THE EFFECT OF AMINO ACID SUPPLEMENTED DIETS ON GROWTH PERFOMANCE OF NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758) FINGERLINGS REARED IN HAPAS SUSPENDED IN EARTHEN POND

BY

ELIZABETH OBADO

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FISHERIES AND AQUATIC SCIENCE IN THE SCHOOL OF NATURAL RESOURCE MANAGEMENT, UNIVERSITY OF ELDORET, KENYA

OCTOBER, 2018

DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret

Elizabeth Odera Obado

______________________________ ______________________________

NRM/PGFI/08/13 **Date**

Declaration by the supervisors

This thesis has been submitted with our approval as University supervisors.

______________________________ ______________________________

Prof. Phillip. O. Raburu **Date**

University of Eldoret

______________________________ ______________________________

Prof. Julius. O. Manyala **Date**

University of Eldoret

DEDICATION

This thesis is dedicated to my late father Mr. Zablon Benjamin Obado, ’ZB’ who always mentored me academically through financial support and advices for greater achievements and to mother Mary Obado and Siblings for their moral support, persistent prayers and encouragement throughout my academic life.

ABSTRACT

Commercial diets are produced in bulk with significant anti-nutritional factors increasing aquaculture production cost. The proximate composition of local feed ingredient is limited by unbalanced dietary amino acid contents, thereby increasing ammonia levels in pond water. This study formulated diets using local ingredients and balanced the Essential Amino Acids (EAAs) to enhance the physical quality and nutritive value for culture of *Oreochromis niloticus*. Three practical diets were formulated at 30% crude protein and one commercial diet with 32% CP was locally purchased and tested at the University of Eldoret fish farm. Diet 1 received both methionine + lysine, diet 2 constituted lysine supplement only while the control diet 3 did not receive any supplement. The supplementation rates were at 5.1 g kg^{-1} lysine and 2.7 g kg^{-1} methionine. The growth performance was conducted in hapas suspended in an earthen pond 150 m^2 in a randomized design for 105 days. There were slight variations in temperature (24 to 26^0C), Dissolved oxygen (4.8 to 6.2 mg L-1) and pH (7.2-7.6) but within optimal range for tilapia during the study period. The diets provided 17.17 MJ kg^{-1} with 22.9% digestible CP level, 8.03% ash and 90.7% dry matter. There were significant differences at ($p<0.05$) in growth perfomance in all tested diets. Lysine supplemented Diet 2 induced the highest mean final weight of 156.05±1.74 g whereas the commercial Diet 4 induced the least mean weight gain of 94.08 ±3.064g. Supplemented Diet 1 indicated better Mean final weight of 125±1.681g while control diet had 114±1.917g at $p<0.05$. All performance indicators confirmed that lysine supplemented Diet 2 was better than other diets with SGR of 2.4, and FCR of 1.42 and Protein Efficiency Ratio of 2.68. Crude lipid content in the carcass were relatively lower in supplemented Diet 1 ($10.371±0.21^a$) and Diet 2 ($10.346±0.27^a$) compared to higher crude lipid contents in commercial Diet 4 ($14.358±0.23^c$).Diet 2 exhibited a high profit index (2.286±0.07) at low incidence cost (0.437±0.05). Commercial diet low profit index (1.652±0.04) at high incidence cost (0.951±0.14) at ($p<0.05$).The study concluded that EAAs supplementation at 5.1 g kg^{-1} Lysine and 2.7 g kg^{-1} Methionine improved growth perfomance in FCR, PER and Weight Gain of *O. niloticus*. The study recommends supplementation of limiting essential amino acid at 5.1 g kg^{-1} Lysine and 2.7 g kg^{-1} Methionine in plant protein feed ingredients to improve the feed nutritive value, growth perfomance of *O. niloticus* at reduced production cost.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND ACRONYMS

AEZ	Agro-Ecological Zone
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BWG	Body Weight Gain
CF	Crude Fibre
CP	Crude Protein
DAP	Di-Ammonium Phosphate
DE	Digestible Energy
DHA	Docosahexaenoic acid
DM	Dry Matter
DW	Durbin Watson Statistic
DWG	Daily Weight Gain
EAAs	Essential Amino Acids
EE	Ether Extract
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Food Conversion Ratio
IC	Incident Cost
KES	Kenya Shillings
LOA	Linoleic acid
LM	Lower Midland Zone
MAE	Mean Absolute Error
MJ	MilliJoules
NARDC	National Aquaculture Research and Development Centre

NfE	Nitrogen free Extract
NRC	National Research Council
P/E	Protein to Energy Ratio
PER	Protein Efficiency Ratio
PGR	Protein Growth Rate
PPV	Protein Productive Value
SE	Standard Error
SGR	Specific Growth Rate
TL	Total Length
UH	Upper Highland Zone
UM	Upper Midland Zone
UNM	University of Maryland
W	Weight (g)

ACKNOWLEGDEMENTS

To God be the glory for the gift of life, wisdom and knowledge in my academic life. No accomplishment in life is without the cooperative effort of many gifted people who willingly and passionately dedicated themselves to the achievement in this project. I sincerely thank Dr. P. Orina and Dr. J. Munguti from the National Aquaculture Research and Development Centre (NARDC) Sagana for donating to us the monosex tilapia fingerlings used in the study. We also thank Mr. Enos for providing us with a commercial feed.

I wish to express my sincere thanks to my Supervisors, Prof. J. O. Manyala and Prof. P. O. Raburu for their support, advice, criticisms, guidance, to complete this thesis successfully. My thanks go to the Department of Fisheries and Aquatic Sciences, University of Eldoret for their time, intellectual effort, inspiration and tolerance.
Many thanks go to Technicians of fisheries laboratory for permitting me to use their Laboratory equipment. I also take this opportunity to thank Mr. Josiah Ani and Tarus Andrew for sparing part of their busy time to take care of the fish and experimental system.

Lastly, I acknowledge the support received from AquaFish Innovation Lab supported in part by United States Agency for International Development (USAID) Cooperative Agreement No. EPP-A-00-06-00012-00 and by contributions from participating institutions.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) is the most cultured freshwater species among the farmed tilapias, contributing about 71 % of the total tilapia production and has considerable potential for aquaculture in many tropical and subtropical regions of the world (Fitzsimmons, 2000: Wantabe *et al.*,2002). In 2009, the production of farmed Nile tilapia reached about 82.1 % of total production of farmed tilapia (FAO, 2009). This species has a disadvantage of prolific breeding under mixed sex culture in ponds where they attain sexual maturity earlier in 2-3 months from fry stage. This characteristic results in the production of large numbers of overcrowded and stunted fish in pond that do not appeal to consumers when harvested for sale (Sule, *et al.*, 1996). Modern Nile tilapia production tries to eliminate prolific breeding since it results into stunted growth of fish. Sule, (2004) compared all-male and all-female *O. niloticus* fed on similar diet and found the growth and survival of all-male to be better than those of the all-female group.

Increased aquaculture production of Nile tilapia is linked to large scale production of formulated diets for fish culture. Formulated diets (aquafeeds) are important in sustaining the expansion of aquaculture production, because feed can make up 50 % or more of the production cost of most aquaculture systems (Wing-Keong, 2002). Increased price and supply of fish meal in the world has led to emphatic search for alternative protein sources in aquafeeds. Much attention has been focused on plant proteins, although they have less essential amino acids, minerals, anti-nutritional factors and complex carbohydrates (Vielma *et al.*, 2003). In Kenya, commercial feeds for tilapia contain highly variable crude protein levels of 18-30% (NRC, 2011; Munguti *et al*., 2014), some of which are considerably below recommended levels, in order to reduce the cost of production.

Sustainability of aquaculture depends on type of feed used and management regime in the culture facility. According to Li *et al.*, (2009), success of intensive fish culture depends to a large extent on adequate information on nutrient requirements, especially dietary protein, which is the most expensive component in artificial diets. With a growing interest in non-fishmeal protein sources for aquafeeds, it is desirable to sustain comparable levels of feed intake, feed conversion efficiency, growth rate and survival in cultured fishes.

Consequently, the expansion of tilapia farming has resulted in establishment of several databases of feed ingredients, their nutrient characteristics and improvements in feed formulations (NRC, 1993; NRC, 2011). Current researches address the importance of proteins and amino acids on fish performance and health, under various experimental and culture condition (Furuya and Furuya, 2010). Variations in the amino acid requirement in tilapia diets has led to continuous research to produce cheap and environmentally friendly diets, improving fish growth, efficiency and fillet yield.

According to Barrows and Hardy, (2001) and Wu, (2010), the digestibility and amino acid profile determine the nutritional and economic value of a protein in fish diet. Selection of a suitable protein ingredient involves knowing its amino acid profile and bioavailability. Proteins in the diet provide nitrogen for the synthesis of non-essential amino acids (Cho, 1980). It is important to have all essential amino acids (EAAs) present at balanced and biologically available levels that meet the targeted species nutrient requirements (Nunes *et al*., 2014). Amino acids are biomolecules that serve as building blocks of proteins and are intermediates in various metabolic pathways. According to Wu, (2010), the essential amino acids include: Methionine, Arginine, Threonine, Tryptophan, Histidine, Isoleucine, Lysine, Leucine, Valine, and Phenylalanine. Amino acids are obtained from proteins in diet (Barrows and Hardy, 2001) and the quality of dietary protein is assessed from essential to nonessential amino acid ratio. Amino acids are crucial for, growth, reproduction, maintenance and for the replacement of worn-out tissues. Also certain amino acids are readily converted to glucose to provide essential energy for some critical body organs and tissues such as brain and red blood cells (Cho, 1980).

Lysine and methionine are the most limiting amino acid in ingredients used for production of commercial fish feeds, especially when fishmeal is replaced by plant protein sources (Mai *et al*., 2006b). Fish feeds prepared with plant protein e.g. soybean meal typically are low in methionine; therefore, extra methionine must be added to soybean protein-based diets in order to promote optimal growth and good health (Craig and Helfrich, 2002). The fish feed manufacturers normally adjust the protein level to suit requirements of the first limiting amino acids in commercial feeds (NRC, 1993).

Appropriate dietary Methionine and Lysine levels improve the use of other essential amino acids because they have the ability to reduce the oxidation rate of other amino acids (NRC, 2011). Fish growth is determined by the first limiting amino acid and reducing the protein content of fish feeds increases the sustainability of tilapia production and reduce the environmental impact (Furuya and Furuya, 2010). The possibility of reducing the dietary protein has been demonstrated in diets for carp (Viola and Lahav, 1991), Nile tilapia (Furuya *et al*., 2005) and rainbow trout (Gaylord and Barrows, 2009).

In Kenya most fish farmers lack the necessary knowledge and skills on feed formulation to ensure profitable fish farming. Commercial manufactures also produce feeds in bulk leaving small fish farmers with the option of buying large quantities of expensive feed, which may go to waste (Pandey, 2013). Small quantities of fish feed required for small scale fish farming can be easily made at home, with local ingredients at low cost. Increased reliance on protein with poor amino acid profile brings the need to pay attention to the essential amino acid (EAAs) requirement of fish. The study determined the effect of commercial and essential amino acid supplemented diet on growth performance of *O. niloticus* in hapas suspended in an earthen pond.

1.2 Problem Statement

Aquaculture production is challenged in improving the production cost and environmental sustainability. Commercially available feed ingredients have significant amounts of anti-nutritional factors, toxic components and complex carbohydrates. Nutrient in commercial diets can be lost during feed manufacturing and improper storage of the aquafeeds. Optimum level of protein in formulated feeds is important because low or high levels of protein may lead to poor growth. The digestibility of the proteins in feed ingredients is highly variable. Fish growth and survival can be compromised by poorly digested feed that can alter the water quality, even with high protein level. Pellet type and manufacturing process also influence water quality and digestion efficiency of the fish species under culture.

Standard nutrient requirement cannot be applied to practical feed formulation since it is not adequate for high density commercial rearing situations. Unbalanced dietary amino acid contents could result in an increased deamination and can increase levels of ammonia released into the water (Robaina *et al*., 1997). Also amino acid deficiency is known to cause reduced growth and poor feed conversion (Wilson and Halver, 1986). Information about proximate composition of locally available feed ingredient for farm made fish feed is usually limited and not reliable. Thus farmers depend only on the existing information about the feed composition given by different fish feeds manufacturers.

Several fish feed ingredients have poor amino acid profile hence the need to pay attention on essential amino acid requirements. Lysine and methionine are the first limiting nutrients in several plant protein sources in fish feeds e.g. soya bean meal, cotton seedcake, sunflower meal, rice bran. Feed ingredient rich in lysine consist of fish meal and blood meal and are often expensive.

1.3 Justification of the Study

Fish is an important protenous diet for a large proportion of the people living in the developing world. *Oreochromis niloticus* is one of the commercially important species among the farmed tilapias in Kenya and many other countries. This is due to its fast growth, resistance to diseases and the ability to feed at the lowest trophic level.

Formulation of suitable Nile tilapia feeds is therefore necessary to provide balanced diet that meets its nutritional requirements for optimum production at minimal cost. Feed formulators are now adopting modern and environmentally-sound formulation techniques based on nutrient value, supplementation with crystalline Essential Amino Acids (EAAs) and on nutrient requirements. In commercial aquaculture production feed costs can be reduced by developing proper feed management and husbandry strategies involving proper suitable stocking densities, nutrient ratios, feeding regimes, aeration and water exchange. Plant proteins that are cheap and locally available are used to supplement animal protein at lower cost. Cheap and locally available feed ingredients are being used globally to replace expensive fishmeal in manufactured fish feeds.

