

**EFFECT OF SUPPLEMENTAL PHYTASE, PROTEASE AND SPROUTED
SORGHUM ON NUTRIENT DIGESTIBILITY, GROWTH AND COST
EFFECTIVENESS OF PRODUCING AFRICAN CATFISH *Clarias gariepinus*
(BURCHELL, 1822)**

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**A RESEARCH THESIS SUBMITTED TO FACULTY OF NATURAL
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AWARD OF DOCTOR OF PHILOSOPHY IN AQUACULTURE AND
FISHERIES SCIENCE.**

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DECEMBER, 2018

DECLARATION

I, Chloe Kemigabo, declare that this thesis is a result of my own original effort and work and that to the best of my knowledge, the findings have never been previously presented to Lilongwe University of Agriculture and Natural Resources (LUANAR) or elsewhere for the award of any academic qualification. Where assistance was sought, it has been accordingly acknowledged.

I also declare that shorter versions of experiment 1, 2 and 3 in this thesis have been published in peer reviewed journals listed below:

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C. (2017) Effect of toasting on phytic acid, protein and pH in a plant-based diet and its ingesta in fed catfish. *African Journal of Rural Development*, 2:77-28.

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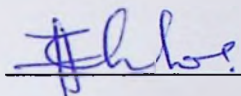
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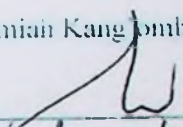
CERTIFICATE OF APPROVAL

We the undersigned, certify that this thesis is a result of the authors own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within Lilongwe University of Agriculture and Natural Resources (LUANAR) or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on 13th November, 2018.

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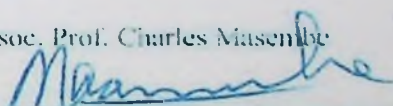
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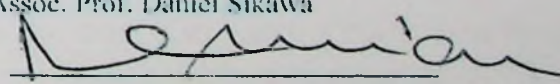
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ABSTRACT

Phytic acid is an anti-nutrient found in almost all feed grade plant materials. Phytic acid binds with nutrients to form complexes that are poorly digested by fish. To increase bioavailability of nutrients from such complexes, diets of 33%, 37%, 50% and 65% crude protein (CP) were made out roasted ingredients. The diets were mixed with phytase, protease and sprouted sorghum and evaluated with African catfish (*Clarias gariepinus*) in four cascading experiments. Phytic acid, CP content, pH, digestibility of protein and phosphorus were determined in the diets. Then growth performance of catfish hatchlings fed on 65% CP diets mixed with protease and that of catfish fingerlings fed on 33% and 37% CP was determined. Results showed that roasting feed ingredients significantly reduced phytic acid (from $5.89 \pm 0.04\%$ to $5.67 \pm 0.04\%$) while dietary phytase in 33% and 37% CP diets and protease in 50% and 65% CP significantly increased digestibility of protein and phosphorus. Phytase increased digestibility of protein from 75.07% to 96.50% and phosphorus from 74.44% to 98.50%. On the other hand, feeding catfish hatchlings on dry diets with 1250 units of protease significantly ($p < 0.05$) enhanced their survival and weight gain from 11% - 42% and 15-296mg respectively. Growth of catfish fingerlings fed on 33% and 37% CP diets mixed with phytase was also significantly enhanced ($p < 0.05$; 174.40-164.04g) in ponds. The cost benefit ratio of pond catfish was significantly higher ($p < 0.05$; 6.30) with 33%CP diets incorporated with 1000 phytase units/kg⁻¹. These results demonstrate that supplemental protease can enhance use of high protein dry diets as catfish starter diets and phytase use of low protein diets as fattening diets. It was therefore recommended that in addition to roasting diet ingredients, protease be used as a micro additive in catfish starter diets and phytase enzyme in grow-out diets.

DEDICATION

To my lovely husband (Charles Damba) and children (Esther Namutangula, Timothy Damba, Christian Damba, Shekinah Tiyamiike and Solomon Damba).

May it be an inspiration to you all!

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LIST OF ABBREVIATIONS AND ACRONYMS

FAO	Food and Agriculture Organization
NARO	National Agricultural Research Organisation
MBAZARDI	Mbarara Zonal Agricultural research and Development institute
DFR	Department of Fisheries Resources
CP	Crude protein
NaFIRRI	National Fisheries Resources Research Institute
NACA	Network of Aquaculture Centers in Asia-Pacific
FTUs	Phytase Units
NRC	National Research Council
FISH	Fisheries Investment for Sustainable Harvest
BecA	Bioscience for Eastern and Central Africa
ILRRI	International Livestock Resources Research Institute

CHAPTER ONE

INTRODUCTION

1.1 Background

The growth in human population is approaching the maximum carrying capacity of the earth estimated to be 9 billion people (FAO, 2018). This has greatly reduced resource availability in all sectors of economic development including the food sector. There is wide spread food shortages especially in developing countries and so is fish scarcity. Fish provides high biological quality protein and oils that are essential for body welfare and brain development especially among children under five years of age. The demand for fish is estimated to be 216,6 million tones yet only 170 million tones is supplied leaving a deficit of about 47 million tons for food fish (FAO, 2018).

Fish farming is the only venture that is hoped to increase fish supply since supply from the wild has been stagnating at about 90.4 million tons each year since 1990's. It is projected that aquaculture will provide up to about 40% of the deficit (18 Million tons). The 60% (28 million tons) that will not be catered for is expected to have negative effect on the economy especially in Africa where fish farming is lowest (2.5%) of the global production. This is anticipated to perpetuate Africa as a net fish importer with fish imports increasing from 14% in 2000 to 33% by 2030 (World Bank, (2013). Malnutrition and nutrient deficiency specially manifested among children of less than five years of age and women of reproductive age are expected to increase (Kikafunda *et al.*, 2012).

1.2 Fish feed quality and sustainable fish production in Africa

Innovations that increase digestibility of fish diets are required to reduce high costs associated with use of fish meal that comprise 40-70% of operating costs in a typical commercial fish farm. According to Hecht (2005), poorly digested fish diets negatively affect productivity and profitability in culture of high value fed species like catfish because of associated phosphorus water pollution that stimulates nuisance plant growth reducing water amenities to various users.

Plant products especially of legumes and cereals are increasingly used as basic ingredients in fish diets instead of the historical high quality fish meal (FAO, 2016.). They are a more sustainable option for fish feed because they are readily available, fairly priced and have protein and oil content comparable to that of fish meal (Kumar *et al.*, 2012). It is however recommended that plant products be pretreated against antinutrients they contain especially phytic acid before they can be used in fish feed (Ayadi *et al.*, 2012). This is in particular important for diets of African catfish which has not evolved an efficient mechanism to breakdown phytic acid (Gabriel *et al.*, 2007; Kang'ombe and Brown, 2008 ; Kumar *et al.*, 2011). To identify the most appropriate pre-treatments however, requires basic information about the content of phytic acid in compound diets and how it is affected by the different pre-treatments. Some pre-treatments may be highly efficient but practically not feasible with regard to affordability, accessibility, handling and or applicability.

