EFFECTS OF SUBSTITUTING FISH MEAL WITH SOYBEAN-BLOOD MEAL MIXTURE ON PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*)

BY

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DECLARATION AND RECOMMENDATION

Declaration by the student

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DEDICATION

I dedicate this work to my wife Angellah Nafula, our children, Clifford, Joy and Gift, my parents, Thomas Simiyu Biketi and Margaret Khasoa Simiyu for their understanding, prayers, moral and financial support**.**

ABSTRACT

There is increase in demand for fish which has accelerated fishing pressure hence dwindling supply from capture fishery resources. This has paved way for rapid growth in fish farming to bridge the gap in demand. Rapid growth in fish farming requires quality fish feeds which are expensive and protein feed ingredients are the most costly. Fish meal (*Rastrineobola argentea)* is used as the major source of protein in fish feed formulation and its price has doubled in recent years due to its scarcity. The study was carried out to determine the possibility of using soybean-blood meal mixture as alternative protein source to fish meal to produce quality feeds for Nile tilapia fish. Five treatment diets were formulated using wheat bran, maize meal, sunflower oil, mineral-vitamin premix and soybean-blood meal (1:2) mixture substituted fish meal at five levels (0, 25, 50, 75 and 100%). Three hundred healthy sex reversed Nile tilapia fingerlings of 0.7±0.1g average weight were selected from among 500 purchased, acclimatized for 14 days and distributed randomly into five groups with three replicates of twenty fingerlings per aquarium in a hatchery at Fisheries and Aquatic Sciences Department, University of Eldoret- Kenya. The five dietary treatments were fed to fish at 5% of body weight at 1000hrs, 1300hrs and 1600hrs for 14 weeks. Substitution demonstrated reduction in ether extracts and ash contents while crude protein, crude fibre and nitrogen free extracts increased ($p<0.05$). Essential amino acid chemical scores reduced ($p<0.05$) except Histidine and Leucine which increased with substitution. Fish group fed 75% level resulted in final mean weight (53.2g) followed by 50% (47.8g), 25% (44.6), 0% (40.53g) and 100% fish meal substitution diet (32.9g) in that order. Average daily gain (ADG), specific growth rate (SGR), relative growth rate (RGR) and feed conversion ratio (FCR) followed similar trend with 75% substitution recording highest ($p<0.05$) values of 0.54g/day, 1.91%, 98.7% and 1.1, respectively. Carcass moisture contents were similar in all diets apart from 25% substitution (73.91%) which had slightly higher value. There was slight increase in carcass protein content with fish meal substitution up to 75%. There was no significant effect on carcass crude protein between 50% (52.22%) and 75% substitution (54.43%) and between 0% (45.69%) and 25% substitution (47.99%) while 100% substitution (35.47%) recorded lowest amount. 25% substitution diet (17.09%), 50%(17.42%) and 75% (18.29%) were similar statistically in carcass ether extracts while 0% (15.32%) and 100% substitution (12.5%) were different statistically ($p<0.05$) to all other diets. There was no effect carcass ash content in all diets. 75% substitution diet had highest gross margin (Ksh. 253.14) followed by 50% which had (Ksh. 196.28). Gross margin for 25% substitution (Ksh. 153.33) and 100% substitution (Ksh. 149.56) were statistically similar while 0% substitution recorded the lowest gross margin (Ksh. 110.71). Based on the results, soybean-blood meal mixture can be used to substitute fish meal up to 75% in fish diets to get highest growth rate and returns from Nile tilapia.

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LIST OF ABREVIATIONS

ADG	Average Daily Gain
AFRIS	Animal Feed Resource Information System
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
APD	Apparent Protein Digestibility
BV	Biological Value
CF	Crude Fibre
CP	Crude Protein
CS	Chemical score
CRD	Completely Randomized Design
DM	Dry Matter
EAA	Essential Amino Acids
EAAI	Essential Amino Acid Index
EE	Ether Extracts
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
GC-MS	Gas Chromatograph-Mass Spectral
GoK	Government of Kenya
HPLC	High Performance Liquid Chromatography
Kcal	Kilocalorie
L	Litre
LSD	Least Significant Difference
MSOP	Manual of Standard Operating Procedures

Mg	Milligrams
NFE	Nitrogen Free Extracts
NRC	National Research Council
pH	Potential of Hydrogen ions
PRI	Protein Requirement Index
PER	Protein Efficiency Ratio
RGR	Relative Growth Rate
SGR	Specific Growth Rate
SBM	Soybean-Blood Meal mixture
SPSS	Statistical Packages for Social Sciences
SR	Survival Rate

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Fish farming is growing at a high rate compared to other segments of food production in the whole world with current production standing at 76.6 million tonnes per year representing 45% of total production (FAO, 2017). The rapid development in the sector is attributed to rise in consumption of fish and its products owing to the change in eating habit for healthy living as fish meat is reported to be cholesterol free and also the increase in human population (GoK, 2010; FAO, 2012).

It has been reported that fish farming currently provide over 45 percent of all consumed sea food and is projected to reach 75% in 20 years (Zaki *et al*., 2016). Continued increase in consumption of fish and its products together with high prices has created a big demand which has accelerated fishing pressure on the natural stock hence the declining supply of fish from capture fisheries resources (Hixson, 2014). This elevates fish farming as the only viable alternative source to satisfy increasing demand for affordable, safe and high quality fish products (Aladetohun and Sogbesan, 2013; FAO, 2014). The projected demand growth for fish and fish products presents to both small and large scale fish farmers with an expanded opportunity for market from which they can enhance their income generation, create wealth and reduce poverty (Omasaki *et al*., 2013).

In Kenya, growth in fish farming sector remained static at subsistence level since independence until when the government recognized it as one of the viable activities

for improving the country's food sector (Nyonje *et al*., 2011). The government established 48,000 fish ponds in the economic stimulus program (ESP) initiated in the year 2009 to 2012 to enhance economic recovery, alleviate poverty and stimulate regional economic development (SDF, 2016). This increased aquaculture space and further encouraged some farmers to construct their own ponds, which increased demand for commercial fish feeds and certified fingerlings for enhanced yields (Musa *et al.,* 2012).

The accelerated growth in fish farming has increased demand for supplemental commercial fish feeds to enhance yields. Fish feeds accounts for about 70% of total production cost (Ahmed, 2007). Despite increase in demand for commercial fish feeds to enhance growth in fish farming, the feeds are very expensive and this is indentified as the major setback to fish farming leading to decline in reported fish production from 24,096 metric tonnes in 2014 to 18,656 metric tonnes in 2015, 14,952 metric tonnes in 2016 and further to 12,000 metric tonnes in 2017 (SDF, 2016; KNBS, 2017; KNBS, 2018). The use of large amounts of protein mainly from the scarce and costly fish meal in compounding fish feeds elevates fish feed production cost (Agbebi *et al*., 2009). low supply of fish meal caused by increased fishing pressure hence dwindling natural stock coupled with high competition for fish as human food and for use in diets for fish, dairy, poultry and pigs indicate that further dependence on fish meal as a single major protein source in formulating fish feeds is not promising for future development of fish farming enterprise (Jacquet *et al*., 2010; Ogello *et al.,* 2014). Therefore search for suitable alternative sources of protein to substitute fish meal is necessary to sustain rapid development of fish farming (Olukayode and Emmanuel, 2012; Abou *et al.,* 2013). Soybean meal which has higher protein content compared to

other plant protein feed ingredients and is also available. Blood is regarded as a waste in slaughter houses therefore can be collected and processed into blood meal and combined with soybean meal to form alternative protein ingredient to enhance fish farming.

1.2 Statement of the Problem

Fish farming is majorly constrained by inadequate supply and costly commercial compounded fish feeds. Use of fish meal in large proportion as a major protein source in formulating fish feeds elevates cost of feeds as price of fish meal has doubled in recent years attributed to increase in demand for fish and dwindling fish stock in available capture fishery resources due to overfishing (FAO, 2013; Hixson, 2014). Feeds take the largest share of about 70% of the total expenditure in semi intensive and intensive fish culture systems (Ahmed, 2007). Due to short supply and high cost of commercial fish feeds, farmers feed fish on complete commercial poultry feeds, wheat bran, rice bran or mixture of the ingredients which do not supply adequate nutrients required by fish to support optimum growth (Munguti *et al*., 2014). This has led to slow growth rate and yields as fish take longer period of time to reach market size and consequently lead to food insecurity and loss of income to farmers. Soybean-blood meal mixture have higher protein content compared to fish meal and other protein ingredients therefore can be utilized as alternative protein source to substitute fish meal in formulating affordable feeds for semi intensive and intensive Nile tilapia culture systems.

1.3 Justification of the Study

Formulation of affordable alternative fish feed using soybean-blood meal mixture as alternative protein ingredient to costly fish meal (*Rastrineobola argentea*) to enhance growth and productivity. Protein is the most expensive fraction of fish feeds accounting for about half of the total production cost and fish meal is usually used in large proportions as the main source of protein in making fish feeds (Agbebi *et al*., 2009). The price of fish meal has doubled in recent years hence its use elevates fish feeds cost and renders commercial compound fish feeds unaffordable to majority of fish farmers (FAO, 2013). Sadiku and Jauncey (1995) recommended mixing of plant protein and animal origin protein feed ingredients to enhance quality of compounded fish feeds. However there is little documentation on the same. The study helped fill the gap by focusing on locally processed blood meal as an animal protein in combination with soybean meal which is a by-product from crop oil extraction industries to form affordable alternative source of protein to replace scarce and costly fish meal to enhance income to fish farmers and also spare fish for human consumption rather than for fish.

1.4 Objectives

1.4.1 Main objective

To formulate affordable quality feeds in fish industry by use of soybean-blood meal mixture as a substitute for fish meal in Nile tilapia diets.

1.4.2 Specific objectives

i) To evaluate effects of substituting fish meal with soybean-blood meal mixture on nutrient composition of Nile tilapia diets

ii) To determine the effect of substituting fish meal with soybean-blood meal mixture on growth performance of Nile tilapia

iii) To determine effects of fish meal substitution with soybean-blood meal mixture on Nile tilapia fish carcass quality.

iv) To analyse gross margin of using soybean-blood meal mixture to substitute fish meal in formulated Nile tilapia diets.

1.5 Hypotheses

i) **H_0**: Fish meal substitution with soybean-blood meal mixture has no significant effects on nutrient composition of formulated Nile tilapia diets

H_a: Fish meal substitution with soybean-blood meal mixture has significant effects on nutrient composition of formulated Nile tilapia diets.

ii) **H_0** Substituting fish meal with soybean-blood meal mixture has no effect on growth performance of Nile tilapia

H_a: Substituting fish meal with soybean-blood meal mixture has effect on growth performance of Nile tilapia

iii) **H_0**: Substituting fish meal with soybean-blood meal mixture in formulated Nile tilapia diets has no significant effect on carcass quality.

H_a: Substituting fish meal with soybean-blood meal mixture in formulated Nile tilapia diets has significant effect on carcass quality

iv) **H_0**: Substituting fish meal with soybean-blood meal mixture in formulated Nile tilapia diets has no effect on gross margin

H_a: Substituting fish meal with soybean-blood meal mixture in formulated Nile tilapia diets has significant effect on gross margin

CHAPTER TWO

LITERATURE REVIEW

2.1 An Overview of Fish Farming

Fish farming refers to raising fish in a controlled environment which include earthen ponds, lined ponds, cages and race ways with the aim of enhancing growth and productivity. It involves breeding, stocking, feeding and water quality management (FAO, 2013). Fish farming is reported to be the fastest growing food production sector the world over at 9.7 percent annual growth with current production standing at 76 million tonnes accounting 45% of total fish production (FAO, 2017).

Asia contributed 92% of the total fish farming production in 2010, while Europe, America and Africa combined accounted for 8%. Fish farming accounted 15% of global total fisheries production in 1988 but tripled to 45% by 2012 almost comparable to capture fisheries production (FAO, 2012). This indicates a declining trend in capture fisheries production and a tremendous increase in fish farming production. The declining supply from capture fisheries resources is a result of accelerated fishing pressure occasioned by increase in demand for fish and fish products (Hixson, 2014).

2.2 Fish Farming in Kenya

Fish farming in Kenya was initiated by the colonialists and since independence in 1963; there has been static growth until recently when the government recognised it as one of the viable activities in Economic Stimulus Programme to promote food security and rural development (Aloo, 2010). In the programme, the government established a total of 48,000 fish ponds, supplied certified fingerlings and commercial fish feeds (Nyonje *et al*., 2011). Many farmers were encouraged to construct their own ponds as a ripple effect of the programme (Munguti *et al*., 2014). There was an increase in reported aquaculture production from 4,895 metric tonnes in 2009 to 12,000 metric tonnes in the year 2010, 21,487 metric tonnes in 2012, 23,501 metric tonnes in 2013 and 24,096 in 2014 (Munguti *et al.*, 2014; KNBS, 2017). When the programme ended in 2013, farmers were left to sustain fish farming but due to high cost and unavailability of quality fish feeds, they feed fish on cereal bran and commercial feeds for terrestrial animals which do not meet nutrient requirement hence declining production from 24,096 MT in 2014 to 12,000 MT in 2017 (SDF, 2016; KNBS, 2018).

Most parts of Kenya are suitable for fish farming but only 0.014% out of 1.4 million hectares of potential fish farming space is utilized for fish farming (Otieno, 2011). Small scale fish farmers account for 95% of fish farming in the country. According to Mbugua (2008), tilapia is the most preferred species of fish in Kenya accounting for 90% of the total farmed fish production. Munguti *et al.* (2014) reported that Nile tilapia (*Oreochromis niloticus*) forms 75% of total farmed fish production, followed by Cat fish (*Clarias gariepinus*) at 18%, Common carp at 6% (*Cyprinus carpio*) and

Oncorhynchus mykiss (Rainbow trout) at 1% in that order. Other farmed fish in very low numbers are ornamental fish, Koi carp and Gold fish.

2.3 Tilapia Fish Farming

Tilapia farming is recorded as an old fish farming practice reported more than 3000 years ago in Africa (Popma and Michael, 1999). It has been farmed in many developing countries at subsistence level in many communities in rural areas mainly to provide protein which is most deficient nutrient (Liti *et al*., 2005; Sumi *et al*., 2014). Cichlidae family is composed of genera, *Sarotherodon, Tilapia* and *Oreochromis* which are given the name tilapia (Santiago and Laron, 2002). Tilapia culture involves the following farmed species; *Orechromis niloticus, O. spirulus, O. aureus, O. mossambica, Sarotherodon mossambicus, Tilapia rendalli and T. zilli.* Tilapia farming has increased tremendously at 12% annual growth rate and is reported as the third most cultured fish group in the world after Salmonids and Carps (Ogello *et al*., 2014).

According to Tacon (1993), tilapia farming in developing countries is practiced majorly in semi intensive fertilized earthen ponds to produce natural food and supplementary feeding to enhance yields. In Kenya, tilapia production forms 90% of the total farmed fish production owing to ease in management, high growth rate, ability to feed on different varieties of feeds, disease resistance and adaptation to tropical and semi tropical climate (Pullin, 1988; Osama *et al*., 2008). Munguti *et al*., 2014) reported that Nile tilapia forms 75% of the total farmed fish species. Nyonje *et al*. (2018) found the same trend in fingerlings production in hatcheries in the country. Fish farmers' preference for Nile tilapia is attributed to its tolerance to a wider margin

of conditions such as; pH, temperature, dissolved oxygen, ammonia levels and its easiness of management (Noor *et al.*, 2010).

2.4 Environmental Requirements for Nile Tilapia

Nile tilapia is less tolerant to salinity than other species of tilapia which grow and reproduce at concentration of 36 parts per thousands (ppt). Optimum growth performance is recorded at salinity concentration of up to 19ppt (El-Sayed, 2006). Salinity tolerance is influenced by the size of the fish rather than age, although resistance to abrupt changes in salinity can be influenced by age (Wetanabe *et al*., 1985; Wetanabe, 1990). Nile tilapia are said to grow well at salinity concentration of up to 15ppt. Unionized ammonia at high concentration is toxic to tilapia and other aquatic animals (Redner and Stickney, 1979; Harris *et al*., 1998) and levels as low as 0.1 mg/L concentration causes reduced feed intake and growth (El-Sherif and El-Feky, 2008). Nile tilapia grows very well at a concentration below 0.05 mg L^{-1} (El-Sherif and El-Feky, 2008).

