diel study of temperature in open pond showed a drastic temperature drop from maximum of 23.52˚C at 1600hrs to 13.86˚C at 0600hrs, a phenomenon which probably negatively affected fish metabolism thereby lowering the growth rates of both Nile tilapia and African catfish in the open pond. On the other hand, the 'greenhouse effect' observed in the greenhouse pond cushioned pond water against abrupt temperature changes that could interfere with fish's metabolism (Ani-Sabwa *et al.,* 2012; Khemakorn, 2015), hence the observed faster growth rate of fish in the greenhouse pond. The SGR of Nile tilapia in the open pond did not vary much from that recorded by Coyle *et al.* (2004) evaluating the growth, feed utilization, and economics of hybrid tilapia in which the

SGR values were in the range of 0.73 to 0.87. Similarly, SGR values of Nile tilapia from the greenhouse pond were comparable with those obtained by El-Saidy and Gaber (2003) of 1.07-1.23 in their study evaluating cottonseed meal supplemented with iron in diets of Nile tilapia.

Evaluation of growth performance of African catfish in this study revealed that, the SGR values were higher in the greenhouse pond than those cultured in the open pond. This differences in growth may be attributed to stable warmer temperatures in the greenhouse pond, unlike the fluctuating temperatures in the open pond resulting to stress in fish (Khemakorn, 2015). The SGR values for African catfish in the open pond were comparable to a range of 0.88 to 1.1 range obtained by Umaru *et al.* (2016) when they evaluated the growth performance of African catfish fed on local and imported feeds. Much lower values of 0.49 to 0.62 were reported by Falaye *et al.* (2016), in their study evaluating the growth response and nutrient utilization of African catfish fed on processed Sunflower. Other studies whose results compare with those obtained for the greenhouse pond in this study include Ani *et al.* (2013) studying the effect of feeding time on the performance of juvenile African catfish which recorded SGR values in the range of 1.26-1.41. Similarly, EL-Haroun (2007) evaluating the growth rate and feed utilization in farmed African Catfish recorded SGR value range of 1.19 to 1.50 which compares to this study.

5.3.2 Length-Weight Relationship (LWR)

The Length weight relationship, b exponent values for Nile tilapia and African catfish in this study differ from those reported by other authors from different regions. This is because, the b-value, can be affected by several factors including stress in the culture environment (Ali & Prasad, 2007), region's ecological conditions (Nehemia *et al.,* 2012), food availability and quality, and feeding characteristics of fish species under study (Kembenya *et al.,* 2014).

Length-weight regression plots indicated that Nile tilapia and African catfish cultured in the greenhouse pond had b values closer to three (3), an indication of isometric growth pattern. This growth pattern observed the Cube law in which the rate of change in weight is proportional to rate of change in biomass. Mean b values of 3.14 and 3.11 were obtained for Nile tilapia and African catfish respectively grown in the greenhouse pond system. In contrast, b values for Nile tilapia and African catfish cultured in the open pond were below three (3), an indication of negative allometric growth.

Growth pattern of fish is influenced by both biotic and abiotic factors (Dan-Kishiya, 2013). Among the abiotic factors, water temperature is key influencer of physiological activities within the body of fish. In this study, the higher and stable water temperatures in the greenhouse may account for the isometric growth of fish. In contrary, the fluctuating temperatures in the open pond may have accounted to the negative allometric growth of fish (Elliot *et al.,* 2015).

The b exponent values obtained for Nile tilapia in the greenhouse pond compares to findings by, Chakravartty and Mondal (2016) who recorded results similar from fish ponds. In their study, there was an isometric growth with b exponent value of 2.98 in Nile tilapia length-weight relationship. Elliot *et al.* (2015) also recorded isometric growth for Nile tilapia with b exponent value of 3.07 in earthen ponds of Egypt, which concur with results in this study.

In the open pond, the b exponent was less than 3, an indication of a negative allometric growth of fish. Several studies have documented that Nile tilapia can assume a negative allometric growth in any given environment. Findings by Otieno *et al.* (2014) on Nile tilapia in Lake Naivasha had b exponent of 2.0 which was less than 3 an indication of negative allometric growth. Dan-Kishiya (2013) also recorded negative allometric growth of Nile tilapia cultured in selected water reservoir in Nigeria. The results agree with the findings of Ibrahim *et al.* (2008), Imam (2010) and Musa *et al*. (2012) whose studies agree that Nile tilapia can adopt a negative allometric growth in earthen ponds.

African catfish cultured in the greenhouse pond had mean b exponent value of 3.11, a value which indicates an isometric growth. In a study conducted by Kembenya *et al.* (2014), African catfish in Lake Baringo had an isometric growth. Akinwole and Faturoti (2007), Davies *et al.* (2013), Kurup *et al.* (2004), Madhusoodanan *et al.* (2016), and Yusuf *et al.* (2013) have also reported same pattern of growth in their studies on African catfish.

African catfish cultured in the open pond had mean b exponent value less than 3, an indication of negative allometric growth. According to Abdullahi *et al*. (2014) and Ratnakala *et al.* (2013) African catfish can adopt a positive or negative allometric growth in any environment.