1.2.1 Pre-treatment and nutrition

The concept of food pre-treatment is as old as human history. Man has used pre-treatments such as roasting, soaking, sprouting and malting/fermentation to improve the palatability features of his food such as texture, taste and shelf life. These pre-treatments especially roasting and sprouting activate a number of enzymes that naturally exist in plant material but in insoluble and low activity ($210 - 670\text{Ukg}^{-1}$) including phytase (Azeke *et al.*, 2011; Raj Kishor *et al.*, 2015). The activated enzymes break down antinutrients to avail bound nutrients for absorption. In a study by Sokrab *et al.* (2012) and Bazaz *et al.* (2016) reduced phytic acid and increased phosphorus and protein digestibility in plant-based fish diets and hypo-allergic baby weaning diets respectively. Sprouting all feed ingredients (cereals and grains) for commercial production of dry fish diets/feed may however be compromised by inappropriate drying facilities in Africa. Poor drying can expose feed ingredients to mould growth thus increasing their susceptibility to contamination with aflatoxin (B1), the most potent toxins for liver cancer.

Roasting is a widely practiced heat pre-treatment because it is less cumbersome compared to sprouting and has been proven to reduce a number of heat labile antinutrients including protease inhibitors, cyanoglucosides while preserving zinc and iron (Omoruyi, 2007). However, limited information on effects of roasting and sprouting on phytic acid content especially in diets compounded from a mixture of unrefined tropical plant products has limited their effective application in aqua diets. The effect of roasting on phytic acid content has been evaluated in some individual feed ingredients

but its effect in compound diets is not well documented. The situation is even made more complex with inadequate knowledge on the content of phytic acid in compound diets. Other heat pre-treatments of phytic acid such as autoclaving and boiling have been found to reduce phytic acid significantly but also reduce other useful nutrients lowering the overall quality of fish diets (Baruah *et al.*, 2004; Hussain *et al.*, 2011).

1.2.2 Advances in enzymatic applications in feeds

Over the last decade, a number of feed additives have been developed to improve efficiency of feed utilisation for growth. One of such advancements has been extraction of enzymes mainly from microbial communities of fungi and bacteria to optimize their usefulness in different industrial processes. For the feed sector, phytase extracts were mainly developed and used in diets of monogastric animals (especially poultry and piggery) primarily to increase phosphorus absorption so as to comply to water discharge standards as a safeguard against phosphorus water pollution. The use of phytase enzyme was later also adopted in aqua feeds for the same reason. However, it has been recognized that other than increasing phosphorus up take through breakdown of phytic ,there are other advantages to be exploited for economic gains and these included enhancing digestibility of dietary protein and growth of fish (Akpoilih *et al.*, 2017; Qiu, 2015; Hussain *et al.*, 2014; Nwanna, 2008; Tudkaew *et al.*, 2008; Debnath *et al.*, 2005; Masumoto *et al.*, 2001).

Phytic acid also binds protein limiting its digestion, so this study anticipated that dietary protease enzyme would improve digestibility of protein especially in dry starter diets for nourishment of catfish hatchlings. Increasing digestibility is essential for protein in diets of catfish hatchling because the hatchlings lack developed and functional digestive system (until 7-9 days) which limits digestion of dry diets after the yolk is used up (Dabrowski, 1984). Live feed organisms such as algae, cladocerans, (daphnia), rotifers and crustacean (brine shrimp ; *Artemia* embryos) contain endogenous enzymes that stimulate proteolytic activity in the stomach of fish hatchlings to initiate auto-digestion of the prey before the digestive system develops (Enyidi and Nduh-Nduh, 2015; Kamarudin, 2011; Pillay, 1993). *Artemia* nauplii are the commonly used starter feed because they remain viable over a long period of time (5 years), are highly digestible, and easy to transport (Garcia-Ortega 1998; Wickins and Lee, 2008). *Artemia* nauplii, was found to be an excellent first feed for rearing catfish larvae under hatchery conditions (Kerdchuen and Legendre, 1994; Lavens and Sorgeloos, 2000; Leger *et al.* 1986). However, raising catfish hatchlings using *Artemia* nauplii in many African countries is constrained by its high cost and limited access as these crustacean embryos are native to marine waters that are less common in Africa. To reduce this constraint, artificial diets incorporated with protease were evaluated with catfish hatchlings as alternatives to *Artemia*. Inclusion of dietary protease targeted to increase hydrolysis of protein so as to make it more digestible and available for fish growth (Drew *et al.*, 2005; Li *et al.*, 2015; Liu *et al.*, 2018).

The trials were also conducted to evaluate effects of phytase enzyme on digestibility of diets by catfish and growth of catfish which has been inconsistent, sometimes none or

even negative in a number of species (Akpoilih *et al.*, 2017; Debnath *et al.*, 2005; Hung *et al.*, 2015; Nwanna and Schwarz, 2007; Sajjadi and Carter, 2004; Yoo *et al.*, 2005). The controversy is attributed to a number of factors including irritation (inflammation) of the gut in some fish species, destruction by the high temperatures during pelleting, feed and fish gut pH and phytic acid content. Differences in fish species, size, phytase sources, diet formulation with its corresponding phytic acid content, and rearing conditions is also among explanatory factors.

This study therefore documented the content of phytic acid in diets whose major ingredients were roasted, effects of phytase, protease and sprouted sorghum on digestibility of diets, growth and cost effectiveness of producing African catfish under hatchery and pond systems.

1.3 Description of the problem and justification of the study

1.3.1 Problem statement

The use of fish meal as a sole protein source to produce quality fish feeds is no longer economically, environmentally and socially viable. Of the 15.6 million tons of fish reduced into fish meal, about 70% is used in aquaculture (FAO, 2018). This leaves insufficient amounts for feeding livestock, poultry and humans (FAO, 2010). Environmentally, fishing activities associated with harvesting of small pelagic fish species used to produce fish meal destroy other non-target organisms. This reduces aquatic biodiversity and the associated ecosystem goods and services putting millions of people at risks of malnutrition and poverty (Tacon, 2009). Plant products such as wheat

and soybean contain protein and oil content comparable to that of fish meal (Lech and Reigh, 2012). However, majority of plant by-products contain antinutrients (Baruah *et al.*, 2004) of which phytic acid is considered the most detrimental (Kumar *et al.*, 2011). Phytic acid binds with nutrients in plant-based feeds including phosphorus and proteins to form indigestible complexes that limit fish growth (Hidvegi and Lasztity, 2002; Kumar *et al.*, 2011). A number of strategies have been tried to reduce phytic acid in plant based diets but with little success. Pre-treatment of cereal and legume-based diets by roasting, sprouting and inclusion of exogenous digestive enzymes have potential to increase palatability and nutrient bio-availability through breakdown of indigestible complexes such as phytic acid but have not been effective. (Hussain *et al.*, 2014; Qiu, 2015). Dietary enzyme supplements (especially, phytase) have shown inconsistent effects on digestibility and growth patterns in fed fish (Wallace, 2015). Effective application of such dietary pre-treatments in fish nutrition is limited among other factors by insufficient information on the minimum tolerable dietary phytic acid, phytic content in compound diets and subsequent effects of the pretreatments on its content and performance of fish fed on pre-treated diets (Baruah *et al.*, 2004 ; Bohn *et al.*, 2008 ; Hussain *et al.*, 2011).

The influence of such pre-treatments on subsequent performance of catfish has also been sparingly evaluated under field conditions. The scanty information on protein digestibility, growth performance and economic returns of farmed fish fed on pre-treated diets is inconsistent and sometimes contradicting (Cao *et al.*, 2007; Kumar *et al.*, 2011; Qiu, 2015). This has made usefulness of these pre-treatments especially use of enzymes

phytase) in fish diets ambiguous (Kim *et al.*, 2006) necessitating further research and exploration of possible alternatives.