Amino acid supplementation in plant based diets is important in reducing the amount of nitrogen released in water especially when dietary nitrogen in properly utilized. This strategy can be improve fish feed conversion ratio while maintaining rapid growth. Supplementation of fish diet with limiting lysine and methionine EAAs improves fish appetite and weight gain. In Kenya protein and amino acids requirements should be considered to improve growth, feed conversion ratio and flesh quality of Nile tilapia. Determining the dietary amino acid requirement of tilapia will allow formulation of suitable, nutritionally balanced and economically profitable Nile tilapia feeds. Accurate determination of lysine and methionine requirement reduces production cost of fish feeds and improves feed utilization in cultured *O. niloticus*. Lysine and methionine are essential amino acids that cannot be synthesized by the body but are obtained from the diet. Lysine is important in carnitine biosynthesis which is required for normal lipid oxidation in mitochondria (Harpaz, 2005). Therefore it is important to supplement lysine in most plant based diets to improve fish growth. Several studies have been done on amino acid nutrition requirements, composition, supplementation and balanced ratios of protein in aquafeeds (NRC, 2011; Munguti *et al.*, 2012; Salama *et al*., 2013) that provided a solid basis for experimentation with EAAs for growth of Nile tilapia with home-made aquafeeds.

1.4 Objectives

1.4.1 Overall objective

The overall objective of this study was to formulate experimental diets using locally available ingredients while balancing the Essential Amino Acids (EAAs) to enhance the physical quality and nutritive value for the culture of *O. niloticus.*

1.4.2 Specific objectives

i) To compare commercial and amino acid supplemented diets on growth performance of monosex male *O. niloticus*

ii) To determine proximate composition of commercial and amino acid diets fed to *O. niloticus*

iii) To determine the proximate composition of the carcass of *O. niloticus* fed on commercial and amino acid supplemented diets

iv) To examine the cost effectiveness of commercial and amino acid supplemented diets in rearing of *O. niloticus.*

1.5 Hypotheses

i) There is no significant difference in the commercial and amino acid supplemented diets on growth of monosex male *O. niloticus.*

ii) There is no significant difference in the proximate composition of diets fed to *O. niloticus*

iii) There is no significant difference in the carcass composition of *O. niloticus* fed to *O. niloticus*

iv) There is no significant difference in the cost effectiveness in rearing of *O. niloticus* fed on different diets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Effect of Diets on Growth Performance of *Oreochromis niloticus*

Growth of tilapia farming has led into the expansion of nutrient requirement data to improve feed formulation. Protein is the most expensive component in fish feeds and plays an important role in growth of fish (NRC, 1993). Several studies has demonstrated that properly balanced ratios of protein to non-protein energy in diets can increase its utilization for fish growth (Wang *et al.,* 2006; Ahmadr, 2008). Improvements of net protein can be accomplished by feeding on a protein of high biological activity. Commercial fish feeds usually have different digestible energy with differences in each species (Kaushik and Medale, 1994). Digestive and absorptive efficiency decreases with an increase in pellet size (Windel *et al.,* 1978). Popma, (1982) reported that *O. niloticus* can digest over 70% of the energy of raw corn starch. Ingredients such as soy bean meal and sunflower are high in ash and fibre content (Maina *et al.,* 2002). Therefore there is need to improve the feed digestibility and utilization of fish feeds by supplementing to improve the growth perfomance of *O. niloticus*.

The growth of cultured fish depends on the nutrient composition of the feed and the ability of the animal to digest and absorb the combined nutrients **(**Riche *et al.,* 2001). Mugo-Bundi *et al*., (2013) reported reduction in growth performance and survival at 100% substitution levels of *Caridina nilotica* with fish meal. The findings suggested lower growth trend curves which may probably indicate limitation of essential amino acid such as arginine, lysine and methionine + cysteine. The result further suggested that it is possible to substitute up to 75% of *C. niloticus* in diets with low fish meal content for *O. niloticus* without affecting growth performance, nutrient utilization and survival. Excessive non-protein energy can reduce feed intake and inhibits the utilization of other nutrients. An optimal dietary Protein to Energy Ratio (P/E) should be taken into account when the fish diet is formulated. Winfree and Stickney, (1981) reported that optimum dietary protein to energy (P/E) ratio for rapid and efficient weight gain of juvenile tilapia reduce with increase in fish size.

Protein Efficiency Ratio (PER), Protein Productive Value (PPV) and protein growth rate (PGR) are used to assess protein utilization and turnover, where they are related to dietary protein intake and its conversion into fish gain and protein gain. Ahmad *et al.,* (2004) conducted a study on the effect of dietary protein levels on growth performance and protein utilization in Nile tilapia with different initial body weights and found out that PER range from 1.58 to 2.35 for fry (~0.5 g), from 1.19 to 1.92 for fingerlings (~20.4 g) and from 0.99 to 1.53 for adult fish (~40.5 g). Studies done by Yan *et al*. (2012) reported growth performance of Nile tilapia to be significantly affected by dietary P/E ratio.

The findings reported that the response of tilapia to protein could still be achieved at lower levels of inclusion. Zhu *et al*., (2010) conducted a research on responses of juvenile Nile tilapia (*O. niloticus*) on experimental diets with graded levels of DL-Methionine at Yangze River Fisheries Research Institute. Their findings suggested improved Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) with increased methionine supplementation. According to Mugo-Bundi *et al.,* (2013), Feed conversion ratio (FCR) is an important indicator of the quality of fish feed, a lower FCR indicate better utilization of the fish feed. Bahnasawy, (2009) reported a decrease in FCR with increasing dietary protein levels that ranged from 2.03 to 2.42 and PER to be significantly affected by protein utilization was obtained at lower protein level and ranged from 1.36 to 2.43. The decrease of PER with increasing dietary protein level have also been reported for different tilapia species (Ahmad *et* *al.*, 2004). Opiyo *et al*., (2014) conducted a study on growth performance, carcass composition and profitability of Nile tilapia (*O. niloticus*) fed commercial and on-farm fish feed in earthen ponds. The findings reported lower FCR of 1.66, an indicator of better feed utilization of the on-farm fish feed.

Several better FCR values have been reported in literature for *O. niloticus* ranging from 1.43 to 2.30 **(**El-Husseiny *et al*., 2008; Khattab *et al*., 2000; Al-Hafedh, 1999).The level of dietary protein to produce maximum growth of fish depends on the energy content of the diet, physiological state of the fish such as age, reproductive state, and environmental factors such as temperature and salinity, amino acid profile and level of food intake (Barrows and Hardy, 2001; Craig and Helfrich,2002). Therefore supplementation of limiting EAAS lysine and methionine in the deficient plant protein sources improves the level of dietary protein content in fish feeds.

2.2 Proximate composition of feed ingredients for *O. niloticus*

Most aquaculture feed are selected based on unit cost of dietary energy, protein and amino acid composition, ingredient digestibility and level of phosphorus. Protein sources for feed formulation should have high protein content, excellent amino acid profiles, and high nutrient digestibility and low cost (Tacon, 1993; El-Haroun, 2007; Munguti *et al*., 2012, Mugo-Bundi *et al*., 2013). Complete fish diets contain all the essential nutrients such as proteins, carbohydrates lipids, vitamins and minerals required for fish growth (Lovell, 1988).

Fish meal has been used as reference protein source in tilapia diets, showing high content of protein with well-balanced amino acids, high palatability and good source of energy, essential fatty acids, minerals and vitamins (Liti, *et al.,* 2006a). Most herbivorous and omnivorous fish require a diet with 25 to 35 % crude protein for growth (Wilson, 2002). Reducing ammonia excretion by fish involve reducing the amount of nitrogen consumed, using highly digestible feedstuffs in diets. It also involves supplementation of amino acids and producing a high quality protein with superior amino acid balance (Furuya and Furuya, 2010).

Freshwater shrimp (*Caridina nilotica*) meal is a rich source of animal protein (Munguti *et al.,* 2006). Furthermore, Fish meal is scarce in the Kenyan market due to seasonal closures of the Omena (*Rastrineobola argentea*) fishery in Lake Victoria. Liti *et al.,* (2005) reported higher costs of fish production with diets containing fish meal compared to those containing all plant protein feedstuffs.

Cotton seed cake residues have high crude protein levels but are low in cysteine, methionine and lysine, which are frequently lacking in plant protein sources (Jauncey and Ross, 1982). The limit in inclusion levels of cotton seed cake is determined by the level of gossypol (El-Saidy and Gaber, 2004), a toxic phenolic compound that is found in the pigment glands of the cotton plant (Berardi and Goldblatt, 1980). Gossypol has been associated with reduced fingerling recruitment in *O. niloticus* (Liti *et al.,* 2005). Wheat bran promotes good growth of Nile tilapia and can substitute other feed ingredients without affecting fish growth, depending on local availability. Cereal brans are generally cheap and readily available in most regions of Kenya, and may therefore be an important feed component in semi-intensive tilapia production (Liti *et al.,* 2006b).

Feeds consisting of soybean, wheat and corn meal, canola meal, extruded pea seed meal, supplemented with methionine have been used in diet formulation for carps, tilapia and catfish without influencing growth performance (Tacon and Metian, 2008). Meat bone meal is recognized as an excellent source of supplemental protein, calcium, vitamin B_{12} and phosphorus with a well-balanced amino acid profile and high protein digestibility for most fish (Kellems and Church., 1998). However, meat bone meal is has lower amino acids content and higher in minerals as compared to fish meal and blood meal (Yang *et al*., 2004).

Feather meal is a by-product from poultry processing industry with complex protein (keratin), which can be hydrolysed to improve bio-availability (Munguti *et al.*, 2014). Feather meal is rich in amino acids such as cysteine, threonine and arginine, and has high level of pepsin digestible protein similar to those of fish meal and soybean meal (Fowler, 1990). Table 1 provides a profile of the most common fish feed ingredients used for formulation of Nile tilapia feeds in Kenya.

Table 1: Proximate composition of different fish feed ingredients analysed in Kenya (±S.E)

Product	**n**	*DM (**gkg-1)**	**DM (g kg-1)**				
			CP	**EE**	**CF**	**NfE**	**Ash**
Fishmeal(*Rastrineobola argentea*)	9	879±0.6	551±1.7	187±1.5	13±0.6	68±1.0	182±1.5
Shrimp (*Caridina nilotica*) meal	7	877±1.7	635±3.3	13±1.3	50±1.8	67±2.1	228±2.5
Maize (*Zea mays*) bran	8	894±3.0	118±4.6	107±2.7	55±0.7	349±3.5	29±1.3
Wheat (*Triticum aestivum*) bran	8	882±1.6	171±6.2	58±2.3	127±2.3	582±6.9	60±2.6
Rice (*Oryza sativa*) bran	5	923±4.2	70±3.8	41±1.6	309±2.4	349±3.5	229±2.2
Arrow root (*Maranta arundinacea*) leaves	6	903±2.6	335±1.0	85±1.5	106±4.6	381±2.1	93±2.3
Banana *(Musa paradisiaca)* peel	5	901±2.1	72±1.7	79±1.3	113±2.6	627±1.7	109±2.8
Banana *(Musa paradisiaca))* stem	7	926±1.0	100±1.8	50±2.2	441±1.7	205±3.5	205±4.5
Banana *(Musa paradisiaca)* leaves	6	899±1.0	170±1.8	127±1.4	241±01.8	337±1.3	124±3.6
Cotton (*Gossypium spp*) seed cake	5	892±2.0	388±7.2	107±1.0	249±4.5	192±2.6	63±4.6
Sunflower (*Helianthus annuus*) seed cake	5	929±0.4	259±0.1	54±0.8	368±0.2	266±0.8	51±0.1
Cassava (*Manihot esculenta*) leaves	5	919±3.6	308±4.8	86±4.1	156±4.0	368±2.1	82±5.2
Papaya (*Carica papaya*) peel	6	839±1.3	179±2.4	18±3.1	194±2.2	456±4.0	154±03.4
Papaya (*Carica papaya*) leaves	7	903±2.9	282±5.0	105±2.5	130±1.3	329±3.3	154±1.2
Papaya (*Carica papaya*) seed meal	4	945±1.7	264±21	316±1.3	119±1.0	203±1.6	98±1.3
Sweet potato (*Ipomoea batatus*) leaves	5	892±1.6	353±3.6	43±3.7	105±3.6	388±1.1	104±3.6
Water fern, (*Salvinia auriculata*)	6	888±2.4	232±1.9	49±0.8	302±3.6	239±1.3	179±3.4
Mango (*Mangifera indica*) seed embryo	2	907±1.4	70±0.7	97±1.4	37±0.7	771±2.1	24±1.4
Coffee (*Coffea arabica)* husks	4	893±1.9	47±1.8	36±0.6	383±2.6	418±3.6	115±2.8
Cotton (*Gossypium spp*) husks	3	906±4.9	173±4.4	55±1.0	587±1.5	153±1.5	36±0.6
Brewery by-product	6	919±1.9	264±0.3	291±0.1	158 ±0.6	221±0.5	66±0.4
Tilapia (*Oreochromis spp*) fillet remains	7	916±1.4	580±1.7	181±1.5	67±1.7	75±1.7	97±1.4
Catfish (*Clarias gariepinus)* fillet remain	6	923±2.5	570±2.4	192±2.3	73±2.4	96±2.4	69±2.2
Sorghum (*Sorghum bicolor) beetle*	4	948±1.2	461±1.6	165±1.5	109±1.4	224±1.5	41±1.2

DM=Dry matter, CP=Crude Protein, EE=Ether Extracts, CF= Crude Fibre, NfE=N-free Extracts (Munguti *et al*., 2012)

2.3 Amino Acid Composition and Supplementation in *O. niloticus* Diets

All fish require essential dietary amino acids and deficiency in any of the amino acid causes reduction in growth. Excess amino acids also cause reduced growth hence the need to balance amino acid in fish diet. Each species has specific requirements for the ratio of essential amino acids (Ketola, 1983). Fish cannot themselves synthesize the 10 indispensable amino acids, so these amino acids must be supplied through the diet. Essential amino acids that can be synthesized from the diet include: Methionine Arginine, Threonine, Tryptophan, Histidine, Isoleucine, Lysine, Leucine, Valine, and Phenylalanine. Lysine and methionine are often the first limiting amino acids ingredients used for manufacturing commercial fish feeds. Dietary lysine levels critically affect fish growth performance and health.