Unionized ammonia readily dissolves in liquid and diffuses through the cell membrane hence causing damage to cells of the gills, liver, kidney, spleen, brain cells and thyroid tissues thus interfering with energy metabolism in fish and other aquatic animals (Smart, 1978). Ip *et al.* (2001) reported hyper excitability, gill hyperplasia, convulsions and death of fish on chronic exposure to ammonia. Ammonia among other end products of protein metabolism, bicarbonates and carbon dioxide is excreted out of the body of fish without the need to synthesize urea or uric acid (NRC, 1993). This implies that fish provided with excess protein in their diets, excrete high amount of ammonia into water and together with ammonia from decomposed unutilized fish

feeds, makes the environment not conducive for optimum fish growth. Tilapia can tolerate a wider range of pH of 3.7 to 11 compared to other species and high growth rate is realized at pH range from 7 to 9 (Ross, 2000). The pH is influenced by the concentration of ammonia, nitrates and nitrites resulting from ammonia oxidation, bicarbonates and carbon dioxide which are linked to nutrition. Nutrition is therefore regarded as a major determinant to enhanced fish production efficiency (Abowei and Ekobo, 2011). Temperature modifies biochemical reactions in the body of fish hence affects reproduction, survival, susceptibility to diseases and growth rate. Nile tilapia is reported to survive at water temperatures from 11 ^{0}C to 42 ^{0}C and optimum growth is attained at 28 ^{0}C to 36 ^{0}C and decreases with decline in temperature (Culton, 1982; Mires, 1995; Teichert *et al*., 1997; FAO, 2012). Dissolved oxygen is another metabolic modifier in fish body as it fuels all biochemical reaction. Tilapia tolerates as low as 0.1mg/L dissolved oxygen (Magid and Babiker, 1975) but optimum growth is achieved at a concentration greater than 3mg/L (Ross, 2000; Boyd, 2004).

2.5 Digestive System of Nile Tilapia

Nutrients are found in feeds which animals ingest and are vital for the growth and development of living beings. Ingested feed passes through digestive tract which is inform of a canal and as it passes down, digestive secretions that contain enzymes are mixed with it to cause its break down to release simple nutrients which are absorbed and metabolized in the body to support growth and development functions in fish. An understanding of fish digestive tract is therefore useful for nutritional studies by researchers, for improvement of fish nutrition and handling of feeding (Banan, 2012).

Smith *et al.* (2000) studied digestive tract of Nile tilapia fish and sub divided it into four distinct topographical regions; the head gut, fore gut, mid gut and hind gut. The head is composed of the mouth and its function is to acquire food and mechanically break it for ingestion. The fore gut is composed of the oesophagus in which the ingested feed passes to the stomach where enzymatic digestion begins. The mid gut is composed of the intestines which accounts for greater proportion of gut length. Enzymatic digestion of feeds is completed and absorption of nutrients occurs mainly in this region (Banan, 2012). The hind gut composed of the rectum and anus forms the final section. Nile tilapia species of fish have a long digestive tract with total intestinal length of 2.5 times its body length reported by Smith *et al.* (2000). They also found out in the study that the intestine is followed by extensive looping and coiling (**Plate2.1**).

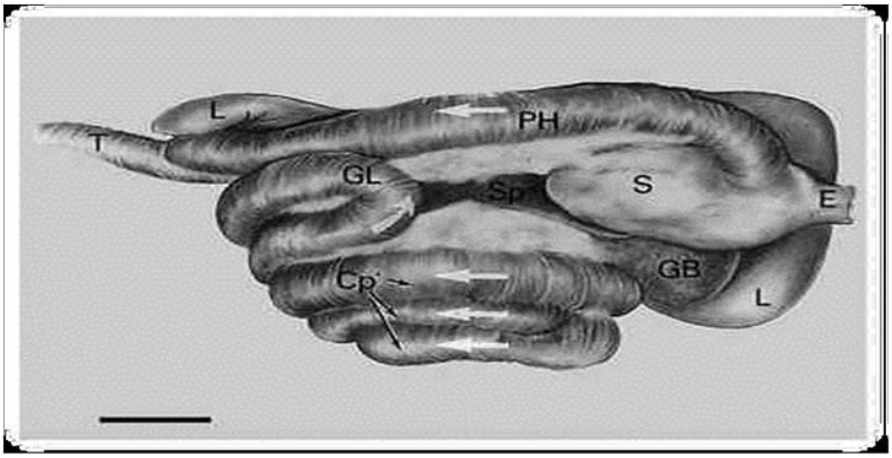

Plate 2.1: Right view of digestive system of tilapia fish. The esophagus (E), Proximal hepatic loop (PH) exit from the stomach (S), centripetal limb of proximal major coil (Cp), terminal section (TS), liver (L), spleen (SP) and gall bladder (GB) (Source smith *et al.*, 2000)

The longer intestinal length which is followed by extensive looping and coiling definitely slows flow rate of the ingested food in order to complete the turns to allow maximum enzymatic digestion and also increases surface area for absorption of

nutrients (Buddington and Diamond, 1986). Intestinal length is variable in different fish species and is strongly correlated with feeding habits. Herbivorous fishes have longer intestines than carnivorous fishes (Genten *et al*., 2009). This was also confirmed by Gilles *et al.* (2008) when they reported that tilapia changes from feeding zooplankton when juvenile to feeding phytoplankton when adults. Longer intestinal length is reported to be related to utilization of diets with high plant feed material (Reinthal, 1989). Based on the digestive tract described in the studies, Nile tilapia fish have relatively high capacity to digest plant material. This is promising for use of plant feed ingredients such as soybean meal, wheat bran and maize meal in relatively high amounts to produce cost effective compound fish feeds.

Moreira *et al*. (2012) studied effect of supplementing natural feeds with commercial compound complete feeds and reported that Nile tilapia fed on natural feeds alone take long time to reach market size. Therefore use of supplemental commercial fish feeds is necessary to enhance growth and consequently increase fish yields.

2.6 Nutrient Requirements of Tilapia Fish

Fish nutrition is an important aspect in production of economically sound and high quality fish products. As fish farming industry expands, demand for species specific compound fish feeds rises to supply adequate nutrients for optimum growth and sound health (Craig and Helfrich, 2007; MSOP, 2015). Nutrients are needed in adequate amounts to support body functions such as growth, development and reproduction (Tacon, 2000). Owing to the establishment of chemical basis of nutrition in 19th Century, the science of nutrition has developed dramatically over the years (Devendra, 1988).

2.6.1 Protein and amino acid requirements

Protein are most critical nutrients in fish as they form large part of fish biomass and are used in synthesis of functional substances such as enzymes. Protein is the most expensive fraction of compound fish feeds and is a major constraint to growth (Reay, 1979; Wilson, 2002). The quality of protein feed ingredients is determined by amino acid profile and their bioavailability for synthesis of body proteins and consequently influence productivity of farmed fish. Ten amino acids out of about 22 that form complex protein cannot be synthesized at all or are in inadequate amounts by fish to support optimum growth and development; therefore they should be supplied in the diets (Wilson *et al*., 1989; Anderson and Lall, 2005). These essential amino acids (EAA) are; Methionine, Tryptophan, Lysine, Valine, Histidine, Isoleucine, Phenylalanine, Leucine, Arginine and Threonine. According to El-Sayed (2006), young tilapia fish require 35% to 40% protein in their diets while adults require 20% to 30% protein for optimum growth and development. Jouncey (1998) recommended 40% to 45% protein for fry, 30% to 35% protein for fingerlings and 25% to 30%protein for grower and adults. Fish meal is the preferred source of protein in compounding fish feeds because of its good amino acid profile, palatability and highly digested by fish (Popma, 1982; Nguyen *et al*., 2009; NRC, 2011). Short supply and high price of fish meal leads to formulating of expensive fish feeds. The major focus has been on the use of plant and animal by-products feed ingredients as alternative protein sources to reduce feed costs (Emani, 2011). The study focused on the possibility of using soybean meal in combination with blood meal to substitute fish meal (*Ratrineobola argentea*).

Table 2.1: Essential amino acid (EAA) requirement for Nile tilapia (*O. niloticus*)

Amino acid	Requirement as a % of total ration	Requirement as a % of dietary protein
Lysine	1.43	5.12
Argenine	1.18	4.2
Histidine	0.48	1.72
Threonine	1.05	3.75
Valine	0.78	2.8
Leucine	0.95	3.39
Isoleucine	0.87	3.11
Phenylalanine + tyrosine	1.05	3.75
Tryptophan	0.28	1
Methionine + cystine	0.75	2.68

Source: NRC, 1993

2.6.2 Alternative protein feed ingredients

Soybean meal

Soybean meal (SBM) is considered as the most nutritious of all plant protein feed ingredients (Lovel, 1988). It has high protein content, good amino acid profile close to fish meal, thus is commonly used in compounding fish feeds (Storebakken *et al*., 2000). Methionine which is the limiting amino acid is deficient in soybean meal, it should be added to fish diets to enhance growth. Studies have indicated that supplementation of deficient EAA in soybean meal diets did not reveal any improvement in growth performance (Teshima and Kanasawa, 1988). Violar and Zohar, 1984) reported no improvement in Nile tilapia growth when deficient EAA was added to the diet. Growth performance of tilapia hybrid (*O. aureus x O. niloticus*) fed on 100% soybean meal diet supplemented with Di-calcium phosphate, limiting amino acid lysine and methionine and was reported to be similar to those fed on 100% fish meal diets (Violar *et al*., 1988). They also reported no effect on growth performance of tilapia when soybean meal diet with 3% Di-calcium phosphate

without including deficient EAA totally replaced fish meal. This indicates that minerals improve soybean meal utilization rather than deficient EAA.

Soybean meal contain anti-nutritional factors such as; trypsin inhibitor, haemagglutinin or lectin and oligoscharides that affect nutrient digestibility and absorption (Dersjant-li, 2002). Trypsin inhibitor is a globular protein that form complexes with enzyme trypsin and reduce its action in protein digestion (Lianar and Kakade, 1980). Trypsin inhibitor is thermo labile hence is deactivated during heat processing and also during pellet making process (Tacon and Jackson, 1985; Tacon, 1993). Phytohaemagglutinin causes agglutination of erythrocytes in animals (Jaffe, 1980). It is destroyed by pepsin enzyme in stomach and therefore it is not a challenge for fish with stomach like tilapia (Mickelsen and Yung, 1966).

Mothwa *et al*. (2013) studied protein digestibility of soybean meal; they found out that Apparent Protein Digestibility (APD) in *Tilapia rendalli* was 96%. With high APD, soybean meal can effectively be utilized in tilapia fish diets. The quality of soybean meal depends on processing during oil extraction but crude protein ranges from 31.2% to 50%. De-hulled solvent oil extraction increases protein content to 50%. Un de-hulled solvent extracted soybean meal has less protein content of around 44% while steam cooked soybean seed have crude protein of 31.2 (NRC, 1993). Fractionation processing and refining of soybean meal removes most of anti-nutritive factors and improves nutrient utilization (Caruso, 2015). Xue *et al.* (2010) conducted 8 weeks growth trials to evaluate the effects of fish meal substitution with Soybean Protein Concentrate (SPC) which is a product of soybean meal fractionation. They reported 100% fish meal substitution with SPC in Nile tilapia fry without significant

effects. Jahan *et al.* (2012) reported 50% fish meal substitution with soybean meal in diets of Cyprinidae, *Labeo rohita* fry without amino acid supplementation. Fagbenro and Davies (2000) recorded 67% fish meal (menhaden) replacement with soybean meal in tilapia diet without including deficient amino acid. Soybean meal is also reported to be a source of linoleic fatty acid 18:2 n-6 which enhances growth in fish (Takeuchi *et al.*, 1983a).

Blood meal

Blood meal is a by-product in meat processing industry obtained during animal slaughter process and blood can be collected in slaughter houses in large amounts and processed into blood meal. Liu (2009) reported that average amount of blood harvested as a percentage of live body weight from cattle, sheep and pigs is 3%-4%, 3.5%-4% and 3%-4%, respectively. Blood meal is rich in lysine containing up to 8% (El-Haroun and Bureu, 2007) with high protein content of 89.2% (NRC, 1993) and Kirimi *et al.* (2016a) recorded 80.4% CP. Kirimi *et al.* (2016a) also found out that blood meal had high amount of isoleucine, phenylalanine and tyrosine while low amount of arginine, alanine and leucine when they treated blood at 100^0C for 45 minutes during processing. Different methods are used in processing blood into blood meal. These are; spray drying, steam tube dried, solar drying, drum drying and oven drying (Bureu *et al*., 1999). These methods of blood meal drying have effect on its nutritive value and digestibility. High amount of heat applied in drying blood meal denature protein and reduce their bioavailability (Batterham *et al*., 1986). Spray dried blood meal has highest nutritional value as least heat is applied in preparation process (Luzier *et al.*, 1995).

Blood meal protein is highly digestible especially for spray dried blood meal and it is a source of EAA, Leucine, Lysine, Phenylalanine and Tryptophan but low levels of methionine and isoleucine (Tacon, 1987; Gaylord *et al.*, 2004). The AFRIS (2010) recommends boiling of fresh blood at 100 ^{0}C for 15 minutes and sun drying or oven drying to produce good quality blood meal. Hussain *et al.* (2011) reported high dry matter digestibility of blood meal compared to fish meal in *Labeo rohita* fingerlings. They recorded APD of blood meal to be slightly lower than that of fish meal at 72% and 80% respectively in *Labeo rohita* (rohu) fingerling. Apparent crude protein digestibility of blood meal was 80% for rain bow trout (El-Haroun and Breau, 2007). Studies indicate various results as Kirimi *et al*. (2016b) recorded 50% of fish meal substitution with blood meal in Nile tilapia formulated diets and same level of blood meal inclusion was reported by Nogueira *et al.* (2012) in diets for sea bream. Another study by Davies *et al.* (1989) found that 75% of fish meal was substituted with blood meal effectively in formulated diets of Mozambique tilapia fry. Blood meal was reported to have completely replaced fish meal in formulated diets for Nile tilapia fish with no effect on utilization of feeds and growth performance by Agbebi *et al.* (2009). Blood meal should be properly processed to destroy disease pathogens and parasites which may pose a challenge to fish consumers (Munguti, 2012; MSOP, 2015).

Since Kenya is a signatory to the World Trade Organization (WTO) agreements, Manual of Standard Operating Procedures (MSOP) for fish inspection and quality assurance in Kenya, was developed to put in place standards which are in line with East African Communities (EAC) common markets establishment Article 45 (3) which calls for application of standards, sanitary measures and regulations within European Union (EU) to promote food safety, security and trade (MSOP, 2015). It

was indicated that mixing plant protein feed ingredients with animal protein feed ingredients enhances quality of complete fish feeds (Sadiku and Jauncey, 1995). However little documentation is available on the same as many studies have focused on single protein ingredients of either plant origin or animal origin to substitute fish meal. In the study soybean meal plant protein feed ingredient was mixed with blood meal to form a mixture to replace fish meal.

2.6.3 Energy requirements of Nile tilapia

Energy is not a nutrient *per se*, fish metabolically oxidize lipids, carbohydrates and protein in absence of carbohydrates to harness energy required to propel all biochemical reactions just like in other animals (NRC, 1993). Tilapia digests most of these nutrients for energy production in feed ingredients efficiently (Kubaryk, 1980). They can relatively utilize high amounts of carbohydrates up to 30% to 40% than most farmed fish but digestibility reduces as percentage increases (Anderson *et al*., 1984; Teshima *et al*., 1985). Tilapia digests lipids and protein more than carbohydrates. Fish requirement for carbohydrate has not been established but its inclusion in fish diets as a source of energy minimizes catabolism of protein to energy (Iqbal *et al*., 2006; Ali *et al*., 2006). Carbohydrates feed stuffs are mainly grains and their milling by-products and are available in large quantities at low prices to be used in fish diets (Tacon, 1993). According to Dupree and Huner (1984), carbohydrates can be utilized as precursors for non essential amino acid and fatty acid metabolism by fish and for fish feed pellet making process as a binder.

Lipids (fats and oil) are important concentrated form of energy and provide essential fatty acids for fish growth and development (Sergent *et al*., 1995). Lipids are also

important carriers of fat soluble vitamins; A, E, D and K. Amount of fat in tilapia fish diets for optimum growth should range from 8% to 12% for young fish and from 6% to 8% for adult fish (Jauncey, 2000). Suresh (2003) recommended lipids concentration in diets for Nile tilapia to range from 5 to 12%. High levels of lipids in excess of 12% in fish diets for young *Oreochromis aureus* and *O. niloticus* hybrid depress growth (Jauncey and Ross, 1982). Dietary lipids should provide adequate amount of between 0.5% to 1% linoleic fatty acids required by tilapia fish (Teshima *et al*., 1982; Takeuchi *et al*., 1983b).

Soybean meal is rich in 18:2 n-6 linoleic fatty acid and Takeuchi *et al.* (1983a) reported that supplementation of soybean oil in tilapia diets recorded better performance than diets containing fish oil which are high in 20:5 n-3 fatty acids. Therefore the use of soybean-blood meal mixture to formulate of diets for Nile tilapia fish not only offer alternative source of protein but also provide linoleic fatty acid for rapid growth and development.