1.3.2 Justification of the study

Dietary pre-treatments such as roasting, sprouting, fermentation and enzyme incorporation are associated with fewer health-related risks compared to use of synthetic drugs and genetic engineering, hence more acceptable to fish consumers and the environment. A number of practices including but not limited to use of drugs (antibiotics, fungicides), growth hormones, genetic engineering and enzymes supplements have been tried (Muir, 2004; Rodgers and Furones, 2009). Through genetic engineering, transgenic crop varieties with low phytic acid or high phytase enzyme have been produced as alternative ingredients but their use is still restricted by the social misconception about genetically modified organisms (GMOs) (Kumar *et al.*, 2012). Antibiotics, fungicides and growth hormones have been associated with human and environmental health risks such as drug and microbial resistance, gigantism and heart complications that need to be addressed for sustainable production of safe and nutritious food fish (Hixson, 2014).

Improving digestibility of diets by catfish will translate into reduced use of fish meal thus improving profitability of fish farming that has been constrained by high feed costs that comprise 60-70% of operating costs in a typical commercial catfish production enterprise. It will also reduce the global challenge of phosphorus leakage to underground deposits where it is hard to retrieve for use in plant growth. Increased phosphorus absorption also reduces water pollution that results in growth of nuisance aquatic plants that reduce social amenities for various water users. It is important to provide an

estimation of phytic acid content that is required to be removed after the common pre-treatment of roasting. This provides nutritionists with information on the content of phytic acid to be further removed after roasting of major ingredients using the common method of open roasting. It is relevant to analyze the phytic acid in compounded diets because practically no single diet is made from a single ingredient. Lack of information on the amount of phytic acid in compound diets might actually be the underlying factor for inconsistent effects observed with use of dietary enzymes due to lack of standardization. Usefulness of phytase enzyme in plant-based diets still ambiguous (Kumar *et al.*, 2012) thus further necessary that further research on alternative approaches be conducted.

Evaluating effects of dietary enzymes under field conditions will guide will provide more dependable results to feed manufacturers and farmers and enable adoption of appropriate pre-treatment of anti-nutrients in plant material to enhance their utilisation as fish diet ingredients. This will gradually translate into reduced use of fish meal to produce quality fish diets, reduce costs thus increase profit margins and the social responsibility of aquaculture as required by a number of policies and conventions (FAO, 2012; FAO, 2010; Gabriel *et al.*, 2007; NEMA, 1999).

The basic concepts behind this study are all in line with majority of the 2030 agenda of Sustainable Development Goals of the United Nations (SDGs) 2015 and the Cape town Call to Action for Aquaculture in Africa (2017) that emphasize promotion of innovations

that increase human nutrition, income generation, employment and sustaining acceptable environmental integrity.

1.4. Research objectives and hypotheses

1.4.1 Goal

The goal of the study was to increase utilisation of plant – based diets for production of high value farmed fed fish species through pre-treatment of major antinutrients.

1.4.2 Main objective

To identify the most beneficial pre-treatment of phytic acid and/or its effects in diets among ingredient roasting and diet supplementation with sprouted sorghum, phytase and protease enzymes to enhance their utilisation by farmed African catfish for growth.

1.4.3 Specific objectives

- i) To determine effect of roasting on phytic acid, crude protein content and pH in catfish diets containing a mixture of tropical plant material.
- ii) To assess the effect of dietary phytase, protease and sprouted sorghum on digestibility of protein and phosphorus in dry diets by catfish.
- iii) To determine effect of varying dietary protease from 750, 1000 and 1250 units/kg of dry starter diets (65%CP) on growth performance and cost effectiveness of catfish hatchlings.

- iv) To determine effect of varying dietary protease from 750, 1000 and 1250 units/kg of dry starter diets (65%CP) on growth performance and cost effectiveness of catfish hatchlings.

1.4.3 Null hypotheses

- i) Roasting ingredients has no significant effect on dietary phytic acid, crude protein content and pH.
- ii) Incorporating phytase, protease and germinated sorghum independently in plant-based diets does not significantly affect digestibility by catfish.
- iii) Varying dietary protease from 750 ,1000 and 1250 in dry starter diets with 65%CP does not significantly affect growth performance and cost effectiveness of catfish hatchlings.
- iv) Increasing dietary crude protein from 33% to 37%CP and phytase from 750 to 1250 units/kg has no significant effect on growth performance and cost effectiveness of catfish fry in earthen ponds.

1.5 Scope of the study

This study focused on pre-treatments of phytic acid and/or its effects in catfish nutrition using roasting and digestive enzymes (phytase and protease) that are naturally produced during such processes like sprouting/germination but have also been recently commercially extracted from microbial bacteria and fungi. Much as plant material are the most accessible and relatively affordable feed grade ingredients, pre-treatment of the heat stable phytic has not been given considerable attention in fish diets. Recent developments

on enzymes have mainly focused on phytase due to its environmental benefits of reducing phosphorus pollution but exploiting enzyme supplements to also improve fish growth is still required to reduce costs. Inconsistent and contradicting performance of fish fed on diets with phytase enzyme is associated with variations in production technologies of plant ingredients, location of phytates in different ingredients (monocots and dicotyledonous seeds), fish species, and age and enzyme source. This therefore calls for case specific studies of phytase inclusion and exploration of alternative interventions for comparison.

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Unlike Akpoilih *et al.* (2016) and Agbabiak *et al.* (2012) who evaluated growth of catfish fed on diets incorporated with phytase respectively in in-door tanks and happas suspended in concrete tanks, this study was conducted under field conditions. Other than nutrient utilisation by catfish, cost effectiveness of its production was also considered to enable adoption of the best practice by farmers. In particular, the study determined effects of roasting, sprouted sorghum and phytase and protease enzyme supplements on crude protein and phosphorus utilisation by catfish and how they translate into cost effectiveness.

1.6 Significance of the study

The study generated information that can support decision making on the pre-treatments of phytic acid and /or its effects found in a number of plant ingredients and diets (Kemigabo *et al.*, 2017). The information forms a basis for further research, raising awareness and training of feed manufacturers and fish farmers on how to reduce phytic

acid and or its effects. The study forms the basis for application of enzyme supplements in diets to improve digestibility, nutrient utilization and cost effectiveness of catfish production. The generated information can enable manipulation of diet pH to match that of catfish gut and the enzyme supplements to for digestive efficiency. The results on phosphorus absorption encourage enforcement of policies and standards for waste discharge from aquaculture due to availability of enabling technology.

1.7 Theoretical framework

This theoretical framework highlights how enzymes work and the role of appropriate pre-treatments in production of enzymes for appropriate application in fish nutrition is to be guided by this study.

Enzymes are globular biological protein catalysts that speed up anabolic and catabolic chemical reactions in the body. Enzymes speed up the rate of the reaction by both lowering the energy required by providing alternate reaction routes at a lower energy level and aiding the molecules to orient themselves correctly during collision. Further to this, the enzyme may also break up one of the reacting molecules into more reactive intermediates, thus speeding up the rate of reaction in this way as well. Without enzymes, chemical reactions would take a lot longer to take place which could negatively affect body functions. Enzymes work by lowering the activation energy needed to start the reaction so that the reaction proceeds much faster and more frequently. They work in many ways: including coupling non-favorable reactions with other more favorable ones, bind substrates in the correct orientation, provide catalytically active groups or stabilizing

the transition state. Each enzyme is specific to its own substance and without a substrate, an enzyme would have nothing to work on. The mode of action /enzyme specificity is explained by two theories "the Victor Henri's quantitative theory of enzyme kinetics" and the "Lock and Key" theory, first introduced by Emil Fischer in 1894.