Lysine is an essential amino acid present in high proportion in fish muscle tissue, promoting growth and maintenance of positive nitrogen balance, also used in "cross-linking" protein (University of Maryland [UNM], 2006). Moreover, it plays an important role in the synthesis of carnitine, which is important for the transport of long-chain fatty acids into the mitochondrion for energy generation (Walton *et al*., 1984). Dietary lysine supplementation is related to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents (Marcouli *et al.,* 2006). With the commercial availability of feed-grade lysine, its addition to plant protein-based diets allows for cost-effective reduction of dietary crude protein without affecting fish growth performance (Mai *et al.,* 2006b). This nutritional strategy can also decrease the excretion of ammonia and soluble phosphorus from fish (Cheng *et al.,* 2003). Mai *et al.,* (2006a) in their study on juvenile Japanese sea bass reported that, the whole body protein and lipid were significantly affected by dietary lysine levels. In practical conditions, it is possible that two fish have the same weight, but produce different values of fillet yield.

Deficiency of lysine causes caudal fin erosion (Ketola, 1983) while deficiency of methionine causes cataracts (Poston *et al*., 1977). Plant proteins do not contain complete amino acid profiles and mostly deficient in lysine and methionine. Soybean and other legume meals are a good source of lysine and tryptophan but are limiting in the sulfur-containing amino acids methionine and cysteine (Miles and Chapman, 2005). Lysine is one of the most limiting amino acids in fish nutrition, not only related to fish growth, but to increase in fillet yield (Furuya *et al*., 2006).

Several studies indicate that commercial amino acids supplemented are effective in promoting growth in Nile tilapia and are also available for diets of different fish species; shrimp, rainbow trout, other cultured tilapias, grass carp, and catfish (Liu *et al*., 2010; Sangsue *et al*., 2010; Wang *et al*., 2010).

2.3.1 Methods used in estimating amino acid requirements of cultured fish

Amino acid requirements of fish may be expressed as diet concentration or diet percentage per unit of digestible energy (g MJ^{-1} DE) or as a proportion of diet content (g 16 g^{-1} N) (NRC, 1993; Bureau and Encarnacao 2006).The quantitative essential amino acid requirements have been established for few fish species and the quantitative dietary requirement of fish is more precisely obtained by dose-response experiments compared to estimates from whole body amino acid profiles (NRC, 2011).The determination of amino acid requirements of the cultured species is of economic importance for purposes of economic analysis in feed production (Furuya *et al.*, 2015). A number of empirical methods have been applied to establish the quantitative (EAAs) dietary requirements of fish. These involve fitting response curves to the experimental data, and non-linear models, linear Broken Line method, High Performance Liquid Chromatography and dose response technique (Lovell, 1988; Meyer and Fracalossi, 2005; Bicudo *et al*., 2009; NRC, 2011).

Bicudo and Cyrino, (2014) studied the evaluation of methods to estimate the essential amino acids requirements of fish from the muscle amino acid profile and recommended the method described by Meyer and Fracalossi, (2005) to be better suited for estimating EAA. Their methodology estimated the lysine requirement of *Piaractus mesopotamicus*, at 13 and 23% above requirement determined using dose-response method. According to studies done by Furuya *et al*., (2015), on estimation of the dietary essential amino acid requirements of Banded astyanax (*Astyanax fasciatus*) by using the ideal protein concept, the regression analysis showed high correlation (r = 0.9710) between whole body essential amino acid profiles of Banded astyanax and Nile tilapia. The study further concluded that the whole body composition of amino acids might be used to estimate the dietary requirements of essential amino acids, including cysteine and tyrosine, for new aquaculture fish species such as Banded astyanax.

Studies done by Salama *et al*., (2013) on sea bass fry recommended addition of lysine, methionine and cysteine to a diet with low protein level to get the best growth and feed utilization. The study further reported amino acids requirements of sea bass to be at 1.8g lysine level with 0.4g methionine and cycteine level at 35% protein and $300kcal100g^{-1}$ diet**.** Davies and Ezenwa, (2010) also reported groundnut cake supplemented with 0.45kg each of lysine and methionine per 100kg to be used in fish feeds. Mugo-Bundi *et al.,* (2013) demonstrated that *Caridina nilotica* can be effectively used to replace up to 75% of fish meal in the diets without compromising growth performance, survival, nutrient utilization and economic benefits in *O. niloticus* culture. Their findings further suggest that at 100% substitution levels of fish meal with *C. nilotica* may probably indicate limitation of essential amino acid such as arginine, lysine and methionine + cysteine. Salama *et al*., (2013) found out that increasing levels of lysine and methionine + cystine in the diet improved the protein efficiency ratio, and FCR. Mai *et al*. (2006a) also reported that PER values were reduced when juvenile Japanese sea bass fed lysine deficient diets (1.28 to 1.86%), while growth response and diet utilization were improved with supplementation of crystalline lysine at 2.46%. Studies done by Furuya *et al.,* (2012) suggested that Nile tilapia fingerlings require diets containing 5.41 g lysine100g^{-1} of digestible protein, in diets balanced for the arginine-to lysine ratio.

2.3.2 Amino acid profile and body composition of Nile tilapia

Amino acids profiles of whole-body tissue of a given fish species resemble its dietary requirements. When the quantitative amino acid requirements of a species are unknown, the approximate essential amino acid requirement can be derived from the essential amino acid pattern of whole-body tissue of that fish (Wilson and Poe, 1985; Tacon 1989). Bahnasawy, (2009) reported a significant increase in muscle protein and a decrease in lipid content with increasing dietary protein and in channel catfish diets with increasing levels of lysine respectively. Furuya *et al*., (2004) reported that a diet with all plant protein source, supplemented with essential amino acids, based on tissue amino acid profile, can replace fish meal without adverse effects on the growth performance, carcass yield and composition. The study further recommends protein composition to be used when the amino acid digestibility from feedstuffs values are known and developed the ideal protein concept.

Whole body essential amino acid profile has been extensively used to estimate simultaneously the requirements for all essential amino acids. Bicudo *et al.,* (2009) reported that the whole body amino acid composition varies across fish species. Thus, estimating the dietary amino acid requirements for each fish species is important for precisely formulating aquafeeds. Furuya *et al.*, (2015) studied the dietary essential amino acid requirements of Banded astyanax (*Astyanax fasciatus*) by using the ideal protein concept.

The study reported that the whole body composition of amino acids might be used to estimate the dietary requirements of essential amino acids, including cysteine and tyrosine, for new aquaculture fish species. Tasbozan *et al*., (2013) carried out proximate analysis and determined the amino acid composition of five different tilapia species from the Cukurova region of Turkey and reported that amino acid composition was significantly different among species when expressed as $g100g^{-1}$. The highest dry matter contents were determined in *T. rendalli* and *T. zillii* as 26.06% and 26.03% respectively, 24.50% in *O. aureus*, 23.54% in *O. niloticus* and 22.47% in *Tilapia* spp. Their findings further reported similar ratios of each essential amino acid content/total essential amino acid content × 1000 (A/E). Leucine, lysine and aspartic acid were higher while methionine, histidine and tyrosine were lower for all species.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at University of Eldoret fish farm (Longitude 35^{o} 18' E Latitude 0^{0}30' N) in Uasin Gishu County, Kenya.

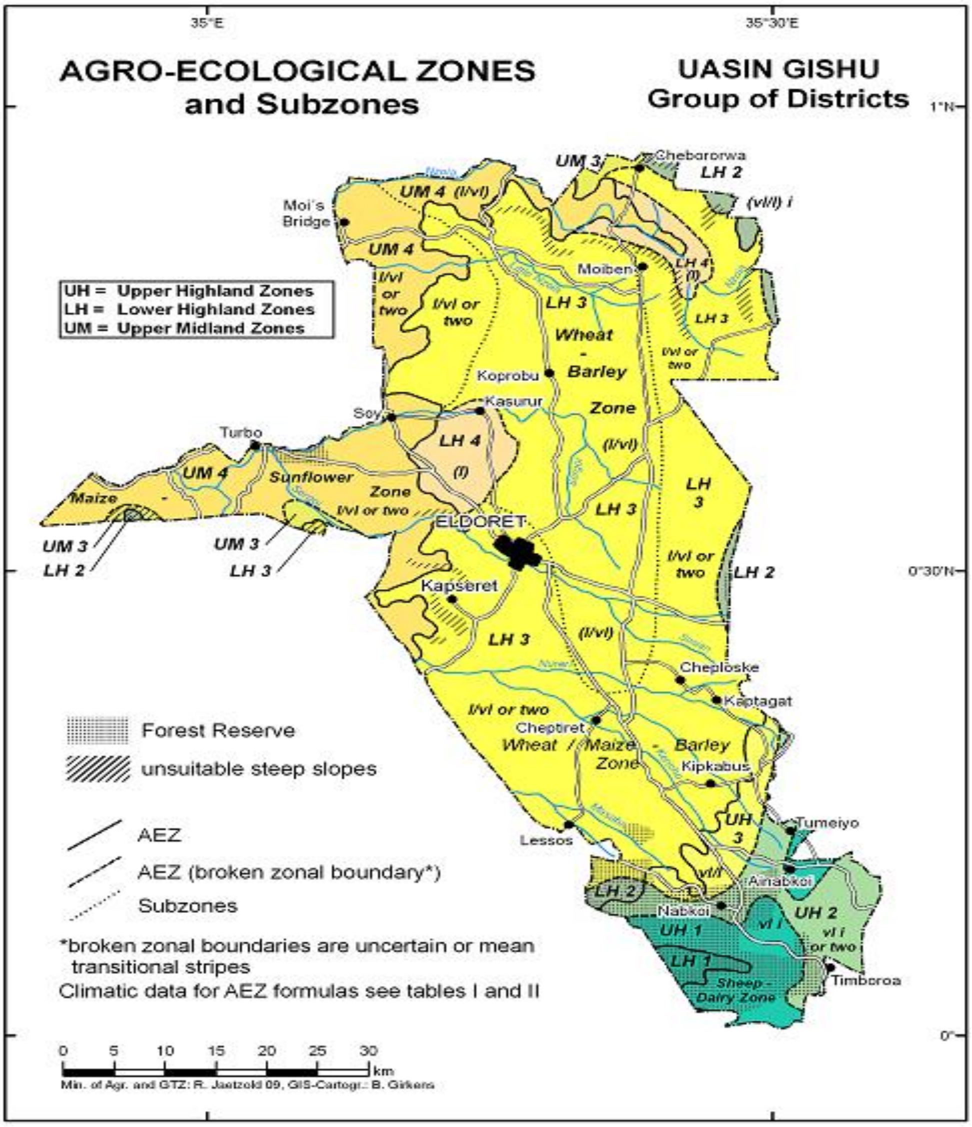

Figure 1: Map of Uasin Gishu County, showing the location of Eldoret Town, University of Eldoret and the Agro-Ecological Zonation of the County (Source: Jaetzold and Schmidt, 1982)

3.2 Study Design

The experiment was conducted for 15 weeks (105 days) from June to September 2015 in twelve hapas of capacity 1 m^3 suspended in an earthen pond of 150 m^2. The four diets were tested using a complete randomized design in triplicates at the University of Eldoret fish farm in hapa nets suspended in an earthen pond.

3.3 Experimental fish and feed Ingredients for *O. niloticus*

3.3.1 Feed ingredient acquisition, formulation and supplementation

Three diets were formulated at 30% CP and one commercial Diet constituting 32% CP was locally purchased. The feed ingredients consisted of freshwater shrimp (*Caridina nilotica*), Cottonseed cake, wheat bran, fish oil, and vitamin/mineral premix. Supplementation rate of the limiting amino acids was according to Santiago and Lovell (1988) with 5.1g kg^{-1} lysine and 2.7g kg^{-1} Methionine. Diet 1 constituted both lysine and methionine while Diet 2 was with lysine supplement only. Diet 3 did not receive any EAAs supplement; Diet 4 was a locally purchased commercial fish feed with 32% crude protein content (Table 2).

3.3.2 Fingerlings source, stocking density and pond fertilization

A total of 300 monosex male *O. niloticus* fingerlings of 1.8 ± 0.25cm were obtained from Sagana National Aquaculture Research and Development Centre (NARDC) and transported to the study area in oxygenated plastic bags. The fingerlings were acclimatized in holding tanks for two weeks prior the experimental stocking in the hapas. The stocking density was of 20 fingerlings per hapa. Experimental hapas nets of 1 m^3 were made of foul resistant synthetic netting of mesh size 1.5 mm and were closed from all sides except the top. The experimental ponds 150 m^2 was subjected to liming at the rate of 500 g m^{-2} with agricultural lime ($CaCO_3$) and fertilized at a rate of 3 g m^{-2} and 2 gm^{-2} with urea and Diammonium Phosphate (DAP) to facilitate primary productivity in the pond.