2.6.4 Protein: energy ratio for Nile tilapia

The ratio of protein in relation to energy should be properly balanced for optimum utilization of protein for growth of fish rather than being metabolized for energy production (Wang *et al.*, 2006; Ahmad, 2008; Schulz *et al*., 2008). Energy deficiency in relation to protein level in diets will result to excessive metabolic oxidation of protein to energy for maintenance and growth. While diets excess in energy in relation to protein will lead to reduced feed intake, utilization of other nutrients for optimum growth is inhibited and excessive deposition of fat in fish body (Winfree and Stickney, 1981). Yan li *et al*. (2012) reported an increase in body weight gain in Nile

tilapia fish with dietary protein to energy ratio up to 25/2800(25% protein and 2800 Kcal/Kg energy). They found no statistical difference in weight gain and body composition between 25% protein to 2800 Kcal/Kg energy and 30% protein to 2800 Kcal/Kg energy ratios. Nile tilapia efficiently digests 70% of non cooked carbohydrates as a source of energy (Popma, 1982). Cho and Slinger, (1979) reported that cooking improved digestibility of starch in fish by 75% higher Digestible Energy (DE) in rainbow trout and 38% higher than uncooked in channel catfish reported by Wilson and Poe, (1985). However, despite efficient digestion of carbohydrates by fish, protein and lipids are highly utilized as energy sources for fish (Smith, 1976). According to studies, the ratio of protein in relation to energy for optimum growth and weight gain should range from 81 mg $Kcal^{-1}$ to 117 mg $Kcal^{-1}$ (NRC, 1993).

2.6.5 Vitamin requirements

No vitamin requirement for tilapia fish has been established and little information on the same is available. This is because most of farmed tilapia fish are raised in semi intensive and extensive earthen ponds where they eat adequate amount of planktons that satisfy body requirements (Robinson *et al*., 1998). Tilapia when reared in culture units where there is no available natural food to feed on show signs of vitamin deficiency which is a clear indication that vitamins are important in fish (Robert, 1978). However, for efficient metabolic functions vitamins must be included in commercial feeds for fish in intensive culture units where there is no or limited planktons. Inadequate amounts of vitamins in diets results in retarded fish growth and less resistance to disease (Robert, 1978).

2.6.6 Mineral requirements

This is a group of nutrients that also have no established requirement for fish since water dissolved minerals diffuse through the gills into the body systems of fish. However, fish show classical requirement for phosphorus because it is found in water in little amount to satisfy body requirement for optimum growth (Wetanabe *et al*., 1980; Gatlin, 2010). Fish require minerals for osmoregulation, tissue development and other body functions (Lall, 2002).

2.7 Effects of Diets on Fish Carcass Proximate Composition

Nutrition is the major factor that influences the quality of fish meat (Baltic *et al*., 2011; Noor *et al*., 2011). Body nutrient retention is dependent on quality and quantity of fish feeds thus influencing carcass composition (Khalid *et al*., 2014). Nutrients present in feeds are broken down in digestion process into simple nutrients which are absorbed and utilized for various body functions or assimilated in the body and consequently influence meat composition. High dietary fiber in feeds for instance interferes with nutrient digestibility and assimilation. Tacon *et al.* (2009) reported high digestibility and assimilation of fish meal than sunflower meal that has high fibre levels. Kissil *et al.* (2000) reported reduction in fish body fat content when they fed fish on plant protein feed ingredients. Rehman *et al.* (2013) recorded reduced dry matter, crude fat and ash while elevated levels of crude protein deposition in *Labeo rohita* (rohu) fed on soybean meal relative to fish meal diets. Protein and fat in fish meat is related to differences in protein feed ingredients and lipid metabolism (Francesco *et al.*, 2007).

Nutrition can be used to influence nutrient levels such as fat content, glycogen, protein, vitamins and minerals that have an impact on texture, flavour and colour to produce nutritious and acceptable meat product to consumers (Rasmessen, 2001; Baltic *et al.*, 2011). The main concern in fish farming industry has been formulation and production of low cost fish feeds to widen profit margin. However, this should be in tandem with production of sufficient quantities of fish meat of high nutritive value for human population as for any product to be sold it must be appealing and acceptable to the consumer. This study sought to determine the effects of soybean-blood meal mixture to substitute fish meal (*Rastrineobola argentea*) at five levels on carcass quality of Nile tilapia fish.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The experiment was conducted at Fisheries and Aquatic Sciences Department Hatchery Unit at the University of Eldoret situated approximately 9 Kilometers along the Eldoret – Ziwa road in Eldoret – Kenya, altitude 2180 m above sea level, Latitude 0^0 57' 02N' and Longitude 35^0 31'42E' with average temperature of 23 ^{0}C, Rainfall 900-1100mm per year and humidity range from 45% to 55% (Jaetzold and Schmidt, 2008). The area is in Uasin Gishu County where main agricultural activities are wheat crop, maize crop, dairy cattle and fish farming.

3.2 Experimental Design

The five experimental diets were formulated and fed to the five groups of fingerlings in 3 replicates under completely randomized design (CRD). Fifteen aquaria (fish holding units) measuring 60 x 40 x 40cm were cleaned, disinfected using potassium permanganate at 5 ppt, dried in the sun and installed on a wooden stand in the fish hatchery (**Plate 3.1**). Each aquarium was filled with clean water, fitted to aerator (8 nozzles, CM 320 W) through air stone and plastic tubule and thermostat (AC-W23Y) set at 28 ^{0}C. Dissolved oxygen (DO) and temperature were measured using digital DO and temperature meter (YSI 550A USA) and pH digital pH meter (pH Tester 20) twice a week throughout the experimental period. Five hundred (500) healthy sex-reversed (males) Nile tilapia fingerlings were purchased from Jewlet Farm in Homa Bay County and transported in oxygenated polythene bags to study site where they were acclimatized for fourteen days in a holding tank before being weighed and

transferred to the fifteen aquaria. Three hundred fingerlings of 0.7±0.1g weight were selected and then distributed randomly into five groups with twenty fingerlings put into each aquarium.

Plate 3.1: Photograph of the experimental set up in the hatchery (Source: Author, 2018)

3.3 Experimental Diets

3.3.1 Sources and processing of feed ingredients

Fresh blood drained from slaughtered cattle was collected in clean containers from a local slaughter house near Chwele Fish Farm. It was collected daily for two consecutive days and transported to the Fish Farm where it was boiled immediately in a container while being stirred at 100 ^{0}C for 15 minutes to get rid of pathogens (AFRIS, 2010; MSOP, 2015). The liquid part of blood was then drained and clots crashed manually into small pieces to increase surface area for drying in the sun. The small pieces of blood lumps were dried in the sun on a polythene liner for two days to reduce moisture content to below 12%. The dried blood lumps were milled into fine powder called blood meal using a hammer mill. Other feed ingredients that were used

in experimental diet formulation were soybean meal, maize meal, wheat bran and mineral vitamin premix and sunflower oil were purchased from animal feed shop in Bungoma town and transported to Chwele Fish Farm.

Sun dried *Rastrineobola argentea*, locally known as "Omena," was purchased from a fish trader at Chwele Market and transported to the farm. All feed ingredients in dry form were ground separately into particle size of approximately 250µm in a hammer mill (**Plate 3.2**) before subjecting samples from each to proximate and amino acid analysis.

Plate 3.2: Photograph of hammer mill for grinding feed ingredients (Source: Author, 2018)

3.3.2 Proximate and amino acid analysis

Samples from blood meal, soybean meal, fish meal (*Rastrineobola argentea)*, wheat bran and maize grain were taken for laboratory analysis at Kenya Industrial Research and Development Institute (KIRDI), Nairobi Station and Fletcher Scientific Solutions Lab for proximate and amino acid profile analysis before formulation of experimental diets. Proximate analysis of Dry Matter (DM), Crude Fibre (CF), Crude Protein (CP),

Ether Extracts (EE) and Ash were done using AOAC (1995) procedures while amino acids were analyzed using High Performance Liquid Chromatography (HPLC) modified AOAC (2005) procedure.

Crude Protein content in each of the feed ingredient was determined using Kjeldahl method in AOAC (1995) procedure in triplicate for accuracy. 0.5 g from each sample of dry milled ingredients was put in a micro-Kjeldahl tube in triplicate. Half Kjeldahl tablet and 15ml of concentrated sulphuric acid (H_2SO_4) was added into the tubes. The tubes were mounted on a digestion chamber and heated for one hour to break down organic matter in the feed sample until the content is clear to bluish green in colour. The solution was left to cool for a period of one hour before reacting it with 40% sodium hydroxide (NaOH) in a distillation chamber. The ammonia liberated in the process was steam distilled, condensed and absorbed into 4% boric acid solution and five drops of methyl red indicator was added in flat bottomed conical flask containing boric acid. The reaction of ammonia and boric acid formed ammonium borate (green in color). Ammonium borate solution in the flat bottomed conical flask was titrated with 0.1N hydrochloric acid until change of colour. Crude protein (CP) was calculated using nitrogen to protein factor of 6.25 as follows.

$$\%\text{CP} = \frac{\text{T x NHCL x 14 x 6.25 x 100}}{\text{1000 x weight of sample}}$$

where,

T = ml of HCL used in titration

NHCL = Normality of HCL used for titration (0.1N)

14- = Molecular weight of nitrogen

6.25 = a factor for conversion of nitrogen content into crude protein content based on assumption that almost all protein contain 16% nitrogen.

Crude fibre (CF): 2 grams of dried milled feed ingredients sample was weighed and put in 500ml glass beaker, 25ml of hot water was added together with 2N sulphuric acid and topped up to 200ml mark. The content was boiled on a heating plate for 30 minutes and filtered in a filter stick packed with glass wool with the help of a vacuum pump and washed three times with hot water. 100ml of hot water was added to filtered content before adding 25 ml of 1.78N KOH (Potassium hydroxide), topped up to a 200ml mark with hot water and again boiled for 30 minutes. The contents were filtered as explained above and washed three times. The residue together with glass wool was transferred into crucible and washed with acetone. The content in the crucible was oven dried for 2hours and placed in a desiccator to cool to avoid absorption of moisture from the atmosphere. After cooling the sample and crucible weight was taken before being incinerated in a muffle furnace at 550 ^{0}C for four hours. The ash and the crucible were placed in a desiccator to cool and weighed for crude fibre calculation as follows.

$$\%\mathbf{CF} = \frac{(\text{weight of crucible + sample residue}) - (\text{crucible weight + ash})\ x100}{\text{Sample weighed (2g)}}$$

Dry Matter (DM) was determined by oven drying 2g of each dried milled feed ingredient on a crucible at 105 ^{0}C for six hours. The sample and crucible were placed in a desiccator to cool before they were weighed. Weight loss recorded will be

moisture content while dry matter will be obtained by getting the difference between dry sample weight and the weight of the crucible.

$$\%\text{DM} = \frac{\text{weight of dry sample} - \text{ weight of crucible x } 100}{\text{Sample weighed (2g)}}$$

Ash content was determined by incinerating 2 g feed sample in a weighed crucible in a muffle furnace at 550 °C for four hours. The muffle furnace was switched off for temperature to reduce to 100 °C before opening it to transfer the ash and the crucible into a desiccator for cooling. Ash was calculated as follows;

$$\%Ash \ = \frac{\text{weight of ash (weight crucible+ ash)} - \text{weight of crucible) x } 100}{\text{Weight sample (2g)}}$$

Ether Extracts (EE) was determined by Soxhlet extraction procedure. Three round bottomed conical flask for each sample were cleaned and oven dried and placed in a desiccator to cool. 2g of each feed sample in triplicate was put in pre-weighed thimbles and corked with cotton. The content in the corked thimble was set in the Soxhlet extraction system fitted with the cleaned dried round bottomed conical flask. The Soxhlet extractor was half way filled with petroleum ether with heating at 40 °C to 60 °C. The crude lipid was extracted in the set up for three hours involving boiling, rinsing and evaporation. The thimble and the remaining sample were oven dried at 60 °C for 5 hours and placed in a desiccator to cool before weighing.

$$\%EE = \frac{\text{wgt of sample before extraction} - \text{wgt of dry sample after extraction) x100}}{\text{Weight of sample (2g)}}$$

where,

Wgt = weight

Nitrogen Free Extracts (NFE) was determined by difference, subtracting the sum of %CP, %CF, %EE, % moisture content and % ash from total wet matter basis of 100% (FAO, 2003).

$$NFE = 100\% - (\%CP + CF + EE + \%Moisture\ content + \%\ ash)$$

Amino acid analysis was done to get nutritionally important essential amino acid (EAA) for formulating and compounding well balanced fish feeds. The EAA considered in analysis were; lysine, methionine, cysteine, tryptophan, threonine, leucine, isoleucine, valine, phenylalanine, tyrosine, histidine and arginine. A modified AOAC (2005) procedure method 982:30, which is HPLC reverse phase method was applied after amino acid hydrolysis.

Extraction of protein was performed as described by Hamilton *et al*. 2012 procedures. 2 mg of each sample were extracted with 4 ml of 2: 5: 1: 1 v/v methanol: water: chloroform. 10 µl of 0.2 mg/ml ribitol was added as internal standard to the extract. The extraction was done at 37^0C with 1200 rounds per minute mixing frequency for 30 minutes using a thermo mixer comfort (model 5355, Eppendorf. AG Hamburg, Germany). Solutions were centrifuged for three minutes and 0.8 ml supernatant was transferred into a new tube, 0.4 ml of water was added to the solution and centrifuged again for three minutes. Methanol/water phase was dried in a centrifugal concentrator (CVE-2000, Eyela, Japan) for two hours then followed by freeze drying for 16 hours.

80µl of 20 mg ml^{-1} methoxyamine hydrochloride in pyridine was added and shaken at 30 °C for 90 minutes.

20 µl of aliquot of extracts was injected directly into Gas Chromatography-Mass Spectoral (GC-MS) analyzer (Sigma St. Louis, MO, USA) for searches with GC fitted with a HP-5 MS low bleed capillary column 30 mm x 0.25 mm1d, 0.25µm (Restek, Bellofonte, PA,USA). Helium was used as a carrier gas at a flow rate of 1.25 ml per minute. Inlet temperature was 270 ^{0}C, transfer line temperature of 280 °C and column oven temperature was programmed from 35 °C to 280 °C with the initial temperature maintained for five minutes then 10 °C per minute to 280 °C for 10 minutes. Electron impact mass spectra were obtained at acceleration energy of 70 Ev.A./o µL. Aliquot of extracts was automatically injected in the split/splitless mode using an auto sampler (7683 Agilent Technologies, Inc, Beijing, China). Amino acids were separated, detected and quantified by GC-MS on standalone gas chromatograph (7890A Agilent Technologies, Inc, Beijing, China) and a mass selective detector (5975 C Agilent Technologies, Inc, Santa clara, CA, USA).

3.3.3 Formulation of experimental diet

The main purpose of formulating a diet is to obtain nutritionally balanced blend of feed ingredients to provide nutrients required for maintenance, growth, reproduction and sound health of the animal at affordable cost (NRC, 1993). After obtaining results on proximate and amino acid analysis, formulation of experimental diets was done as follows; soybean meal was first mixed with blood meal at a ratio of 1:2 (one part of soybean meal in two parts of blood meal). The protein concentration of soybean-blood meal mixture was calculated before being used to fish meal (*Rastrineobola*

argentea) for protein balance together with other feed ingredients. Five experimental diets with each containing 300g/kg protein (30% CP) with test protein ingredients providing 23% CP of the total 30% CP were formulated using computer excel spread sheet program. The other feed ingredients that were used in formulation include, wheat bran, maize meal, vitamin-mineral premix and sunflower oil. 0% fish meal substitution diet which was used as control diet was formulated with fish meal as the main source of protein. 25% substitution fish meal was substituted by 25% soybean-blood meal mixture, 50% substitution fish meal was substituted at 50% with soybean-blood meal mixture, 75% substitution diet fish meal was substituted at 75% with soybean-blood meal mixture and lastly total fish meal substitution (100%) soybean-blood meal mixture was the main source of protein as indicated in Table 3.1.

Table 3.1: Experimental diets

Feed ingredient (%)	**Formulated dietary treatments (fish meal substitution with soybean-blood meal %)**				
	0	**25**	**50**	**75**	**100**
Fish meal	38.27	28.7	19.14	9.57	0
Soybean-blood meal	0	8.26	16.52	24.77	33.03
Maize meal	20.73	21.84	22.94	23.96	24.97
Wheat bran	39	39	39	39	39
Sunflower oil	1	1.2	1.4	1.7	2
Mineral-vit premix	1	1	1	1	1
Total (Kg)	**100**	**100**	**100**	**100**	**100**

The finely ground dried feed ingredients were weighed and put in a motor driven feed mixer (**Plate 3.3**) in required proportions as indicated in the formulations for mixing to get a homogenous blend.

Plate 3.3: Photograph of feed mixer (Source: Author, 2018)

The feed ingredients were mixed for three minutes then stopped, 25% clean water mixed with sunflower oil and vitamin-mineral premix was added into the feed and preceded with mixing for three minutes to form dough. The dough was put through the pellet making machine (**Plate 3.4**) in which it was pressed through dies at high pressure to produce 3.5 mm floating pellets. The pellets were properly dried in the sun for one day (**Plate 3.5**) and then packed in labelled water proof and airtight bags to prevent moulds (MSOP, 2015).

Plate 3.4: Photograph of pellet making machine (Source: Author, 2018)

Plate 3.5: Photograph of experimental feeds being sun dried (Source: Author, 2018)

The pellets were crushed into crumbles prior to feeding fingerlings up to week six when they were able to consume the pellets. Proximate and amino acid analyses were determined in triplicates. Chemical score, essential amino acid indices (EAAI) and protein requirement indices of the diets were calculated from the laboratory results using the following formula in triplicates.