Growth rate results obtained for African catfish cultured in the open pond had a negative allometric growth indicating that the rate of change in length was faster than rate of change in weight. These results are comparable to studies of Abubakar (2006) and Aderibigbe and Olurin (2006) who observed a negative allometric growth of African catfish in Nigerian earthen ponds.

5.3.3 Fish Condition Factor (K)

Fish condition factor describes the health and well-being of fish in relation to their culture environment. The K factor is used to compare the condition, fatness or wellbeing of the fish based on the fact that heavier fish of a particular length are in a better physiological condition (Ahmed *et al*., 2011; Alhassan *et al.,* 2015). Furthermore, condition factor is a useful tool for monitoring feeding intensity, age and growth rates of fish in aquaculture (Ndimele *et al.,* 2010). In this study, Nile tilapia and African catfish cultured in the greenhouse pond recorded mean K values of 2.15 and 3.63 respectively. These values were higher than 0.88 and 0.88 recorded for Nile tilapia and African catfish respectively in the open pond. The K value >1 obtained in the greenhouse pond implies that fish grew in healthier and better conditions than in the open pond (Chakravartty & Mondal, 2016). The K-value for Nile tilapia in the open pond in this study agrees with those of Anyanwu *et al.* (2007) who recorded value of 0.89±0.02. On the other hand, K-value for African catfish in the open pond compared favorably to findings by Fafioye and Oluajo (2005), who recorded mean K-values of 0.76±0.04 respectively in Abuja ponds in Nigeria. The low K-values recorded in the open pond compared to greenhouse pond could be explained by the relatively low temperatures beyond the optimum level obtained in the open pond ranging from 13.8 to 23.5˚C.

The temperature differences in the two environments could explain the difference in the K factors of the two pond systems since other recorded physico-chemical parameters did not vary significantly. Temperature greatly influences the metabolic rate of fish and has a profound effect on the food conversion ratio hence the condition factor. Unlike the open pond, the greenhouse pond recorded a temperature range of 22.3-27.5˚C which is within the recommended temperature range for raising warm water fish species. Optimum temperature range recorded in the greenhouse provided a more favourable environment for fish growth explaining the high condition factor observed due to improved Food Conversion Ratio (Davison & Piedrahita, 2015; Kausar & Salim, 2006).

Condition factor (K) in any aquatic ecosystem is influenced by both biotic and abiotic environmental conditions (Anene, 2005), therefore, the K values obtained may differ from other reported studies probably due to variations which include but not limited to (i) physico-chemical factors of production ponds (Arthington & Canonico, 2005) especially temperature, feeding regimes, nutrients composition and amount of fat accumulated (Deekae *et al.,* 2010), (ii) environmental stressors such as pollution (Aderibigbe & Olurin, 2006) and (iii) sex which has also been documented to affect fish condition factor (K) (Shabir *et al.,* 2012). The 1st and 2nd factors could have greatly influenced the results in study unlike the 3rd factor since, only male fingerlings were used.

5.4 Food Conversion Ratio (FCR)

The cost of fish feeds is the largest expenditure in aquaculture, consuming upto 60% of the operation costs (El-Sayed, 2004). This is because of the extensive reliance on fish meal and other animal protein sources such as shrimps and squid meal to meet the high dietary protein requirements of cultured fish, making them expensive (Ogunji *et al.,* 2008). Fish farmers therefore need to have a reliable indicator to determine the level of performance of the feed or a feeding strategy employed by the farmer. The FCR provides a good indication of efficiency of a particular feed or feeding strategy to allow the aquaculturist to make wise choices in selecting and using feed to maximize profitability (Nakamura & Pandit, 2010).

The FCR values of feeds fed to fish in the greenhouse and open ponds varied significantly in this study. The FCR values for both Nile tilapia and African catfish were lower in the greenhouse pond than in the open pond. However, evaluation of the five redworm experimental diets in this study indicated that there were no significant differences in diets performance in terms of FCR when fed to Nile tilapia and African catfish in the two ponds.

Evaluation of five experimental diets showed no significant difference among the FCR for the five redworm of the 5 feeds for fed to both fish species in the greenhouse pond. However, the FCR values were lower than the range of 2.65 to 3.65 reported by El-Sayed (2007), after evaluating the effect of stocking density and feeding level on the growth of Nile tilapia in Egypt. The ration containing 50% redworms recorded the lowest FCR value of 1.81 while 75% redworm ration recorded the highest FCR value of 1.86. Similar results ranging from 1.65 to 1.94 were obtained by Ali *et al*. (2016), after evaluating the performance of alternative protein sources to fish meal in the diets of Nile tilapia raised in earthen ponds.

In the open pond environment, the FCR values of the five redworm rations fed to African catfish showed no significant difference. Similar results with FCR value range of 2.15 to 2.58 were obtained by El-Haroun (2007) in Egyptian ponds where African catfish were fed on a biogen enriched diet. However, the FCR ranges of 2.18 to 2.22 obtained in this study were lower than those of Goda *et al.* (2007) whose FCR values were in the range of 2.31 to 3.32 in a study where the effect of replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) cultured in Nigeria.

