EFFECT OF REPLACING SHRIMP SHELL MEAL WITH HYDROLYZED FEATHER MEAL ON GROWTH, DIET DIGESTIBILITY AND BODY COMPOSITION OF *Oreochromis mossambicus* (Peters, 1852)

BY

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DECLARATION

Declaration by the student

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DEDICATION

I dedicate this work to all stakeholders in aquaculture; researchers, fish farmers, managers and fish feed nutritionists

ABSTRACT

This study was conducted to determine the effect of replacing shrimp meal with hydrolyzed feather meal on growth, apparent digestibility and body composition of tilapia *Oreochromis mossambicus*. Five hundred fish were distributed in a completely randomized design with five treatments in quadruplicates with 25 fish (average weight 3.42±1.02g) per tank. The fish were fed isoproteinous diet with increasing inclusion levels of hydrolyzed feather meal (HFM) (0%, 4%, 8% 10% and 12%) designated as HFM0, HFM4, HFM8, HFM10 and HFM12 respectively. The fish were fed tjrough automated feeding protocol. Fish fed the diet containing 10% and 12% HFM exhibited a significantly higher growth and nutritional parameters ($P<0.05$) in terms of mean final weight; $P=0.006$ (8.05± 2.56 g) and, specific growth rate; $P = 0.042$; (3.67±0.29 %), food conversion ratio (1.97±0.11) and mean weight gain (4.9±0.33g), compared to the other diets. Final body composition was influenced significantly by increasing the level of HFM through decreasing carcass moisture and lipids. Diet containing 12% HFM had significantly lower protein; $P=0.004$, (11.75±0.05%) and ash $P= 0.012$, (8.43±0.51%) compared to diet HFM0. Inclusion levels of HFM also improved digestibility and degree of hydrolysis of the formulated diets. The diet recommended for *O. mossambicus* in this study is HFM10; this is because it exhibited improved nutritional factors such mean growth, food conversion ratio, specific growth rate and digestibility which are significant factors and considerations in aquaculture nutrition.

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ACRONYMS AND ABBREVIATIONS

ADC	Apparent digestibility
ANF	Anti- nutritional factors
ANOVA	Analysis of Variance
CF	condition factor
CHO	carbohydrates
CP	Crude Protein
DH	Degree of hydrolysis
DM	Dry matter
DO	Dissolved Oxygen
DWG	Daily Weight Gain
EFA	Essential fatty acids
ESP	Economic Stimulus Program
FAO	Food and Agricultural Organization
FCR	Food Conversion Ratio
FFEPP	Fish Farming Enterprise Productivity Program
FM	Fish meal
FWS	Fresh water shrimp
GDP	Gross Domestic Product
HFM	Hydrolyzed feather meal
KNBS	Kenya National bearuea of statistics
L	lipids

MWG	Mean weight gain
NFE	Nitrogen free extracts
NRC	National research council
OPA	O-phthaldialdehyde
SGR	Specific growth rate
SSM	Shrimp shell meal
TME	True Metabolizable Energy

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CHAPTER ONE

INTRODUCTION

1.1 Kenyan fisheries sector

The Fisheries industry plays an important role in the economic and social development of Kenya. The contribution of fisheries to local incomes, subsistence and nutrition is significant as it occurs in areas with the highest incidences of poverty. Fish contributed 0.5% to the Kenyan economy in the year 2013 (KNBS, 2014). The contribution of aquaculture is 1% of the total fisheries production. Lake Victoria produces over 90% of all fish consumed and exported from Kenya. However, the increased fish supply in Kenya will depend on aquaculture production because the production from capture fisheries sector has been declining over the last few years (Manyala, 2011). Between 2011 and 2012 the capture fisheries production decreased by 15% (FAO, 2010). Lake Victoria has been facing enormous challenges including; invasion by water hyacinth, loss of biodiversity, eutrophication and increased fishing effort associated with low catch per unit effort (Regional frame survey, 2013). The frame survey indicates that illegal and destructive fishing gears are increasingly being used. This has affected mainly the juveniles and the broodstock since illegal gears are operated on the breeding grounds. This therefore calls for fish production through aquaculture to augment capture fisheries and improve dietary protein availability and food security.

Aquaculture in Kenya began as early as 1920 when it was introduced to produce trout for sport fishing by the colonial government (Bowman *et al.*, 2007). However it remained at subsistence level upto the year 2007 (Jacobi & Colombi, 2013). In the year 2009, the government of Kenya launched an economic stimulus programme as a strategy to reduce

poverty as defined in the vision 2030 blue print (GOK, 2007). The aquaculture component of the stimulus package, the fish farming enterprise productivity programme FFEPP, aimed at increasing fish production in Kenya by helping small scale fish farmers in 140 constituencies (Musa, 2013). These are areas which have all the conditions needed for sustainable fish farming including; Nyanza, Western, Rift valley, Eastern, coast and Nairobi province. The programme was executed in phases and 28,000 fish ponds, each measuring 300m^2, had been dug by 2010 which catapulted aquaculture output from 4452 MT in 2008, to an estimated 12,151 MT in 2010 and 21488 MT in 2012. Consequently, this created demand for catfish and tilapia fingerlings of upto 28,000,000; and formulated fish feed of upto 14000 metric tonnes (Musa, 2013). The demand for fish feed and seeds is expected to rise as more farmers adopt aquaculture as an economic activity in Kenya.

Nile tilapia makes up 72% of farmed fish in Kenya (Figure 1.1, FAO, 2014). The fish is cultured in semi intensive pond system and require relatively less technology to culture. Besides, there are ideal climatic conditions in Kenya of warm climate with temperature ranging between 22-25°C.

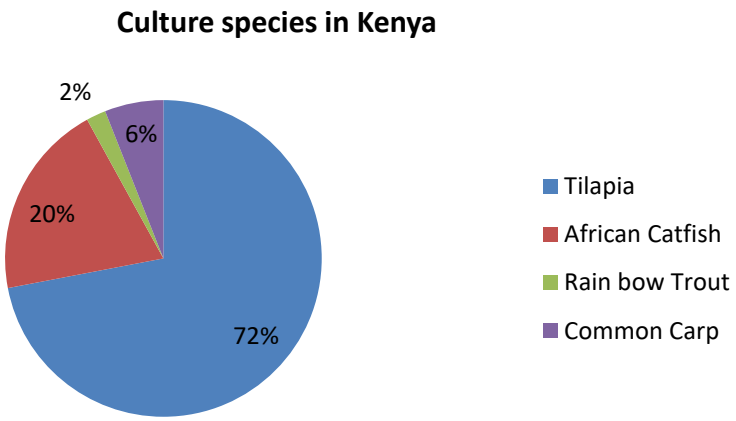

Figure 0.1: Production by species in 2012 (FAO, 2014)

1.2 Local feed ingredients used as aquafeeds in Kenya:

The most commonly used ingredients in Kenya include: fresh water shrimp (FWS) (*Caridina nilotica*), dagaa (*Rastrineobola argentea*), wheat or rice bran, sunflower or cotton seed cake and cassava for binding. The other animal based ingredients that are promising but not used currently include: blood meal, feather meal, meat and bone meal (Munguti, *et al*,. 2014).

Despite the economic importance of aquaculture in Kenya, research on production of cost- effective nutritionally balanced feed is inadequate. Information on digestibility of different feedstuff is lacking and these combined together has resulted in lack of fish feed production technology to produce aquafeeds on commercial scale. The objective of this study is to evaluate the effect of replacement of shrimp meal with hydrolyzed feather meal on growth, survival and body composition of tilapia. Also to determine the relative digestibility of the diets formulated.

1.3 Problem statement and Justification.

Sustainability of profitable aquaculture sector in Kenya requires availability and accessibility of standardized cost effective fish feeds. The key animal protein sources in formulated fish feeds in Kenya are the *dagaa* (*R. argentea)* and fresh water shrimp (FWS) *Caridina nilotica (Roux).* However, dagaa is used for human consumption while the supply of fresh water shrimp is not reliable since it is low in supply during the *dagaa* closure seasons in Lake Victoria. The cost of transporting these raw materials to other areas in Kenya is very high considering that many fish farms are located over 1000 kilometers from Lake Victoria. This has made the cost of these ingredients very high and perhaps for this reason only two companies in Kenya have met the standard protein requirement for farmed tilapia (Charo-Karisa *et al*., 2014).