Chemical score (CS) = is the ratio of each essential amino acid in the test diets relative to the same essential amino acid requirements (Bunda *et al.*, 2015).

$$\mathrm{CS} = \frac{\%\ \text{EAA in formulated diet}}{\%\ \text{corresponding EAA required level by Nile tilapia}}$$

Essential amino acid index (EAAI) = is the mean geometrical ratio of all essential amino acid in the formulated diet to their required levels in Nile tilapia (Bunda *et al.*,

2015). The formula for calculating essential amino acid was shown below as used by Mune-Mune *et al*. (2011):

$$\text{EAAI} = \text{n}\sqrt{\frac{100\text{a} \times 100\text{b} \times ..100\text{j}}{\text{a1} \times \text{a2} \times \ldots . \text{a10}}}$$

where,

a, b..j…an = the essential amino acid level in the formulated diets while

a_1, a_2…a10 = the corresponding dietary essential amino acid requirement levels by Nile tilapia (**Table 2.1**) and n is number of amino acids used. Protein Requirement Indices (PRI) were calculated using same EAAI mathematical model except that values of individual essential amino acid chemical scores that exceeded 100% were reduced to 100% as used by Rao *et al.* (1964).

3.4 Feeding and Data Collection

The diets were fed to fish at a rate of 5% of total average body weight per day for fourteen weeks. They were hand fed thrice a day at 10.00hrs, 13.00hrs and 16.00hrs. Fish sampling was done fortnightly and feed provided was adjusted basing on weight gain. At every sampling, ten fish were sampled using scoop net from each aquarium, weighed and amount of feed consumed was recorded throughout the experimental period.

3.4.1 Fish growth performance

The effect of fish diets on growth performance of fish was determined using five response parameters:

a) Survival rate was calculated by subtracting the number of fish that died during the experimental period from fish stocked divided by number stocked multiplied by 100 to convert the figure into percentage (Charo *et al.,* 2006)

$$\text{Survival rate (SR \%)} = \frac{\text{Initial number of fish stocked (20) – dead fish x 100}}{\text{Initial number stocked}}$$

b) Average daily gain (ADG g/day) was calculated by getting the difference between the final body weight (g) and the initial body weight in grams over a period of experiment time in days.

$$\text{ADG} = \frac{\text{Final body weight – Initial body weight}}{\text{Days of experiment}}$$

c) Relative growth rate (RGR)

$$\text{RGR (\%)} = \frac{\text{Final average fish weight– initial average fish weight x 100}}{\text{Final average fish weight}}$$

d) Specific growth rate is described by Khalafalla *et al.* (2010) as the instantaneous percentage change in body weight of fish per day at a given time interval and is calculated by taking natural logarithm of body weight change expressed as percentage per day.

$$SGR = \left\{\frac{\text{Logn final body weight} - \text{Logn initial body weight}}{\text{Experimental period}}\right\} X\ 100$$

Logn = natural logarithm

e) Feed conversion ratio (FCR) is a measure of feed utilization efficiency by the fish and is defined as the amount of feed utilized to produce a unit weight gain. The lower the FCR, the less feed utilized to produce a unit of weight gain and the higher the growth rate (Guroy *et al.*, 2005).

$$FCR = \frac{\text{feed intake in grams}}{\text{Weight gain in grams}}$$

3.4.2 Fish carcass composition

At the end of the experimental period two fish were sacrificed from each aquarium, washed in clean tap water, gutted, removed scales and washed again in clean tap water. They were labelled and packed with ice in a cool box and taken to laboratory at the University of Eldoret Chemistry laboratory for proximate carcass composition analysis (Moisture content, CP, EE and Ash). The fish were removed from the cool box, weighed on a weigh balance, wrapped in aluminium foil and labelled with HP pencil. They were put in an oven and dried for four days at 60 ^{0}C. After four days, dry weight was taken and percentage moisture content was determined by getting the difference between initial weight when wet and weight after drying divided by initial weight multiplied by 100. The dried fish were ground separately into powder using a mortar and pestle before they were subjected to proximate analysis. Crude protein, crude lipids (Ether extracts) and ash contents were performed on dry ground fish carcass sample as described in section 3.3.2.

3.4.3 Gross margin determination

The cost of feeds was considered in this study for gross margin evaluation and other costs were assumed to be constant. The cost of the feeds was calculated using the prevailing market prices which were recorded when the feed ingredients were purchased as shown in (**Table 3.2**). Gross margin was calculated as follows:

$$Gross\ margin = QP - Cost\ of\ feed$$

Where Q is quantity in kilograms of fish produced and P is the unit market price

Table 3.2: Price of feed ingredients used in formulating experimental diets

Feed ingredient	Price per Kilogram (Ksh.)
Fish meal (*Rastrineobola argentae*)	250.00
Blood meal	15.00
Soybean meal	70.00
Wheat bran	17.00
Maize	30.00
Sunflower oil	140.00
Mineral-vitamin premix	250.00

(Source: Author, 2018)

3.5 Data Analysis

Data on proximate and amino acid analysis, chemical score (CS), essential amino acid indeces (EAAI), protein requirement indices (PRI), survival rate (SR), average daily gain (ADG), relative growth rate (RGR), specific growth rate (SGR) and feed conversion ratio (FCR) for nutrient composition and growth response parameters respectively, proximate carcass composition values on moisture content (MC), crude protein (CP), ether extracts (EE) and ash and data on gross margins were subjected to

analysis of variance (ANOVA) using SPSS statistical package version 22.0 to establish significant differences at $p \leq 0.05$ level of significance. Where there were differences, mean separation was carried out by least significant difference (LSD) at $p \leq 0.05$. General linear model for completely randomized design (CRD) was;

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where,

Y_{ij} = Total observation on j^{th} fish and i^{th} treatment

μ = the overall population mean

T_i = the effect due to soybean-blood meal mixture level (0, 25, 50, 75 and 100%)

ε_{ij} = is the error term

CHAPTER FOUR

RESULTS

4.1 Nutrient composition of Nile Tilapia Diets

4.1.1 Proximate nutrient composition

Results for proximate nutrient composition of feed ingredients used in formulation of Nile tilapia diets are indicated in (**Table 4.1).** Blood meal had highest crude protein content (81.49%) while maize flour had the lowest crude protein content (7.57%). Wheat bran recorded the highest crude fibre content of 8.19%. Fish meal was reported to have the lowest fibre content of 0.56%. Fish meal recorded highest amount of ether extracts (8.86%) and blood meal had the lowest ether extracts content of 1%. Fish meal had the highest ash content of 20.57% with maize flour recording the lowest (0.91%) ash content. Nitrogen free extracts content was reported in this study to be highest in maize flour while lowest in blood meal. Proximate nutrient compositions for the formulated fish feeds were shown in (**Table 4.2).** Results indicated a slight increase ($p<0.05$) in crude protein levels with total fish meal substitution (100%) recording 30.5% of fish meal substitution with soybean-blood meal mixture, increased ($p<0.05$) crude fibre levels and nitrogen free extracts (NFE) while ash and ether extracts content reduced.

Table 4.2: Proximate nutrient composition of feed ingredients (%)

Nutrient	Maize meal	Wheat bran	Soybean meal	Blood meal	Fish meal	SEM	p-value
DM	87.74 [d]	88.42 [c]	90.44 [b]	90.82 [a]	90.48 [b]	0.33	<0.001
CP	7.57 [e]	14.45[d]	45.29 [c]	81.49 [a]	60.09 [b]	7.41	<0.001
CF	2.54 [c]	8.19 [a]	6.03 [b]	0.92 [d]	0.56 [e]	0.80	<0.001
EE	4.44 [b]	4.04 [c]	1.70 [d]	1.00 [e]	8.86 [a]	0.74	<0.001
Ash	0.91[e]	3.71 [d]	6.13 [c]	7.14 [b]	20.57 [a]	1.81	<0.001
NFE	72.27[a]	58.02[b]	31.27[c]	0.27 [d]	0.40 [d]	7.84	<0.001

Mean values with different superscripts in the same row are different statistically (p<0.05)

Table 4.2: Proximate nutrient composition (%) of the treatment diets

Nutrient	Formulated Diets (% fish meal substitution)					SEM	p-value
	0	25	50	75	100		
DM	89.85 [e]	90.1 [d]	90.91[a]	90.63 [b]	90.35 [c]	0.01	<0.001
CP	30.23 [e]	30.3 [d]	30.38 [c]	30.43 [b]	30.5 [a]	0.03	<0.001
CF	3.95 [e]	4.13 [d]	4.32 [c]	4.51 [b]	4.7 [a]	0.07	<0.001
EE	6.89 [a]	6.39 [b]	5.9 [c]	5.49 [d]	5.1 [e]	0.17	<0.001
Ash	9.52 [a]	8.12 [b]	6.78 [c]	5.32 [d]	3.92 [e]	0.58	<0.001
NFE	39.25 [e]	41.16 [d]	43.54 [c]	44.87 [b]	46.14[a]	0.67	<0.001

Values with different superscripts in the same row are different statistically (p<0.05)

4.1.2 Essential amino acid composition

Amino acid composition of feed ingredients are shown in (**Table 4.3**) which revealed that blood meal had highest amount of Lysine (81.4mg/g protein) with maize flour recording the lowest amount of 14.23mg/g protein. Fish meal recorded highest level of Arginine (58.7mg/g protein) and maize flour had lowest amount of 24.17mg/g protein. Blood meal had highest amount of Histidine (54.43mg/g protein) and lowest amount was recorded in soybean meal (12.5mg/g protein). Fish meal had highest amount of Threonine (42.83mg/g protein) and soybean meal recorded the lowest amount of 19.57mg/g protein.

Table 4.3: Amino acid composition (mg/g protein) of the feed ingredients

Amino acid	Maize meal	Wheat bran	Soybean meal	Blood meal	Fish meal	SEM	p-value
Lysine	14.23 ^{e}	17.53 ^{d}	30.1 ^{c}	81.4 ^{a}	78.06 ^{b}	0.79	<0.001
Arginine	24.17 ^{e}	28.13 ^{d}	33.87 ^{b}	29.57 ^{c}	58.7 ^{a}	0.57	<0.001
Histidine	21.2 ^{c}	18.13 ^{d}	12.57^{e}	54.43 ^{a}	24.33 ^{b}	0.39	<0.001
Threonine	25.97^{d}	31.57 ^{c}	19.57 ^{e}	38.67 ^{b}	42.83 ^{a}	0.22	<0.001
Valine	40.87 ^{d}	49.3 ^{c}	22.37 ^{e}	62.73 ^{a}	54.03 ^{b}	0.37	<0.001
Leucine	71.53 ^{c}	68.53 ^{d}	36.87 ^{e}	100.6 ^{a}	75.53 ^{b}	0.54	<0.001
Isoleucine	32.6 ^{c}	38.3 ^{b}	30.23 ^{c}	9.7 ^{d}	45.5 ^{a}	0.33	<0.001
Ph + Tyr	74.97 ^{a}	61.07 ^{b}	27.01 ^{e}	58.3 ^{c}	51.56 ^{d}	0.42	<0.001
Met + Cyst	32.03 ^{b}	32.53 ^{b}	12.77^{d}	20.5 ^{c}	38.4 ^{a}	0.25	<0.001
Tryptophan	2.37^{d}	2.53 ^{d}	6.83 ^{c}	9.4 ^{b}	11.53 ^{a}	0.10	<0.001

Mean values with different superscripts in the same row are different statistically ($p<0.05$)

Blood meal had highest amount of Valine (62.73mg/g) while soybean meal had lowest amount (22.37mg/g). Blood meal recorded highest Leucine content (100.6mg/g protein) and soybean meal had lowest amount (36.87mg/g protein). Fish meal was reported to have highest amount of Isoleucine (45.5mg/g protein) and blood meal recorded the lowest figure (9.7mg/g protein). Statistically, the amount of Isoleucine in soybean meal and maize flour were similar ($p>0.05$). Maize flour recorded the highest amount of Phenylalanine+ Tyrosine (Phe + Try) and soybean meal recorded the least (27.01mg/g protein). Methionine+Cysteine (Met + Cyst) was reported to be highest in fish meal (38.4mg/g protein) and lowest in soybean meal (12.77mg/g protein). Tryptophan was highest in fish meal (11.53mg/g protein) and lowest in maize flour with no statistical difference ($p>0.05$) with wheat bran. Essential amino acid composition in formulated diets shown in (**Table 4.4**) indicated that fish meal substitution with soybean-blood meal mixture significantly reduced amounts of essential amino acids except Histidine and Leucine which increased ($p<0.05$).

Total substitution showed no difference statistically (p>0.05) with 75% substitution and between 50% substitution and 75% fish meal substitution in the concentration of Leucine.

Table 4.4: Amino acid composition (Mg/g protein) of treatment diets

Amino acid	Formulated dietary treatments (% fish meal substitution)					SEM	p-value
	0	25	50	75	100		
Lysine	39.67[a]	37.66 [b]	35.67 [c]	33.65 [d]	31.64 [e]	0.76	<0.001
Arginine	38.45 [a]	35.66 [b]	32.87 [c]	30.06 [d]	27.24[e]	1.06	<0.001
Histidine	20.78 [e]	22.03[d]	23.28 [c]	24.51[b]	25.74 [a]	0.47	<0.001
Threonine	34.07[a]	32.94 [b]	31.80 [c]	30.63 [d]	29.47[e]	0.44	<0.001
Valine	48.38 [a]	47.73 [b]	47.08 [c]	46.39 [d]	45.71[e]	0.25	<0.001
Leucine	70.46 [d]	70.89[cd]	71.29 [bc]	71.29[ab]	71.99[a]	0.16	<0.001
Isoleucine	39.11[a]	36.30 [b]	33.49 [c]	30.35[d]	27.81[d]	1.07	<0.001
Ph + Tyr	68.13 [a]	65.73 [b]	63.32 [c]	60.84 [d]	58.35 [e]	0.93	<0.001
Met + Cyst	34.02[a]	32.19 [b]	30.35 [c]	28.48 [d]	26.57 [e]	0.71	<0.001
Tryptophan	5.89 [a]	5.52 [b]	5.15 [c]	4.78 [d]	4.41[e]	5.33	<0.001

Mean values with different superscripts in the same row are different statistically (p<0.05)

4.1.3 Chemical score, essential amino acid, protein requirement indices

Results for amino acid Chemical Scores, Essential Amino Acid Indeces (EAAI) and Protein Requirement Indices (PRI) are presented in (**Table 4.5).** Chemical scores for amino acids lysine, arginine, threonine, valine, isoleucine, phenylalanine+tyrosine, methionine+cysteine and tryptophan significantly reduced (p<0.05) with fish meal replacement. Scores for amino acid histidine and leucine significantly increased (p<0.05) with fish meal substitution. Chemical scores for amino acid leucine were statistically similar (p>0.05) between 100% substitution and 75% substitution and between 50% substitution and 75% substitution respectively. Essential Amino Acid Indices (EAAI) and Protein Requirement Indices (PRI) indicated slight reduction with

fish meal substitution; 0% substitution recorded highest indices and 100% substitution recorded lowest indices.

Table 4.5: Chemical scores, EAAI and PRI of treatment diets (%)

Amino acid	Formulated dietary treatments (% fish meal substitution)					SEM	p-value
	0	25	50	75	100		
Lysine	77.47[a]	73.56 [b]	69.66 [c]	64.72 [d]	61.79 [e]	1.54	<0.001
Arginine	91.54 [a]	84.90 [b]	78.26 [c]	71.57[d]	64.87 [e]	2.52	<0.001
Histidine	120.8 [e]	128.1[d]	135.3 [c]	142.5 [b]	149.6 [a]	2.73	<0.001
Threonine	90.86 [a]	87.85 [b]	84.80 [c]	81.69 [d]	78.58 [e]	1.16	<0.001
Valine	172.8 [a]	170.5 [b]	168.1[c]	165.7 [d]	163.2 [e]	0.90	<0.001
Leucine	207.9 [d]	209.1 [cd]	210.3 [bc]	211.3 [ab]	212.4 [a]	0.46	<0.001
Isoleucine	125.7 [a]	116.7 [b]	107.7 [c]	98.6 [d]	89.4 [d]	3.44	<0.001
Ph + Tyr	181.7 [a]	175.3 [b]	168.8 [c]	162.2 [d]	155.6 [e]	2.47	<0.001
Met + Cyst	127 [a]	120.1[b]	113.2 [c]	106.3 [d]	99.2 [e]	2.63	<0.001
Tryptophan	58.9 [a]	55.20 [b]	51.50 [c]	47.77[d]	44.1 [e]	1.38	<0.001
EAAI	116.9 [a]	113.01[b]	108.90 [c]	104.35[d]	99.91[e]	1.61	<0.001
PRI	90.77 [a]	88.74 [b]	86.63 [c]	84.15 [d]	81.08 [e]	0.05	<0.001

Mean values with different superscripts in the same row are different statistically (p<0.05)

4.2 Effect of Substituting Fish Meal with Soybean-Blood Meal Mixture on Growth of *O. niloticus*

4.2.1 Water quality parameters

Water temperature in the aquaria was maintained by the thermostats at 28 ± 1^0C. Dissolved oxygen (DO) varied from 8.5 mg L^{-1} to 8.7 mg L^{-1}, this was supplied and maintained by the aerators connected to the aquaria. The pH ranged from 7.05 to 7.1 (**Table 4.6)**. All measured water parameters were statistically similar (p>0.05).