An enzyme has a 3-D shape called it's active site, it has a specific shape for its substrate to fit. It can break a substrate, build it up, or even modify it. Each type of enzyme has specific optimum condition under which it works. Environmental conditions such as temperature, pH, light, availability or absence of Oxygen, minerals like Fe or Ca, influence enzymatic activity. Being protein in nature, high temperature and extreme change in PH can cause enzyme to denature and stop working. The optimal temperature for most enzymes is 35°C-45°C.

Enzymes exist naturally in plant materials but at low concentrations and dormant state. They are known to be activated by pre-treatments such as roasting and sprouting. Of recent, enzymes have been extracted, concentrated and packaged for a number of industrial applications including feed. However limited information on the extent to which such pre-treatments improve digestibility of feed to boost fish growth limited their adoption in aqua feeds. Their use has been associated with poor choice, use and applications under farmer conditions have (Kumar *et al.*, 2011). These gaps have compromised fish feed quality, fish growth, productivity and profitability of fish farming in Uganda. This study explored these gaps by undertaking laboratory analyses of phytic acid concentrations, crude protein and pH in diets made from roasted and un roasted ingredients, determination of protein digestibility in diets incorporated with phytase,

protease and sprouted sorghum, determination of growth for catfish hatchlings and fingerlings under hatchery and grow-out pond conditions fed on diets incorporated with phytase and protease enzymes. Analysis of results described appropriate use of dietary enzymes to improve utilization of low value feeds to enhanced catfish growth and productivity.

1.8 Conceptual framework for this study/ research

This conceptual frame work provides the background of the research, the problem addressed, the specific gaps that retained the problem in place, what was done towards addressing the gaps, the results and recommendations and suggestions for research (Figure 1.1).

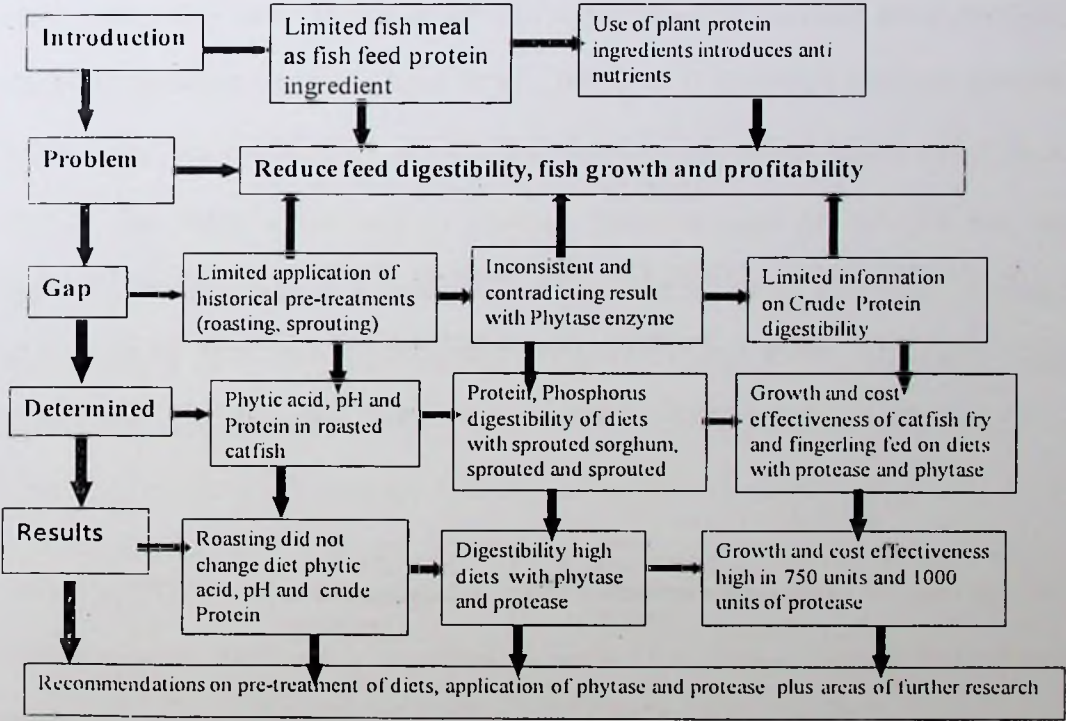


Figure 1.1 Conceptual frame work of the study

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter comprises a review as a background for this study on the growth performance of African catfish fed on formulated enzyme incorporated diets. It contains sections on nutrient requirements of catfish, feed constituents and antinutrients, characteristics of phytic acid, its diagnostic methods, a description of how it is analysed and treated.

2.2 Fish feed production in Africa

Feed is the major input in production of farmed fish that accounts for at least 60-70% of the total operating costs (Gabriel *et al.*, 2007). It is therefore the sole determinant of productivity and profitability of most fish farming enterprises (Jamu and Aymila, 2003). Quality fish feed is required to produce extra tonnage of fish for the expanding population in especially in Africa (FAO, 2012 ; Hecht, 2006). However, poor quality and highly priced fish diets predominate (Gabriel *et al.*, 2007), frustrating aquaculture development (Hecht, 2006).

Global production of commercial fish diets increased from 587 000 tons in 1995 to 2.4 million tons in 2007 and is projected to reach 11.7 million tons by 2020 (FAO, 2010). However, in Uganda feed manufacturing has gone commercial with only one private company that produce floating (extruded) fish feeds (Cocker, 2014). Production of

African catfish alone required about 343,000 metric tonnes but only 235,000 metric tonnes of feed were produced due to low purchasing capacity of farmers (FISH, 2009). Effective demand was reduced by high feed costs associated with use of the costly fish meal. Previously underutilized small pelagic fish species were directly consumed by humans leaving less for production of fishmeal. This led to search for alternative protein sources to replace fish meal in feed formulations beyond the level at which fish meal becomes a limiting factor of production (De Silva and Anderson, 1995; El-Sayed, 1999; FAO, 2009). Plant protein especially from oil seeds, legume grains and cereals are considered a more reliable source of protein for partial or total replacement of fish meal (Kumar *et al.*, 2012). They are widely distributed, fairly priced and more easily adapted.

2.3 Catfish (*Clarias gariepinus*) farming in Uganda

From the biological perspective, catfish is undoubtedly the ideal aquaculture species in the world (Hecht *et al.*, 1996). They are the third largest cultured freshwater species comprising about 23.1% of global fish production (FAO, 2009) and about 72% of global catfish are fed on commercial complete diets (Tacon, 2009). They are popularly cultured species in sub-Saharan Africa and thus of economic importance to social economic development. Production of farmed catfish accounts for about 70% of farmed fish products in Uganda followed by Nile tilapia with about 27% (FAO, 2010; Chepkwemai, 2013; Mwanja, 2005-2016). It tolerates high stocking densities; gains biomass fast under near to ideal conditions and is easily marketable live (Nabulime *et al.*, 2015). Other than food, catfish production also supports the bait industry in the Nile perch fishery. Its effective demand as bait had reached three (3) million fingerlings per day by the year

2007 making it one of the most lucrative fish farming enterprise in the region (Isyagi, 2007).