Partial substitution of the shrimp meal and fish meal with hydrolyzed feather meal (HFM) could be a remedy to the supply of cheaper animal protein which in effect will reduce the cost of the fish feed and diversify animal protein sources for the Kenyan aquafeeds industry. The poultry industry in Kenya is vibrant and contributes 0.7% to the gross domestic product (GDP). There is high production of feather from this industry which causes environmental pollution due to poor disposal of feathers. Utilization of feather as a protein source in fish feeds could be a remedy to the pollution problem. Feather meal hydrolyzed from poultry feather is very high in protein content. However, nutritional factors like essential amino acid composition, digestibility, palatability and anti-nutritional factors (ANF) are critical and therefore should be determined through experimental study. Therefore the need to lower the cost of tilapia feed by replacing (marine protein) fish meal and shrimp meal with cheaper protein sources such as feather meal forms the focus of this study.

1.4. Objectives of the Study

1.4.1. General objective

To evaluate the potential of HFM in aqua feeds in Kenya with respect growth, iet digestibility and body composition of *O. Mosambicus*

1.4.2. Specific objectives

i. To investigate the effect of replacement of shrimp meal with feather meal on growth and survival of *O. mossambicus* juveniles.

ii. To determine the effect of replacement of marine protein with feather meal on carcass composition of *O. mossambicus*

iii. To determine the effects of increasing levels of HFM on the digestibility of the formulated diets hydrolyzed feather meal.

1.5 Hypothesis

i. Hydrolyzed feather meal does not have effect on the growth and survival of *Oreochromis mossambicus*

ii. Hydrolyzed feather meal does not have effect on the carcass composition of *O. mossambicus*

iii. Hydrolyzed feather meal does not improve digestibility in a formulated diet of *O. mossambicus*

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutritional requirements of fish

Fish species differ in their nutritional needs and this must be taken care of through provision of appropriate diet. Fish feed accounts for at least 50-70% of the total variable production cost in a farm enterprise (Munguti, *et al.*, 2014 , Miles & Chapman, 2014). The price of feed and its utilization are therefore of major importance in aquaculture production. In fish diets containing moderate to high level protein, the protein part of the inclusion is commonly around 50% of the total feed production cost.

Nutritional requirements of fish depend on the species and life history stage. Larval stages of fish are normally fed high protein diet of between 40-50% while the protein levels are reduced as the fish grows bigger and the metabolic rate decreases (Abowei and Ekubo, 2012). The particle size of the feed must also change as fish grows. Small fish larvae require live feed when they are hatched and powdered diet at fingerling stage; this is due to the small size of their mouth and the difficulties to process nutritionally balanced and stable small particle dry feed. To avoid overfeeding or underfeeding, fish should not only be fed a percentage relative to body weight (Abowei and Ekubo, 2012). Overfeeding causes adverse effects on water quality and farm profitability while underfeeding leads to loss of weight and reduced economic returns.

Intensification of the culture system affects the kind of diet to be fed to the fish. In intensive culture systems such as closed tank, raceway systems or recirculation aquaculture systems where stocking density is very high, fish should be fed complete diet with high protein level (Craig, 2009). This is because fish in these systems cannot forage

freely for natural food like the fish in semi intensive pond culture systems which are fed supplementary diet.

2.2 Nutritional requirements of tilapine species.

Oreochromis mossambicus, (Plate 2.1) is a benthopelagic fish inhabiting fresh and brackish water conditions. This species can tolerate high salinity conditions up to and above that of normal sea water (35ppm) and temperature of between 8 to 42°C. Naturally, they are distributed in southern Africa (Patricio, 2004)**.** Information on the growth and aquaculture potential of these species in East Africa is still scanty and therefore there is need to conduct research to assess their potentiality in a bid to diversify on the fish species for aquaculture.

Plate 0.1: Picture of mature *Oreochromis mossambicus* (Source: Fish base.com)

2.2.1 Protein and amino acid requirement of tilapia

In fish nutrition, protein is the most important and expensive ingredient. Therefore there is a need to accurately determine the correct inclusion levels of different protein sources in feed formulation. It must be balanced in the amino acid composition in relation to the

requirement of the species. The nutritive balance and the ingredient cost is affects the cost effectiveness of the feeds (Bureau & Encarnação, 2006).

Many studies reveal varied results on the effect of protein on the growth of tilapia, findings, for example by Abdel-Tawwab, (2012) shows better growth of juvenile tilapia (average weight 2.5g) when fed 45% crude protein compared to 25% crude protein. The average final body weight of the fish was 10.1g and 7 g respectively after 10 weeks in aquaria tanks. However, Loum *et al*., (2013), had a different result in which they obtained a final average weight of 14.92 g compared to 9.55 g when tilapia fry were fed diet containing 37.5% CP and 45% CP respectively for 42 days in a recirculatory system. Similar result were reported by Islam & Hossain(1994), who found that *Oreochromis mossambicus* require 30-40% of protein for optimal growth. Protein requirement for fish decreases with increasing size. El-Sayed (2004), also reports that adult tilapia require 20-30% protein while the juveniles require 30-40% protein for maximum growth.

Amino acids are the end product of protein digestion and are the fundamentals in the protein metabolism in the fish. There are 10 essential amino acids that must be supplied in fish diet. They include: Methionine, Arginine, Threonine, Tryptophan, Histidine, Isoleucine, Lysine, Leucine, Valine, and Phenylalanine. Furuya *et al.*, (2012), in a study to determine the digestible lysine requirements of tilapia, fingerlings reported the best growth at 1.6% of lysine inclusion levels. The amino acid requirements for tilapia according to the NRC, (2011) are shown in Table 2.1.

Table 0.1 Comparison of Amino acid profiles of fish meal, feather meal and the amino acid requirements of tilapia (% protein).

Amino acid	HFM(Bishop,*et al*., 1996)	HFM (Feedipedia.org.)	Fish meal (NRC,201)	Amino acid requirements of tilapia (NRC,2011)
Lysine	1.91	2.1	5.10	1.6
Arginine	5.81	6.7	3.68	1.4
Threonine	3.38	4.6	2.28	1.1
Histidine	0.66	0.8	1.56	1.0
Valine	6.39	7.2	3.51	1.5
Leucine	6.76	8.0	5.00	1.9
Isoleucine	4.00	4.9	3.06	1.0
Methionine	0.64	0.7	1.95	0.9
Phenylalanine	3.98	4.7	2.66	1.1
Tryptophan	0.46	0.6	0.76	0.3
Cysteine	4.9	4.3	1.6	1.0

2.2.2 Lipid requirements

In a formulated diet for aquaculture fish, lipids (L) are important source of energy. They have structural roles, component in hormones and source of essential fatty acids (EFA) (Tidwell *et al*, 2007). Lipids imparts palatability, enhances food consumption and also improves food conversion ratio (FCR) (Miles & Chapman, 2014). Fish require EFA

which are unsaturated and must be provided in the diet. Juvenile tilapia require 10% while adult tilapia require 6-8% lipid in the diet (Boyd, 2005). A study conducted by Chou and Shiau (1996) to determine the optimal dietary lipid level of juvenile hybrid tilapia, *Oreochromis niloticus X Oreochromis aureus,* recommended inclusion levels of 5% lipid since there was no significant difference with those fed lipid inclusion levels of 10 and15%. Lipids also have protein sparing functions in fish nutrition as reported in a study by De Silva *et al* (1991) on a hybrid *Oreochromis niloticus X Oreochromis mossambicus* (mean weight 1.185 g), where it was reported that the best growth was obtained at 18% inclusion levels of lipid in all the three levels of dietary protein (15%, 20% , 30% protein content). However there was reduced growth when inclusion levels was above 30%. In a similar study by Orire and Sadiku (2014), the protein sparing ability was obtained with 10% lipid inclusion in the diet of *Oreochromis niloticus* thereby bringing down crude protein inclusion to 30%.