Table 4.6: Water quality parameters, Dissolved oxygen (DO) pH and Temperature

Parameter	Aquaria allocated to different dietary treatments					SEM
	0	25	50	75	100	
DO (mg/L)	8.7 [a]	8.6 [a]	8.6 [a]	8.5 [a]	8.5 [a]	0.01
pH	7.1 [a]	7.06 [a]	7.07[a]	7.05 [a]	7.06 [a]	0.04
Tempe [o] C	28.3 [a]	28.3 [a]	28.1[a]	28.01[a]	28.2 [a]	0.06

Mean values with same superscripts in the same row are statistically similar (p<0.05)

4.2.2 Growth response parameter

Fish group fed on 75% fish meal substitution with soybean-blood meal had highest (p<0.05) final average weight (53.2g) followed by 47.8g 44.6g and 40.5g for 50, 25 and 0% substitution levels and the least mean weight was recorded at 100% (32.9g). Highest average daily gain (p<0.05) was recorded at 75% fish meal substitution level (0.54g/day) compared to 50% (0.48g/day), 25% (0.45g/day), 0% (0.41g/day) and 100% fish meal substitution level (0.33g/day) respectively. 75% substitution was found to have highest (p<0.05) specific growth rate (SGR) of 1.91% while 50% (1.87%), 25% (1.83%), 0% (1.79%) and 100% substitution had the least (1.71%). Slight difference (p<0.05) was realized in relative growth rate (RGR) with 75% (98.7%) having the highest and 100% fish meal substitution was reported to have the least (97.9%). Feed conversion ratio (FCR) followed the same trend with 75% substitution (1.1) recording the lowest (p<0.05) and 100% substitution (1.8) the highest (**Table 4.7**). Survival rates were high and statistically similar (p>0.05) for all the five diets (**Table 4.7**). Growth curves shown in **Figure 4.1** indicated that 75% substitution had higher growth rate followed by 50, 25, 0% and 100% had poor performance in growth rate.

Table 4.7: Growth parameters of Nile tilapia fed on formulated diets

Parameter	Dietary treatment					SEM	p-value
	0	25	50	75	100		
Initial weight (g)	0.71 [a]	0.71 [a]	0.70 [a]	0.71 [a]	0.70 [a]	0.005	0.998
Final weight (g)	40.53 [d]	44.60 [c]	47.80 [b]	53.20 [a]	32.90 [e]	1.83	<0.001
ADG (g/day)	0.41 [d]	0.45 [c]	0.48 [b]	0.54 [a]	0.33 [e]	0.02	<0.001
SGR (%)	1.79 [d]	1.83 [c]	1.87 [b]	1.91 [a]	1.71 [e]	0.02	<0.001
RGR (%)	98.2 [d]	98.4 [c]	98.5 [b]	98.7 [a]	97.9 [e]	0.07	<0.001
FCR	1.4 [b]	1.3 [c]	1.2 [d]	1.1 [e]	1.8 [a]	0.06	<0.001
Survival Rate (%)	95 [a]	93 [a]	95 [a]	97 [a]	92 [a]	0.96	<0.596

Mean values with different superscripts in the same raw are statistically different while same superscripts indicate insignificant difference

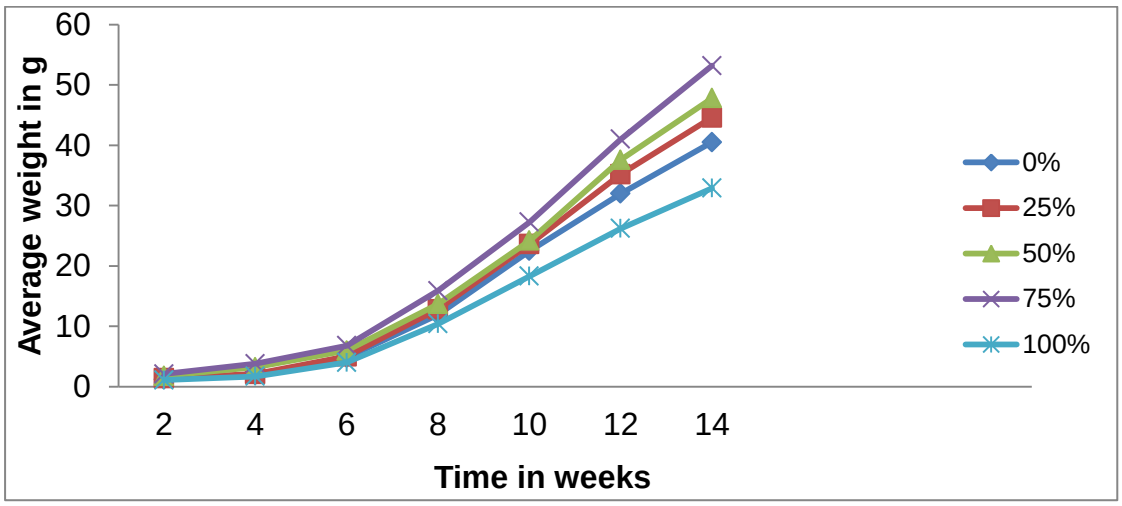

Figure 4.1: Growth curves of Nile tilapia fish fed treatment diets with fish meal substitution with soybean-blood meal mixture

4.3 Effects of Fish Meal Substitution with Soybean-Blood Meal Mixture on Nile Tilapia Fish Carcass Nutrient Composition

Results for carcass proximate nutrient composition are shown in (**Table 4.8**). Carcass moisture content was similar ($p>0.05$) in all diets apart from 25% fish meal substitution diet (73.91%) which had slightly higher ($p<0.05$) value. Substitution of fish meal with soybean-blood meal mixture caused slight increase in carcass crude protein content, though there was no significant effect ($p>0.05$) between 0%

substitution diet (45.69%) and 25% substitution diet (47.99%) and between 50% substitution diet (52.22%) and 75% substitution diet (54.43%) while 100 % substitution diet (35.47%) recorded lowest ($p<0.05$) (**Table 4.8**). 50% and 75 substitution diet had significantly ($p<0.05$) higher values than 0% and 25% fish meal substitution diet.

Table 4.8: Proximate carcass composition (DM basis) of Nile tilapia

Proximate composition	Formulated diets (fish meal substitution,%)					SEM	P-value
	0	25	50	75	100		
Moisture content	72.61 [b]	73.91[a]	72.44 [b]	72.93 [b]	72.74 [b]	0.15	0.013
Crude protein	45.69 [b]	47.99 [b]	52.22 [a]	54.43 [a]	35.47 [c]	1.32	<0.001
Ether extracts	15.32 [b]	17.09 [a]	17.42 [a]	18.29 [a]	12.50 [c]	0.42	<0.001
Ash	19.90 [a]	19.30 [a]	19.54[a]	20.18 [a]	20.46 [a]	0.31	0.822

Mean values with different superscripts in the same row are different statistically ($p<0.05$)

75%substitution diet (18.29%), 50% substitution diet (17.42%) and 25% substitution diet (17.09%) recorded statistically similar ($p>0.05$) results of carcass ether extracts but were highest and different ($p<0.05$) from 0% substitution diet (15.32%) and 100% fish meal substitution diet (12.5%) which had lowest value of fat. Substitution revealed no significant effect in carcass ash content in fish fed on all experimental diets.

4.4 Effects of Fish Meal Substitution on Gross Margin

Diet 1 had highest ($p<0.05$) feed cost per kilogram (Ksh. 112.42) and total feed cost (Ksh. 120.29) which reduced with fish meal substitution levels with soybean-blood meal mixture (**Table 4.9**). Harvested weight and value of harvested fish increased ($p<0.05$) with fish meal substitution levels from 0 to 75% but dropped drastically in 100% fish meal substitution diet. Gross margin for 75% fish substitution diet (Ksh. 253.14) was highest ($p<0.05$) followed by 50% substitution diet (Ksh. 196.28). Gross margin for fish meal substitution diet (Ksh. 153.33) and 100% substitution diet (Ksh. 149.56) were statistically similar ($p>0.05$) while 0% fish meal substitution diet recorded the lowest gross margin (Ksh. 110.71).

Table 4.9: Gross margin analysis for all formulated diets

Parameter	Dietary treatments					SEM
	0	25	50	75	100	
Feed cost/Kg (Ksh)	112.42	91.86	71.33	50.88	30.43	7.75
Feed fed (Kg)	1.07	1.05	1.07	1.09	1.03	0.01
Total feed cost (Ksh)	120.29 ^{a}	96.45 ^{b}	76.34 ^{c}	55.46 ^{d}	31.34 ^{e}	8.32
Harvest weight (Kg)	0.770 ^{d}	0.833 ^{c}	0.909 ^{b}	1.03 ^{a}	0.603 ^{e}	0.04
Fish price/Kg (Ksh)	300	300	300	300	300	-
Value of fish (Ksh)	231.0 ^{d}	249.8^{c}	272.6^{b}	308.6 ^{a}	180.9^{e}	11.61
Gross margin (Ksh)	110.71^{d}	153.3^{c}	196.28 ^{b}	253.14^{a}	149.56^{c}	13.06

Mean values with different superscripts in the same raw are different

CHAPTER FIVE

DISCUSSION

5.1 Effects of Fish Meal Substitution with Soybean-Blood Meal Mixture on Nutrient Composition of Formulated Nile Tilapia Diets

Crude protein, ether extracts and ash contents found in soybean meal and fish meal were in agreement with results reported by previous researchers (Anjum *et al.,* 2014; Prado *et al.,* 2016). Similarly proximate nutrients values in maize were slightly lower than values recorded in their studies. Values for crude protein and ether extracts for wheat bran were 14.45% and 4.04% respectively in this study which was lower than figures reported by Asadujjaman *et al.* (2014) of 17.13% and 6.69%. It was noted that differences in agronomic practices and processing methods used bring about differences in nutrient composition of feeds and feed stuffs (Anjum *et al.,* 2012).

Prado *et al.* (2016) noted that fish meal processed from whole fish is of high protein quality with high protein content of above 60%. In this study whole fish (*Rastrineobola argentea*) was used to process fish meal hence the high protein content of 60.09% reported. Variations in fat and protein contents in fish meal occur as a result of species of fish used, freshness and processing method (Anjum *et al.,* 2014). Amount of crude protein in blood meal (81.49%) was within range reported in literature (NRC, 1993). Values for crude protein content in blood meal also agree with those obtained by Otubusin *et al.* (2009). Kirimi *et al*. (2016a) in their study reported 80.4% crude protein in blood meal when they treated fresh blood at 100 ^{0}C for 45 minutes. Exposure of fresh blood to high temperatures for a long period of time during processing denatures protein and reduces their bioavailability (Batterham *et*

al., 1996). This may have resulted to the slightly lower amount of crude protein than values recorded in the current study when fresh blood was treated at 100 °C for 15 minutes in blood meal processing.

Increase in crude fibre with increase in fish meal substitution levels was because of increase in amounts of soybean meal which had high levels of crude fibre since wheat bran with highest crude fibre content was constant in all diets. Ether extracts and ash contents reduced with fish meal replacement due to increase in amount of soybean-blood meal mixture which was low in the same proximate nutrients. Nitrogen free extracts (NFE) increased with fish meal replacement as a result of increase in amount of maize flour and soybean meal which had high NFE values. Formulated diets had all proximate nutrient values within range recommended for *Oreochromis niloticus* fish. Recommended crude protein range from 30% to 35% for fingerlings and 25% to 30% for growers and adult Nile tilapia and the lowest amount in formulated diets had 30.23% (Jouncey, 1998). Ether extracts was highest in 0% fish meal substitution with the lowest figure of 5.1% reported in total substitution of fish meal (100%) which was within recommended range of 5% to 12% for Nile tilapia fish (Suresh, 2003). De Silva and Anderson (1995) recommended crude fibre levels from 8% to 12% in tilapia fish diets and diets used in this study had lower figures.

Increase in amino acids histidine and leucine amounts with fish meal substitution in the diets was due to high amount of blood meal and maize meal which had high levels. Blood meal had very low amount of isoleucine, this was blended with high levels in soybean meal, maize meal and wheat bran to surpass tilapia requirement level of 31.1 mg g^{-1} protein (NRC, 1993). Methionine+cysteine was low in blood

meal and soybean meal, this was reflected in its reduction with fish meal replacement in the diets. Wheat bran and maize meal boosted the level of methionine+cysteine above 26.8 mg g^{-1} protein requirement recommended in literature (NRC, 1993).

Methionine and cysteine are sulfur amino acids and it is reported that cysteine can meet about 50% of all sulfur based amino acids in diets for pigs (Chung and Baker, 1992). Metabolic requirement of cysteine can be met by methionine and cysteine can supply 50% methionine hence they are combined in nutritional evaluation of tilapia feeds (NRC, 1993). Aromatic amino acids phenylalanine and tyrosine exhibit the same metabolic relationship. Aromatic amino acid can be synthesized metabolically from phenylalanine and its availability in diets for fish reduces phenylalanine requirement (NRC, 1993). Highest chemical scores were recorded in diet with 0% fish meal substitution because fish meal was the main source of protein and is known to have high and balanced essential amino acids (NRC, 1993). This was reflected in the formulated diets which caused reduction with replacement in chemical scores for essential amino acids apart from histidine and leucine which increased due to increase in blood meal. Chemical scores revealed that tryptophan was the first limiting essential amino acid while lysine and arginine were second and third respectively in the diets.

All amino acids should be available at protein synthesis site in adequate amounts for proper metabolism of amino acids (Svier *et al.*, 2001). Oser (1959) reported that each amino acid is crucial in building of stable protein hence all amino acids should be considered in evaluating nutritional value of a protein feed. Oser (1959) developed Essential Amino Acid Index (EAAI) model using whole egg protein essential amino

acids as a standard protein and he found it to be highly correlated to Biological Value (BV) and Protein Efficiency Ratio. Hepher (1988) noted that essential amino acid requirement provides a good standard protein in calculation of chemical scores and EAAI than whole egg protein. Rao *et al.* (1964) derived another protein evaluation method from EAAI mathematical model called Protein Requirement Index which they defined as the geometrical mean ratio of essential amino acid in test feed to their requirement levels but values which exceed 100% are reduced to 100%. Kapour and Heiner (1982) used the model and found a high correlation (r = 0.88) with Biological Value in wheat. Formulated diets had high EAAI exceeding 100% except (total substitution of fish meal) which had a mean of 99.91%. Protein Requirement Index also had high values with the lowest in total fish meal substitution (100%) which recorded 81.08%. With correlation between PRI and biological value of 0.88 obtained by Kapour and Heiner (1982) it w,as found that total substitution of fish meal by soybean-blood meal mixture produced a ration with 71.35% biological value. Ijarotimi and Keshinro (2011) stated that a protein feed is considered to be of good nutritive value when its Biological Value is from 70% to 100% and EAAI is above or equal to 90%, useful nutritive value that is about 80% and incomplete or poor protein quality when is less than 70%. All experimental diets had EAAI above 90% and estimated Biological Value above 70%, therefore were of good nutritive value.

5.2 Effect of substituting fish meal with soybean-blood Meal Mixture on Growth Performance of Nile tilapia (*O. niloticus*)

5.2.1 Growth performance

Growth improved significantly with increase in fish meal substitution levels with soybean-blood meal mixture up to 75% fish meal substitution level which recorded the highest growth rate and dropped drastically at total fish meal replacement diet (100%) with total fish meal replacement. This could be as a result of presence of important nutrients specifically digestible essential amino acids and lipids in soybean meal, blood meal and fish meal which formed a blend that met nutrient requirement of fish. Fuller *et al.* (1989) pointed out that excess dietary essential amino acids relative to requirement are catabolised to energy and ammonia at the expense of growth and development as excretion of ammonia needs energy. 75% fish meal substitution diet had EAAI close to 100% which is exact to requirement which may have had minimal energy expenditure on catabolism of excess essential amino acids and enhanced growth realized. Soybean meal contain linoleic fatty acids which when fortified with other nutrients especially from animal protein feed ingredients enhance growth of Nile tilapia fish compared to fish meal alone which has arachdonic fatty acids (Takauchi *et al*., 1983a). Soybean meal was reported to have good amino acid profile close to fish meal and is highly digestible with apparent protein digestibility (APD) of 96% reported in *Tilapia rendalli* (Mothwa *et al.,* 2013). Blood meal was also reported to have high dry matter digestibility compared to fish meal in *Labeo rohita* fingerlings, apparent protein digestibility of 80% in *Oncorhynchus mykiss* (El-Haroun and Breau, 2007; Hussain *et al.,* 2011).