The FCR values for the five redworm diets when fed to the African catfish fed in the greenhouse pond showed no significant difference. The African catfish fed on feed ration containing 50% redworm had the lowest FCR value. These results were higher than the ones obtained by Oyelere *et al.* (2016) who recorded FCR in the range of 0.96 to 1.57 while evaluating the growth and nutrient utilization of African catfish fed on varying levels of *Albizia* leaf meal. On the other hand, Davies *et al.* (2013) evaluating the effect of total or partial replacement of fish meal by alternative protein sources on growth of African catfish obtained FCR values ranging from 2.25 to 3.21, were higher than those obtained in this study.

The FCR results obtained from both greenhouse pond and open pond indicated that a 50% redworm ration had the lowest FCR compared to the other four redworm formulated rations fed to Nile tilapia and African catfish. Furthermore, it was observed that the FCR values recorded in the greenhouse pond system were lower than those of the open pond. This scenario can be explained by temperature differences observed in the two pond systems.

In aquaculture, rising water temperatures to optimum levels increases metabolic activities of fish, this in turn increases food demand hence improves food conversion ratio (lower FCR) hence faster growth (Kausar & Salim, 2006). Li and Robinson (2015) recommends an FCR range of 1.5 to 2 as an ideal ratio for profitable farming of Nile tilapia and African catfish. However, in aquaculture, FCR can be influenced by several factors which include water quality, pond management, temperature, how and when feed is presented to the fish, and the health of the fish. Therefore, the low FCR values recorded in the greenhouse could be attributed to the optimum temperatures that were attained when compared to the open pond.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From objective ONE (1), the findings of this study concluded that there was a significant difference in water temperature with greenhouse pond recording the highest temperatures compared to open pond. However, comparison for other physical-chemical characteristics in the greenhouse and open pond revealed that there was no significant difference in the recorded dissolved oxygen, electrical conductivity, water pH, Ammonia, Nitrite, Nitrates, Soluble Reactive and Total Phosphorus. From these findings, the study ended up rejecting the null hypothesis that there is no significant difference in the water physico-chemical characteristics in the greenhouse pond and open pond.

From objective TWO (2), this study concluded that there were significant variations in the proximate composition of redworm meal and fish meal. For instance, redworm meal had higher crude protein content of 62.29±1.13% than 57.71±0.77% of fish meal which is the conventional protein ingredient used in fish feeds. Furthermore, redworm meal had higher crude lipid content of 6.34±0.48% than fish meal which had 5.51±0.19%. Redworms had highest level of moisture and ash content (7.97±0.06 and 12.23±0.12 respectively) compared to fish meal with moisture and ash content of 6.07±0.06 and 9.25±0.23 respectively. However, fish meal had higher crude fibre content of 5.15±0.08% than 3.02±0.80% of the redworm meal. From these findings, the null hypothesis that there is no significant difference in the proximate composition of redworm meal and fishmeal was therefore rejected.

From objective THREE (3), this study concluded that;

i. There were no significant differences in the growth performance indices of Nile tilapia and African catfish when fed on the five formulated feeds containing redworm meal in the rations of 0%, 25%, 50%, 75% and 100%. In both pond systems, there were no significant differences in the absolute growth (AG), specific growth rate (SGR) and mean weight gain (MWG) of both Nile tilapia and African catfish, although a ration with a combination of 50% redworm and 50% fish meal had the highest values in terms of AG, SGR and MWG in both ponds.

ii. There were significant differences in the length-weight relationships (LWR) and fish condition factors (K) of Nile tilapia and African catfish cultured in the greenhouse and open pond system. Nile tilapia and African catfish grown in the greenhouse pond had isometric growth an indication of favourable growth conditions. One of the factors explaining this growth is the '*greenhouse effect'* accounting for the higher mean temperatures in the greenhouse throughout the study period as compared to the open pond.

The study therefore rejected the null hypothesis that there is no significant difference in the growth rate, length-weight relationship and the condition factor of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in the greenhouse and open pond when fed on fish feeds formulated using redworm and fishmeal.

From objective FOUR (4), this study concluded that there were no significant differences in food conversion ratio (FCR) of the formulated feeds fed to Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in Greenhouse and Open Pond systems. From the results of FCR evaluation of the five redworm experimental diets containing redworm meal in the ratios 0%, 25%, 50%, 75% and 100%, it was established that in the individual pond environment, there were no significant differences in diets performance in terms of FCR when fed to both Nile tilapia and African catfish. This study therefore failed to reject the null hypothesis; there were no significant differences in food conversion ratios (FCR) of the formulated feeds fed to Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) cultured in the open pond and greenhouse pond systems.

6.2 Recommendations

i. This study recommends adoption of greenhouse technology in cold areas to promote aquaculture of warm water fish species such as Nile tilapia and African catfish. The *greenhouse effect* retains solar radiations thus maintaining and cushioning the greenhouse pond against drastic temperature changes on daily basis. Throughout the production cycle greenhouse ponds will maintain the warm temperature favouring the growth of warm water fish species. Warm temperatures in aquaculture promotes fish metabolism which improves feed intake and food conversion ratio (FCR) resulting into improved growth rates of fish.

ii. This study recommends the use of redworms, *Eisenia foetida* as a protein feed ingredient in aquaculture feeds.

   a. Redworms had slightly higher crude protein and crude lipid content than fish meal.

   b. There was no significant difference in growth performance of Nile tilapia and African catfish when fed on diets containing 100% redworm meal and 100% fish meal protein.