2.2.3 Carbohydrates requirements

Carbohydrates (CHO) are the least expensive nutrient and provide dietary energy (Wang *et al*., 2005). Carbohydrates also have binding properties and hence make aquafeeds more stable and buoyant for example when processing extruded floating pellets. The dietary carbohydrates requirements vary amongst species; omnivorous and herbivores species digest carbohydrates better than carnivorous fish (Miles and Chapman, 2014b). In tilapia diet, inclusion levels of 20% is adequate (Boyd , 2005). Like lipids, carbohydrates have protein sparing ability. El Hammady, (2002) reported that hybrid tilapia (*Oreochromis niloticus* X *Oreochromis aureus)* utilized carbohydrates for growth when CHO: L ratio was increased up to 6. Growth however reduced with ratios above 6, the author argued

that CHO: L of 6 at 25% CP was able to offer sparing effect to a higher protein diet of 30% CP at CHO: L of 4. Practically, the former combination is cheaper and may be feasible to many farmers.

2.2.4 Vitamins and minerals.

Fish also have requirements for vitamins and minerals which are availed as premixes in formulated diets (Abowei and Ekubo, 2012). The premixes are added to a diet mixture in amounts that are adequate to provide the required levels of vitamin and mineral.

Vitamins are either water soluble or fat soluble. The water soluble include: B -vitamins, choline, folic acid, Pantothenic acid, biotin and vitamin C. Vitamin C and E acts as antioxidants and also help the immune system in fish (Craig, 2009). The recommended levels of vitamin E in tilapia diets is 80 $mgkg^{-1}$ according to Ispir *et al.*, (2012), an increase on the red blood cells and hemoglobin concentration was reported with increasing dietary vitamin E upto 80 $mgkg^{-1}$. Others include; vitamin C, 60 $mgkg^{-1}$, 9.5 and 16.5 mg vitamin B6/kg diet are required for tilapia fed diets with 28 and 36% protein, respectively (Nzonga *et al*,. 2013). The fat soluble vitamins include: A vitamins, retinols, D vitamins, cholecalsiferols, E vitamins, the tocopherols and K vitamins. Dietary vitamin A requirements of tilapia is 5,850 to 6,970 IU/kg diet (Shiau & Lin, 2006).

Minerals are divided into macro and micro minerals. The macro minerals are important in maintaining osmotic balance and bone formations. They include: potassium, chloride, sodium and phosphorus. These are required in high quantities while the micronutrients are required in small amounts in hormone and enzyme systems (Craig, 2009). The common trace elements include: copper, chromium, iodine, selenium and zinc.

2.3. Feather meal in tilapia diet

Hydrolyzed feather meal is a product from poultry feathers and has been recommended by many nutritional experts as a possible replacement for the more expensive fish meal (FM) and shrimp meal (Zhang *et al*., 2014). This is because of its high protein level, commonly in the range of 70- 82%, high lipid level in the range of (8.3-15%) and low fiber (0.68%) (Bishop *et al.*, 1996; Feedipedioa.org) Hydrolyzed feather meal is deficient in lysine and methionine but is adequate in cysteine and arginine which are important in tilapia nutrition (Bureau, *et al.,* 2000). Comparisons of feather and fishmeal amino acid profile are shown in the Table 2.1. Despite proven applicability of HFM in aquaculture it has not been incorporated as an ingredient in tilapia diet in Kenya. This is perhaps due to lack of knowledge on available hydrolyzing process, its effects on apparent digestibility and its impact on growth and survival of tilapia

Earlier studies on replacement of animal protein with HFM have indicated satisfactory results. Up to 66% replacement of animal protein by feather meal did not have significant effect on growth of *O. niloticus* fry (Bishop, *et al*., 1996). However, total replacement of animal protein with HFM leads to reduced growth in tilapia due to deficiency of essential amino acid lysine and methionine as shown in Table 2.1. In another study, up to 50% dietary protein was successfully replaced by HFM in a trial experiment on *Labeo rohita* (Hasan, *et al*., 1997), further confirming the practicality of feather meal as a protein source in aquafeeds.

The quality and nutritional value of HFM depends on the processing method. When hydrolyzed by steam cooking the interaction of pressure/temperature, and cooking time is important. In a study to determine indicators of the nutritional value of HFM, true metabolizable energy (TME) of HFM significantly decreased with increasing pressure

(Moritz and Latshaw, 2001). The authors found that at 207 kPa, TME was 3.51 while the TME was 3.03 and 2.95 at 414 kPa and 517 kPa respectively. Increased pressure also significantly lowered Cystine and its equivalents, from 3.99% to 2.44 to 2.21 and to 1.48% at increasing pressure of 207, 310, 414 and 517kPa respectively.

2.4 Digestibility consideration in fish nutrition

Evaluation of digestibility provides a measure of the nutritive value of a feedstuff and a rationale for the formulation of a diet in fish nutrition. Digestibility assays can be done *in vitro* or *in vivo. In vivo* digestibility involves inclusion of indigestible indicator such as yttrium oxide or chromic oxide as an inert marker in the diet (Bureau and Cho, 1999). Feaces are then collected to allow for measurement of the ratio of energy and nutrient to the marker in the feaces and nutrient in the diet (Satiye and Sener, 2005). For *in vitro* digestibility, the assay is done by simulation of gastrointestinal digestion in fish using commercial enzymes and adjustment of pH as required (Satiye and Sener, 2005).

One of the commonly used methods for *in vitro* digestibility assay is the degree of hydrolysis (DH) of protein. This is defined as the proportion of peptide bonds cleaved (Nielsen *et al.,* 2001). Some methods for monitoring DH include: pH-stat, osmometry, soluble nitrogen content, trinitrobenzene sulfonic acid (TNBS) and the O-phthaldialdehyde (OPA) method (Nielsen *et al.,* 2001). The DH increases with increasing hydrolysis time and enzyme concentration and vice versa (Huang and Liu, 2010). The OPA method is fast and convenient and based on the reaction of OPA (prepared by reaction of OPA and ethanol) with amino groups released during proteolysis of a protein substrate (Nielsen, *et al*., 2001)

Generally, factors affecting digestibility in fish nutrition include feed ingredients and ratio, fish size, feed intake water temperature and feed processing conditions (NRC, 2011).

Apparent digestibility coefficients (ADC) of some selected feedstuff in tilapia diet as outlined in National Research Council, (2011) include: Anchovy fish meal (91.6%) soy bean meal (90.9%), corn grain (75%), cotton seed meal (82%), hydrolyzed feather meal (79%), rape seed meal (85%). Generally, animal based protein have a higher digestibility compared to plant based protein in fish diets (Zhou and Yue, 2012). For example, a study on ADC of selected feed ingredients for hybrid juvenile tilapia, ADC range of 71.88-89.53% for animal protein and 65.89- 79.98% for plant protein was reported.

2.5.Effects of formulated diets on whole body composition of fish.

Studies have demonstrated dietary influence on the whole body composition of tilapia which depends on the age of the fish (Al Hafedh, 1999). Percentage protein of the smallest fish (0.56g) was higher (16.95%) in fish fed 40% crude protein (CP) than those fed 25% CP (15.60%) whereas percentage body lipid decreased with increasing dietary protein content (Al Hafedh, 1999). In this study, bigger fish (264g) did not show changes in body protein due to dietary protein levels, lipid content decreased with increasing dietary protein while there was no definite trend in ash content with increasing dietary lipid. Similar results on protein and lipid were reported by (Ahmad *et al*., 2004), however variations in ash contents among fingerlings and adult fish were insignificant.

Dietary lipids also affect body composition of tilapia as reported by (Kasheif and Ibrahim, 2012). In their study, the biochemical analysis of whole tilapia fish bodies indicated that moisture, ash and protein contents are unaffected by the lipid levels in the

diet, however, whole body lipid content increased with the dietary lipid levels. Fish fed diets with no lipid had 23.49% of body lipid compared to 25.69% of body lipid at 9% inclusion levels.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The growth trial experiment was carried out for a period of 30 days at the Verid laboratory in Saudarkrokur, Iceland located at latitude 65^0 44' 27.40"N, longitude 19^0 38' 25.71 W and elevation of 48 ft above these level (Google Earth © 2015) between January and February 2015. The protein and fat proximate analysis was done in Matis laboratories located at latitude 64^0 07' 49.29"N, longitude 21^0 45' 58.26" W.

3.2 Experimental diets

The different ingredients were chosen in consideration of their similarities to the ingredients commonly used for tilapia feeds in Kenya. Shrimp meal, rapeseed meal, soya meal, fish meal, yttrium oxide and the premix was sourced from Laxa feed mill Ltd, Iceland, wheat-bran from Lifland Ltd, while wheat and plant oil bought from the local stores in Saudarkrokur.