High digestibility of soybean meal and blood meal may have also contributed to increase in growth rate with increase in fish meal substitution levels with soybean-blood meal mixture up to 75%. Results concur with reported 75% fish meal substitution level with blood meal without affecting growth performance in Mozabique tilapia by researchers (Agebebi *et al*., 2009; Bekibele *et al*., 2013). Kirimi *et al*. (2016b) in their study reported that blood meal substituted fish meal up to 50% in Nile tilapia fish when blood was subjected to cooking at 100^0C for 45 minutes. Exposure of blood to high temperatures for a long period of time during processing denatures proteins and reduces their bioavailability (Batterham *et al.,* 1996). This may be the cause of lower growth rate with increase in fish meal substitution levels with blood meal than results reported in this study. It was noted that mixing plant protein feeds with animal protein feed ingredients enhances quality of compounded fish feeds (Huet, 1994; Sadiku and Jauncey, 1995)

Total fish meal substitution (100%) with soybean-blood meal mixture resulted to drastic drop in growth performance. Nogueira *et al.* (2012); Kirimi *et al*. (2016b) also observed drop in growth performance with total fish meal substitution with blood meal in sea bream and Nile tilapia fish respectively. Poor growth performance reported may have been caused by imbalanced essential amino acids in the diets as soybean meal and blood meal feed ingredients are known to have low levels of amino acid methionine (NRC, 1993). Crude fibre in total fish meal substitution diet was highest due to increased amount of soybean meal which had high amount of fibre. High fibre content in fish diets interfere with digestion of feeds leading to poor growth performance and increased feed conversion ratio (De Silva and Anderson, 1995). De Silva and Anderson (1995) recommended crude fibre content of 8% to 12%

in tilapia diets. Though total fish meal substitution formulated diet had less than recommended amount of crude fibre, it may have played part in lowering digestibility of feeds hence poor growth performance and high feed conversion ratio realized in this study. There could be animal growth factors in fish meal that are not present in soybean meal and blood meal that when fish meal is not part of the fish diet causes sharp drop in growth performance. Growth curves indicated low growth rate at the initial stage up to week six after which there was an exponential growth rate for all treatment diets (**Figure 4.1**). This may be attributed to acclimatization to new feed types. High growth rate of tilapia fish is realized at temperatures ranging from 28^0C to 36^0C, pH from 7 to 9 and dissolved oxygen at concentration greater than 3 mg L^{-1} (Ross, 2000; Boyd, 2004; FAO, 2012). The water parameters were within the range for optimum biochemical reactions in digestion and metabolic utilization of feeds to enhance growth rate.

5.2.2 Effect of substitution on survival rate

Survival rate was high ranging from 92% in total fish meal substitution diet (100%) to 97.7% in 75% substitution diet and statistically similar ($p>0.05$) for all diets which were slightly lower than results of 98% reported by Kirimi *et al*. (2016b) when Nile tilapia of initial weight (12 g) were fed on fish meal substituted with blood meal diets in a fertilized pond. They also reported high and statistically similar survival rate in their study where fish meal was substituted at 0, 50 and 100% with blood meal. Results were lower in this study because the fingerlings were younger and smaller (0.7 g) initial weight which are more susceptible to stress than bigger fish. The high rate of survival may be as a result of proper management and handling practices put in place to minimize fish stress. Water pH was maintained by siphoning faeces at the

bottom of aquaria every day and refilling with fresh clean water. Dissolved oxygen was supplied and maintained by water aeration while optimal temperature was maintained by thermostats. During sampling fish were scooped and placed in a container with some amount of fresh clean water. Eyo and Olatunde (1999) reported high mortality in fish group fed on high blood meal levels in their study. It was not clearly understood as to what caused the high mortality in fish. Results reported agree with findings by Agbebi *et al*. (2009) in their study in which blood meal completely replaced fish meal in juvenile Cat fish (*Clarias gariepinus*). A hundred (100%) survival rate was reported in an experiment in which blood meal totally substituted fish meal in Nile tilapia fingerlings (Aladetohun and Sogbesan, 2013).

5.3 Effects of Fish Meal Substitution with Soybean-Blood Meal Mixture on Carcass Composition of Nile Tilapia Fish

Carcass moisture content was close to figures reported by authors in their studies (El-Sayed *et al*., 2013; Sumi *et al*., 2014). Carcass crude protein and crude lipids content slightly increased with increase in fish meal substitution up to 75% substitution and reduced at 100%. The findings of Sumi *et al*. (2014) contradicted the study results in which Nile tilapia fingerlings were subjected to animal (bone & meat meal) and plant (soybean meal) based diets for 30 days in their study. They found slightly higher figures of carcass protein in diets with high amounts of animal protein. However, figures varying from 35.47% to 54.43% reported in this study were slightly lower than figures reported by Mohamed (2009) who found values ranging from 64.5 to 66.53% when Nile tilapia fingerlings were fed on varying levels of dietary protein content (17, 25, 30 and 35%) for 180 days. Dietary protein in the formulated treatment diets in the study was 30% and reported carcass crude protein ranging from

34.47% to 54.43% when they were fed for fourteen weeks. Carcass crude lipids were inversely proportional to crude protein in Mohamed (2009) study while the study recorded a slight increase with replacement level up to 75% and a drop in total fish meal substitution level indicating a directly proportional relationship contrary to findings by Muhamed (2009).

The carcass lipid content at 30% dietary crude protein levels in Muhamed (2009) study was close to figures found in the current study ranging from 12.5% to 18.29%. Total fish meal substitution diet (100%) performed poorly on growth rate and also had lowest values of carcass crude protein and crude lipids. This may have been caused by low digestibility and utilization of the diet as reflected in higher feed conversion ration reported. 75% fish meal substitution diet on the other hand had higher growth rate and lowest feed conversion ratio. This confirms that 75% fish meal substitution was highly digested and utilized by the fish and was reflected in the slightly higher figures of carcass protein and lipid content. High dietary crude fibre interferes with feed digestion and nutrient assimilation which in turn is reflected in carcass nutrient composition as reported by Tacon *et al*. (2009) who found high digestibility and nutrient assimilation in fish meal compared to sunflower meal with high dietary fibre content. Though the study did not cover digestibility of the formulated treatment diets, it is suggested for investigators to put digestibility tests into consideration as they have bearing on nutrient assimilation and carcass nutrient composition. Khalid *et al*. (2014) noted that nutrient retention depends on the quality and quantity of feeds fed which are reflected in carcass nutrient composition. Rehman *et al*. (2013) in their study on carcass nutrient composition in *Labeo rohita* fish fed on soybean meal as alternative protein source to fish meal found reduced dry matter, crude fat and ash

contents with high crude protein content. There was no effect on carcass ash content in this study indicating that fish meal substitution with soybean-blood meal mixture has no effect on carcass ash content. Results on carcass ash content were in agreement with findings which indicated no significant difference by authors (Muhamed, 2009; Opiyo *et al*., 2014). Ngugi *et al*. (2017) found no significant effect on carcass moisture and protein content while lipids and ash content reduced when they replaced fish meal with amaranth leaf protein concentrate in formulated Nile tilapia diets.

5.4 Effect of Using Soybean-Blood Meal Mixture to Substitute Fish Meal in Nile Tilapia Diets on Gross Margin

Gross margin analysis of the diets used showed high returns at 75% fish meal substitution diet and low returns realized at 0% fish meal substitution level. It was established that 75% fish meal substitution with soybean-blood meal mixture in compounding fish diets is more profitable than to use fish meal as a single protein feed ingredient in Nile tilapia farming. Kirimi *et al*. (2016b) reported high incidence cost in fish meal-based diets, low incidence cost and high profit index in blood meal based diet in Nile tilapia. Blood is considered a waste in abattoirs, slaughter houses and slaughter slabs in Kenya and often leads to pollution of the environment. Small amount of money was paid to people who assisted in blood collection. Soybean meal was less expensive than fish meal therefore combination of the two resulted into low cost diets hence contributing to high gross margin realized.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

i) EAAI indices and PRI indices of the treatment diets reveal that soybean-blood meal mixture can be used as alternative protein source to expensive fish meal (*Rastrineobola argentea*). Amino acid chemical score indicated tryptophan to be the most limiting amino acid in all diets.

ii) Growth performance, however, indicated that 75% fish meal substitution had highest growth rate while total fish meal substitution showed poor performance.

iii) Fish carcass nutrient composition revealed that 50 and 75% fish meal substitution had highest carcass crude protein. Slight difference was reported in carcass crude fat content while no effect on carcass moisture and ash content.

iv) Gross margin analysis demonstrated better returns at 75% fish meal substitution.

6.2 Recommendations

i) Addition of the amino acid tryptophan is recommended in fish meal substitution with soybean-blood meal diets to improve its dietary concentration.

ii) 75% fish meal substitution is recommended to get highest growth rate and better returns.

iii) For higher carcass protein, 50 and 75% fish meal substitution is recommended. Digestibility study is recommended as it has a bearing on nutrient utilization.

iv) Bigger Nile tilapia fingerlings and longer experimental period in ponds is recommended. Further research is also recommended on fish meal substitution with soybean-blood meal on other farmed fish species

REFERENCES

Abou, Y., Fiogbe, D., Aina, M. P., & Micha, J. (2013). Growth and Fatty Acid Composition of Nile tilapia (*Oreochromis niloticus L.*) Fed Azolla- Diets in Tanks and Earthen Ponds. A comparative Study. *Natural Sciences* 5(1) 77-83

Abowei, J. F. & Ekubo A. K. (2011).A Review of Conventional and Unconventional Feeds in Fish m. Agricultural Engineering Service Publication, No. NRAES – 106

Agbebi, O. T., Otubusin, S. O., & Ogunleye, F. O. (2009). Effect of Different Levels of Substitution of Fishmeal with Blood Meal in Pelleted Feeds on Cat Fish *Clarias gariepinus* (Burchel,1822) Culture in Net Cages. *European Journal of Scientific Research.*31 (1):6-10.

Ahmad, M.H (2008) Response of African Catfish, *Clarias gariepinus*, to different dietary protein and lipid levels in practical diets. *J World Aquac Soc* 39(4):541–548

Ahmed, N. (2007). Economics of aquaculture feeding practices: Bangladesh. In M.R. (ed.). Economics of aquaculture feeding practices in selected Asian countries. FAO. Fisheries Technical Paper. No. 505. FAO, Rome, PP. 33-64

Aladetohun, N. F. and Sogbesan, O. A. (2013): Utilization of blood meal as a protein ingredient from animal waste product in the diet of *Oreochromis niloticus*. *International Journal of Fisheries and Aquaculture*, 5(9):234 – 237.

Ali, M., Iqbal, F., Salam, A., Sial, F., &Ather, M. (2006).Comparative Study of Body Composition of Four Fish Species in Relation to Pond Depth. *International Journal of Environmental Science and Technology 2: 359-364*

Aloo, O. P. (2010). Contribution of the Fisheries Sector Towards Food Security and Poverty Alleviation in Kenya. Tropentag: *World Food Systems- A Contribution from Europe*

Anderson, J., Jackson, A., Matty, A. J., & Capper, B. S. (1984).Effects of Dietary Carbohydrate and Fibre on Tilapia *Oreochromis niloticus* (Linn).*Aquaculture* 37: 303- 314.

Anderson, S., &Lall, S. P. (2005). Amino Acid Nutrition of Salmonids: Dietary Requirement and Bio availability In: Montero.(ed) Basuroo B (ed).Al exi M.(ed) Izquierdo M(ed). *Mediterranean Fish Nutrition.Zaragoza:CIHEAM, 2005.P73-90 (Cahiers Options Mediterranean n.63*

Animal Feed Resources Information System (AFRIS), 2010. file://C:Documents%20and %20Setting/ADMIN/My%Document Retrieved 26/1/2017

Anjum, M. A, Hussain, Z., Khan, S.H., Ahmad, N., Muhammad, Y. A. and Iftikhar, N. (2014). Assessment of Poultry Feed Ingredients Used in Commercial Compound Feeds. *Pakstan Journal of Life and Social Sciences.*, 12(2): 69-73

Anjum, M. A, Khan, S.H, Sahota A. W. and Sardar R (2012). Assessment of aflatoxin B1 in commercial poultry feed and feed ingredients. *The Journal of Animal and Plant Sciences*, 22: 268-272.

AOAC.(1995).Official Methods of Analysis 16th Ed; Association of Official Analytical Chemists International, Arlington VA, USA.

AOAC.(2005) Official methods of Analysis 18 ed. Association of official Analytical chemist.

Asadujjaman, M., Biswas, S., Manirujjaman, M., Rahman, M., Hossain, M.A, Islam, M. A. (2014) Determination of Protein, Lipid and Carbohydrate Contents of Conventional and Non-Conventional Feed Items Used in Carp Polyculture Pond. *Fisheries and Aquaculture Journal*, 5: 109. doi: 10.4172/2150-3508.1000109

Baltic , Z. M., Markovic, R. &Dordevic, V. (2011). Nutrition and Meat Quality.*Plenary paper on International 56 Meat Industry Conference held on Tara mountain;*

Banan Khojasten (2012). The Morphology of the post gastric alimentary canal in teleost fishes. *International Journal of Aquatic Science*, ISSN: 2008-8019,Vol 3, No. 2, 2012

Batterham, E. S., Lowe, R. F., & Darnell, R. E. (1986). Availability of Lysine in Meat Meal, Meat and Bone Meal and Blood Meal as Determined by the Slope-Ratio Assay with Growing Pigs, Rats and Chicks and by Chemical Techniques. *British Journal of Nutrition. 55:427-440.*

Bekibele, D. O., Ansa, E. J., Agokei, E. O., Opara, J. Y., Alozie-chidi, V. C. Arango, A. A., Ibemere, I. (2013). The comparative effect of fish and blood meal based diets on growth and survival of juvenile tilapia (*Oreochromis niloticus*) in concrete tank. *Journal of Fisheries and Aquatic Science, 8*(1)184-189

Boyd, E. C. (2004). Farm- Level Issues in Aquaculture Certification: Tilapia. Report by WWF-US- in 2004. Auburn University, Alabama.Brussels, Belgium

Buddington R. K. and Diamond J. (1986) Pyloric caeca of fish: A new absorptive organ. *Am. J. Physiology*, 252: 665-676

Bureau, D. P., Harris, A. M. and Cho, C. Y. (1999). Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 180, 345–358.

Bunda, M.G.B., Tumbokon, B.L.M. and Serrano Jnr, A.E. (2015). Composition, Chemical Score (CS) and Essential Amino Acid Index (EAAI) of the Crinkle Grass (Rhizoclonium spp.) as Ingredient for Aqua Feeds. Aquaculture, aquarium, conservation and legislation. *International Journal Bioflux* 8, pp 411–420.

Caruso, G. (2015). Use of Plant Products as Candidate Fish Meal Substitutes: An Emerging Issue in Aquaculture Productions. *Fish Aquac J* 6: pp 123.

Caulton, M. S. (1982). Feeding, metabolism and growth of tilapias- some quantitative considerations; *In*: Pullin R.S.V. and Lowe-McConnell R.H. (eds), The Biology and Culture of Tilapia. ICLARM, Manila pp.157–184.

Charo, H., Komen, H., Reynolds, S., Rezk, M. A., Ponzoni, R. W., &Bovenhuis, H. (2006). Genetic and Environmental Factors Affecting Growth of Nile Tilapia (*Oreochromis niloticus*) Juveniles: Modelling Spatial Correlations between Hapas. *Aquaculture* 255:586-596.

Cho, C. Y., and S. J. Slinger. (1979). Effect of water temperature on energy utilization in rainbow trout. Proceedings of the Eighth Symposium on Energy Metabolism in Farm Animals, S. E. Mont, ed. Cambridge, U.K.: Cambridge University Press, Pp. 287-291

Chung, T. K., and D. H. Baker. (1992). Maximal Portion of the Young Pig's Sulfur Amino Acid Requirement that can be Furnished by Cystine. *Journal. Of Animal. Science*. 70:1182–1187.

Craig S., and Helfrich L. (2007) Understanding Fish Nutrition, Feeds, and Feeding.*Virginia state university publication* No 420-256

Davies, S. J., Williamson, J., Robinson, M., Bateson, R. I. (1989): Practical inclusion levels of common and by products on complete diets for tilapia (Oreochromis mossambicus). In proc. Third Intl. Symp.On feeding and Nutri.In Fish, Tisa Japan. 325 - 332.

De Silva, S. S. & Anderson, T. A. (1995). Fish Nutrition in Aquaculture. *Chapman & Hall Aquaculture series. London.* pp 319

Dersjant-Li, Y., (2002).The use of soy protein in aquafeeds. In: L. E. Cruz- Suárez, D. Ricque-Marie, M. Tapia-Salazar, M. G. Gaxiola-Cortés and N. Simoes, eds. *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola.* Cancun, Mexico: pp. 541

Devendra, C. (1988) General approaches to Animal Nutrition Research and their relevance to Fish Production in the Asian Region. In Fin Fish Nutrition Research in Asia: Proceedings of the Second Asian Fish Nutrition Network Meeting, De Silva, S.S(1988); pp 7-22.

Dupree, H. K. &Huner, J. V. (1984). Third Report to the Fish Farmers: Nutrition, Feeds and Feeding Practices: US Fisheries and Wild Life pp 141-147.

El-Haroun, E. R. and Bureau, D. P. (2007).Comparison of the bioavailability of lysine in blood meals of various origins to that of L-lysine HCL for rainbow trout (*Oncorhynchus mykiss*).*Aquaculture*, 262, pp. 402–409.