   From these findings, redworm meal and fish meal had similar nutritional composition and diet performance, thus, promoting redworm meal as a protein ingredient in fish feeds can be a strategy towards farm's sustainability. The redworms, *Eisenia foetida* have faster reproduction rates with their juveniles reaching maturity in 2 to 3 months. Furthermore, it is cheap to culture the redworms on-farm utilizing the available organic matter thus assuring farms' sustainability. This makes redworms an excellent replacement to fish meal whose economies of scale has become unsustainable due to compounded effect of scarcity in its supply as a result of seasonal availability, increased competition from livestock sector and increased transportation costs.

iii. This study recommends further research to be carried out on redworm meal to inform on other nutritional components like amino acid profile that this study did not focus on.

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APPENDICES

Appendix A: Photographs showing the Greenhouse and Open earthen fish ponds where Nile tilapia and African catfish were cultured

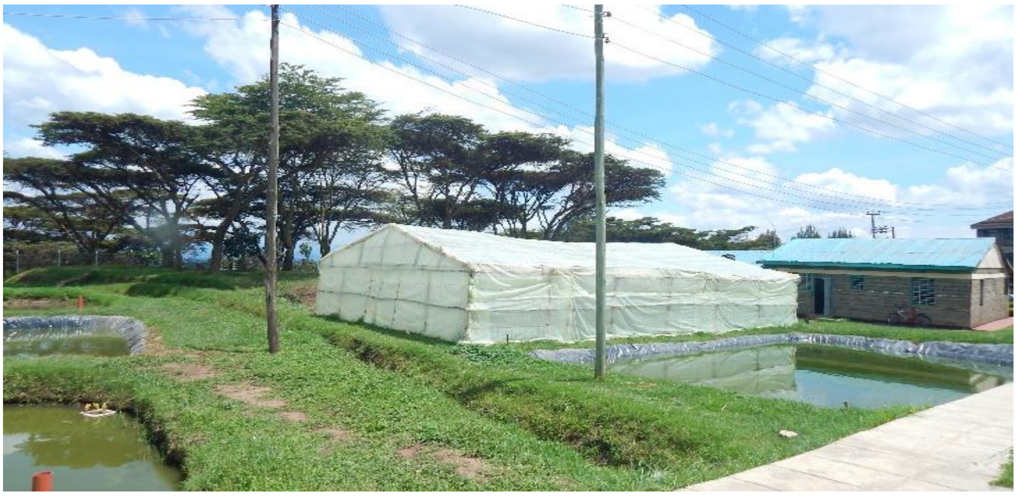


Plate 1: A locally constructed greenhouse used for culturing and feeding fish in the study.

Plate 2: A layout of Hapa nets used for fish culturing and feeding inside the greenhouse pond.

Plate 3: Hapa nets for fish culturing and feeding in the open pond.

Appendix B: Photographs showing the various structures used for culturing the redworms used for feeding during the fish feeding study

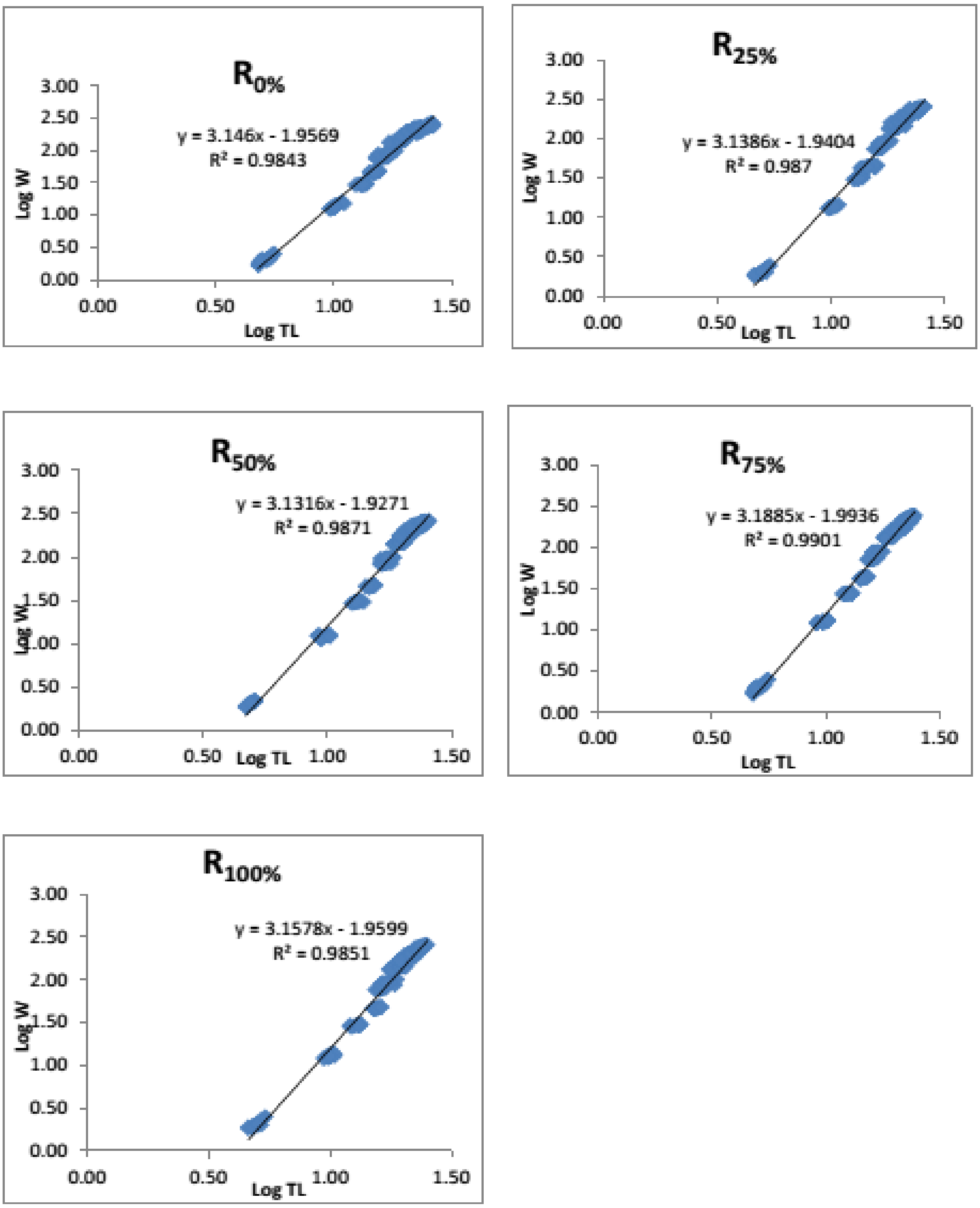
Plate 4: Cut broken plastic tanks used for culturing the redworms.