2.4 Aqua feeds and water quality

Freshwater is limited and its quality is under constant pressure from anthropogenic activities, including aquaculture (WHO Water, 2008). Poorly formulated, un-digestible and underutilized feed is the main source of water pollution in aquaculture. It is noted that most fish kills, disease outbreaks, poor growth, poor feed conversion and other management problems are associated with poor water quality (Boyd and Tucker, 1998). About 37% of the feeds eaten is retained as biomass and the rest is discharged as particulate and inorganic dissolved wastes (Jobling, 1994). This makes particulate organic matter, inorganic phosphorus and nitrogen major components of polluted water (Boyd and Tucker, 1998; Davidson *et al.*, 2008). During decomposition of uneaten and poorly digested feed in water by bacteria, most of the dissolved oxygen is utilized leaving little for fish to breath; turbidity and eutrophication are also increased in public waters (Lin and Yi, 2003).

Water nutrient enrichment reduces carrying capacity of aquatic systems, microhabitats diversity, benthic communities and nutrient recycling. Beyond the threshold, public water use is limited for a variety of amenities including drinking, crop irrigation, fishing and recreation. These negative impacts of fish farming on natural ecosystems have been severely criticized and attracted global responsibility of balancing fish production with sustainable aquatic ecosystem goods and services (FAO, 2012 ; FAO, 2010). To effect

this responsibility a number of countries including Uganda have established regulations/standards for waste water discharge as shown in Table 2.1 (NEMA, 1999).

Increased aquaculture production is expected to be achieved by intensification. This makes efforts in maintaining acceptable water quality both in rearing facilities and receiving public waters indispensable to save many livelihoods from its adverse effects. This therefore makes enhancement of digestibility and utilisation of low value feeds such as those used in Uganda timely (Hecht, 2005; 2006).

Table 2.1 Minimum permissible limits for waste water discharge into water or land for Uganda

Parameter	Minimum Permissible Limits
Turbidity	300 NTU
Total suspended Solids	100 mg l ⁻¹
TDS	1200 mg l ⁻¹
Phosphate (soluble)	5.0 mg l ⁻¹
Phosphate (total)	10 mg l ⁻¹
Nitrite –nitrogen	20 mg l ⁻¹
Nitrate nitrogen	2.0 mg l ⁻¹
Total nitrogen total	10 mg l ⁻¹
Ammonia Nitrogen	10 mg l ⁻¹
BOD5	50 mg l ⁻¹
pH	6.0-8.0

Source: NEMA (1999)

2.4.1 Important water quality parameters and their assessment

For sustainability, the ecosystem approach has been adopted over the last few years for water quality determination and monitoring. It is hinged on a criterion that an aquatic ecosystem whose structure and functions (ecological integrity) are not disrupted is immediately suitable or suitable after simple treatment, for a variety of uses. Water quality is determined by a wide range of physical, chemical and biological parameters which affect its use for a defined purpose. For long term impact assessment, benthic invertebrates and fish are commonly used as biological indicators of water quality because they live longer, are less mobile and integrate pollutant effects into their systems over a long period. They are cheaper than chemical measurements and toxicological bioassays. For short term studies however, physical and chemical parameters are used due to limited time involved. Most physico-chemical water parameters are determined by titration following standard methods of American Public Health Association, American Water Works Association, Water Environment Federation (APHA, AWWA and WEF) (Rice *et al.*, 2012). However, some electronic gadgets and reagent kits have been developed to enable rapid measurements for alkalinity, pH, dissolved oxygen, turbidity, and water temperature in situ. Proper calibration is however important for reliable results and this makes kits and electronic meters less accurate than laboratory titration methods though.

Alkalinity is defined as the total concentration of bases expressed as milligrams per litre of equivalent calcium carbonate practically referred to as the total amount of acid required to reduce the pH of a water sample mixed with phenolphthalein indicator from

pH 8.3 to pH 4.5. In natural waters bicarbonate and carbonates bases dominate with alkalinity of above 40mgL^{-1} consider productive. The phosphorus and other nutrients are said to increase with alkalinity reaching as high as 120 mgL^{-1} in fertilized ponds. Most fish species are reported to be tolerant to high alkalinity and its buffering effects minimize pH variability for stable metabolism. For ammonia, the toxic ammonia species in natural waters is unionized ammonia ($\text{NH}_3\text{-N}$) which exists in a temperature – pH dependant equilibrium with the ammonium ion (NH_4^+) and is easily absorbed by algae and plants in natural waters than in tanks.

In fresh water, phosphorus (P) is the major limiting essential nutrient for plant growth (Wetzel and Likens, 2000). This makes even a modest increase in phosphorus to result in excessive plant and algal growth which increase turbidity and depletion of dissolved oxygen during respiration especially at night and decomposition. Excessive nitrogen is toxic and as early as 1940s consuming water with high nitrate levels caused methemoglobinemia ("blue baby" syndrome) in infants due to impaired ability of blood to carry oxygen (Knobeloch *et al.*, 2000). Effluents in small-scale, low-volume fish farming operations, effluents are relatively dilute in wastes and therefore not cost-effective for conventional treatments as in intensive systems.

2.5 Nutrient requirements of catfish

One of the most important aspects in aquaculture is the nutrition, as for optimum development of organisms, it is essential to have all the necessary nutrients, in terms both of quantity and quality. Fish in confined systems need a diet that is properly balanced for the specific requirements of species; although it must also be considered that nutritional

requirements are affected by the growth rate, growing conditions, and environmental factors. This chapter summarizes information concerning the the energy and nutritional requirements of catfish fish.

Catfish like other fish, require nutrients for energy, health, somatic growth and reproduction irrespective of the culture system. Major nutrients required by catfish are proteins, lipids and carbohydrates with protein as the main constituent (Jobling, 1994). Protein requirement is linked to general energy needs of fish at a given water temperature. Vitamins and minerals are also essential but in small quantities. The quantity and quality of nutrients supplied depends upon the goal of the farmer but as intensification of fish farming increases like in cage farming, complete diets become paramount. According to Hecht (2005) and Tacon and Methian (2009) the nutritional requirements of African catfish (*Clarias gariepinus*) differ among age groups (Table 2.2).

Table 2.2 Important dietary requirements of African catfish (*Clarias gariepinus*) at different life stages

Nutrient	Hatchlings (12-14 days)	Nursery (0.5- 10g)	Grow out (10-100g)
Crude protein (% min)	55	50	40-43
Least cost & or appetite feeding requirements			
Crude protein (% min)	55	50	35-38
Lipids	9		9.2-17
Carbohydrates	21		15 -35
Digestible energy (kJg ⁻¹)			12.7-16
Metabolisable energy (kJg ⁻¹)			13
Gross energy (kJg ⁻¹)			24-11.0
Protein-energy ration (mgkj ⁻¹)			20-136
Lipid to carbohydrate ratio (g/g)			2.47 (13% lipid in 33.42% carbohydrate)
Calcium (%)			0.8 -1.8
Phosphorus (%)			0.6 -1

Source: Adapted from Hecht (2005); Tacon and Methian (2009).

2.5.1 Requirements for protein and amino acids

According to Jobling (1994) and Xu *et al.* (2001) omnivorous fish such as catfish prefer protein as an energy source to lipids and carbohydrates. This implies that their diets need higher protein with well balanced amino acids in terms of quality and quantity. Amino acids are required as major constituents of tissues, organs, vital nitrogenous compounds including hormones, nucleic acids, and vitamins and are responsible for repair of decomposed tissue proteins. Only ten (10) amino acids have been well studied and found

not to be manufactured by the body of catfish (Jobling, 1994) and thus have to be supplied through diet (Table 2.3).