Poultry feather was procured from ISfugl harvesting factory and transported to the MATIS laboratory located in Reykjavik. The feathers were washed in running tap water and pressure cooked in an autoclave at 220 Kpa at 121ºC for 35 minutes. The hydrolyzed feather was then dried by spreading a thin layer in trays for 24 hours at 30ºC (Bureau, et al., 2000). The feathers were then blended and oven dried at 75ºC for 12 hours and milled to make the meal.

Winmix software as described by Nogueira, *et al.*, (2012) was used to derive, the formula for the test diets as provided in Tables 3.1 and 3.2. Five isoprotein (36% CP) diets (**HFM0, HFM4, HFM8, HFM10 and HFM12**) were formulated with increasing

inclusion levels of feather meal partially replacing Shrimp shell meal (SSM). The inclusion level of fish meal and soya meal was kept constant but inclusion ratio of other ingredients was varying for keeping good amino acid balance in the diets for tilapia.

All the ingredients were ground into fine powder and mixed as per the formulation for each treatment until homogenous. Water was added to the mixture to produce dough and pelletized into 1.5mm pellets using laboratory pelletizer then oven dried for 24 hours at 75 ºC.

Table 0.1 Formulation of ingredients composition in the experimental diets (g/100g)

Ingredient	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12
Fish meal	7	7	7	7	7
Shrimp shell meal	60	49	39	16	0
Hydrolyzed feather meal	0	4	8	10	12
Soya meal	10	10	10	10	10
Rapeseed meal	0	0	0	25	43
Wheat Bran	8	18	19	0	0
Fish oil	3	3	7	0	1
Wheat	10	8	8	30	25
Laxa premix	1	1	1	1	1
Yttrium oxide	1	1	1	1	1
Total	100	100	100	100	100
Estimated composition (g/kg)					
Crude protein	360.0	360.0	360.0	360.0	360.0
Crude fat	60.0	60.0	100.0	60.0	95.4
Crude ash	197.4	166.7	141.3	91.3	55.7

Crude fiber	17.9	29.2	31.2	45.5	68.0
NFE – fiber**	266.1	284.7	271.8	333.1	312.9
Dry Matter	900.9	901.3	904.6	889.6	892.3
Calculated gross energy(MJ/kg) in DM*	16.3	16.9	18.2	18.2	19.7

***Gross energy value is calculated according to gross energy constants in nutrients: fat= 39.5MJ/kg; protein= 23.6MJ/kg; NFE= 17.3MJ/kg.**

**The Nitrogen Free Extracts values are calculated estimates of CHO with fibers excluded.

Table 0.2: Estimated Amino acid composition (%) of the ingredients.

Amino acid (%)	HFM0	HFM4	HFM8	HFM10	HFM12
Lysine	1.8	1.7	1.6	1.6	1.6
Methionine	0.6	0.6	0.6	0.6	0.7
Arginine	2.2	2.8	3.0	2.1	2.2
Isoleucine	1.7	2.1	2.1	1.6	1.0
Histidine	0.9	1.1	1.1	0.8	0.9
Threonine	1.5	2.0	1.9	1.4	1.5
Phenylalanine	2.0	2.4	2.4	1.7	1.7
Tryptophane	0.9	1.3	1.3	0.7	0.8
Leucine	2.4	2.6	2.7	2.7	1.9
Valine	2.0	2.5	2.7	2.1	2.0
Met+Cyst	1.0	1.1	1.2	1.4	1.5

3.3 Proximate analysis

The chemical composition of experimental diets and feces samples were determined based on the methods of AOAC, (1995)

Protein was analyzed by micro-Kjeldahl method where the sample was digested in sulphuric acid then put into a distillation unit, 2400 Kjeltec auto sampler system. The acid

solution was made alkaline by NaOH and ammonia distilled into boric acid and titrated with H_2SO_4.The nitrogen was multiplied by a factor of 6.25 to obtain the crude protein content of the sample thus:

$W_p = 6.25*W_n$

Where, $W_{p\ is}$ the crude protein content in grams per kilograms of the test sample while W_n is the nitrogen content in grams per kilograms of the test sample

Crude fat was extracted by soxhlet method by boiling samples in petroleum ether at temperature range of 40-60ºC.

The moisture was determined by drying 2 g of diet samples in an oven at 105 ºC for 4 hours, cooled in a desiccator and reweighed. The moisture content was calculated as:

Ash content of the diets was analyzed by burning 2g samples of each diet in a muffle furnace (Griffin and George ltd) at a temperature of 550 ºC for 4 hours then cooled in a desiccator and reweighed. Ash content was calculated as:

$$\text{Ash, \%} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100$$

Gross energy of the diets and feces were determined with the help of oxygen bomb calorimeter (IKA C 200 model). A dried sample weighing 0.5g was put into a crucible and then a cotton string was tied to connect the firing wire and the food sample in the crucible. The calorimeter vessel was filled up with oxygen and placed into the water jacket filled with water of 25 ºC. The Gross energy of the diet samples and the feces was recorded after 13 minutes, after detecting the heat created in total combustion of the sample. The calorimeter reading of the gross energy was then recorded.

3.4 Experimental design

Tilapia *O. mossambicus* mixed sex juveniles were obtained from a private fish farm south of Reykjavik and acclimatized at the Verid Laboratory for 14 days before commencement of the experiment. During acclimation they were fed a commercial diet (40% crude protein). Juveniles numbering 25 of average weight 3.4 g and length 5.84±0.03 g were randomly stocked in 20 buckets, each of capacity 17 liters and supplied with aerated fresh water (flow rate 1 liter min^{-1}) (Figure 3.1). Five isoprotein (36% CP) *diets* were fed to the fry in quadruplicates to satiation for 30 days through an automatic feeder set to dispense the feeds every 10 minutes for 25 seconds, during constant light period (24L: 0D). Water temperature was maintained at 26.4 ºC ±0.67. The estimated ingredient and amino acid composition of the diet is shown in Table 3.1 and 3.2 respectively.

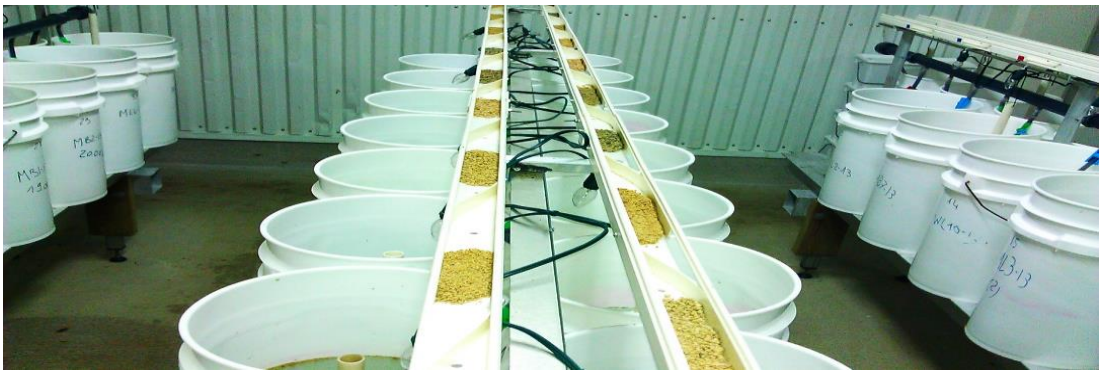

Figure 0.1: Experimental tank setup showing automatic feeding protocol of the diets. (Source: Author, 2015)

3.5 Determination of Growth Performance

The weight and length of the tilapia fingerlings were recorded at the commencement and end of the experiment and at the end. The specific growth rate (SGR), condition factor (K-Factor), feed conversion ratio (FCR) and mean weight gain (MWG) were used as the growth performnace parameters and were calculated using the formula:

- Mean weight gain (g) = (mean final weight – mean initial weight)
- Specific growth rate (SGR); %/day $100\times \ln (W_2) - \ln (W_1)/\Delta T$.

  Where:

- W1 = initial body mass in grams
- W_2 = final body mass in grams
- ΔT = time between measurements in days.
- SGR= specific growth rates (daily % growth)

Survival = Number of fish harvested

Condition factor (CF),

$K= 100W/L^3$. Where:

K = condition factor,

L = total length of fish in cm

W = weight of fish in grams.