Plate 5: Cut Plastic containers used for culturing redworms.

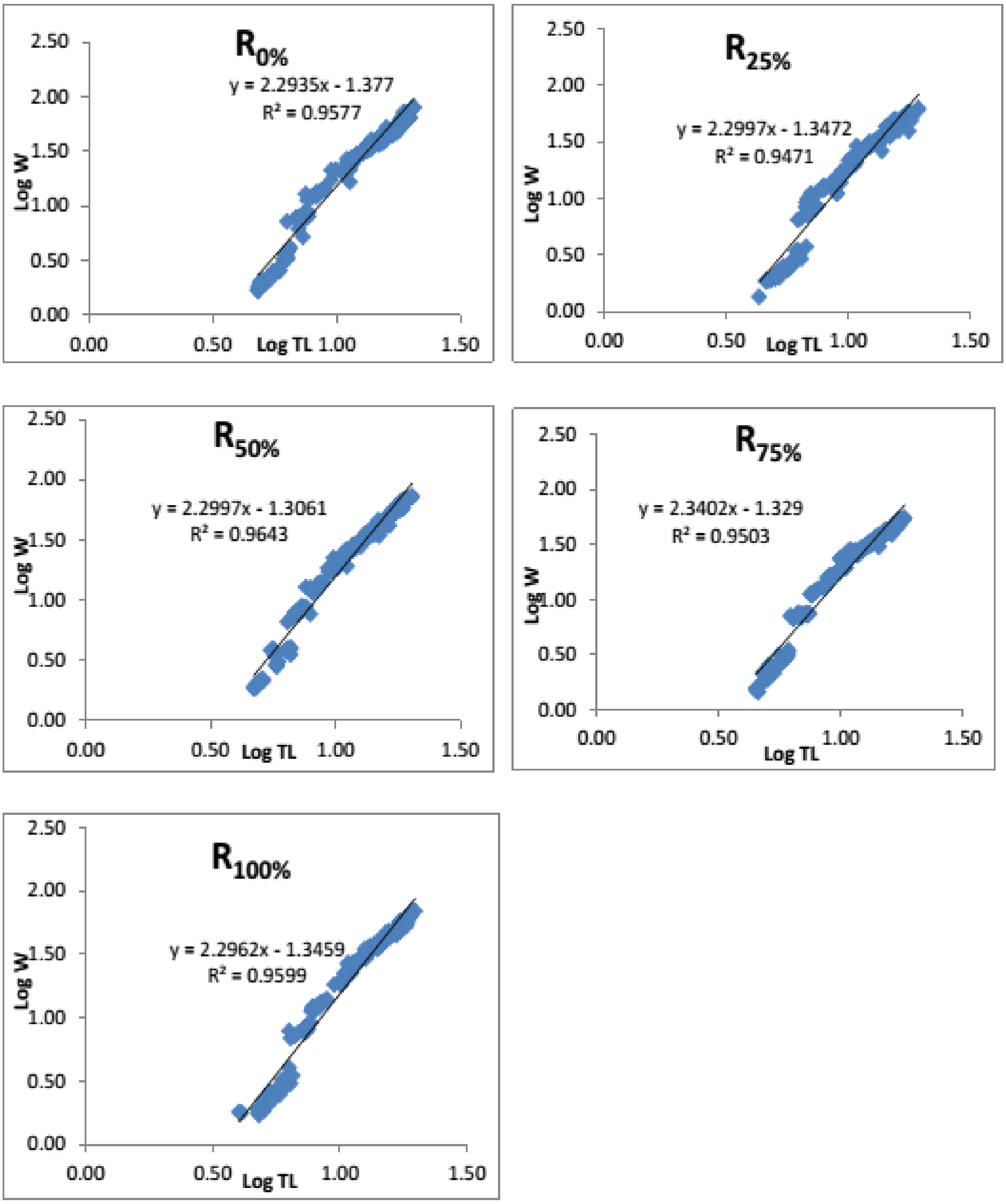
Plate 6: Improvised wooden beds used for culturing redworms.