Feeding fish diets with excess protein is, however, uneconomical and environmentally unsound because protein is the most expensive dietary component and major water pollutant. Total protein requirements generally increase with fish size while the reverse is true for virtual protein requirements (FAO., 2010; Guillaume *et al.*, 2001; Wilson, 2002).

Table 2.3 Essential amino acid requirements for grow out catfish

Amino acid	Optimum level (% Protein)	Total (mg/100mg)	Free amino acids (mg/mg)
Argenine	4.3	1.64	0.00895
Histidine	1.5	0.923	0.000542
Iso Leusine	2.6	0.992	0.000185
Leusine	3.5	1.76	0.000243
Lysine	5.1	3.84	0.0249
Threorenine	2.1	0.928	0.000142
Tryptophan	0.5	-	-
Valine	3.0	1.10	0.000483
Methionine + Cystine	2.3	0.448	-
Phenylamine	5	1.10	
+Tyrosine			

Source: NRC (1993)

Deficiency of indispensable amino acid results in poor utilization of dietary protein; poor feed efficiency, low live weight gain, growth and immunity. In severe cases many tryptophan-deficient fish become scoliotic while Methionine deficiency results to lens cataracts (Lovell, 1998).

2.5.2 Requirements for lipids

Lipids are oily organic compounds that are insoluble in water but soluble in organic solvents at standard temperature and pressure (De Silva and Anderson, 1995). They are components of body structures especially the cell membrane where they maintain membrane flexibility and permeability together with carbohydrates and proteins. Dietary lipids should provide essential highly unsaturated fatty acids (HUFAs) such as linoleic acid (n-6, C18:2) and linolenic acid (n-3 C18:3). They are sources of highly digestible energy and a vessel for absorption of fat soluble vitamins during digestion, precursors of hormones, enzymes and growth factors (Guillaume *et al.*, 2001; Jobling, 1994; Lim and Webster, 2006). Lipids from by-products of terrestrial animal and plant ingredients are rich in omega 6 (n-6 series) fatty acids while lipids from water based ingredients especially of marine origin are rich in omega 3 (n-3 series) (Jobling, 1994). Catfish being an omnivore fresh water fish require dietary omega 3 fatty acids but in lower quantities than carnivorous and marine fish whose prey/ concentrate omega 3 fatty acids from micro algae and bacteria through bioaccumulation (personal opinion).

Highly saturated lipids are more digestible (90-98%) than poly unsaturated fatty acids (PUFAs) such as stearate (of 18carbons) which may require dehydrogenation (Guillaume *et al.*, 2001; Webster and Lim, 2002). High dietary inclusion of lipids (15-18%) reduces protein requirement by providing most of the required energy thus sparing proteins for tissue deposition. In grow out catfish, better growth and feed efficiency was observed in 37% CP diets with 10-25% lipid (Bilguven and Ibrahim, 2013; Kim *et al.*, 2006; Watanabe *et al.*, 1988). This makes considerations of protein-energy ratio important in

fish feed formulation rather than just protein content only. However, high lipid content in feeds results in sticky feed which reduces pelleting efficiency. Therefore, after the minimum requirement is met, some oil is included after pelleting, in a process referred to as the over oiling to reduce feed dust. However lipid inclusion above 18% results in fatty and low weight fish (Jobling, 1994) and should be born in mind during fish fattening.

2.5.3 Requirements for Carbohydrates

In the aqueous water environment, fish digestive and metabolic systems are better adapted for proteins and lipids use as energy sources than carbohydrates which are scarce (Guillaume *et al.*, 2001). Fish is therefore known to maintain satisfactory growth with no symptoms of nutritional diseases when fed carbohydrate-free diets (Lim and Webster, 2006). However, carbohydrates have several practical and physiological functions in plants and animals including serving as major energy reservoirs (starch, glycogen), structural components like chitins in exoskeletons, cellulose in cell walls that they are always included fish diets (Lim and Webster, 2006; NRC, 1993). Although relatively few studies have addressed the use of carbohydrates in the diets, most fish are sensitive to high inclusion levels (> 36%).

2.5.4 Requirements for vitamins

Vitamins are organic compounds required in trace amounts from the diets and other external sources for normal growth, reproduction and health (Lim and Webster, 2006; NRC, 1993). They are categorized into water soluble and fat soluble (NRC, 1983). Eight of the water soluble vitamins, thiamin, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid, vitamin B12, and biotin are collectively called vitamin B complex and

primarily serve coenzyme roles (Lovell, 1998). Choline, inositol, and vitamin C are also water soluble but are needed in larger quantities and in addition to playing coenzymes roles, have other functions (Lovell, 1998; NRC, 1993). Fat soluble vitamins A, D, E, and K function independent of enzymes although they may have coenzyme roles (NRC, 1993). Very few studies however exist on vitamin requirements for catfish and fish premix is usually added onto diets to cater for minimum requirements (Table 2.4).

Table 2.4 Vitamin requirements of catfish at different growth stages

Vitamins	Hatchlings (12-14 days)	Nursery (0.5-10g)	Grow out (10-100g)
Vitamin A IUkg ⁻¹		1000-2000	
Vitamin D IUkg ⁻¹		500-1000	
Vitamin E (min mgkg ⁻¹)		25-50	
Thiamine (min mgkg ⁻¹)		1	
Riboflavin (min mgkg ⁻¹)		9	
Pyridoxine (min mgkg ⁻¹)		3	
Pantothenic acid (min mgkg ⁻¹)		10 to 15	
Niacin (min mgkg ⁻¹)		33.1	
Folic acid (min mgkg ⁻¹)		1.2	
Choline (min mgkg ⁻¹)		400	
Biotin (min mgkg ⁻¹)			2.49
Ascorbic acid (min mgkg ⁻¹)	150-500		11 to 60

Source: NRC (2011).

2.5.5 Requirements for mineral elements

Fish require minerals to maintain normal life processes, as components of hormones, enzymes skeletal structures, electron transfer system, regulation of acid-base equilibrium and osmoregulation (NRC, 1993). Meeting mineral requirements of fish is challenged by

ionic exchange and their absorption from water, tissue storage and formulation of mineral-free diets (Lovell, 1998 ; NRC, 1993). For this matter, both macro and micro mineral elements are included in catfish diets even when not all of them are essential (Table 2.5). Calcium and phosphorus are found in fish bone, scales in the ratio of 2:1 (Chow and Schell, 1980). Calcium, sodium, potassium, and chloride are largely absorbed from water except in calcium poor water, where about 34mg/g or less may be required for fish such as carp, eel, tilapia, and catfish. Optimum dietary Ca: P ratio was observed to range from 1:1-1:2 with a higher Ca: P ratio interfering with phosphorus absorption. Dietary trace element requirements (mg/kg) include iron, 30-170; copper, 1-5; manganese, 2-20; zinc, 15-40; cobalt, 0.05-1; selenium, 0.15-0.5; iodine, 1-4 (Lall, 2002).