FCR = net feed intake / increase in body mass

3.6 In vivo digestibility evaluation of ingredients.

Fecal collection began seven days after fish had begun feeding experimental diet. Feces were collected from each experimental tank every morning by siphoning through a 100µm mesh material. The Feces were dried for 4 hours in an oven set at 50 ºC then frozen at -26 ºC (Allan *et al*., 2012). The Fecal samples from each diet treatment were pooled together in the course of the experimental period until sufficient quantity was obtained for digestibility determination.

Apparent digestibility coefficient of each diet was calculated thus:

$ADC\ (\%) = 100-[100(F/D \times YO_d / YO_f)]$, where;

- ADC = apparent digestibility coefficient
- F = percent of nutrient or energy in the feaces,
- D = percent of nutrient or energy in the diet,
- YO = percent of yttrium oxide in the diet while
- YO_f = percent of yttrium oxide in the feaces (Allan *et al.*, 2012).

3.7 Evaluation of degree of hydrolysis of proteins in the diets.

The degree of hydrolysis was carried out in two steps: Samples weighing 0.1g of each diet were dissolved in 10ml of distilled water and pH was adjusted to 2.0 using 2N hydrochloric acid (HCl). Pepsin 0.0029g was added to the mixture and shaken in an incubator for 1hour at 37 ºC. The pH of the mixture was again adjusted to 5.3 using $NaHCO_3$ and finally to 7.5 using 2N NaOH. In the mixture was added 0.004g of pancreatic enzyme and shaken in an incubator for 2hours at 37 ºC. The digestion was terminated by submerging the samples in boiling water for 10 minutes. The samples were kept in the refrigerator until determination of the DH. The degree of hydrolysis assay was determined by O-phthaldialdehyde (OPA) method as outlined in the procedure by Nielsen (2001). O-phthaldialdehyde reagent was prepared by dissolving 1.905g of di-Na-tetraborate decahydrate and 50mg of SDS (Na-Dodecyl-sulfate) in 35ml of distilled water and stirred until completely dissolved before adding 40mg of OPA dissolved in 1ml of ethanol and 44mg of DDT(Dithiothreitol 99%) dissolved in 50ml of Distilled water. A standard solution was also prepared by dissolving 5mg of serine in 50 ml of water and adding 30mls of OPA reagent. A blank solution was prepared in deionized water using the same procedure as the standard. A sample from enzymatic digestion measuring 30µl

was added into the microplate and mixed with the same quantity of OPA reagent and allowed to stand for two minutes before spectrophotometer reading performed at 340nm. The calculation for DH was determined according to the formula of (Nielsen, 2001).

3.8 Evaluation of carcass composition

Samples of 10 fish were taken from each treatment at the beginning and end of the study to evaluate the initial and final proximate body composition respectively. The Samples were ground using a blender. Each content was put in plate and placed inside FOSS scan Near Infrared spectrophotometer (Foss Hillerod, Denmark). The parameters analyzed for included: moisture, fat, protein and ash

3.9 Statistical analysis

Statistical analyses on SGR, FCR, CF and survival values was done using Sigmaplot version 13 programme. Shapiro-Wilk test indicated no deviation from normality ($P>0.05$) for the replicates. One-way analysis of variance was used to test for significant differences at $\alpha=0.05$ between the means of the treatments. The results were considered significantly different at $p<0.05$ and where there was significant difference, Tukey multiple comparison test was used to compare the variance amongst the parameter means of the test diets.

CHAPTER FOUR

RESULTS

4.1 Proximate analysis of the feed

Proximate analysis of the dietary treatments are shown in Table 4.1 Hydrolyzed feather meal was also analyzed and the biochemical composition was; 3.6±0.1 and 1.2±0.1% for moisture and ash respectively while protein and lipid were 72.8 and 18.1% respectively. The analyzed protein content in the diets was in general below the approximated 36% CP level, with minimal fluctuations between diets. The lipid level did fluctuate more from the approximated 6%, where the HMF0 diet had lowest value (4.5%) but HFM12 the highest value (9.3%). The analyzed lipid level of HFM was higher than expected (6%) but that fact does not explain the whole variance between diet types. The ash content is varying between diets, in the range of 20.2%-4.6%, most probably affected by inclusion level of shrimp shell meal. The calculated content of Nitrogen free extracts (fibers + other carbohydrates) was high in general and in the range of 37.3-48.2%. The HMF10 and HMF-12 have the highest NFE value. The inclusion of wheat is also high in these two diets. The calculated gross energy content is reflected in measured GE content, but with some aberrance in diet HFM0 and HFM4.

Table 0.1 Proximate composition of the diets (% as fed basis)

Ingredient (%)	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	HFM
Protein	32.9	35.2	33.8	34.9	34.4	72.8
Lipid	4.5±0.8	6.1±0.8	7.3±0.8	5.6±0.8	9.3±0.8	18.1±0.8
Ash	20.2 ±6.9[a]	17.9±2.0[a]	15.3±0.1[a]	9.0±0.2[b]	4.6±0.2[b]	1.6±0.6
Moisture	1.8 ±0.8[a]	3.5±0.3[b]	3.4±0.1[b]	2.3±0.2[b]	3.6±0.6[b]	4.4±0.4
NFE*	40.6	37.3	40.2	48.2	48.1	40.6
GE-calculated**	16.3	16.6	17.2	18.4	19.4	23.8
Gross Energy (KJg^{-1}) measured	13.70±0.1[a]	15.42±0.0[b]	17.05±0.04[b]	18.13±0.0[c]	19.13±0.10[c]	23. 7±0.13

*Values with the same superscript denotes no significant difference while those with different superscript shows significant difference amongst them

*NFE: calculated= 100-(%CP+CF+%ash+%moisture); where:

NFE= nitrogen free extracts

CP = crude proteins

CF= crude fiber

**Gross energy values in DM (dry matter) are calculated according to gross energy constants in nutrients: fat= 39.5MJ/kg; protein= 23.6MJ/kg; NFE= 17.3MJ/kg.

4.2 Effect of hydrolyzed feather meal on growth and survival of *O. mossambicus*

Growth performance parameters for *O. mossambicus* fed increasing inclusion levels of hydrolyzed feather meal during the 30 days experimental period are presented in Table 4.2 and Fig. 4.1. The initial weight of the fish did not differ significantly (P = 0.791). There were significant differences in the final mean weight, SGR and mean weight gain of the fish amongst the dietary treatments (P<0.05). Fish fed diet containing 10% and 12% hydrolyzed

Feather meal (HFM10 and HFM12) exhibited significantly higher final mean weight P=0.006; (8.05± 2.56 and 7.61±2.14 respectively) and specific growth rate (3.67±0.57 and 3.36±0.14 respectively), (P=0.042) compared with those fed diets HFM0, HFM4 and HFM8. Fish fed diet HFM10 and HFM12 (P=0.697) were not significantly different. Fish fed diet HFM0, HFM4 and HFM8 showed similar response in SGR and final mean weight (FMW). Mean weight gain increased with increasing levels of HFM from 3.6±0.22g for diet HFM0 and HFM8 to 4.9±1.18 g for diet HFM10 as shown in Figure 4.2

Table 0.2: Growth performance, survival and feed conversion of *O. mossambicus* fed diets with increasing inclusion levels of hydrolyzed feather meal (Mean ± SEM)

Parameter	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	P-Value
Number of fish stocked	100 (4 x25)	100 (4x 25)	100 (4x 25)	100 (4 x 25)	100 (4x 25)	P = 1.000
Initial length (cmfish^{1})	5.9±0.61^{a}	5.8±0.63^{a}	5.8±0.58^{a}	5.8±0.61^{a}	5.8±0.57^{a}	P = 0.791
Final length (cm fish^{-1})	7.26±0.68	7.21±0.65	7.22±0.65	7.51±0.79	7.29±0.74	P = 0.008
Initial mean wt.(g)	3.43±1.02^{a}	3.43±1.02^{a}	3.42±0.95^{a}	3.42±0.97^{a}	3.42±0.95^{a}	P = 0.875
Mean final wt.(g)	6.97±1.91^{a}	7.19±1.95^{a}	7.06±1.74^{a}	8.05± 2.56^{b}	7.61±2.14^{a}	P = 0.006
SGR (% day^{-1})	2.97±0.07^{a}	3.08±0.06^{a}	2.99±0.16^{a}	3.67±0.29^{b}	3.36±0.1^{b}	P = 0.042

Condition Factor	1.77±0.45	1.82±0.58	1.83±0.61	1.88±0.57	1.91±0.63	P = 0.674.
Survival (%)	94±0.5	77±1.8	85±1.0	87±2.4	83±2.8	P = 0.488

Values are Mean ± S.E of four replicates. Means having the same letter in the same row are not significantly different at P<0.05

Food conversion ratio was lower in diet HFM10 and HFM12; than the other diets. FCR was affected by increasing levels of HFM. The diet containing 10% and 12% HFM (HFM10 and HFM12) had a significantly lower FCR (2.08±0.20 and 2.1±0.14 respectively), while diet 4% (HFM4) had the highest FCR (3.72±1.56) as shown in Figure 4.1 Survival rate was not significantly affected by the dietary treatments. In all the treatments, survival was above 75%.