Table 2: Fish Feed Ingredients and essential amino acid supplementation

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	30% crude protein			**32% Crude protein**
Wheat bran	48.0	48.0	48.0	A Commercial diet
C. nilotica	30.0	30.0	30.0	
Cotton seed meal	18.0	18.0	18.0	
Fish oil	2.0	2.0	2.0	
Trace mineral	1.0	1.0	1.0	
Lysine%	1.96% + Supplement 5.1 g kg^{-1}	1.96% + Supplement 5.1 g kg^{-1}	No supplement	
Methionine%	0.95%+ Supplement 2.7 g kg^{-1}	No supplement	No supplement	

3.3 Preparation of the experimental feed for *O. niloticus*

Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into a compounded diets. The ingredients were weighed and ground to small particle size (approximately 250 µm) thoroughly mixed with warm water and partially cooked. Practical diets were prepared using warm water to reduce disintegration and leaching of nutrients in water. Oil, vitamins, and minerals mixture were added to the diets. Fish oil was included in diet formulations to serve as source of lipids and to enhance feed floatability. The diet was dried for 8 hours in the open air and broken to appropriate size of 1.5 to 2 mm crumbles. Feeds were offered daily in hapas by broadcasting method at 10:00 am and 4:00 pm. Initial feeding was offered at 10% of body weight adjusted to 5% and to 3% body weight on monthly basis.

3.4 Determination of Nutrient Composition of the experimental feeds

Feed ingredients were subjected to proximate analysis to determine the crude protein, moisture content, lipid, crude fibre, ash, and digestible carbohydrate. Experimental diets were formulated using Winfeed Version. 2.8 software. The chemical compositions of the formulated diets was determined following Association of Official Analytical Chemists [AOAC] (1990) procedures Dry matter was determined by drying in an oven at 105 $^{\circ}$C for 8 hours. Crude lipid content was determined by Soxhlet extraction with petroleum ether. Ash content, by incineration in a muffle furnace at 580 $^{\circ}$C for 8 hour. Crude protein was determined by measuring nitrogen (N× 6.25) using the Kjeldahl method.

3.5. Determination of growth for O. *niloticus* and water quality parameters

20 fish were sampled fortnightly to measure the Total Length (TL) in centimetres (cm) while the weighing balance was used to measure weight in grams (g) using a 0.01g sensitive weighing balance using. The mean weights and total lengths of fish sampled from each hapa were recorded. Fish Growth parameters were calculated using the following formulae according to Ricker (1975):

i) $Body\ Weight\ Gain\ (BWG) = \frac{[Final\ weight\ (g) - Initial\ weight\ (g)]}{Initial\ weight\ (g)}$

ii) $Daily\ Weight\ Gain\ (DWG) = \frac{[Final\ weight\ (g) - Initial\ weight\ (g)]}{Time\ interval\ (days)}$

iii) $Specific\ Growth\ Rate\ (SGR) \frac{[Ln\ (Final\ weight\ (g)) - Ln\ (Initial\ weight\ (g))]}{Time\ interval\ (days)}$

iv) $Food\ Conversion\ Ratio\ (FCR) = \frac{Weight\ of\ dry\ feed\ (g)}{[Final\ weight\ (g)] - [Initial\ weight\ (g)]}$

v)$Protein\ Efficiency\ Ratio\ (PER) = \frac{[Final\ weight(g)] - [Initial\ weight\ (g)]}{Protein\ consumed\ (g)}$

Water quality parameters measured included dissolved oxygen, temperature and pH. Three readings were taken daily at the surface, mid depth and at the pond bottom and their means were calculated.

Dissolved oxygen was measured using an Oxymeter (YSI 200), pH was measured with a glass electrode-pH meter. The water temperature was measured with a digital thermometer. A probe attached to the thermometer was immersed directly in the pond and recorded in degree Celsius, °C.

3.6 Statistical Analysis

Feed indices; Feed conversion ratio, protein efficiency ratio, Specific growth rate and water quality parameters were subjected to one way Analysis of Variance (ANOVA) to test the effects of the diets and to determine whether there were any significant differences among the means for the above indices at P = 0.05 using the Minitab statistical package (software).

Linear regression was used to compare the regression lines relating the growth in length or weight as the y-variable against the grow-out period as the x-variable with the different diets D1-D4 as categorical factor using Statgraphics Ver. 16. The test was performed to determine whether there were significant differences between the slopes at the different levels of the factor (diets) assuming that the intercept was constant and implying that the average size of all the fish were similar at the start of the experiment.

3.6.1 Economic analysis of the commercial and amino acid supplemented diets

A simple economic analysis was used to assess the cost effectiveness of diets used in the feed trial. The cost of feed was calculated using market prices, taking into consideration the cost of feed and transport, assuming all other operating costs remained constant (cost of fingerlings, hapas and labour). Indices for economic evaluation are according to Yakubu *et al.,* (2014).

$$Incidental\ Costs = \frac{Cost\ of\ feeds\ (KES)}{Weight\ of\ fish\ produced\ (g)}$$

And,

$$Profit\ Index = \frac{Weight\ of\ fish\ produced\ (g)}{Cost\ of\ feeds\ (KES)}$$

CHAPTER FOUR

RESULTS

4.1 Growth Performance of *O. niloticus* on Different Diets

4.1.1 Growth in length of *O. niloticus* during the experimental period

The initial mean length of experimental fish was 3.37±0.032 cm. Diet 2 recorded the highest final mean length of 18.61±0.381cm followed by supplemented Diet1 with 17.12±0.413 cm. The non-supplemented control diet had 16±0.330 cm, while commercial Diet 4 had the least mean length of 14±0.374 cm (Figure 2). There were significant differences for growth in lengths with different diets as evidenced in the regression slopes of Diet 1=0.6122, Diet 2=0.6127, Diet 3=0.6062 and Diet 4=0.6013 (p=0.00005) at 95% confidence interval (Table 3 and Figure 2).

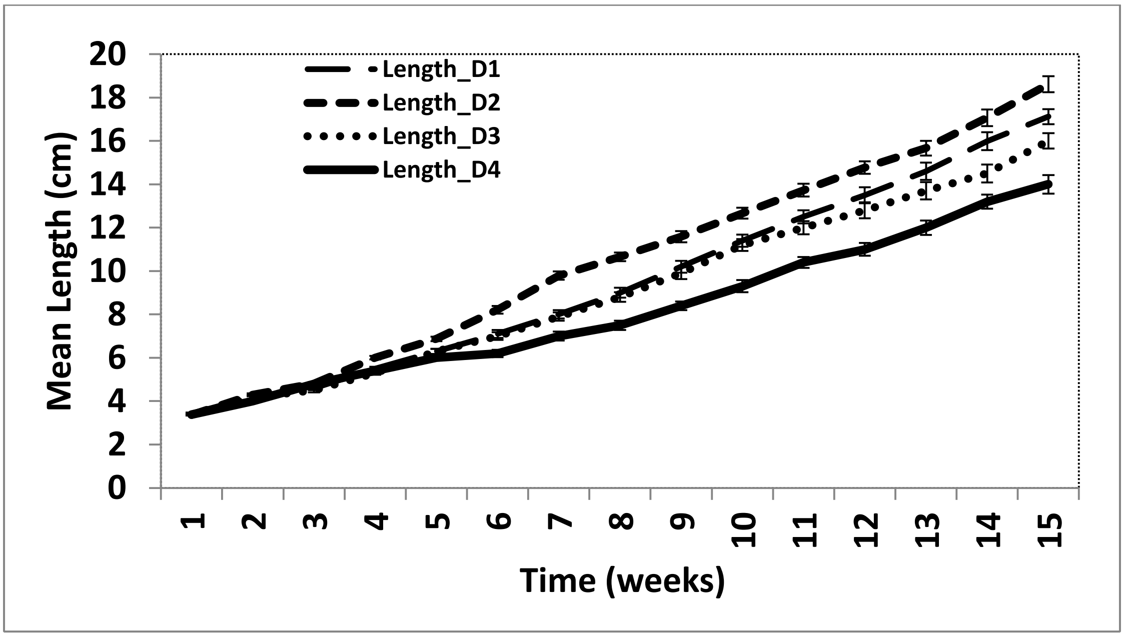

Figure 2: Growth in length of *O. niloticus* during the experimental period

Table 3: Multiple Regression Analysis based on the log transformed data on total length and grow out days with diets as treatment of *O. niloticus*

		Standard	T	
Parameter	**Estimate**	**Error**	**Statistic**	**P-Value**
CONSTANT	-0.043165	0.005053	-8.542	<0.00005
Log Days	0.612212	0.003455	177.204	<0.00005
Log Days*Treatment=Length D2	0.000519	0.002226	0.233	0.8156
Log Days*Treatment=Length D3	-0.006012	0.002226	-2.701	0.0069
Log Days*Treatment=Length D4	-0.010885	0.002226	-4.889	<0.00005
Treatment		**Intercept**		**Slope**
Length D1		-0.043		0.6122
Length D2		-0.043		0.6127
Length D3		-0.043		0.6062
Length D4		-0.043		0.6013

There is a statistically significant relationship between the variables (Log Length, Log Days and Treatment) at 95.0% confidence level. The R-Squared statistic indicated that the model as fitted explains 91.7405% of the variability in Log Length in different dietary treatments. The adjusted R-Squared statistic, compared different independent variables (dietary treatment and days) and indicated adjusted R-Squared=91.7305%. Durbin-Watson (DW) Statistic indicated a serial correlation with P=0.00005 at the 95.0% confidence level. (Table 4).

Table 4: Analysis of Variance of log length of *O. niloticus* with diets as treatment during the study period

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	190.975	4	47.7437	9182.98	<0.00005
Residual	17.194	3307	0.0052		
Total (Corr.)	208.169	3311			
ANOVA Diagnostic parameter					**Values**
R-Squared					91.7405%
R-Squared (adjusted for d.f.)					91.7305%
Standard Error of Estimate (SE)					0.0721052
Mean Absolute Error (MAE)					0.0581309
Durbin-Watson (DW) Statistic					0.851358
p-value associated with Durbin-Watson Statistic					<0.00005)
Lag 1 residual autocorrelation					0.574277

Table 5 tested the statistical significance of the terms in the model. At 99% confidence level there is statistically significant difference among the slopes of different dietary treatments of commercial and amino acid supplemented diets with p=0.00005 during the study period.

Table 5: Further ANOVA for Variables in the Order Fitted for Log Length of *O. niloticus* during the study period

Source	Sum of Squares	Df	Mean Square	F-Ratio	p-value
Log Days	190.791	1	190.791	36696.49	<0.00005
Slopes	0.184	3	0.0614291	11.82	<0.00005
Model	190.975	4			

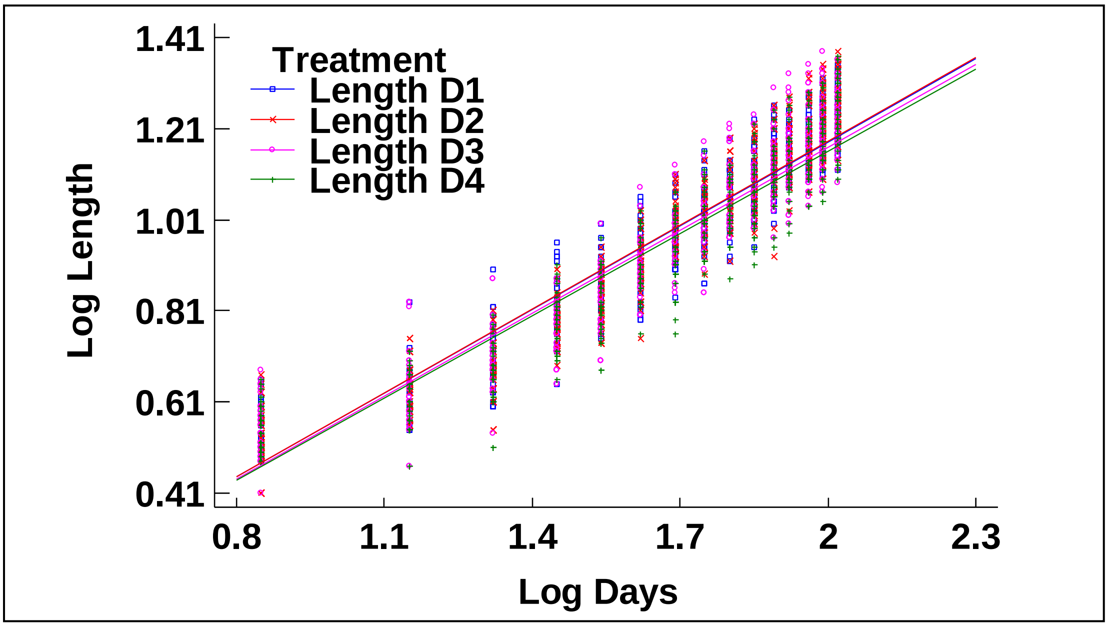

Figure 3: Growth in length (Log-Log transformed) of *O. niloticus* with diets as treatment during the study period

4.1.2 Growth in weight of *O. niloticus* during the experimental period

There was a general increase in body weight of all the experimental fish fed on different diets during the study. There were significant differences in all the tested diets as evidenced in the linear regression slopes for Diet1=2.073, Diet2=2.056, Diet3=2.049 and Diet4=2.041 with p=0.00005 at 95%confidence level. A superior growth in weight was recorded in the amino acid supplemented Diet 1 and 2 indicating relatively higher linear regression slope. Relatively lower linear regression slopes were evidenced in non-supplemented control Diet 3 and Commercial Diet 4.

The overall growth performance parameters (final mean weight, body weight gain and Specific growth rate and feed conversion ratio) are presented in Table 6 and Figure 4. Diet 2 induced the highest mean weight of 156.05±1.741 g within 15 weeks whereas the commercial Diet 4 induced the least mean weight gain of 94.083 ±3.064g. Lysine and Methionine supplemented Diet1 indicated a better mean final weight of 125±1.681g while the control diet had an average perfomance of 114±1.917g.