El-Sayed, A. M. (2006). Tilapia Culture in Salt Water: Environmental Requirements, Nutritional Implications and Economic Potentials. Eighth Symposium on Advances in Nutrition Aquaculture. November 15-17, Nuevo Leon, Mexico

El-Sayed A. F. M, El-Ghobashy, A.E. and El-Mezayen, M.M. (2013). Effect of feed colour on growth and feed utilization of Nile tilapia (*Oreochromis niloticus*) larvae and fingerlings. *Aquaculture Nutrition*; 19, pp 870-876

El-Sherif, M. S., and A. M. EL-Feky.(2008). Effect of ammonia on Nile Tilapia (*O. niloticus*) performance and some hematological and histological measures.Eighth International Symposium on Tilapia in Aquaculture. Cairo, Egypt

Enami, H. R. (2011). A Review of Using Canola/ Rape Seed Meal in Aquaculture Feeding.*Journal of Fisheries and Aquatic Science, 6*(1):22-36.

Eyo, A. A. and Olatunde, A. A. (1999). The Effect of Replacement of Soyabean Meal with Blood Meal on the Growth of Mudfish (*Clarias anguillaris L.)* fingerlings, *13th Annual Conference of the Fisheries Society of Nigeria* (*FISON*), (pp. 232-240). Retrieved from http://aquaticcommons.org/3861/1/13P232.pdf

Fagbenro, O. A. and Davies, S. J. (2000**):** Use of oilseed meals as fish meal replacer in tilapia diets. Proc.5th Inter. Symp.On Tilapia in Aquaculture, Rio Dejaneiro-Brasil, 3-7 September, 2000.

FAO, (2012).The State of World Fisheries and Aquaculture.Food and Agriculture Organization United Nations.Rome Italy.

FAO, (2013): Food and Agriculture Organization. The State of Food Insecurity in the World.The multiple dimensions of food security. Rome, 56

FAO, (2014). The State of World Fisheries and Aquaculture: Opportunities and Challenges, Food and Agriculture Organization of the United Nation, Rome, Italy, pp 243.

FAO, (2017). World Aquaculture 2015. A Brief Overview by Rohana Subasinghe, Fisheries and Aquaculutre Cicular No. 1140, Rome, Italy. pp 47

Francesco, M. DE., Paris, G., Perez-Sanchez, J., Gomez- Requeni, P., Medale, F., Kaushik, S.J. , Mecatti, M. and Poli, B.M. (2007).Effect of high level fish meal replacement by plant proteins in gilthead seabram *Sparus aurata* on growth and body/filet quality traits. *Aquaculture Nutrition*, vol. 13, pp. 361–372

Fuller, M. F., McWilliam, R., Wang, T.C & Giles. L. R. (1989). The Optimum Dietary Amino Acid Pattern for Growing Pigs 2. Requirements for Maintainance and for Tissue Protein Accretion. *British journal of nutrition.* 62, 255-267

Gatlin, D. M. (2010).Principles of Fish Nutrition. Southern Regional Aquaculture Center.Publication No. 5003.

Gaylord, T. G., Rawles, S. D., & Gathin, D. M. (2004). Amino Acid Availability from Animal Blended and Plant Feed Stuffs for Hybrid Striped Bass (*Moronechrysops x M. saxatilis*). *Aquaculture Nutrition,* 10(5): 345-352

Genten F., Trewinghe E. and Danguy A. (2009) Digestive System. In: Atlas of Fish Histology,Science Publishers,75-91.

Gilles, S.; Lacroix, G.; Corein, D.; Ba, N.; Luna, C. L.; Nandjul, J.; Ouattara, A.; Oue´Draogo, O.; Lazzaro, X. (2008) Mutualism between euryhaline tilapia *Sarotherodon melanotheron heudelotii* and *Chlorella* sp.— Implications for nano-algal production in warm water phytoplankton-based re-circulating systems. Aquacultural Engineering, v. 39, n. 2-3, pp. 113-121.

Government of Kenya (2010). Agriculture Sector Development Strategy 2010-2020.Nairobi: Kenya.

Guroy, D., Emrah, D., Kut, G., & Adem, A. (2005). Influence of Feeding Frequency and Feed Intake, Growth Performance and Nutrient Utilisation in European Sea Bass (*Dicentrarchus labrax*) Fed Pelleted or Extruded Diets. *Turkish Journal of Veterinary Animal Science. 30(2006) 171-177.*

Hamilton B. S., Xiangjie, S. & Changik, C. (2012). Acquisition of a Novel Eleven Amino Acid Insertion Directly N terminal to a Tetra Basic Cleavage Site Confers Intracellular Cleavage of an H7N7 Influenza Virus Hemagglutinin. *Journal of Virology. 434(1): 88–95*

Harris, J. O., Maguire, B. G., Edwards, S., & Hindrum, S. M. (1998). Effect of Ammonia on the Growth Rate and Oxygen Consumption of Juvenile Greenlip Abalone, *Haliotis laeuigata Donovan. Aquaculture 160, 259-272.*

Hepher, B. (1988).Nutrition Requirements in Nutrition of Pond Fishes. Cambridge University Press, Cambridge, UK. PP. 175-216.

Hixson S. M. (2014). Fish Nutrition and Current Issues In Aquaculture: The Balance In Providing Safe and Nutritious SeaFood, In An Environmentally Sustainable Manner. *Journal of Aquaculture Resource Development,* 5(3): 234

Huet, M. (1994). Textbook of fish culture: Breeding and cultivation of fish. *Fishing International 2004. Nutrition Abstracts and Reviews: Series B, 74*(9), 23-32.

Hussain, S. M., Afzal, M., Salim, M., Javid, A., Khichi, T. A. A., Hussain, M. (2011): apparent digestibility of fish meal, blood meal and meat meal for *labeo rohita* fingerlings. *The Journal of Animal & Plant Sciences,* 21(4): 807 – 81

Ijarotimi, O. S. and Keshinro, O. O. (2011). Determination of Amino Acid, Fatty Acid, Mineral, Functional and Choking Properties of Germinated and Fermented Popcorn (*Zea mays everta*) Flour. *European Journal of Food Research & Review,* 1(2): 102-122

Ip, Y. K., Chew, S. F., & Randall, D. J. (2001).Ammonia Toxicity, Tolerance and Excretion. In: Wright, P.A, Anderson, P.M (Eds.) Fish Physiology, Vol.20. Academic Press, New York; pp 109-148.

Iqbal, F., Ali, M., Umer, K., & Rona, S. A. (2006).Effect of Feed Cycling on Specific Growth Rate, Condition Factor, Body Composition and RNA/ DNA Ratio of *Cirrhinus mrigala.Journal of Applied Sciences and Environmental Management.10 (2): 129-133.*

Jaetzold, R., and Schmidt, H. (2008).Farm management handbook of Kenya. Natural conditions and farm management information-Western Kenya. Nairobi.

Jacquet, J., Hocevar, J., Lai, S., Majluf, P., Pelletier, N., Pitcher, T. (2010): Conserving wild fish in a sea of market-based efforts. Oryx: The International Journal of Conservation, 44 (1): pp 45 – 56.

Jaffe, W. G. (1980). Toxic constituents of plant foodstuffs, I. E. Liener, ed. New York: Academic Press; pp. 73-102

Jahan, D. A., Hussain, L., Islam, A. M., Khan, M. M. and Nima, A. (2012).Use of soybean as partial substitute of fish meal in diets of *Rohu, Labeo rohita* (Ham) fry.*A scientific Journal of Krishi foundation*; The Agriculture 10 (2); 68-76.

Jauncey K. and Ross B., (1982) *A guide to Tilapia feeds and feeding*. Institute of Aquaculture Stirling. Uk. 111pp

Jauncey, K. (1998). Tilapia Feeds and Feeding. Stirling, Scotland: Pisces Press

Jauncey, K. (2000). Nutritional Requirements. In: Beveridge, M.C.M & McAndrew, B. J (eds.) Tilapias: Biology and Exploitation, Fish and Fisheries Series 25, Kluwa Academic Publishers, Dordrecht. The Netherlands, pp. 327- 375

Kapour, A. C. And Heiner R. E. (1982). Biochemical changes in developing wheat grains. Changes in nitrogen fractions, amino acids and nutritional quality. *Journal of. Science. Food Agriculture*. 33: 35

Kenya National Bureau of Statistics (KNBS). (2017). Economic Survey 2017, Kenya National Bureau of Statistics, Nairobi, Kenya, pp 333.

Kenya National Bureau of Statistics (KNBS). (2018). Economic Survey 2018, Kenya National Bureau of Statistics, Nairobi, Kenya

Khalafalla, M. M., Bassiouni, M. I., Eweedah, N. M., & Elmezyne, H. M. (2010).Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings Fed Diets Containing Different Levels of Amecozyme. *Journal of Agricultural Research.Kafer El- sheikh University.*,36 (2):2010

Khalid, J. I., Muhammed, A., Farzana, A., Arshad, J., Muhammed, H., Sumaira, A., Fayyaz, R., Noor khan, A & Muhammed, A, (2014). Effect of Plant - Fishmeal and Plant By- Products Based Feed on Growth, Body Composition and Organoleptic Flesh Qualities of *labeo rohita*. *Pakistan Journal of Zoology;* vol 46(1):253-260

Kirimi, J. G., Musalia, L. M., & Munguti, J. M. (2016a).Effect of replacing fish meal with blood meal on chemical composition of supplement for Nile tilapia (*Oreochromisniloticus*).*East African Agricultural and Forestry Journal*, 8(1):1-9. http://dx.doi.org/10.1080/00128325.2016.1158898

Kirimi, J. G., Musalia, L. M., Magana, A. and Munguti, J. M. (2016b). Performance of Nile tilapia (*Oreochromis niloticus)* fed diets containing blood meal as replacement of fish meal. *Journal of Agricultural Science*; Vol. 8 (8) 79-87

Kissil, G.W., Lupatsch, I., Higgss, D.A. and Hardy, R.W. (2000).Diatray substation of soy and rapeseed meal protein concentrates for fish meal and their utilization in gilthead seabram*Sparusaurata*. *Aquaculture Research*, vo. l31, pp. 595–601.

Kubaryk, J.M (1980) Effect of diet, feeding schedule and sex on food consumption, growth and retention of protein and energy by tilapia. PH.D. Diss., Auburn University, Auburn, AL.

Lall, S. P. (2002). The Minerals in Fish Nutrition.Hawer, J. E & Hardy, R.W. (Eds) 3rd edition. London: Academic Press pp 308-359

Liener, I. E., and. Kakade. M. L. (1980). Protease inhibitors. Pp. 7-71in Toxic Constituents of Plant Foodstuffs, 2d ed., I. E. Liener, ed. NewYork: Academic Press.

Liti, D.M., Cherop L., Munguti, J.M., and Chhorn L. (2005) Growth and Economic performance of Nile tilapia (*Oreochromis niloticus*) fed on two formulated diets and two locally

Liu, D.C. (2009). Better Utilization of By-Products from the meat industry. Department of Animal Science. National Chung-Hising University Taichung Taiwan Roc.www.agnet.org/library/teb/515/eb/515.pdf

Lovell, R. T. (1988). Use of Soya Bean Products in Diets for Aquaculture Species.*Journal of Aquaculture Production,* 2:27-52.

Luzier, J. M., Summerfelt, R. C., &Ketola, H. G. (1995). Partial Replacement of Fish Meal with Spray Dried Blood Powder to Reduce Phosphorus Concentrations in Diets of Juvenile Rainbow Trout, *Oncorhynchus mykiss*(Walbaum).*Aquaculture Nutrition* 26: 577-587

Magid, A., &Babiker, M. M. (1975).Oxygen Consumption and Respiratory Behaviour of Three Nile Fishes.*Hydro- biologia,* 46.359-367.

Manual of Standard Operating Procedures for Fish Inspection and Quality Assurance in Kenya, Government of Kenya (2015), *Government printer*

Mbugua, H. M. (2008). Aquaculture in Kenya, Status, Challenges and Opportunities, Directorate of Aquaculture, Kenya.

Mickelsen, O., and Yang, M. G (1966).Naturally-occurring toxicants in foods. FASEB25: 104-123.

Mires, D. (1995).The tilapias.. *In*: Production of Aquatic Animals: Fishes (eds Nash, C. E., and A. J. Novotony. Elsevier, New York, pp. 133–152

Moreira, L. R., Seveira, P. L., Teixeira, G.E., Moreira, L.G. A, Moura, S. P. and Farias, L. R. W. (2012). Growth and gastrointestinal indeces in Nile tilapia fed with different diets; *Acta Scientiarum*, No. 3, v 34: pg 223-229

Mothwa, M., Jacobs, A. and Dlamini, R. (2013). Protein digestibility of soybean, canola, and sunflower, and its effect on growth performance and body. Aquaculture conference, Stellenbosch, pp 9-13

Muhamed, H. B. (2009). Effect of Dietary Protein Level on Growth Performance and Body Composition of Monosex Nile tilapia (*Oreochromis niloticus L.*) Reared in Fertilized Tanks. *Pakistan Journal of Nutrition*, 8 (5) pp 674-678

Mune-Mune, M.A., Minka, S.R, Mbome, I.L., Etoa, F.X. (2011). Nutritional Potential of Bambara Bean Protein Concentrate. *Pak. J. Nutrition*. 10 (2), pp 112-119.

Munguti, J., Charo-Karisa H., Opiyo, M. A., Ogello, E. O., Marijani, E., Nzayisenga, L & Liti, D. (2012). Nutritive Value and Availability of Commonly Used Feed

Munguti, J. M., Mugiranea, J. K., Ogello E. O. (2014). An Overview of Kenyan Aquaculture Sector; Current status, Challenges and Opportunities for Future Development. *Fisheries and Aquatic sciences* 2014; 17(1):1-11.

Musa, S.Aura M.C, Owiti G, Nyonje B, Orina P and Charo-Karisa H. (2012). Fish farming enterprise productivity program (FFEPP) as an impetus to *Oreochromis niloticus*(L.) farming in Western Kenya: lessons to learn. *Afr J Agric Res*, 7, 1324-1330. http://dx.doi.org/10.5897/AJAR11.1608.

Ngugi, C.C., Okoth, O.E., Manyala, J.O., Fitizsimmons, K. and Kimotho, A. (2017). Characterization of nutritional quality of amaranth leaf protein concentrate and suitability of fish meal replacement in Nile tilapia feeds, *Elsevier Aquaculture Reports*, 5:62-69

Nguyen, T. N., Davis, A., & Saoud, I. P. (2009). Evaluation of Alternative Protein Sources to Replace Fishmeal in Practical Diets for Juvenile Tilapia, *Oreochromis spp*. *Journal of the World Aquaculture Society,* 40(1): 113-121.

Nogueira, N., Cordeiro, N., Andrade, C. and Aires, T., (2012). Inclusion of low levels of blood and feathermeal in practical diets for gilthead seabream (*Sparus aurata*).*Turkish Journal of Fisheries and Aquatic Sciences,* 12, pp. 641-650.

Noor, E. L., Deen, A. I., & Mona, S. Z. (2010).Impact of Climate Changes (Oxygen and Temperature) on Growth and Survival Rate of Nile Tilapia (*Oreochromis niloticus*) Report and Opinion 2:192- 195.

Noor, K., Aziz, N., Nasir, M., Rasool, F., &Iqbal, F. (2011). Effects of Artificial Diet and Culture Systems on Sensory Quality of Fried Fish Flesh of Indian Major Carps

NRC.(1993). Nutrients Requirements of Fish.National Academy Press, Washington, DC USA.

NRC.(2011) Nutrient requirements of fish and shrimp. Washington, DC, USA: National Academies Press, pp 376

.

Nyonje, B.M, Charo-Karisa H, Macharia SK and Mbugua M. (2011). Aquaculture Development in Kenya: Status, Potential and Challenges. In Samaki News: Aquaculture Development in Kenya towards Food Security, Poverty Alleviation and Wealth Creation. Vol. 7.No. 1. pp. 8-11.

Nyonje, B.M., Opiyo, M.A., Orina, P.S., Abwao, J., Wainaina, M., and Charo-Karisa, H. (2018). Current status of freshwater fish hatcheries, brood stock management and fingerlings production in the Kenya aquaculture sector. Livestock Research for Rural Development. (1) pp. 30.

Ogello, E. O., Safina, M., Aura, C. M., Abwao, J. O., Munguti, J. M. (2014): A critical appraisal of feasibility of tilapia production in earthen ponds using biofloc technology, a review. *International Journal of Aquatic Sciences*, **5** (1): 21 – 39.

Olukayode, A. M and Emmanuel, B. S. (2012): The potential of two vegetable-carried blood meals as protein sources in African catfish (*Clarias gariepinus* Burchell) juvenile diet. *Open Journal of Animal Science***, 2**(1):15 -18.

Omasaki, S. K., Charo-Karisa, H & Kosgey, I. (2013). Fish Production Practices of Smallholder Farmers in Western Kenya

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

Osama, M. El-Husseiny, Galal El- Din, M. Abdul-Aziz & Rania S Mabroke (2008). Effect of mixed protein schedules combined with choline and betaine on the growth performance of Nile tilapia *Oreochromis niloticus. Aquaculture Research* 39, 2910-300

Oser, B. L., (1959). An Integrated Essential Amino Acid Index for Predicting the Biological Value of Proteins. In: Protein and Amino Acid Nutrition. Albanese A. A. (ed), Academic Press, New York, USA, pp. 281-295.