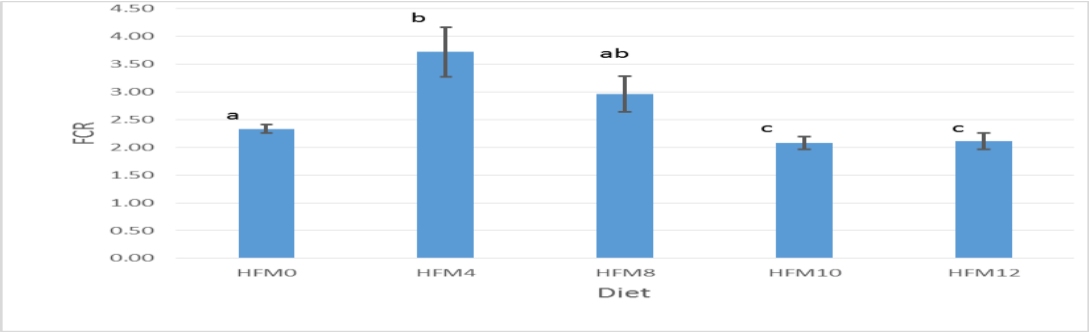

Figure 0.1: Comparison of mean food conversion ratio (FCR) of *O. mossambicus* fingerlings fed diets containing increasing inclusion levels of hydrolyzed feather meal (Mean±SEM). Bars with the same letters have no significant difference.

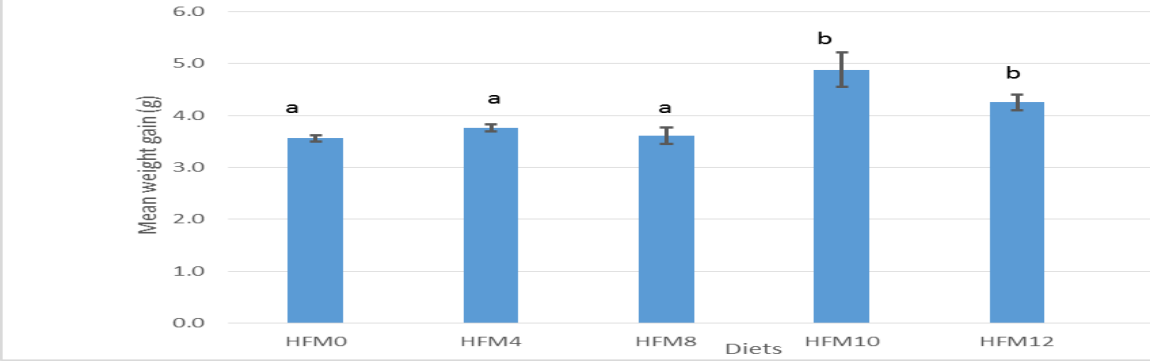

Figure 0.2: Comparison of mean weight gain of *O. mossambicus* fingerlings fed diets containing increasing inclusion levels of hydrolyzed feather meal (Mean±SEM). Bars with the same letters have no significant difference

The water temperature monitored during the experimental period ranged from 25.3 to 27.8 ºC while dissolved oxygen (D.O) ranged from 6.5 to 8.6 mg/l, (Figure 4.3 and 4.4).

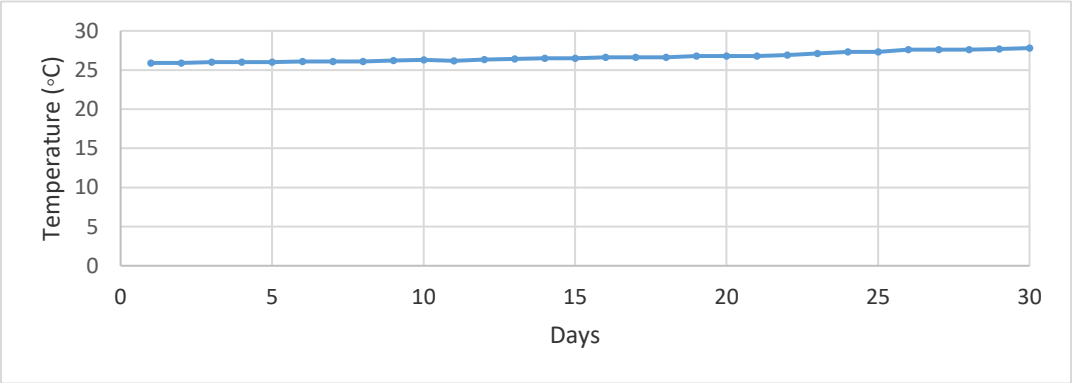

Figure 0.3: Temperature in the rearing system during the growth period.

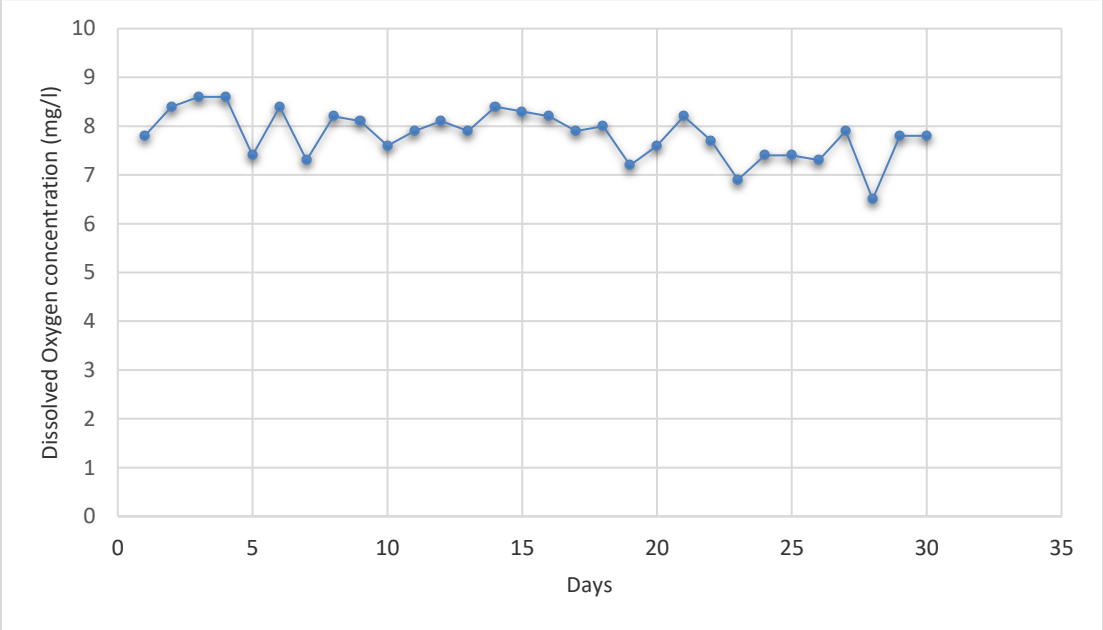

Figure 0.4: Dissolved oxygen concentration (mg/l) in the experimental system during the trial period.

4.3 Whole body composition of fish

Initial and final carcass compositions of *Oreochromis mossambicus* fed on the test diets are presented in Table 4.3. All fish displayed a change in the whole body composition

(compared with the initial composition). There were no significant variations between the final and initial protein content of the carcass even though there were significant differences carcass composition amongst the dietary treatments. Final fat content was higher in all the diet treatments than the initial content and increased with increasing levels of the dietary HFM in the experimental diets. Diets HFM12 and HFM10 exhibited significantly higher fat content (P=0.001) than HFM0, HFM4 and HFM8. The final moisture content of the carcass was lower in all the treatments compared to the initial moisture content. There was a significant difference in the final moisture content amongst the treatments and it decreased with increasing inclusion levels of HFM (P<0.001)

Table 0.3: Proximate carcass composition of *O. mossambicus* fed increasing inclusion levels of HFM at the start and end of the experiment.