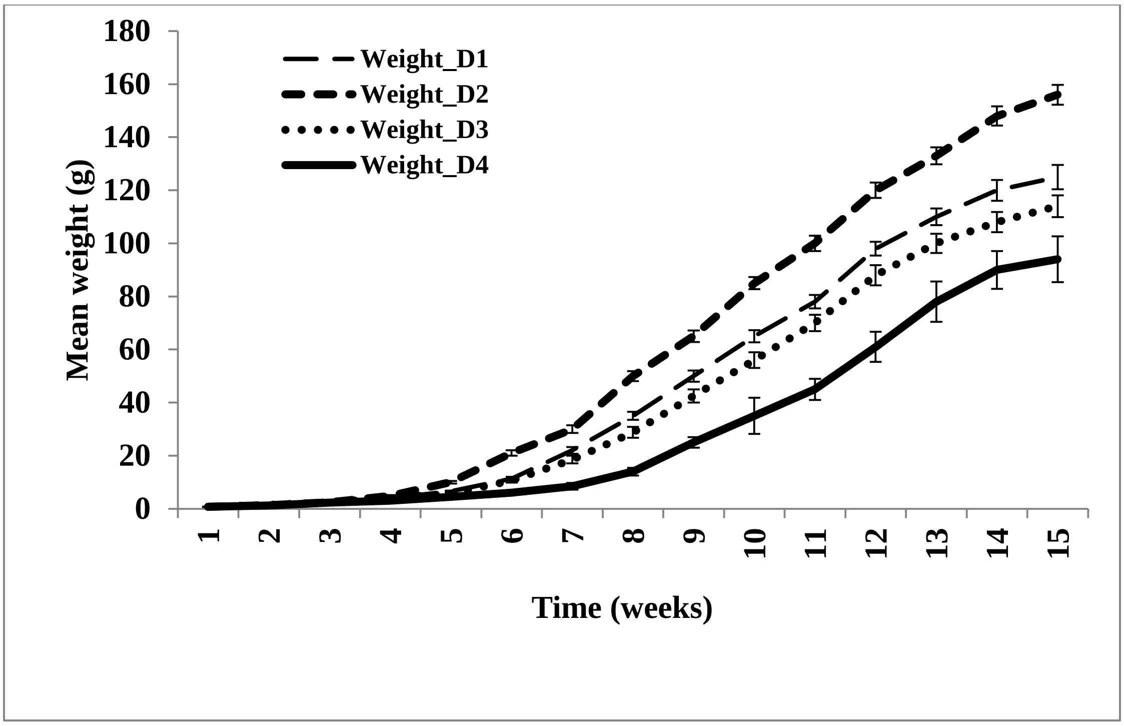

Figure 4: Growth in weight of *O. niloticus* over 105 days on the four tested diets

All the other performance indicators; Body Wight Gain (BWG) 156.12±1.74, Daily Weight Gain (DWG) 0.274±0.03, Food Conversion Ratio (FCR) of 1.21 and Protein Efficiency Ratio (PER) 2.68 indicated that the lysine supplemented Diet 2 had a superior growth perfomance and better feed digestibility compared to other diets. Protein utilization was assessed through protein dependant parameters indicating a significance difference at p=0.00005. High PER values were indicated in the supplemented Diets 2 and Diet 1 while lower PER values were obtained in non-supplemented Diet 3 and commercial Diet 4 as shown in Table 6.

Table 6: Growth performance parameters of *O. niloticus* over 105 days on the four test diets

Parameter	Diet 1	Diet 2	Diet 3	Diet 4
Initial average weight (g)	1.24±0.018	1.25±0.019	1.24±0.021	1.25±0.021
Final average weight (g)	125.05 ± 1.68[a]	156.12 ± 1.74[b]	114.03 ± 1.92[c]	94 ± 3.064[d]
Weight of dry feed (g)	250.00	250.00	250.00	250.00
BWG	179.12. ± 0.02[a]	215.67 ± 0.01[b]	153.26 ± 0.01[c]	136.03 ± 0.01[d]
DWG	0.265 ± 0.05[a]	0.274 ± 0.03[b]	0.247 ± 0.04[c]	0.236 ± 0.05[d]
SGR	2.34±0.01a	2.40±0.03a	2.25±0.02a	2.28±0.11a
FCR	1.42 ± 0.23[a]	1.21 ± 0.15[b]	1.61 ± 0.24[c]	1.80 ± 0.45[d]
PER	2 .60±0.06[a]	2 .68±0.12[b]	2 .42±0.09[c]	2. 32±0.16[d]

Values are expressed as mean± SE. The superscripted letters indicate significant difference at p<0.05

Table 7: Multiple Regression Analysis based on the log transformed data on total Weight and grow out days with diets as treatment

Parameter	Estimate	Standard Error	T Statistic	p-value
CONSTANT	-2.12993	0.0168579	-126.346	<0.00005
Log Days	2.07296	0.0115254	179.860	<0.00050
Log Days*Treatment=Weight D2	-0.0170288	0.0074265	-2.29298	0.0218
Log Days*Treatment=Weight D3	-0.0237672	0.0074265	-3.20033	0.0014
Log Days*Treatment=Weight D4	-0.0318076	0.0074295	-4.28124	<0.00005
Treatment		**Intercept**		**Slope**
Weight D1		-2.12993		2.07296
Weight D2		-2.12993		2.05594
Weight D3		-2.12993		2.04920
Weight D4		-2.12993		2.04116

There is statistically significant relationship between the variables; weight, days and dietary treatment with p=0.00005 at 95.0% confidence level (Table 7).The R-Squared statistic indicates that the model as fitted explains 91.93% of the variability in Log Weight. The adjusted R-Squared statistic compared different independent variables (grow out days and dietary treatment) indicated adjusted R- squared=91.92%.The Durbin-Watson (DW) statistic indicated a serial correlation at 95% confidence level with a p=0.00001 (Table 8).

Table 8: Analysis of Variance based on the log transformed data on total length and grow out days with diets as treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	p-value
Model	2179.13	4	544.782	9415.85	<0.00005
Residual	191.278	3306	0.0578579		
Total (Corr.)	2370.4	3310			
ANOVA Diagnostic Parameter					**Value**
R-Squared					91.9306%
R-Squared (adjusted for d.f.)					91.9208%
Standard Error of Estimate (SE)					0.240537
Mean Absolute Error (MAE)					0.199004
Durbin-Watson (DW) Statistic					0.59899
p-value associated with Durbin-Watson (DW) Statistic					0.0000)
Lag 1 residual autocorrelation					0.700459

Table 9 indicated a significant differences for the slopes with p=0.0002 at 99% confidence level with different dietary treatment of commercial and amino acid supplemented diets of *O. niloticus*.

Table 9: Further ANOVA for Variables in the Order Fitted for Log Weight of *O. niloticus*

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Log Days	2177.98	1	2177.98	37643.51	<0.00005
Slopes	1.15072	3	0.383572	6.63	0.0002
Model	2179.13	4			

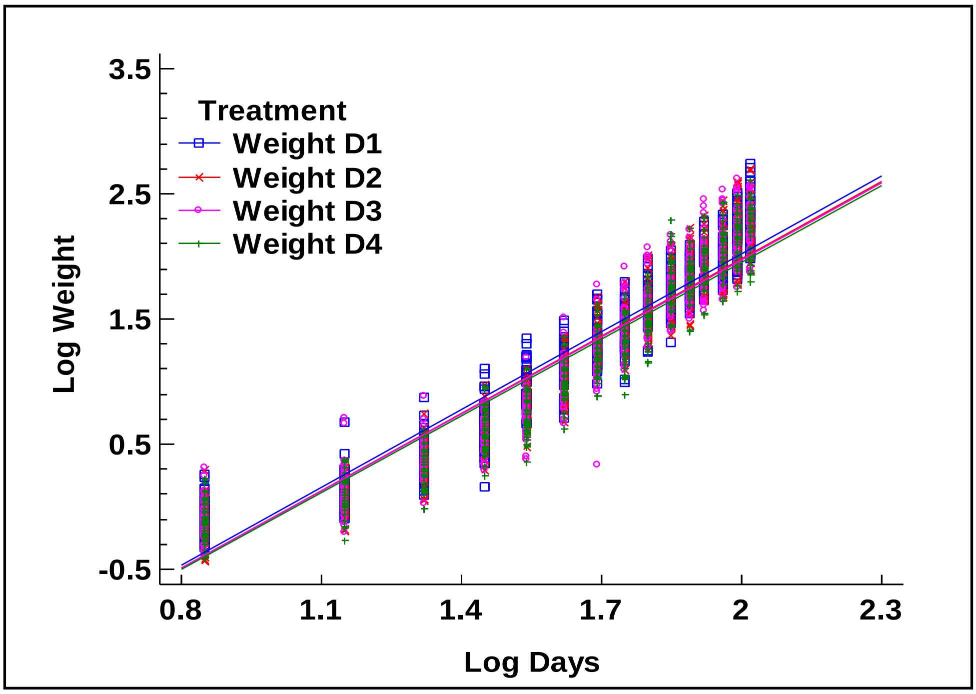

Figure 5: The growth performance in weight (g) for *O. niloticus* for 105 Days

4.1.3: Water quality Parameters on growth performance of *O. niloticus* in the experimental pond during the study period

For temperature, there were variations with time ($F_{0.5,\ 15,\ 80} = 4.27$; p-value<0.05).Temperatures were maintained at a range of 24 ± 1.16 to 26 ± 1.26°C in all the sampling weeks as shown in Figure 6.

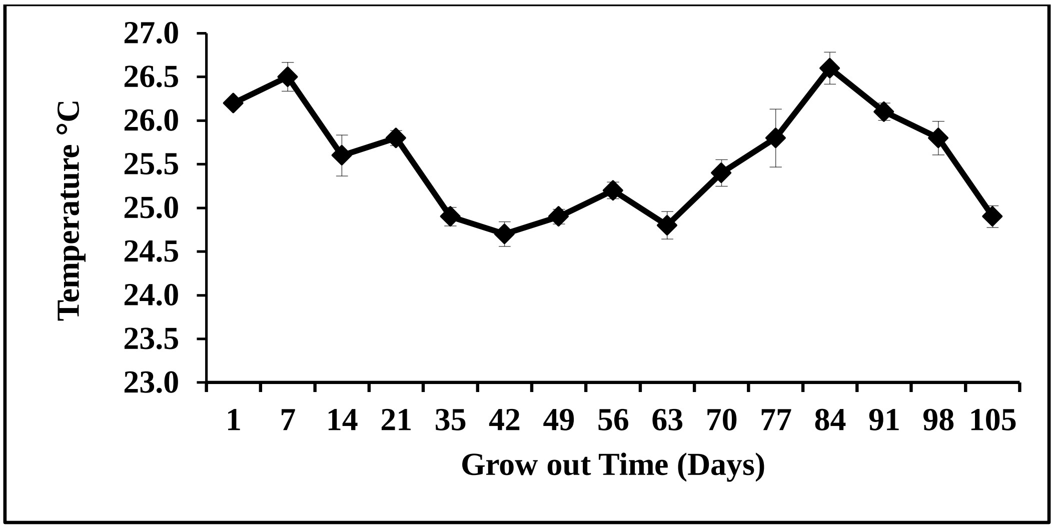

Figure 6: Temperature of the experimental pond for the culture of *O. niloticus* during the study period.

The Dissolved Oxygen (DO) concentration did not show any significant variation with time ($F_{0.5,\ 15,\ 80} = 1.31$; p-value=0.217). Dissolved oxygen levels were at a range of 4.8±1.13 to 6±1.24 mg L^{-1} (Figure 7).

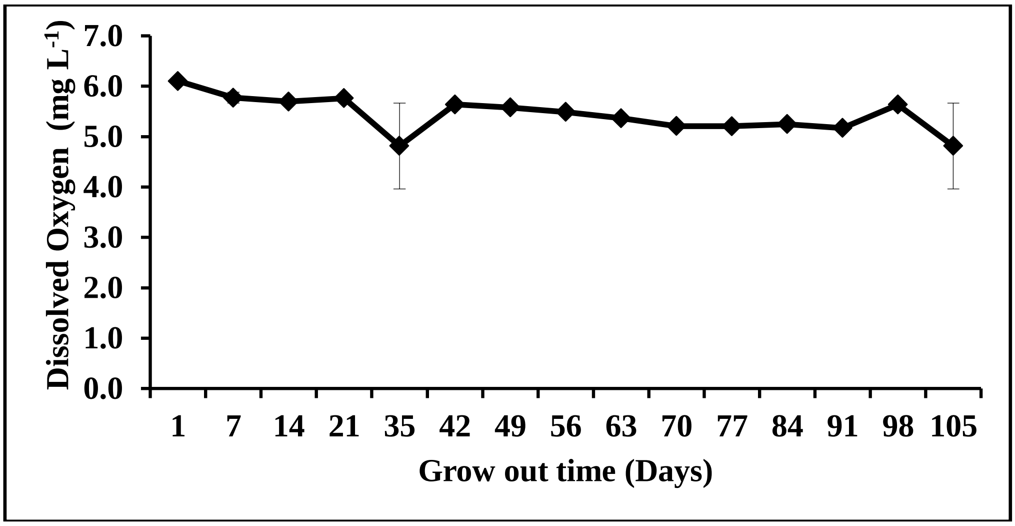

Figure 7: Dissolved Oxygen levels of the experimental pond for the culture of *O. niloticus* during the study period.

The water pH was statistically significant with time ($F_{0.5,\ 15,\ 80} = 7.63$; p-value<0.00005). The pH range was 7.2 ± 0.987 to 7.6 ± 1.250 throughout the study

(Figure 4.8). The range of these critical water quality parameters were within suitable range for tilapia culture.