Table 2.5 Mineral requirements of catfish at different growth stages

Macro elements (%)	Hatchlings (12-14 days)	Nursery (0.5-10g)	Grow out (10-100g)
Calcium		0.45	1.5
Phosphorus		0.45	0.5
Magnesium		0.04	
Sodium		0	
Potassium		0.26	
Micro elements(mg/kg) dry matter			
Iron		30	
Copper		5	
Manganese		≤ 2.40	
Zinc		20	
Selenium		0.25	

Source: Adapted from Hecht (2005) and Tacon and Methian (2009).

Numerous symptoms including anorexia, poor growth, loss of equilibrium, high mortality, hypo chromic microcytic anaemia, low hepatic iron content, low hematocrit and hemoglobin content, and lethargy (Lim and Webster, 2006) and skeletal deformities (vertebral and spinal malformations, lordosis, scoliosis, and platyspondyly (short-tail, compressed vertebrae) have been linked to mineral and/ or vitamin deficiencies.

Solubility of trace metals in natural waters is dependent on pH, type and concentration of ligands and chelating agents, oxidation state of the mineral components and its redox state. High dietary mineral content on the other hand may induce sub-lethal effects such as morphological, physiological (growth, swimming performance, respiration and

reproduction), and behavioral changes. Absorption of minerals by fish is influenced by feed digestibility, water pH, pellet size, interaction with other nutrients, chelators, inhibitors, physiological and pathological states of the fish, water chemistry, feed processing, to mention but a few.

2.5.6 Requirements for energy

In practical terms, energy is the ability to do work (Jobling, 1994). Although not a nutrient, low intake of dietary energy results in breakdown of body tissues leading to emaciation. There is priority energy allocation for maintenance activities while reproduction and somatic growth are considered last as a luxury. Because fish feed to certify energy requirements, an optimum energy level should be observed otherwise when dietary energy is high less feed is consumed resulting in reduced growth while at sub optimal energy more feed is consumed thereby increasing feed costs. The influence of energy on growth and nutrient uptakes was also reported by Kang'ombe *et al.* (2007) when energy below and above 20.5 kJ/g diets reduced growth and nutrient uptake in *Oreochromis shiranus*. For African catfish diet containing 33.5% digestible protein and 2.75 kcal/g digestible energy with a protein to energy ratio of 121 .8 (mg protein/kcal) appeared to be best utilized for growth (Hossain *et al.*, 1998). Increasing proportions decreased growth due to increased ammonia excretion and oxygen requirement as a result of decrease in energy efficiency. Thermoregulation and physical activity however increase energy requirements thus making age, season and environment influential factors. In studies by Jobling (1994) and De Silva and Anderson (1995), energy losses in form of faecal matter range from 10-40% but can increase to 60-80% with inclusion of uncooked starch such as wheat middlings and yellow corn.

2.6 Antinutrients in fish feed resources affect feed efficiency

Anti-nutrients are naturally occurring compounds within organisms classified under secondary metabolites (Kumar *et al.*, 2012). Antinutrients include substances such as protease inhibitors, lectins, phytic acid, saponins, phyto-estrogens, anti-vitamins, allergens, protease inhibitors, glucosinolates, tannins, alkaloids, protease inhibitors, allergic proteins, oxalates, phytohaema-gglutinins, cyanides, toxic non protein amino acids and aflatoxins (Table 2.6). They impart varying negative effects including reduced growth, poor feed conversion, appetite loss (anorexia), impaired reproduction, immunity suppression, nutrient bio-availability reduction and sometimes death (Krishnankutty, 2005). Fish lacks sufficient amounts of enzymes to break them down and therefore succumb to negative -physiological effects they impart at high levels in fish feeds (Baruah *et al.*, 2007).

Plant feed resources contain more anti-nutrients per unit weight than animal resources. For practical purposes, anti-nutritional factors are grouped into two large categories; heat-stable (e.g. phytic acid, condensed tannins, alkaloids, gluconosilates, phyto-oestrogens) and heat-labile groups (lectins, proteins inhibitors, cyanogens, α – galactosides and poisonous free amino acids), (Kumar *et al.*, 2012). Soybean and rape seed are major plant protein ingredients but are relatively rich in phytic acid, bitter taste, toxic proteins, phytic acid salts, haemagglutinins and trypsin inhibitors (Martin *et al.*, 2010) that require pre-treatment before use (Kang'ombe and Brown, 2008). Heat treatments have been used to deactivate a number of antinutrients but in some cases different approaches are required for heat stable anti-nutrients (Table 2.6).

Table 2.6 Classification of major anti-nutritional factors commonly present in plant-derived feedstuffs used in aquaculture diets

Anti-nutrient	Plant-derived nutrient source	Means of alleviation
Interaction with protein nutrition		
Protease inhibitors	Soybean, Jatropha kernel meal, rapeseed meal, lupin seed meal, pea seed meal, sunflower oil cake, alfalfa leaf meal, sesame meal	Heat, autoclaving
Heamagglutinins (lectins)	Soybean, Jatropha kernel meal, pea seed meal	Heat, autoclaving
Saponins	Peas, alfalfa, Jatropha kernel meal, lupin seed meal, pea seed meal, sunflower oil cake, alfalfa leaf meal	
Polyphenols (Tannins)	sorghum, Jatropha kernel meal, pea seed meal, mustard oil cake	
Chlorogenic compounds	Supplementary methionine or choline	
Interaction with mineral availability		
Phytic acid	Soybean, Jatropha kernel meal, pea seed meal, cottonseed meal, sesame meal	Use of phytase
Oxalic acid	Leaf proteins	Heat treatment
Glucosinolates	Rapeseed, mustard oil cake	Genetic improvement
Gossypol	Cottonseed meal	Genetic improvement
Interaction with vitamin availability		
Vit A (lipooxygenase)	Soybean	Heat treatment
Vit D Soybean	Soybean	Autoclaving
Autoclaving		
Vit E (oxidase)	Kidney beans	Autoclaving, addition of Vit E
Anti-nicotinic acid (niacinogen)	Corn	
Anti-pyridoxine	Linseed meal	Water extraction, heating
Anti-vitamin B12	Raw soybean	Heat treatment
Cyanogens	Cassava, sorghum, pea meal,	Heat treatment
Mimosine	Leucaena leaf meal	Heat treatment
Arginase inhibitor	Sunflower oil cake	Heat treatment
Cyclopropenoic acid	Cottonseed meal	Heat treatment
Antivitamins	Alfalfa leaf, cottonseed, pea seed and soybean meals	Heat treatment
Alkaloids	Lupin seed meal	Heat treatment
Phytoestrogens	Soybean and lupin seed meal	Heat treatment
Allergens	Soybean	Heat treatment

Source: Kumar *et al.* (2012).

2.6.1 Phytic acid and its effects

Phytic acid refers to myo-inositol 1, 2, 3, 4, 5, 6- hexakisphosphate (Abdoulaye *et al.*, 2011; Baruah *et al.*, 2007; Baruah *et al.*, 2004; Cao *et al.*, 2007; Dechavez and Serrano Jr, 2012; Garc  a-Esteva *et al.*, 1999b; Hidvegi and Lasztity, 2002; Hussain *et al.*, 2011; Hussain *et al.*, 2014; Norhaizan and Ain, 2009). It is a colourless to pale yellow viscous liquid with molecular formula $C_6H_{18}O_{24}P_6$ and weight of $660.04 \text{ g mol}^{-1}$. Phytic acid is formed naturally as plants mature mainly in seeds and grains to serve as an antioxidant and energy reserve during germinating. The salts of phytic acid are known as phytates and they are not digested by monogastric animals including fish due to lack of phytase enzyme (Pointillart *et al.*, 1987). Phytic acid is found in almost all plant protein feed ingredients that have potential to replace fish meal. Phytic content in plant feed ingredients ranges from 0.7- 2.5 %.with the highest content reported to be in rapeseed meal (5-7.5%) and 1-1.5% (Adeola and Sands, 2003; Table 2.7).