Otieno. M. J. (2011). Fishery Value Chain Analysis: Background Report – Kenya. FAO, Rome, IT, pp. 2-10.

Otubusin, S. O., Ogunleye, F. O., & Agbebi, O. T. (2009). Feeding Trials Using Local Protein Sources to Replace Fish meal in Pelleted Feeds in Cat fish (*Clariasgariepinus* Burchell 1822) Culture. *European Journal of Science Research, 31*(1), 142-147

Popma, T.J., (1982) Digestibility of selected feedstuffs and naturally occurring algae by tilapia. Ph.D. dissertation.Auburn University, Auburn, Alabama.

Popma, T. and Michael. M. (1999) *Tilapia life history and biology*. SRAC Publication No. 23. Pp. 1-5.

Prado, S. P. J., Cavalheiro, O. M. J., Silva, A. J., Cavalheiro, B. T. and Silva, G. V. F. (2016). Amino Acid Profile and Percent Composition of Meals and Feeds Used In Shrimp Farming. *Gaia Scientia*, 10(4): 347-360.

Pullin, R. S. V. (1988).Tilapia Genetic Resources for Aquaculture.ICLARM Conference Proceedings 16. International Centre of Living Aquatic Resources Management, Manila, Philippines., pp 108.

Rasmussen, R. S. (2001). Quality of Farmed Salmonids with Emphasis on Proximate Composition, Yield, and Sensory Characteristics.*Aquaculture Research*.32: 767- 786.

Rao, P. B. R., Norton H. W. And Johnsn, B. C. (1964).The Amino Acid Composition and Nutritive Value of Proteins: The Amino Acid Requirements As A pattern For Protein Evaluation. *Journal of Nutrition*. 82: 88

Rehman, T., Asad, F., Qureshi, A.N. and Iqbal, S. (2013): Effects of Plant Feed Ingredients (soybean and sunflower meal) on Growth and Body composition of *Labeo rohita. American Journal of life science,* vol. 1, No. 3, pp 125-129

Redner, B. D., and R. R. Stickney. (1979). Acclimation to ammonia by Tilapia aurea. Trans. Am. Fish. Soc.108:383–38

Reinthal, P. N. (1989). The gross intestinal morphology of a group rock-dwelling Cichlidae fishes from Lake Malawi. *Neth j zool* 39:208-225

Reay,P.J. (1979) Aquaculture. The institute of Biology's Studies in Biology no.106. pp 31-40.

Roberts, R. J. (1978). Fish Pathology.A BailliereTindall Book, Publ. Cassell Ltd.

Robinson,E., Li, M., & Brunson. (1998). Feeding Cat Fish in Commercial Ponds. Southern Regional Aquaculture center publication 181.

Ross, L. G. (2000). Environmental Physiology and Energetic.pp.89-128.In:M.C.M Beveridge and B.JMcAndrew (eds.).Tilapia Biology and Exploitation, Fish and Fisheries 25, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sadiku, S. O. E., Jauncey, K. (1995): Soybean flour–poultry meat meal blends as dietary protein source in practical diets of Oreochromis niloticus and Clarias gariepinus. *Asian Fisheries Science*, **8** (2): 159 –168.

Santiago, C. B. And Laron, M. (2002): Growth and fry production of Nile tilapia Oreochromis niloticus (L) on different feeding schedules. Aquaculture Research**,** 33: 129 – 136

Schulz, C., Huber, M. and Ofunji J. (2008). Effects of varying dietary protein to lipid ratios on growthperformance and body composition of juvenile pike perch (Sander lucioperca). *Aquac Nutr* 14:166–173. doi:10.1111/J.1365-2095.2005.00369.

Sergent, J. R., Bell, J. G., Bell, M. V., Handerson, R. J., &Tocher, D. R. (1995).Requirement Criteria for Essential Fatty Acids.*Journal of Applied Ichthyology, 11: 183-198.*

Smart, G. R. (1978). Investigations on the Toxic Mechanisms of Ammonia to Fish Gas Exchange in Rainbow Trout (*Salmogairdneri*) Exposed to Acutely Lethal Concentrations. *Journal of Fish Biology* 12: 93-104.

Smith, R. R.(1976). Metabolizable energy of feedstuffs for trout. Feedstuffs 48: 16-21.

Smith, B. J., Smith, S. A., Tengejaroenkul, B. and Terry, A. L., (2000). Gross Morphology and Topography of the adult intestinal tract of theTilapia fish (Oreochromis niloticus). Cell tissue organs, 166:294-303

State Department of Fisheries (SDF). (2016). Kenya Fish Farming Enterprise Productivity Capacity Assessment and Gap Analysis Report, Directorate of Aquaculture Technologies Development, Kenya, pp 68. (Unpublished)

Storebakken, T., Refstie, S., &Royter, B, (2000). Soy Products as Fat and Protein Sources in Fish Feeds for Intensive Aquaculture. Soy in Animal Nutrition (Ed. by Drackley, J. K).*Feed Animal Science Society,* Savoy, IL, USA, 127-170.

Sveier, H., Nordas, R., Berge, G. E. & Lied, E. (2001). Dietary Inclusion of Crystalline D- and L-Methionine: Effects on Growth, Feed and Protein Utilization, and Digestibility in Small and Large Atlantic Salmon (*Salmon salar* L.). *Aquaculture Nutrition* 7 (3):169-181.

Sumi, K. R., Pilot, A. I., Sharker, M. R., Mandal, S. C., Mokhlasur R. and Alam, M. R. (2014). Effect of Different Live Feeds on The Growth Performance and Flesh Quality of Nile Tilapia (Oreochromis niloticus) Fingerlings. *International Journal of Live Sciences and Biotechnology and Pharma Research*, 3 (2): pp 182-188

Suresh, V. (2003). Tilapias. In: Luc as, J.S. and Southgate, P.C. (eds). Aquaculture: Farming of aquatic animals and plants. *Blackwell Publishing, Oxford*, pp. 321–345.

Tacon, A. G. J., Jackson, A. J. (1985): Utilization of conventional and unconventional protein sources in practical fish feeds. In Nutrition and Feeding in Fish, Cowey CB, Mackie AM, Bell JG. (eds). Academic Press, London, 119 – 145

Tacon, A. G. J. (1987). The Nutrition and Feeding of Farmed Fish and Shrimp- A Training Manual. 1. The Essential Nutrients FAO Field Document, Project GCP/RLA/075/ITA Field Document No. 117.

Tacon, A. G. J. (1993): Feed ingredients for warm water fish. Fish meal and other processed feedstuffs, FAO Fisheries Circular 856: 64.

Tacon, A.G. J (2000).Standard Method for the Nutrition and Feeding of Farmed Fish and Shrimp.*Washington D.C, U.S.A Argent Laboratories press p. 454*

Tacon, A. G. J., Metian, M. and Hasan, M. R. (2009). Feed ingredients and fertilizers for farmed aquatic animals: sources and composition. FAO Fisheries and Aquaculture Technical Paper No. 540., Rome, Italy

Takeuchi T., Satoh S., and Watanabe W. (1983a). Dietary lipids suitable for practical feed of *Tilapia nilotica. . Bulletin of Japanese Society of Fisheries Sciences Fish*. *49* (9): 1361-1365.

Takeuchi, T., Satoh S., and Watanabe W. (1983b). Requirement of *Tilapia niloticas* for essential fatty acids.*Bull.Jap.Soc. Sci*. Fish. 49(7): 1127-1134

Teichert, D.R., Popma, T.J., &Loushin, L.L. (1997). Attributes of Tropical Pond-Cultured Fish pp183-198.In: EgnaH.S& Boyd, C.E. (eds.). Dynamics of Pond Aquaculture.CRC Press, Boca Raton, Florida, USA.

Teshima, S., Kanazawa, A., &Sakamato, M. (1982). Essential Fatty Acids of (*Tilapia nilotica*).*Memoirs of the Faculty of fisheries, Kagoshima University.31.201-204.*

Teshima, S., Kanazawa, A., & Uchiyama, Y. (1985). Optimum Protein Levels in Casein- Gelatine Diets for *Tilapia nilotica*Fingerlings. *Memoirs of the Faculty of Fisheries, Kagoshima University .34: 45-52*

Teshima, S. and Kanazawa, A. (1988).Nutritive value of methionine-enriched soy plastein for *Oreochromis niloticus* fry.*In*: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L. (eds.). 2nd Intl. Symp.on Tilapia in Aquaculture, ICLARM Conf. Proc. No. 15, Manila, Philippines. pp. 393-399

Viola, S. and Zohar, G. (1984): Nutrition studies with market size hybrids of tilapia (Oreochromisniloticus) in intensive culture. *Israel Journal of Aquaculture,* 36: 3 –15.

Viola, S., Arieli, Y. and Zohar, G. (1988). Animal-protein-free feeds for hybrid tilapia (Oreochromisniloticus X O. aureus) in intensive culture. Aquaculture, 75: 115 – 125.

Wang, Y., Guo, J.L. and Li., K. (2006) Effects of dietary protein and energy levels on growth, feed utilizationand body composition of cuneate drum (Nibeamiichthioides). Aquaculture 252:421–428.

Watanabe, W. O., Kuo, C. M., & Huang, M. C. (1985). The Ontogeny of Salinity Tolerance in the Tilapias *Oreochromisaureus*and O. *mossambicus x. O. niloticus*Hybrids, Spawned and Reared in Freshwater. *Aquaculture* 47:353-367.

Watanabe, W. O., Ellington, L. J., Olla, B. L., Ernst, D. H., &Wicklund, R. I. (1990). Salinity Tolerance and Sea Water Survival Vary Ontogenetically in Florida Red Tilapia. *Aquaculture* 87:311-321

Watanabe.T., Takeuchi T., Murakim A., and Daino C. (1980).The availability to Tilapia of phosphorus in white fish meal.*Bulletin of Japanese Society of Fisheries Sciences* 46(7): 897-899.

Wilson, R. P., and W. E. Poe. (1985). Apparent digestibility of protein and energy in feed ingredients for channel catfish. Prog. Fish-Cult.47:154-158.

Wilson, R. P., Robinson, E. H., Gatlin, D. M., & Poe, W. E. (1989). Dietary Phosphorus Requirement of Channel Cat Fish, (*Ictalurus punctatus). Journal of Nutrition, 112(6).*

Wilson, R. P. (2002). Amino acids and Proteins.Fish Nutrition. J. E. Halver and R.W. Editors Fish Nutrition, 3rd Edition. Academic press, San Diego, California, USA.

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

Xue, M., Zhao, H., Xie, S., Wu, X and Guo, L., (2010). Fish meal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) of less than 2g. *Aquaculture Nutrition*, 16: 648-653

Yan, l., Bordinhon, A. M., Davis, A. D. and Zhu, X. (2012). Protein : energy ratio in practical diets for Nile tilapia (Orechromis niloticus). *Aquaculture International Journal*. D01 10.1007/s10499-012-9616-3

Zaki, M. A., Mostafa, A.R., Khalafalla, M. M., Dorina, M., Adrian, T. R., Maria, M. and Ayman, A. H. (2016). Physiological and Nutritional Studies on Improving Growth of Nile Tilapia (Oreochromis niloticus) Fingerlings Using Alpinia Meal (*Alpinia galanga*) As a Feed Additive. *Agriculture and Agricultural Science Procedia*, 8 (10):604-614

APPENDICES

ANOVA FOR PROXIMATE ANALYSIS OF TREATMENT DIETS

Parameter	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	2.114	4	.528	139.066	<0.001
	Within Groups	.038	10	.004		
	Total	2.152	14			
CP	Between Groups	.136	4	.034	62.329	<0.001
	Within Groups	.005	10	.001		
	Total	.142	14			
CF	Between Groups	1.054	4	.264	278.460	<0.001
	Within Groups	.009	10	.001		
	Total	1.064	14			
EE	Between Groups	6.094	4	1.523	9140.560	<0.001
	Within Groups	.002	10	.000		
	Total	6.095	14			
ASH	Between Groups	69.830	4	17.457	2663.905	<0.001
	Within Groups	.066	10	.007		
	Total	69.895	14			

ANOVA FOR AMINO ACID IN TREATMENT DIETS

Parameter	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
LYS	Between Groups	120.882	4	30.221	375.286	<0.001
	Within Groups	.805	10	.081		
	Total	121.687	14			
ARG	Between Groups	235.257	4	58.814	1480.724	<0.001
	Within Groups	.397	10	.040		
	Total	235.655	14			
HIST	Between Groups	46.104	4	11.526	1477.704	<0.001
	Within Groups	.078	10	.008		
	Total	46.182	14			
THR	Between Groups	39.838	4	9.960	454.636	<0.001
	Within Groups	.219	10	.022		
	Total	40.057	14			
VAL	Between Groups	13.363	4	3.341	797.975	<0.001
	Within Groups	.042	10	.004		
	Total	13.405	14			
LEU	Between Groups	4.365	4	1.091	14.413	<0.001
	Within Groups	.757	10	.076		
	Total	5.122	14			
ISLEU	Between Groups	239.365	4	59.841	828.979	<0.001
	Within Groups	.722	10	.072		
	Total	240.087	14			
P+T	Between Groups	179.598	4	44.900	509.489	<0.001
	Within Groups	.881	10	.088		
	Total	180.479	14			
M+C	Between Groups	103.906	4	25.976	553.711	<0.001
	Within Groups	.469	10	.047		
	Total	104.375	14			
TRY	Between Groups	5955.151	4	1488.788	117535.868	<0.001
	Within Groups	.127	10	.013		
	Total	5955.277	14			

ANOVA FOR CHEMICAL SCORES, EAAI AND PRI

	Sum of Squares	Mean Square	df	F	Sig.	
Lysine	Between Groups	487.310	4	121.827	133.985	<0.001
	Within Groups	9.093	10	.909		
	Total	496.402	14			
Arginine	Between Groups	1333.607	4	333.402	1474.750	. <0.001
	Within Groups	2.261	10	.226		
	Total	1335.868	14			
Histidine	Between Groups	1558.842	4	389.710	1483.029	<0.001
	Within Groups	2.628	10	.263		
	Total	1561.469	14			
Threonine	Between Groups	283.376	4	70.844	453.741	<0.001
	Within Groups	1.561	10	.156		
	Total	284.938	14			
Valine	Between Groups	170.398	4	42.600	789.174	<0.001
	Within Groups	.540	10	.054		
	Total	170.938	14			
Leusine	Between Groups	38.027	4	9.507	14.417	<0.001
	Within Groups	6.594	10	.659		
	Total	44.621	14			
Isoleusine	Between Groups	2475.054	4	618.764	828.043	<0.001
	Within Groups	7.473	10	.747		
	Total	2482.527	14			
Phen+tyr	Between Groups	1276.838	4	319.209	509.773	<0.001
	Within Groups	6.262	10	.626		
	Total	1283.099	14			
Met+cyst	Between Groups	1446.521	4	361.630	556.834	<0.001
	Within Groups	6.494	10	.649		
	Total	1453.015	14			
Tryptophan	Between Groups	392.527	4	98.132	111.937	<0.001
	Within Groups	8.767	10	.877		
	Total	401.293	14			
EAAI	Between Groups	546.831	4	136.708	3786.221	<0.001
	Within Groups	.361	10	.036		
	Total	547.192	14			
Protein index	Between Groups	173.497	4	43.374	1425.843	<0.001
	Within Groups	.304	10	.030		
	Total	173.801	14			

ANOVA FOR GROWTH PARAMETERS

One-way ANOVA: wgt gain (g) versus DIET

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
DIET	4	703.216	175.804	6450.73	<0.001
Error	10	0.273	0.027		
Total	14	703.48			

One-way ANOVA: ADG (g/day versus DIET

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
DIET	4	0.072760	0.018190	1364.25	<0.001
Error	10	0.000133	0.000013		
Total	14	0.072893			

ANOVA: SGR (%) versus DIET

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
DIET	4	0.072893	0.018223	911.17	<0.001
Error	10	0.000200	0.000020		
Total	14	0.073093			

One-way ANOVA: RGR versus DIET

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
DIET	4	0.000360	0.000090	900.00	<0.001
Error	10	0.000001	0.0000001		
Total	14	0.000360			

One-way ANOVA: FCR versus DIET

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
DIET	4	0.806293	0.201573	7559.00	<0.001
Error	10	0.000267	0.000027		
Total	14	0.806560			

ANOVA FOR PROXIMATE CACARSS COMPOSITION

	source of variation	Sum of Squares	df	Mean Square	F	Sig.
MC	Between Groups	8.001	4	2.000	3.948	0.013
	Within Groups	12.666	25	.507		
	Total	20.667	29			
CP	Between Groups	1308.201	4	327.050	40.850	<0.001
	Within Groups	200.154	25	8.006		
	Total	1508.354	29			
LIPIDS	Between Groups	126.468	4	31.617	26.259	<0.001
	Within Groups	30.101	25	1.204		
	Total	156.569	29			
ASH	Between Groups	4.692	4	1.173	.379	0.822
	Within Groups	77.414	25	3.097		
	Total	82.106	29			