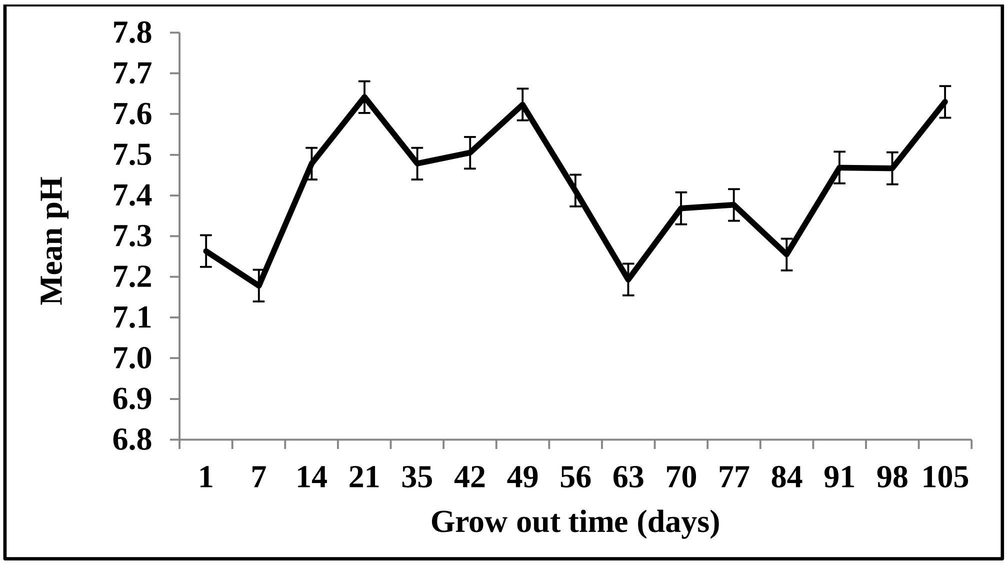

Figure 8:The pH ranges of the experimental pond for the culture of *O. niloticus* during the study period.

4.2. Proximate Composition of Diets fed to *O. niloticus* and its Carcass

4.2.1 Proximate Composition of Practical Diet before Amino Acid Supplementation

The practical diets were formulated at 30% crude protein content using cheap and locally available feed ingredients indicated in Table 10. The commercial diet was indicated to constitute 32% crude protein content. According to the practical diet formulation in this experiment, the following nutritional values were calculated based on the ingredient profiles from tropical areas on as-is basis of dry feedstuff.

Table 10: Proximate composition of the experimental feed ingredients before amino acid supplementation for *O. niloticus*

Parameter	Nutritive value	Parameter	Nutritive value
DM	90.72	Cholesterol	0.12
Ash(mg kg^{-1})	8.03	Astaxanthin	804.05
Gross Energy (MJ kg^{-1})	17.17	Arginine	2.53
Digestible Energy(MJ kg^{-1})	9.67	Histidine	0.81
Crude Protein	30.15	Isoleucine	1.04
Digestible Crude Protein	22.91	Leucine	2.24
Lipid	5.53	Lysine	1.96
Fibre	6.87	Methionine	0.95
LOA (18:2n-6)	0.72	M+C	2.27
LNA (18:3n-3)	0.03	Phenylalanine	1.57
ARA (20:4n-6)	0.02	P+T	2.62
EPA (20:5n-3)	0.64	Threonine	1.21
DHA (22:6n-3)	0.40	Tryptophan	0.30
Total n-3	1.07	Valine	1.34
Total n-6	0.75	Ca	1.44
n3:n6	1.43	Available P	1.14
Total phospholipids	1.77		

The proximate composition of commercial and amino acid supplemented diet are presented in Table 11. Laboratory proximate analysis after supplementation indicated a slight increase in the crude protein levels at $P<0.05$ in all the dietary treatments. Amino acid supplemented diet 2(32.3±0.12%) and Diet 1 (32.1±1.21%) indicated improved Crude protein content. The non-supplemented control Diet 3 at 30.3±0.14% and the commercial Diet 4 indicated reduced crude protein level of 28.9±0.18%.There was no significant difference in the moisture content in all the dietary treatments. A relatively higher Ash content in the commercial diet compared to all other dietary treatments at $P<0.05$

Table 11: Proximate composition of the experimental feed ingredients after amino acid supplementation

Composition (g/kg)	Diets			
	Diet1	**Diet 2**	**Diet 3**	**Diet 4**
Dry Matter	90.6±1.12	90.6±1.18	90.5±1.21	90.3±1.15
Moisture content	0.38±0.12	0.48±0.04	0.42±0.03	0.47±0.12
Crude Protein	32. 3 ± 0.18[a]	32.1± 1.21[a]	30.3 ± 0.14[b]	28.9 ± 0.18[c]
Crude Lipid	0.054±0.002[a]	0.052±0.001[a]	0.05±0.002[a]	0.036±0.05[b]
Ash Content	7.07±1.09[a]	7.06±1.15[a]	7.07±1.11[a]	8.02±1.19[b]

Values are expressed as mean± SE. Superscripted letters indicate significant difference at p<0.05

4.2.2 Carcass composition of *O. niloticus* fed on commercial and amino acid supplemented Diets

There was a significant difference the crude protein, lipid and ash contents between the supplemented and non-supplemented diets at p=0.005 at 95% confidence level. There was no significant difference in the moisture content of all the carcasses fed on different diets. Supplemented Diet 1 and 2 indicated crude protein content of (64.21±0.20) and (64.12±0.18) respectively. The Crude Protein and lipid contents in fish carcass were affected by dietary protein level. Crude lipid content was relatively lower in the supplemented Diet 1 (10.371±0.21) and Diet 2 (10.346±0.27) as compared to higher crude lipid contents in commercial (14.358±0.23) and non-supplemented Control Diet (12.704±0.26). The control Diet 3 recorded a relatively lower ash content (13.375±0.93) compared to the supplemented diets as shown in Table 12.

Table 12: Carcass composition of *O. niloticus* fed on Commercial and amino acid supplemented diets

Composition (%)	Diets			
	Diet 1	Diet 2	Diet 3	Diet 4
Moisture content	7.77±0.12[a]	7.74±0.19[b]	7.69±0.14[b]	7.75±0.16[c]
Crude Protein	64.21±0.20[a]	64.12±0.18[a]	63.10±0.23[b]	58.9±0.19[c]
Crude Lipid	10.371±0.21[a]	10.346±0.27[a]	12.704±0.26[b]	14.358±0.23[c]
Ash Content	14.750±0.52[a]	15.125±0.66[a]	13.375±0.93[b]	14.042±0.55[c]

Values are expressed as mean± SE. Superscripted letters indicate significant difference at p<0.05

4.3 The Cost effectiveness of Commercial and amino acid supplemented Diet fed to *O. niloticus*

The Simple economic analysis of test diets exhibited a significant variation at P<0.05 for different tested diets. Simple economic analysis indicated that higher profit index was achieved in the amino acid supplemented Diet 2 (2.973±0.02[a]) at a low incidence cost (0.336±0.09[b]). Diet 1 also constituted a relatively higher profit index (2.286±0.07[a]) with (0.437±0.05[a]) incidence cost. The control Diet 3 had an average profit index of 2.272±0.06[c]. Diet 4constituted of low profit index (1.052±0.04[d]) at high incidence cost (0.951±0.14[d]). The cost per kilogram of purchasing commercial diet was expensive with lower yield in terms of final mean weight gain compared to the formulated practical diet where the ingredients were locally purchased, formulated and supplemented. as shown in Table 13.

Table 13: Economic analysis of *O. niloticus* fed on different dietary treatment in hapas for 105 days

Diets	**Cost/Kg feed (KES)**	**Harvested Biomass**	**Incidence Cost**	**Profit Index**
Diet1	54.70±0.01	125.05±0.04^{a}	0.437±0.05^{a}	2.286±0.07^{a}
Diet2	52.75±0.02	156.8±1.738^{b}	0.336±0.09^{b}	2.973±0.02^{b}
Diet3	50.25±0.02	114.19±1.917^{c}	0.440±0.11^{c}	2.272±0.06^{c}
Diet4	90±0.12	94.66±3.064^{d}	0.951±0.14^{d}	1.052±0.04^{d}

Values are expressed as mean± SE with different superscript letters are significantly different ($p<0.05$).

CHAPTER FIVE

DISCUSSION

5.1 Growth Performance of *O. niloticus* on Commercial and amino acid supplemented diet

The present study revealed a significant effect of commercial and amino acid supplemented diets on growth performance of monosex Nile tilapia fingerlings reared in hapas suspended in an earthen pond. The variability in the composition of four tested diet is reflected in growth and development of *O. niloticus.*

Mean Weight Gain (MWG) was statistically different ($P<0.05$) among all the diets, Fingerlings of *O. niloticus* could be raised to about 156 g with any of the diets used and that the slight variations could be due to chance. High Weight Gain, Protein Efficiency Ratios, Feed Conversion Ratio, Specific Growth Rates were obtained in the EAAs supplemented Diets 1 and 2 compared to the non-supplemented 3 and Commercial Diet 4. The final average weight for Lysine supplemented Diet 2 (156±1.738g) was higher than both lysine and methionine supplemented Diet 1 (125±1.681g) because methionine is usually limiting in plant proteins but can be partially spared by non-essential amino acids cysteine. Wilson, (2002) estimated that cysteine can spare 40–60% of methionine in the diets for various fishes.

Dietary lysine supplementation is reported to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents (Marcouli *et al.,* 2006).This is concurrent with the results of the present study reporting better Feed Conversion Ratio, Specific Growth Rate, Daily Weight Gain and Protein Efficiency

Ratio. Salama *et al*., (2013) found out that increasing levels of lysine and methionine + cystine in the diet improved the protein efficiency ratio, and the feed conversion ratio (FCR).

The final Average weight for non-supplemented and commercial Diet 4 (94±3.064g) had least growth performances among the tested diets because of the dietary content and EAAs was deficient in the diet. The commercial Diet 4 caused poor digestibility and more of uneaten feeds were found in the hapas at the end of the study. Diet 3 and 4 showed relatively higher Food conversion Ratio (FCR) and Protein Efficiency Ratio (PER) and this is linked to the nutritive constituent of the diets hence poor feed utilization. Davies and Wareham (1988) observed that Essential amino acids (EAAs) imbalance could reduce efficiency in growth and feed utilization in plant protein diets. Mai *et al*., (2006a) also reported that PER values were reduced when juvenile Japanese sea bass fed lysine deficient diets, while growth response and diet utilization were improved with supplementation of crystalline lysine. Murai *et al.*, (1982) also reported improved growth of fish after amino acid supplementation.

Therefore there has been a worldwide increasing effort to supplement many fish diets with EAAs and other additives in order to improve diet quality and enhance growth (Ketola, 1983; Ruscoe *et al.,* 2005; Tacon and Metian, 2008; Nunes *et al.,* 2014). Commercial supplemented amino acids are effective and are consequently available to different fish species; shrimp, rainbow trout, tilapia, grass carp, and catfish (Liu *et al*., 2010; Sangsue *et al*., 2010; Wang *et al*., 2010).

5.1.1 Feed Conversion and Protein Efficiency Ratio of the Cultured *O. niloticus* fed on commercial and amino acid supplemented diets

Feed conversion ratio (FCR) involve finding out how much feed given actually goes into building fish flesh. The lower the FCR the better the quality of feed applied. This is because a smaller quantity of feed is needed to build a unit of fish flesh at low costs. In the present study the FCR obtained in all the tested diets were within the recommended growth for most species. The FCR 1.42 ± 0.23 and 1.21 ± 0.15 obtained in Diet 1 and 2 suggested better feed utilization and are economical for commercial tilapia culture. The lower FCR showed in Diet 1 and 2 is contributed by supplementation with limiting Lysine and Methionine that improved the feed nutritive value and fish appetite thus more of the diet was converted into flesh. Lysine and methionine are essential amino acids that cannot be synthesized in the body but obtained from protein in the diet; improving fish appetite and fillet yield (Barrows and Hardy, 2001; Furuya *et al*., 2006).

The FCR 1.61 ± 0.24 and 1.80 ± 0.45 obtained in commercial and non-supplemented diet had relatively higher FCR meaning a larger quantity of the diet and a lot of money is needed to produce a unit of flesh. The highest FCR indicated the lowest growth in Diet 4 meaning that the fish converted small amount the diet to flesh. Relatively higher FCR values obtained may be dependent on the dietary content of the feeds. Diet 3 and 4 did not constitute adequate levels of Lysine and Methionine supplement leading to the high FCR values recorded. According to Barrows and Hardy (2001) if a feed is deficient in any one of the essential amino acids, it can cause poor growth and increase feed conversion ratio even in cases of adequate protein levels.

Protein efficiency ratio is a measure of growth using the dietary protein as an index. Lysine and methionine supplementation in diet 1 and 2 improved the protein efficiency ratio (PER) and the feed conversion ratio (FCR). PER was higher in the supplemented Diets1 (2.60±0.06) and Diet 2 (2.68±0.12) and lower in non-supplemented and commercial Diets 3(2 .42±0.09) and Diet 4 (2. 32±0.16). Thus the final weight gain of the experimental fish is linked to different protein content of the tested diets.

The present study is consistent with the findings of previous researchers that reported improvement in growth performance and feed utilization with supplementation of limiting lysine and methionine in rainbow trout, cat fish, grass carp and sea bass diets. (Cheng *et al*., 2003; Mai *et al*., 2006a; Davies and Ezenwa, 2005; Yang *et al*., 2010; Salama *et al*., 2013). The growth of cultured fish showed a progressive performance and did not attain their maximum growth. The possible reason is quality of the fish seeds influenced by environment and genetic factors. Therefore good quality fingerlings results into good growth performance and it is important for fish farmers to identify reliable sources for good quality fish seeds.