Plate 7: Use of green organic matter in feeding of the redworms.

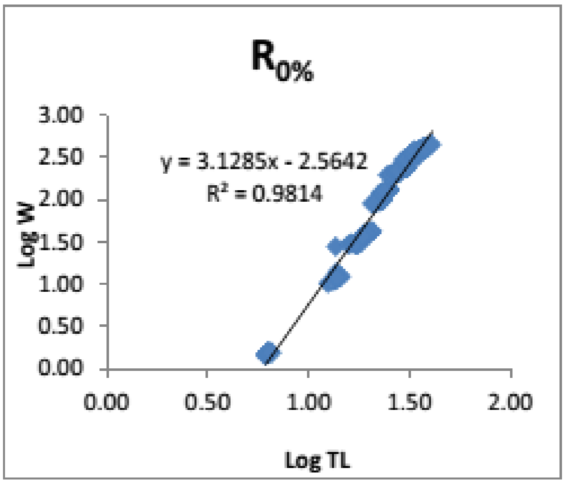
Appendix C: Length-weight relationships of Nile tilapia and African catfish fed on five redworm rations in the greenhouse and open pond

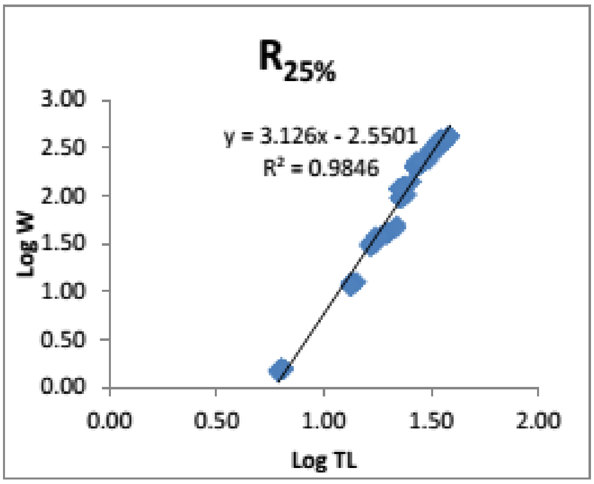


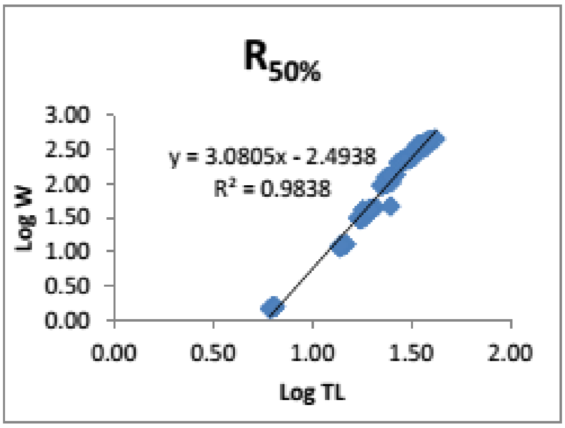
Appendix D: Length-weight relationships of Nile tilapia cultured in greenhouse pond.

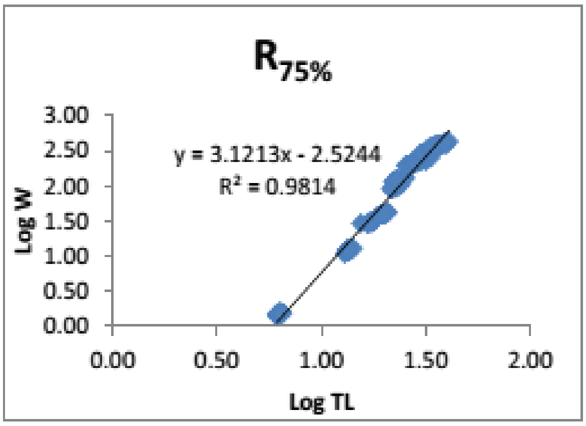


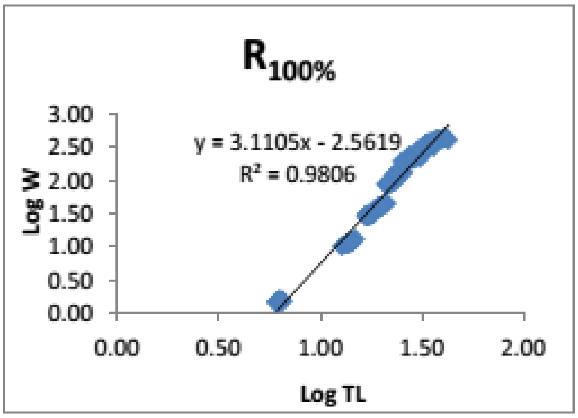
Appendix E: Length-weight relationships of Nile tilapia cultured in open pond.