Parameter (%)	Initial body composition	Final body composition					
		HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	P -Value
Moisture	72.72±0.88	71.1±0.79^{a}	69.5±0.15^{a}	69.7±0.18^{a}	68.5±0.06^{a}	66.4±0.16^{b}	P<0.001
protein	13.72±0.06	13.40±0.51^{a}	11.84±0.05^{b}	12.88±0.04^{ab}	12.23±0.11^{ab}	11.75±0.05^{b}	P=0.004
Fat	11.58±0.06	13.01±0.54^{a}	13.47±0.14^{a}	14.38±0.21^{ab}	15.15±0.08^{ab}	16.58±0.17^{b}	P<0.001
Ash	12.04±0.53	11.55±0.39^{a}	10.75±0.56^{a}	9.67±0.07^{a}	10.29±0.54^{a}	8.43±0.51^{b}	P=0.012

Values are Mean ± S.E of three replicates. Means having the same letter in the same row are not significantly different at P<0.05

4.4 Apparent digestibility of protein and the degree of hydrolysis

The *in vivo* %ADC of CP showed that diet HFM12 and HFM10 had a significantly higher ADC (80 and 78.8% respectively) than the other diets. The lowest ADC was

observed in diet HFM4 and HFM8 HFM0, (Table 4.4). A similar trend is observed in the ADC of gross energy (GE) of the different diets although the highest ADC is recorded for diet HFM10.

Table 0.4: Apparent digestibility coefficient of crude protein, gross energy and the degree of hydrolysis of protein in the diets

Digestibility (%)	HFM0	HFM4	HFM8	HFM10	HFM12	SEM
ADC of CP	73.9	70.9	73.4	78.8	80.0	0.87
ADC of GE	67.1	65.0	69.2	77.5	75.3	1.21
Degree of hydrolysis (%DH)	24.4	18.7	18.2	25.6	26.1	0.87

The results of the DH of protein in the ingredients are shown in Figure 4.5. There were no significant differences amongst diet HFM0, HFM10 and HFM 12 indicating values of 24.4, 25.6 and 26.1% respectively. HFM4 and HFM8 showed significantly lower DH of protein (18.7 and 18.2% respectively) than in the other diets.

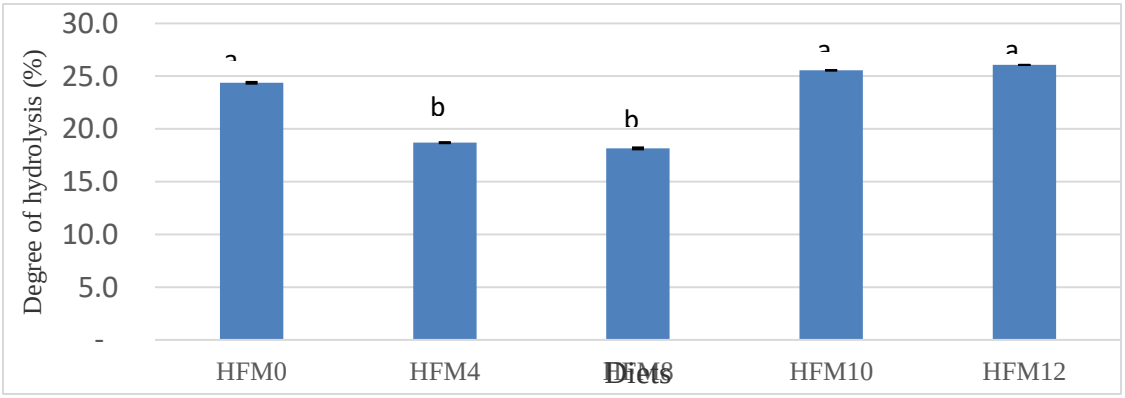

Figure 0.5: The degree of hydrolysis (%DH) of protein in the diets (values are Mean±S.D) Bars with the same letters have no significant difference

There was a significant correlation (R^2= 0.7187) between in vivo ADC of CP and in vitro DH of protein, Figure 4.6.

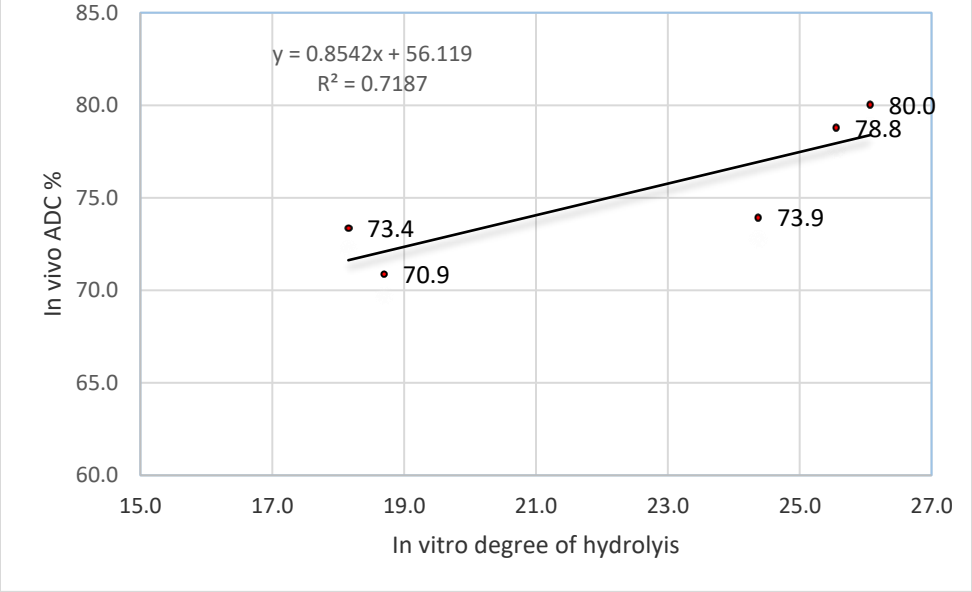

Figure 0.5: **Relation of Degree of hydrolysis (*in vitro*) and apparent digestibility coefficient of crude protein (*in vivo*)**

CHAPTER FIVE

DISCUSSION

5.1 Effect of Hydrolyzed feather meal on growth and survival of *O. mossambicus*

Under the experimental conditions of the present study, survival of *O. mossambicus* was high (above 75%) during the 30 days trial and was probably contributed by the overall quality and stability of the experimental conditions. Other studies have reported similar survival of tilapia while attempting to replace fish meal with HFM. Hasan *et al*., (1997) evaluated HFM as a protein source in the diet of *Labeo rohita* and observed a survival of between 95-99%. Similarly, Suloma, *et al.*, (2014), reported 97.5-98% survival of *O. niloticus* while a survival of 97-100% was observed in *Heterobranchus longifilis* fingerlings when fish meal(FM) was replaced with crab meal (Keremah, 2013). In the present study the lowest survival (77±1.8) was recorded in fish fed diet HFM4 but not significantly different from the rest.