5.2 Proximate Composition of Commercial and amino acid supplemented Diets

Proximate analysis in the laboratory indicated a slight increase in the amino acid supplemented Diets (32.3±0.12% and 32.1±1.21%) compared to non-supplemented Diet3 (30.3±0.14%) and commercial Diet4 (28.9±0.18%). The slight increase in crude protein level was as a result of supplementation of the diet with crystalline Lysine and Methionine hence improving the nutritive value of the diet and yielding better growth performance. Commercial Diet 4 indicated reduced crude protein content of 28.9% and this could be linked to the dietary protein content lacking the limiting essential amino acid hence inducing the least growth perfomance among the tested diets. Balarin and Halfer (1982) reported that fry of tilapia <1g require diet with 35-50% protein, 1-5g fish require diet with 30-40% protein and 5-25g fish require diet with 25-35% protein. The Moisture content did not vary significantly among the tested diets.

In the present study, the moisture content did not vary in the dietary treatments. A higher ash content was determined in commercial Diet 4 as compared to the experimental diets. This caused poor digestibility of the diet generating more fish wastes and also uneaten feed particles were found in the hapas during sampling. The amino acid supplemented diet consisted of 0.05-0.054(gkg^{-1}) crude lipid content equivalent to 5-5.4% dietary content that is consistence with the within the recommended levels. According to Lim *et al.,* (2011), the optimum dietary lipid requirement for tilapia is 5 to 12%. This indicates that higher ash content in fish diets causes poor digestibility of the feed thereby reducing growth perfomance of *O. niloticus.*

5. 2. 3 Carcass composition of *O. niloticus* fed on commercial and experimental diets

Chemical analysis of fish carcass at the end of the experiment is shown in (Table 4.11) the Dry matter, crude protein, body fat and body ash content were slightly fluctuated among all the test diets with a significant differences at $P<0.05$. The present study revealed increased crude protein levels with a low fat content in the fish carcass for the supplemented Diets 1 and 2. The non- supplemented Diet 3 and commercial Diet 4 showed a reduced crude protein level with an increased level of the lipid content. This is consistent with the findings of Cheng *et al.,* (2003) from their study on rainbow trout (*Oncorhynchus mykiss*) reported that Lysine supplementation in the plant protein-based diets reduced fat in fish body. Marcouli *et al.,* (2006) also reported that dietary lysine supplementation is related to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents. Furuya *et al.*, (2006) reported that lysine is one of the most limiting amino acids in fish nutrition, not only related to fish growth, but to increases fillet yield.

5.3 Cost effectiveness of Commercial and amino acid supplemented Diets in rearing of *O. niloticus*

The economic benefits of using the test diets lies in the fact that the cost of feed (in terms of Incidence Cost (IC) in raising *O. niloticus* to an average weight of 156g was lowest for fish fed on amino acid supplemented Diet 1 and 2.Therefore it is economical to raise fingerlings of *O. niloticus* when fed on Essential Amino Acid supplemented diets at reduced cost and high profit. The cost per kilogram of Diet 4 was higher than Diets 1, 2 and 3**.**The higher IC for fish fed on diet 4 shows higher cost of the feed with low biomass produced. Mai *et al.,* 2006b reported similar findings on supplementation of Lysine to plant protein-based diets to enhance cost-effective reduction of dietary crude protein without affecting fish growth performance. Opiyo *et al.,* (2014) reported a cost benefit analysis results for the on-farm formulated feed, to be economically viable for semi-intensive system rearing of *O. niloticus* in the earthen ponds.

Fish feeds account for the highest operational costs in aquaculture with protein being the most expensive diet. The production cost can be reduced by supplementing animal protein source with cheap and locally available plant protein sources supplemented with limiting amino acids at lower costs. Also several authors report that cheap and locally available feed ingredients are being used to replace expensive fishmeal (Tacon *et al*., 1983: Liti *et al*., 2006a: Tacon and Metian, 2008: Munguti *et al*., 2012) hence reducing production costs in aquaculture

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1Conclusion

Dietary supplementation with specific amino acids lysine and methionine are beneficial for increasing nutritional value of aquafeeds. The present study has demonstrated that:

- Essential Amino acid supplementation (EAAs) rate at 5.1 g/kg lysine and 2.7 g/kg methionine in Diet 1 and 2 indicated better feed utilization and improved growth perfomance.
- EAAs supplemented Diets 1 and 2 indicated improved nutritive value in crude protein content, Feed conversion ratio and high protein efficiency ratio than commercial and control diets were deficient of EAAs supplements
- The EAAs supplemented Diet 1 and 2 induced good flesh quality attributed by low fat content in the carcass
- Furthermore it is more economical to raise *O. niloticus* on EAAs supplemented diet that indicated better feed conversion ratio, a higher profit index compared to commercial diet that was relatively expensive but with higher feed conversion ratio and low profit index.
- On the other hand proper pond fertilization provides natural food that can improve the essential amino acid deficiency in plant based diets supply of amino acids from natural food may be an economically attractive strategy of supplementing limiting amino acids in tilapia diets.

6.2 Recommendations

Lysine and methionine are essential amino acids that cannot be synthesized in the body but obtained from protein in the diet. Therefore it is essential to balance the plant-based feed ingredients with essential amino acid supplements to meet the nutrient requirement for the targeted species. The study therefore makes the following recommendations:

- There is need for studying amino acid requirement for various fish species, fish sizes and at different stages of growth
- The EAAs supplemented diets 1 and 2 with superior growth perfomance should be adopted for trials in earthen ponds at University of Eldoret to assess its potential.
- The amino acid supplemented Diets 1 and 2 should be on mixed sex population of *O. niloticus* to determine the effect on growth perfomance and reproduction.
- Further research should incorporate EAAs supplements in *Clarias gariepinus* Diet.
- Further studies should determine whether the same effect of amino acid supplementation can be observed at high fish stocking densities
- Further studies should consider quantification of amino acids from natural food as a result of pond fertilization in a semi intensive system.

REFERENCES

Adeparusi, E. O. (2011). Water Temperature and Surface Coating Effect on Floatability, Water Absorption and Thickness Swelling of Feed. Journal of Agricultural Science 4(3), 1916-9752.

Ahmad, M. H., Abdel-Tawwab, M. & Khattab, Y. A. (2004). Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (*Oreochromis niloticus*) with different initial body weights. The sixth international symposium on tilapia in aquaculture, Manila, Philippine 249-263.

Ahmadr, M. H. (2008). Response of African Catfish, *Clarias gariepinus*, to different dietary protein and lipid levels in practical diets.World Aquaculture Society 39(4), 541–548.

Al-Hafedh Y. S. (1999). Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus.* L. Aquaculture. Research.30, (5), 385-393.

Association of Official Analytical Chemists [AOAC] (1990). Official methods of analysis of the association of official analytical chemistr*y* (15th ed., p. 17). Arlington, VA: Association of Official Analytical Chemists.

Bahnasawy, M. H. (2009). Effect of Dietary Protein Levels on Growth Performance and Body Composition of Monosex Nile Tilapia, *Oreochromis niloticus L.* Reared in Fertilized Tanks. Pakistan Journal of Nutrition 5(8), 674-678.

Balarin, J. D. & Halfer, R.D. (1982). The intensive culture of tilapia in tanks, raceways and cages. In: J. F. Muir and R.J. Roberts (Eds.). Recent Advances in aquaculture, Crom Helm, London. 265-356.

Barrows, T. F. & Hardy, W. R. (2001). Nutrition and Feeding. Fish Hatchery Management, 2nd Edition. American Fisheries Society, Bethesda, Maryland. 483-556.

Behnke, K. C. (1996). Feed manufacturing technology: Current issues and challenges. Animal Feed Science and Technology 62, 49-57.

Berardi, L. C. and Goldblatt, L. A. (1980). Gossypol. In**:** Toxic Constituents of Plant Foodstuffs (Ed. Liener I. E.). Academic Press, New York, USA. 184-238.

Bicudo, A. J. (2009). Estimating amino acid requirement of Brazilian freshwater fish from muscle amino acid profile. J. World Aquaculture. Society. 40, 818-823.

Bicudo, A. J. A., & Cyrino, J. E. P. (2014). Evaluation of methods to estimate the essential amino acids requirements of fish from the muscle amino acid profile. Latin. America. Journal of Aquaculture Research. 42(1)**,** 271-275.

Bureau, D.P. & Encarnacao, P.M. (2006). Adequately defining the Amino Acid Requirement of Fish: The Case example of Lysine. International Aqua feed. Vol.1 University of Guelph Ontario Canada. 29-54.

Cheng Z. J, Hardy R. W & Usry, J. L. (2003). Plant protein ingredients with lysine supplementation reduce dietary protein levels in rainbow trout *Oncorhynchus mykiss* diets, and reduce ammonia nitrogen and phosphorus excretion. Aquaculture 218, 553–565.

Chervinski, J. (1982). Environmental physiology of tilapias. In**:** Pullin, R. S. V. and Lowe, R. H. (eds.). The biology and culture of Tilapia, ICLARM Conference Proceedings. 119 – 128.

Cho, C. Y. (1980). Recent advances in diet formulation and nutrition of salmonid fishes: Type of fat and its quality. Progress report of Conference for Canadian Feed Manufacturers, 1980. University of Guelph, 23-27.

Craig, S. & Helfrich, L.A. (2002). Understanding Fish Nutrition, Feeds and Feeding. Cooperative Extension Service, publication. Virginia State University, USA. 420–256.

Davies, O. & Ezenwa, N. C. (2010).Groundnut Cake as Alternative Protein Source in the Diet of *Clarias Gariepinus* Fry. International Journal of Science and Nature. 1(1),73-76.

Davies, S. J. & Wareham, H. (1988). A preliminary evaluation of an industrial single cell protein in practical diets for tilapia (*Oreochromios mossambicus* Peters). Aquaculture. 73, 189–199.

EL-Haroun, E. R. (2007). Improved growth rate and feed utilization in farmed African cat fish *Clarias gariepinus* (Burchell 1822) thought a growth promoter Biogen supplementation. Journal of Fisheries and Aquaculture Science. 2, 319-327

El-Husseiny, O. M, El-Din, G., Abdul-Aziz, M., Mabroke, R. S. (2008). Effect of mixed protein schedules combined with choline and betaine on the growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Research. 39(3), 291-300.

El-Saidy, D. M. S. and Gaber, M. M. A. (2004). Use of cotton seed meal supplemented with iron for detoxication of gossypol as a total replacement of fishmeal in Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture. Research 34(3**)**, 1119-1127.

Fitzsimmons, K. (2000). Future trends of tilapia aquaculture in the Americas. World. Aquaculture Society In***:*** Tilapia Aquaculture in the Americas, Baton Rouge, LA, USA. 252-264

Food and Agriculture Organization of the United Nation (2009). FAO Fishery and Aquaculture Statistics. Aquaculture Production. 44 (49), 50

Fowler, L. G. (1990).Feather meal as a dietary protein sources during parr-smolt transformation in Fall Chinook salmon diets. Aquaculture. 89, 301 - 314.

Furuya, W.M & Sakaguti, E.S. (2006). Digestible lysine requirements of Nile tilapia juveniles. Rev. Bras. Zootec. 35, 937–942

Furuya, M. W. & Furuya, V. R. B. (2010). Nutritional innovations on amino acids supplementation in Nile tilapia diets. R. Bras. Zootec. 39, 88-94

Furuya, W. M, Pezzato, L. E, Barros, M. B, Pezzato, A. C, Furuya, V. B. R. & Miranda, E. C. (2004). Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.) Aquaculture Research. 35, 1110-1116

Furuya, W. M., Botaro, D., Macedo, R. M. G. (2005). Aplicação do conceito de proteína ideal para redução dos níveis de proteína em dietas para tilápia-do-Nilo (*Oreochromis niloticus*). Revista Brasileira de Zootecnia. 34, 1433-1441.

Furuya, W. M., Graciano, T. S, Vidal, L.V, Tadeu Xavier, O., Gongora, D. L., Righetti, J. S., & Furuya, V. R. B., (2012). Digestible lysine requirement of Nile tilapia fingerlings fed arginine-to lysine- balanced diets. R. Bras. Zootec. 41, (3), 485-490.

Furuya, W. M., Michelato, M., Salaro, A. N., da Cruz, T. P., & Furuya, R. V. B. (2015). Estimation of the dietary essential amino acid requirements of colliroja *Astyanax fasciatus* by using the ideal protein concept. Latin. America Journal of Aquaculture Research. 43(5) 888-894.

Gaylord, T. G. & Barrows, F. T. (2009). Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. Aquaculture. 287, 180-184,

Harpaz, S. (2005). L-Carnitine and its attributed function in fish culture and nutrition: a review. Aquaculture. 249, 3–21.

Jaetzold, R. & Schmidt H. (1982). Farm Management Handbook of Kenya VOL. II - Natural Conditions and Farm Management Information, 2nd Edition. Part B Central Kenya Subpart B1b, Northern Rift Valley Province. Publisher: Ministry of Agriculture, Kenya, in Cooperation with the German Agency for International Cooperation (GIZ)

Jauncey, K. (1998). Tilapia feeds and feeding. Pisces Press Ltd, Stirling, Scotland. 13-105.

Jauncey, K. & Ross, B. (1982). A guide to tilapia feeds and feeding. Institute of Aquaculture. University of Stirling, Scotland. U.K.

Kaushik, S. J. & Medale, F. (1994). Energy requirements, utilization and dietary supply to salmonids. Aquaculture. 124, 81-97.

Kellems, R. O., Church, D. C. (1998). Livestock Feeds and Feeding. 4th edition, Prentice Hall, Upper Saddle River, New Jersey, USA. 146

Ketola, (1982). Amino acid Nutrition of fishes requirements and supplementation of diet. Comp. Biophisiol, 73B (1), 17-24.