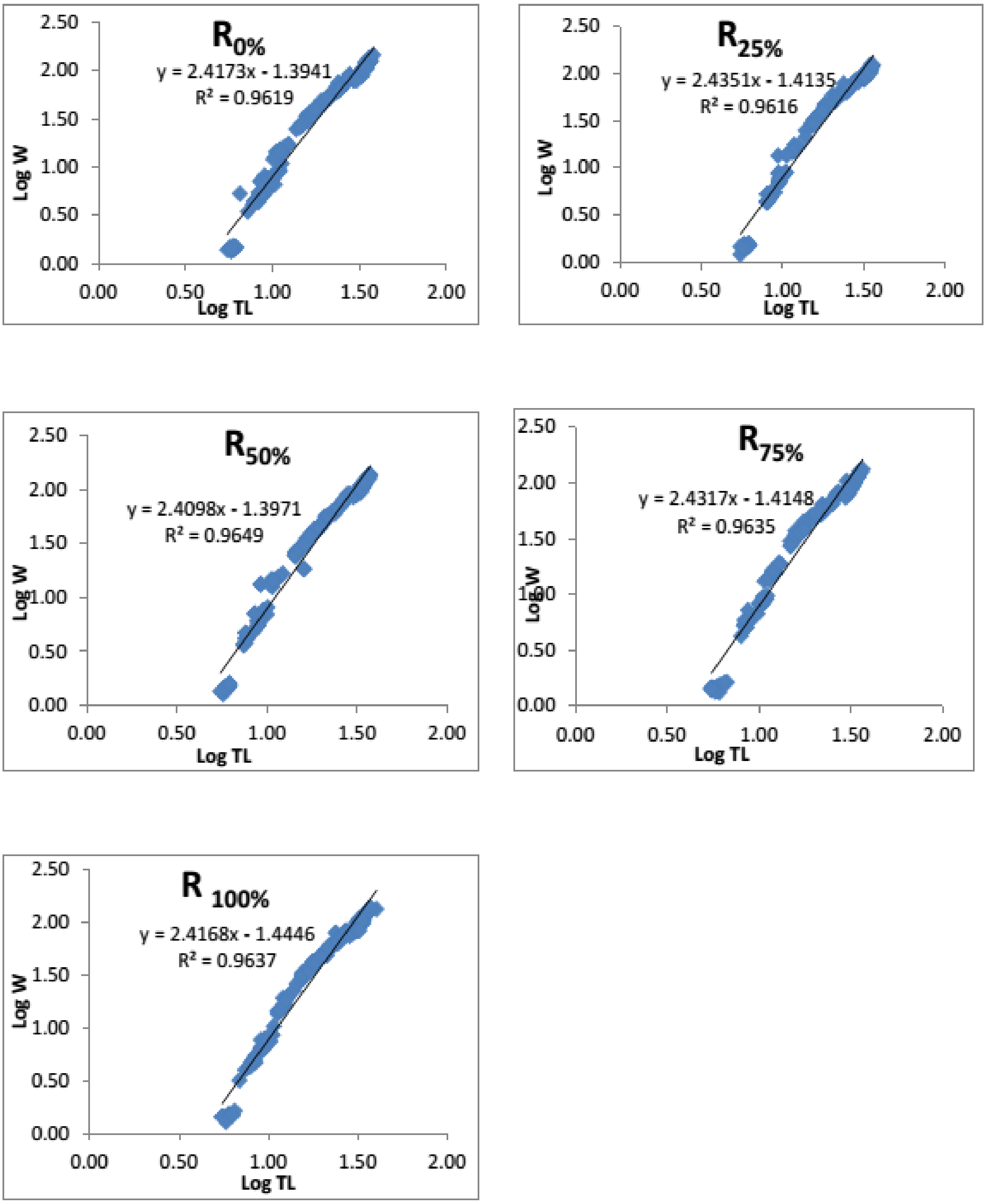








Appendix F: Length-weight relationships of African catfish cultured in greenhouse pond.



Appendix G: Length-weight relationships of African catfish cultured in open pond.

Appendix H: Conference presentation on the findings; Suitability of Redworms As Protein Feed Ingredient in Fish Feeds for Nile Tilapia and African Catfish cultured in Greenhouse and Open Pond Aquaculture Systems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Prof. (Eng.) Abel N. Mayaka, PhD
Co-Convenor, Conference Org Committee

Appendix I: Certificate awarded by the conference committee for participation and presentation of research findings in the annual international interdisciplinary conference at Multimedia University.

Appendix J: Manuscript/Articles publication

Appendix K: Proximate evaluation of redworms (*Eisenia foetida*) as an alternative protein ingredient to fish meal.