The lowest mean weight gain (3.6±0.22g**)** was observed in diet HFM0 (control) while diet HFM10 and HFM 12 had a significantly higher MWG of 4.9±1.18g and 4.3±0.52g respectively. This shows that the fish responded positively to all the diets. The present study showed that inclusion of hydrolyzed feather meal in substitution of shrimp meal sources in the diet of *O. mossambicus* is feasible. The results indicated that inclusion of at least 12% HFM had positive effect on growth rate and weight gain in comparison to other tested diets, formulated with lower HFM inclusion. Fish fed the diet containing 10% and 12% HFM had a significantly higher FMW and SGR compared with the other diets (HFM0, HFM4, HFM8). A significantly lower FMW and SGR observed in the control diet (HFM0) , HFM4 and HFM8) might be due to high proportion of SSM (60%) and

progressively higher inclusion of wheat bran (Köprücü & Özdemir, 2012). Feeds containing high ash content may have high protein content and favorable essential amino acid profile but still have poor digestibility. In this study diets, HFM0, HFM4 and HFM8 had a relatively high ash content of 20.02, 17.9 and 15.3% respectively. The growth performance recorded in diet HFM10 and HFM12 which replaced the animal protein by 30% and 63% respectively concurs with a similar study by Bishop,*et al*.,(1996) which demonstrated that the growth of *O. niloticus* was not affected by the replacement of up to 66% of the animal protein (9.9% of the total diet) by feather meal. Studies on replacement of fish meal and shrimp meal with HFM have been done on *Oreochromis niloticus* but few if any on *O. mossambicus*. Results of this study differ with those of Munguti, *et al.*, (2014) who found significant decline in growth of *O. niloticus* fed diet containing 8.6% HFM. The highest weight gain (69.5%) was recorded for fish fed 4.5% HFM. This may have been attributed to different processing methods of the HFM and the different combinations of the ingredients in the treatments. Bureau, *et al*.,(2000) reported 15% replacement of FM with HFM in the diet of rain bow trout and they found no significant differences in weight gain and feed efficiency in fish fed the diet containing HFM (15%) and those fed the control diet of 50% HFM

Food Conversion Ratio is an important economic indicator of how efficiently the fish utilizes the feed thereby reducing wastage. The FCR was generally high in this study due to the uneaten feeds due to the relatively bigger sizes of pellets fed to the fish. The lowest FCR (2.0±0.11and 2.11±0.14) was observed in the fish fed diet HFM10 and HFM12.This was significantly lower than those for the fish fed diet HFM0, HFM4 and HFM8 and therefore indicates the best utilized diet compared to the other diets. This could be because

of the diets being relatively digestible as demonstrated by the high degree of hydrolysis and ADC of CP in diet HFM10 and HFM12 (Table 4.4). This was followed by the FCR of 2.33±0.07, 2.96±0.32 and 3.72±0.44 for diets, HFM0, HFM8 and HFM4 respectively. When Poultry feather meal was used as a single animal protein at inclusion levels of 48%, Bag *et al*.,(2012) realized an FCR of 2.28 which had no significant difference with the other dietary treatments (earthworm meal and slaughter offal meal). This compares to the FCR recorded on fish fed the control diet in this study. This is perhaps due to the high inclusion HFM in the former hindering growth due to low levels of lysine and methionine amino acids in HFM.

Temperature and oxygen are critical parameters in fish culture systems and in this study, the temperature ranged between 25.3 to 27.8 ^{o}C while dissolved oxygen (D.O) measured ranged between 6.5 to 8.6 mg/l. These values are within the recommended range for tilapia culture (Soto-Zarazúa *et al*., 2010).

5.2. Proximate composition of the test diets

When formulating diets one can always expect some variations in exact chemical composition of ingredients from the approximated one. Additionally there are always some possible aberrance in the weighing and processing procedure of the experimental diets. The proximate composition of the ingredients in this study differed with the estimated proportion as shown in Table 2.1 The protein content of the diets were near isoproteinous although diet HFM4 had the highest percentage of 35.2% while diet HFM0 had the lowest protein percentage of 32.9%.This however did not reflect on the variation in growth parameters. The same trend is repeated in the proportion of lipid in all the diets.

This is probably due to the high lipid content in HFM of 18.1%. The proximate lipid content in HFM in this study is much higher than that reported by NRC, 2011 of 5.4. %.

There was a difference in the gross energy in the diets. Diet HFM12 had the highest gross energy of 19.13±0.10 KJg^{-1} while diet HFM0 had the lowest gross energy of 13.7 KJ^{-1}. This observation could be due to the increasing lipid content as a result of the increasing level of HFM which is high in lipid (18.1%) in this study. HFM had gross energy of 23.7±0.13KJ^{-1} which is similar to that recorded by (Bureau,*et al*.,1999). The high ash content in diet HFM0 and HFM4 and HFM8 is as a result of the high proportion of SSM, HFM is low in ash content and this is reflected in the proximate ash content of diet HFM10 and HFM12. HFM meal had protein content of 76.1% which is lower than the value of 80.26% reported by (Bishop, *et al.*, 1996). This could be because of different processing methods of HFM.

5.3. Effect of HFM on the Body composition of *Oreochromis mossambicus*

At the end of the experiment the body moisture content was lowest in fish fed diet HFM10 and HFM12; 68.5±0.06 and 66.4±0.16% respectively, indicating better quality of flesh than the other diets which were significantly higher in the body moisture content. Body protein did not differ much from the initial composition in all the diet treatments. These values have similar trend as in the study by Bag *et al.*, (2012) on *O. mossambicus* using poultry feather meal where they recorded moisture and protein contents of 75.91% and 11.01% respectively at the beginning of the experiment and 75.28% and 11.03% respectively at the end of study.

Final body lipid increased with increasing level of dietary HFM and was highest in diet HFM12, further explaining the high weight gain in fish fed diet HFM10 and HFM12 and the lower moisture content compared to the other diets which had significantly lower lipid content in the carcass.

5.4.Digestibility of Hydrolyzed feather meal in the diets of *O.mossambicus*

The ADC of protein increased with increasing inclusion levels of HFM. Diet HFM12 and HFM10, had higher ADC of protein than HFM0, HFM4 and HFM8. This indicates that the inclusion of HFM in this study improved digestibility of the diets. The significantly lower ADC of protein in diet HFM4 and HFM8 could be a result of the high fiber content resulting from the high proportion of wheat bran. The digestibility of shrimp shell meal can be poor due to high chitin content Tibbetts, *et al*, (2006) and its inclusion is relatively high in in the first three diets.

The ADC of CP reported in this study are higher than the ones reported by (Munguti, *et al*., 2014), probably due to the different plant protein sources used in the diet formulations. HFM improved the digestibility of the diets and had no adverse effects on digestibility in this study. The same scenario is reported by Zhang *et al*., (2014) in a study cotton seed meal and soy bean meal were partially replaced by HFM at inclusion levels of 12% in the diet of hybrid tilapia (*O. niloticus* × *O. aureus*) without any adverse effect on digestibility.

Degree of hydrolysis assays have been used before in aquafeeds to ascertain feed quality and to measure digestibility (González-Félix *et al.*, 2013, Yasumaru & Lemos, 2014,Nielsen, 2001). Following in the trend and consistent with growth parameters, diet HFM10 and HFM12 had the highest DH of 25.6±0.01 and 26.1±0.01% respectively while

the rest had lower DH as shown in figure 4.5. It indicates, together with the highest measured ADC in this study, that the processing method of steam hydrolysis of the feathers did create reasonably good protein source. The high proportion of wheat bran in diet HFM4 and HFM could be the reason for the low DH as argued by (Alarcón, *et al*, 2002) where they realized that the DH decreased with increasing levels of plant proteins. High fiber content in diets might also affect the protein hydrolysis, both in vitro and in vivo. The significant correlation between DH and the ADC of CP (R^2= 0.7187) confirms that DH is a reliable indicator of the digestibility of protein in tilapia diet in this study. González-Félix *et al*., (2013) reported a non-significant correlation of R^2=0.6 in the diet of Nile tilapia while evaluating the impact of replacing fish meal with different plant protein sources. This confirms that HFM improved diet digestibility in the formulation.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

- Results from this study have shown that feather meal is a feasible ingredient in shrimp in Kenya.
- It is also clear from this study that feather meal can replace up to 63% (at inclusion levels of 12%) of shrimp meal in the diet of *O. mossambicus* when formulated together with plant protein such as rapeseed meal. this confirms the alternative hypothesis
- Degree of Hydrolysis assay by O-PA method can be an accurate and quicker way of assessing the digestibility of ingredients and this should be done for all the ingredients to ascertain their quality.
- Inclusion of hydrolyzed feather in diet formulation in this study improved the digestibility of the formulated diets
- Hydrolyzed feather meal does not have negative effect on the carcass composition of the *O. mossambicus*, there the null hypothesis is rejected

6.2 Recommendations

- In this study the author recommends the ideal diet formulation for O. *mossambicus* diet to be HFM10 or HFM12 for best growth, feed efficiency and survival. For sustainable aquaculture to be realized, up scaling of research on fish nutrition

should be ensured through incorporation of the non-conventional protein sources like hydrolyzed feather meal.

- Hydrolyzed feather promotes digestibility of the formulated diet at inclusion levels of 10 %, hence should be incorporated in diet formulation.
- The study recommends inclusion levels of 12% hydrolyzed feather meal to promote body lipid composition

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