Ketola, H. G. (1983). Requirement for dietary lysine and arginine by fry of rainbow trout. An.Sci. 56, 101-107

Khattab Y. A. E, Ahmad M. H, Shalaby A. M. E, Abdel-Tawwab, M. (2000). Response of Nile tilapia (*Oreochromis niloticus* L.) from different locations to different dietary protein levels. Egypt. J. Aquat Biol. and Fish. 4(4), 295-311.

Lemme, A. (2010). Availability and effectiveness of free amino acids in aquaculture. In: Avances en Nutrición Acuícola X-Memorias del Décimo Simposio Internacional de Nutrición Acuícola. 8-10.

Li, P Mai, Trushenski, K, & Wu, J. G (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds Amino Acids Texas University College Station, USA. 37(1), 43–53 DOI 10.1007/s00726-008-0171.

Lim, C., Oksoy, M. & Klesius, P. (2011). Lipid and fatty acid requirements of tilapia. North American Journal of Aquaculture 73(2)188-193.

Liti, D.M., Kerogo, L., Munguti, J. & Chhorn, L. (2005): Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on two formulated diets and two locally available feeds in fertilized ponds. Aquaculture Research. 336, 746-752.

Liti, D. M., Waidbacher, H., Straif, M., Mbaluka, R. K, Munguti, J. M. & Kyenze, M. M. (2006a). Effects of partial and complete replacement of freshwater shrimp meal (*Caridina nilotica* Roux) with a mixture of plant protein sources on growth performance of Nile tilapia (*Oreochromis niloticus* L.) in fertilized ponds. Aquaculture Research. 336, 1535 -1543.

Liti, D. M., Mugo, R. M., Munguti, J. M & Waidbacher, H. (2006b): Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. Aquaculture Nutrition. 12, 239-245.

Liu, T., Li, H., Zhu, X., & Lemme, A. (2010). Responses of grass carp (*Ctenopharyngodon idellus*) to supplemental methionine and lysine. 14th International Symposium on Fish Nutrition and Feeding, Qingdao, China, 485.

Lovell, T. (1988). Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, 260.

Mai, K., Wan, J., Ai Q., Xu W., Liufu, Z., Zhang, L., Zhang, C. & Li, H. (2006a). Dietary methionine requirement of juvenile yellow croaker *Pseudosciaena crocea* R. Aquaculture. 251, 564–572

Mai, K., Zhang, L., Ai Q, Duan, Q., Zhang, C., Li, H., Wan, J. & Liufu, Z. (2006b). Dietary lysine requirement of juvenile sea bass (*Lateolabrax japonicas*). Aquaculture. 258, 535–542

Maina, J.G., Beames, R.M., Higgs, D., Mbugua, P.N., Iwama, G. & Kisia, S.M. (2002). Digestibility and value of same feed ingredients fed to tilapia *Oreochromis niloticus* (L). Aquaculture Research. 33,853-862.

Mambrini, M. & Kaushik, S. J. (1995). Indispensable Amino Acid Requirements of Fish: Correspondence between Quantitative Data and Amino Acid Profiles of Tissue Proteins. Journal of Applied Ichthyology. 11**,** 240-247.

Marcouli, P. A., Alexis, M. N. Andriopoulou, A. (2006). Dietary lysine requirement of juvenile gilthead sea bream *Sparus aurata* L. Aquaculture Nutrition. 12, 25-33.

Meyer, G. & Fracalossi D. M. (2005). Estimation of Jundia (*Rhamdia quelen*) dietary amino acid requirement based on muscle amino acid composition. Sci. Agr., 62(4), 401-405.

Miles, R. D & Chapman F. A, (2005). The Benefits of Fish Meal in Aquaculture Diets. Fisheries and Aquatic Sciences Department, UF/IFAS EDIS .University of Flourida website at http://edis.ifas.ufl.edu accessed on 21st July, 2016.

Mugo-Bundi, J., Oyoo-Okoth, E., Ngugi, C. C., Manguya-Lusega, D., Rasowo, J., Victoria Chepkirui-Boit, V., Opiyo, M. & Njiru, J. (2013) Utilization of Caridina nilotica (Roux) meal as a protein ingredient in feeds for Nile tilapia (*Oreochromis niloticus)* Aquaculture Research Blackwell Publishing Ltd. 1–12 doi:10.1111/are.12181.

Munguti J. M., Liti D.M, Waidbacher H, Straif, M & Zollitsch W. (2006). Proximate composition of selected potential feedstuffs for fish production in Kenya. Austrian Journal of Agriculture. Research. 57(3)131 -141.

Munguti, J. M., Charo-Karisa, H., Opiyo, M. A., M. Ogello, E. O., Marijani, E., & Nzayisenga, L., (2012). Nutritive value and availability of commonly used feed ingredients for farmed Nile Tilapia (*Oreochromis niloticus* L.) and African catfish (*Clarias gariepinus* Burchell) in Kenya, Rwanda and Tanzania. African Journal of Food Agriculture, Nutrition and Development. 12(3), 1 – 22.

Munguti, J. M., Ogello, E. O., Liti, D., Waidbacher, H., Straif, M. & Zollitsch, W. (2014). Effects of pure and crude papain on the utilization and digestibility of diets containing hydrolysed feather meal by Nile tilapia (*Oreochromis niloticus* L.). International Journal of Advanced Research. 2(6), 809 – 822.

Munguti J.M., Musa, Orina, P.S., Kyule, D.N., Opiyo, M. A., Charo-Karisa, H. & Ogello, E.O. (2014): An overview of current status of Kenyan fish feed industry and feed management practices, Challenges and Opportunities International Journal of Fisheries and Aquatic Studies. 1(6), 128-137.

Murai, T., Akiyama, T, Ogata, H., Hirasawa Y, & Nose, T. (1982). Effect of coating amino acids with casein supplemented to gelatin diet on plasma free amino acids of carp. Bull Jpn Sot Sci Fish. 48, 703–710.

National Research Council, Committee on Animal Nutrition, Board of Agriculture [NRC] (1993). Nutrient Requirement of fish. Washington D.C., National Academic Press.

National Research Council, Committee on Animal Nutrition, Board of Agriculture [NRC] (2011). Nutrient requirements of fish and shrimp. Animal Nutrition Series National Research Council of the National Academies. The National Academies Press, Washington, D.C., USA. 376.

Nunes, A, Marcelo V. C. Browdy. L. and Vazquez-Anon, M. (2014). Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture. 431, 20–27

Pandey, G., (2013). Feed formulations and Feeding Technology for Fishes. International Research Journal of Pharmacy. Moksha publishing House, 4(3) ISSN 2230-8407.

Popma, T. J. (1982). Digestibility of selected feed stuff on naturally occurring algae by Tilapia. Ph.D. Aurburn University. Aurburn Alabama.

Poston, H. A., Riis, R. C., Rumsey, G. L. & Ketola, H. G. (1977). The effect of supplemental dietary amino acids, minerals and vitamins on Salmonid fed cataractogenic diets. Cornell Veterinarian. 67(4), 472-509.

Opiyo, M.A., Githukia, C.M., Munguti, J.M., & Charo-Karisa, H.(2014). Growth performance, carcass composition and profitability of Nile tilapia (*Oreochromis niloticus* L.) fed commercial and on-farm made fish feed in earthen ponds. International Journal of Fisheries and Aquatic Science. 1(5), 12-17.

Riche M., Trottier N. L., Ku P. K. & Garling D. L. (2001). Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. Fish Physiol Biochem. 25 (3), 181-194.

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191, 1-382.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M. & Montero, D. (1997). Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. Aquaculture, 157, 347-359. Doi: 10.1016/S0044-8486(97)00174-9.

Ruscoe, I. M., Jones, C. M., Jones, P. L. & Caley, P. (2005). The effects of various binders and moisture content on pellet stability of research diets for freshwater cray fish. Aquaculture Nutrition. 11(2), 87-93.

Salama, M., Fatma, H., EL-Abed**,** Alaa, A. & El-Dahhar (2013). Effect of Amino Acids (Lysine and Methionine + Cyctine) Supplementation Rate on Growth Performance and Feed Utilization of Sea Bass (*Dentrarshus laborax*) Larvae. Arabian Aquaculture Society. 8(1**),** 1-12.

Sangsue, D., Jintasataporn, O., Triwutanon, O. & Lemme, A. (2010). Reduction of protein in diets for hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) with special focus on amino acid supplementation. 14th International Symposium on Fish Nutrition and Feeding, Qingdao, China. 388.

Santiago, C. B. & Lovell, R.T. (1988). Amino acid requirements of growth of Nile tilapia. Journal of Nutrition. 118, 1540–1546.

Strahm, B. & Plattner, R. (2001). Put the Right Tools in your Toolbox to ease Aquafeed Extrusion. Feed Management. 52(3), 19-22.

Sule, O. D. (2004). Growth Performance of Mono-Sex and Mixed Sex Population of *Oreochromis niloticus* fed similar Diet. Animal Research International. 1(2**)**, 106 – 109.

Sule, O. D., Okwundu, E. C. & Okaeme, A. N. (1996). Utilization of free flow borehole pond for fish culture in the north-east zone of Nigeria. National Institute for Freshwater Fisheries Research Annual Report. 160 – 165.

Swich, R. A. (2001). Feed based tilapia culture. ASA Technical Bulletin, AQ49-2001**.** 5-7.

Tacon, A. J. (1989) Nutricion y alimentacion de peces y camarones culivados: Manual de Capacitacion. FAO, Brasilia, Brazil, 572.

Tacon, A. G. J. (1993). Feed ingredients for warm water fish. Fishmeal and other processed feedstuffs. FAO Fisheries Circular No. 856, FAO, Rome, Italy.

Tacon, A. G. J. & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aqua feeds: Trends and future prospects. Aquaculture. 258, 146 –158.

Tasbozan, O., Ozcan, C. Erbas, E. Undag, A. Atici, A. & Adakli, E (2013). Determination of proximate and amino acid composition of five different Tilapia Species from the Cukurova Region (Turkey). J. Appl. Biol. Sci. **7,** 17-22.

University of Maryland (2006). Lysine. Edu. Maryland, 2006. Available at: <http://www.unm>. Accessed on: May 17, 2016.

Vielma, J., Koskela, J., Ruohonen, K., Jokinen, I. & Kettunen, J. (2003). Optimal diet composition for European whitefish (*Coregonus lavaretus*): carbohydrate stress and immune parameter responses. Aquaculture. 225, 3-16.

Viola, S. & Lahav, E. (1991). Effects of lysine supplementation in practical carp feeds on total protein sparing and reduction of pollution. The Israeli Journal of Aquaculture. 43, 1112-1128.

Walton, M. J., Cowey, C. B. & Adron, J. W. (1984). The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). British Journal of Nutrition. 52,115-122.

Wang, Y., Guo J. L., & Li, K. (2006). Effects of dietary protein and energy levels on growth, feed utilization and body composition of cuneate drum (*Nibea miichthioides*). Aquaculture. 252**,** 421–428.

Wang, S., Encarnacao, P. M., Payne, R. L., & Bureau, D. P. (2010). Estimating dietary lysine requirements for live weight gain and protein deposition in juvenile rainbow trout (*Oncorhynchus mykiss*). 14th International Symposium on Fish Nutrition and Feeding, Qingdao, China. 4,124.

Watanabe, W.O., Losordo, T.M., Fitzsimmons, K. & Hanley, F. (2002). Tilapia production systems in the Americas: technical advances, trends, and challenges. Reviews in Fisheries Sciences. 10(3), 465-498.

Wilson, R. P. &. Halver, J. E, (1986). Protein and amino acids requirements of fishes, Ann. Rev. Nutr. 6, 225 – 244.

Wilson, R. P. & Poe, W. E. (1985). Relationship of whole and egg essential amino acid patterns to amino acid requirement patterns in channel catfish (*Ictalurus punctatus*). Comparative Biochemistry and Physiology. 80B, 385-388.

Wilson, R. P. (2002). Amino acids and proteins. p. 143-179. **In.** Halver, J.E.; Hardy, R.W., eds. Fish Nutrition. 3ed. Academic Press, San Diego, CA, USA.

Windel, J. T., J. W. Foltz & J. A. Sarokon. (1978). Effects of body size, temperature and ration size on the digestibility of a dry pelleted diet by rainbow trout. Trans. Am. Fish. Soc.107, 613-616.

Winfree, R. A, & Stickney RR (1981) Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. Journal of Nutrition. 111, 1001–1012

Wing-keong, N. (2002). Potential of palm oil utilization in aquaculture feeds. *Asia Pacific J.* Clin. Nutr. 11, 473–476.

Wu, G. (2010) "Functional amino acids in growth, reproduction, and health," Advances in Nutrition. 1 (1), 31–37.

Yakubu A. F, Nwogu, N.A, Apochi, J.O, Olaji, E.D Adams, T.E. (2014). Economic Profitability of Nile Tilapia (*Oreochromis niloticus* Linnaeus *1757*) in Semi Flow through Culture System." Journal of Aquatic Science 2.1 1-4.

Yan. L., Moreira, A., Bordinhon, D., Davies, A., Zhang, W & Xuezhi Zhu (2012). Protein: energy ratio in practical diets for Nile tilapia *Oreochromis niloticus* Aquaculture international. 21(5), 1109-1119.

Yang, Y., Xie, S., Cui, Y., Lei, W., Zhu, X. & Yu, Y. (2004): Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp, Carassius *auratus gibelio*. Aquaculture Nutrition, 10, 289 – 294.

Zhu, X., Wenhua, J., M. & Lemme, A. (2010). Responses of juvenile Nile Tilapia (*Oreochromis niloticus*) to supplemental methionine. 14th International Symposium on Fish Nutrition and Feeding, Qingdao, China, 4, 486.