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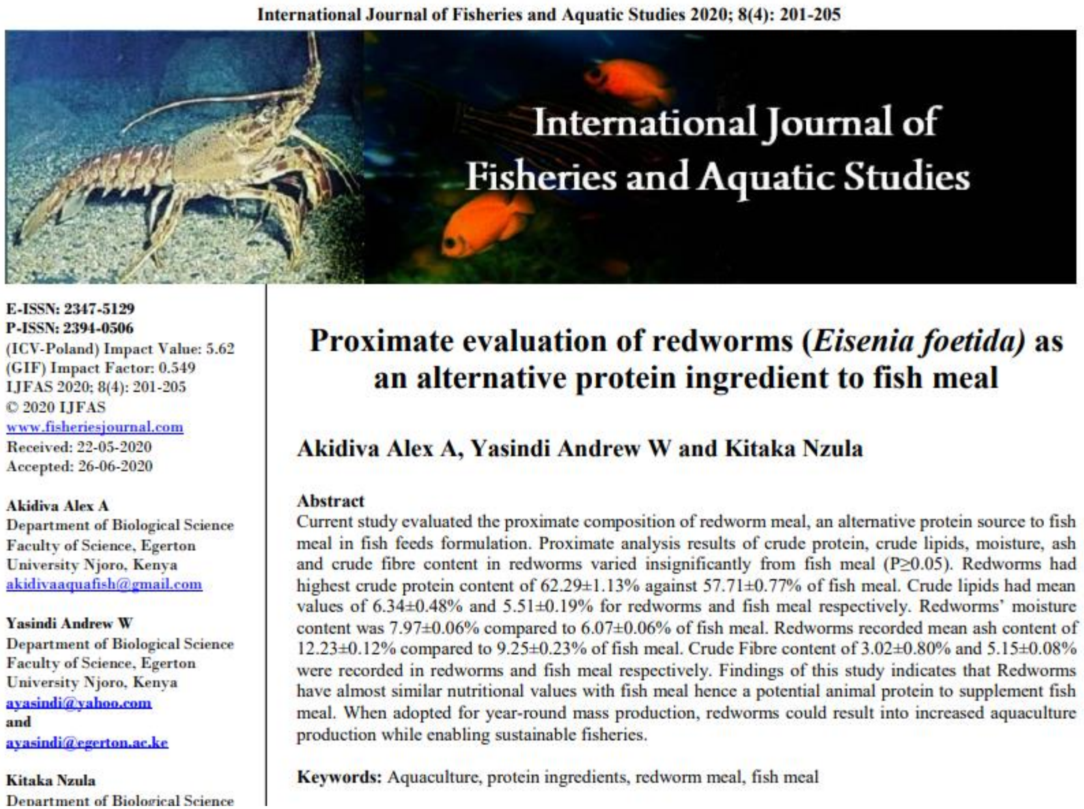

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Proximate evaluation of redworms (*Eisenia foetida*) as an alternative protein ingredient to fish meal

Akidiva Alex A, Yasindi Andrew W and Kitaka Nzula

Abstract
Current study evaluated the proximate composition of redworm meal, an alternative protein source to fish meal in fish feeds formulation. Proximate analysis results of crude protein, crude lipids, moisture, ash and crude fibre content in redworms varied insignificantly from fish meal ($P \geq 0.05$). Redworms had highest crude protein content of 62.29±1.13% against 57.71±0.77% of fish meal. Crude lipids had mean values of 6.34±0.48% and 5.51±0.19% for redworms and fish meal respectively. Redworms' moisture content was 7.97±0.06% compared to 6.07±0.06% of fish meal. Redworms recorded mean ash content of 12.23±0.12% compared to 9.25±0.23% of fish meal. Crude Fibre content of 3.02±0.80% and 5.15±0.08% were recorded in redworms and fish meal respectively. Findings of this study indicates that Redworms have almost similar nutritional values with fish meal hence a potential animal protein to supplement fish meal. When adopted for year-round mass production, redworms could result into increased aquaculture production while enabling sustainable fisheries.

Keywords: Aquaculture, protein ingredients, redworm meal, fish meal

Appendix L: Journal certificate confirmation for publishing a manuscript titled 'Proximate evaluation of redworms (*Eisenia foetida*) as an alternative protein ingredient to fish meal'.

Appendix M: Influence of greenhouse technology on selected pond water quality parameters and growth performance of Nile tilapia in high altitude areas.

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Influence of greenhouse technology on selected pond water quality parameters and growth performance of Nile tilapia in high altitude areas

Akidiva Alex A, Yasindi Andrew W and Kitaka Nzula

Abstract

Current study investigated the impact of greenhouse on selected water quality and growth parameters of Nile tilapia. Fingerlings were cultured and uniformly fed on isonitrogenous diet for six months in greenhouse and open ponds. At the end of experiment, Greenhouse pond had significantly higher temperatures than open pond. Mean dissolved Oxygen, pH and conductivity in the two ponds compared insignificantly. The concentrations of ammonium and Nitrate were higher in the greenhouse pond while Nitrites, soluble reactive phosphorus and total phosphorus in two ponds compared insignificantly. Final weight, Specific and Absolute growth rates and condition factor of Nile tilapia in greenhouse pond were significantly higher compared to open pond. This study recommends adoption of greenhouses in Nile tilapia aquaculture in high altitude areas. Current findings will be useful to aquaculture practitioners aiming to improve Nile tilapia performance and other warm water fish species in high altitude areas characterized by low temperatures.

Keywords: Greenhouse, water quality, growth parameters

Appendix N: Journal certificate confirmation for publishing a manuscript titled 'Influence of greenhouse technology on selected pond water quality parameters and growth performance of Nile tilapia in high altitude areas.'