2.6.2 Phytic acid and phytates as antinutrients

Phytic acid is classified among the heat stable anti-nutrients and is considered the most powerful and detrimental anti-nutrient in plant-based fish diets (Kumar *et al.*, 2012). Phytic acid is considered an anti nutrient because its high negative charge enables it to interact with nutrients to form indigestible complexes (Papatriphon *et al.*, 1999; Papatriphon and Soares, 2001). It reacts with starch, proteins, fats and multiple minerals, over a wide pH range to form phytate complexes that cannot be broken down by fish (Kumar *et al.*, 2011; Norhaizan and Ain, 2009) thus reducing their digestibility (Mugendi *et al.*, 2010; Reddy *et al.*, 1989). Phytic acid also inhibits protease enzymes thereby

reducing their ability to break down proteins into absorbable amino acids (Von Der Haar *et al.*, 2014).

Based on this background supplementation of protease enzymes in fish diets might counteract effects of phytic acid on protein digestion and probably serve the same purpose as phytase enzyme. Phytic acid is estimated to bind about 50-80% of organic phosphorus in plants (Figure 2.1) making it responsible for about 14.4 million tonnes (65%) of annual global phosphorus fertilizer sales (Lott *et al.*, 2000). Phytic acid also chelates with divalent and trivalent mineral cations [potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), copper (Cu)] forming insoluble mineral salts. Poor protein, lipid and mineral utilization culminates into poor fish growth and mineral enrichment of water beyond threshold to sustain aquatic ecosystem goods and services. The combination of such these scenarios reduce profitability and social credibility of catfish farming.

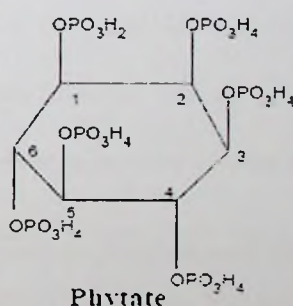


Figure 2.1 Structure of phytate.

Source: Kumar *et al.* (2011).

Phytic acid is found in almost all plant products that have potential to be used as feed ingredients with the highest content recorded in Sesame and rape seed (Table 2.7).

Table 2.7 Phytic acid content of common feed ingredients (% dry weight)

Ingredient	Average phytic acid content (%)
Sesame seeds dehulled	5.36
100% wheat bran cereal	3.29
Soybeans	1-2.22
Pinto beans	0.60-2.38
Parboiled brown rice	1.6
Navy beans	0.74-1.78
Peanuts	1.03-1.76
Barley	1.19
Coconut meal	1.17
Whole corn	1.03
Rye	1.01
Wheat flour	0.96
Brown rice	0.84-0.94
Chickpeas	0.28-1.26
Lentils	0.27-1.05
Milled white rice	0.2

Source: Kumar (2012).

2.6.3 Strategies to reduce phytic acid in plant-based diets

Efforts have been made to reduce phytic acid especially in plant material to improve palatability and digestibility. Historically, man has used soaking, sprouting, cooking, malting and fermentation. However there are recent advancements that include genetic manipulation and incorporation of enzyme supplements. Plants with high content of

phytic acid have been genetically modified to have reduced phytic acid and or to increased phytase enzyme to break down to phytic acid. Mutant maize and barley with normal phosphorus but reduced phytic acid content and transgenic pigs with phytase-like saliva have been bred (Kumar *et al.*, 2011). The salivary phytase increased digestion of dietary phytic acid, reducing inorganic phosphate supplementation, and faecal phosphorus content up to 75% which exceeded 40% reported for pigs fed on phytase enzyme supplements. However, genetic engineered organisms received low acceptability due to the misconceptions about genetically modified organisms (GMOs). Instead incorporation of enzyme supplements especially microbial phytase as feed additives has gained popularity. This enzyme supplementation also offers a basis for exploration and exploitation/ of other widely acceptable pre-treatments such as roasting and sprouting that involve enhancement of enzymatic activity.

2.6.3.1 Roasting

Heat processing especially roasting is a historical pre-treatment of food and feeds. According to (Sandhu *et al.*, 2015), roasting improves texture, palatability, color, flavor, shelf life, gelatinization of starch, inactivation of heat labile toxic compounds and enzyme inhibitors. Ovando-Martinez *et al.* (2013) observed that oat grains kilned at 88–115 °c for 90–120 minutes had intensified flavor and denatured about 60 – 80% of lipase in the grain. Autoclaving, soaking and boiling reduced phytic acid in grains and legumes but had a setback of reducing other non-target nutrients essential for fish growth (Baruah *et al.*, 2004; Hussain *et al.*, 2011). Roasting activates innate phytase enzymes that naturally occur in plant seeds and grains to breakdown phytic acid at low temperatures. As temperatures increase, the phytase get destroyed stopping further breakdown of phytic

acid. In the studies of Ari *et al.* (2012) and Ndidi *et al.* (2014), they reported that roasting had no effect on crude protein content in Bambara nuts but reduced fat and fiber content.

The observations differed from the 40.77% (from 552.3-327mg/100g) reduction in phytic acid reported by Pandey and Awasthi (2015) in fenugreek seeds roasted at $130 \pm 5^{\circ}\text{C}$. 64.42% (from 319.55-113.71mg /100g) by Ndidi *et al.* (2014) in Bambara nuts roasted at 300°C , 48.12% and 71.7% for soybean for roasted at 100°C for 30minutes by Ari *et al.* (2012). The variations may have risen due to different temperatures and plant material involved.

Roasting was reported to deactivate trypsin inhibitors, reduce cyanoglucosides while preserving zinc and iron by Omoruyi (2007). Excessive heating was however reported to reduce nutrient availability (FAO, 2012). Roasting of major ingredients in soybean based diets increased feed intake and reduced phytic acid concentration by only 3.68 %. However, applications of roasting on phytic acid in diets from unrefined plant materials especially in the tropics have remained limited. Prior understanding of the phytic acid content in locally produced diets is thus important to guide of efficient phytic acid pre-treatment strategies.

2.6.3.2 Sprouting

Another food pre-treatment commonly practiced for plant material especially legumes and cereals is sprouting. According to Azeke *et al.* (2011) and Raj Kishor *et al.* (2015), sprouting activates a number of enzymes including phytase that naturally exist in plant material but at low activity ($210\text{-}670\text{Ukg}^{-1}$). The activated enzymes then break down salts of phytic acid (phytates) releasing bond nutrients/elements for digestion and absorption.

Sokrab *et al.* (2012) and Bazaz *et al.* (2016) reported that sprouting reduced phytic acid in plant fish diets and hypo-allergic baby weaning diets with subsequent increase in phosphorus and protein respectively. Chitra *et al.* (1996) reported up to 60% reduction of phytic acid in sprouted chickpea and pigeon pea seeds and 40% reduction in sprouted mung, Urd (black gram) and soybean. Sprouting sorghum for five -seven days significantly increased total crude protein, reduced lipase inhibitors, lectins, and phytic acid and increased mineral content (Hwei-Ming *et al.*, 1997). Inoculating diets (mainly cereal beverages) rich in carbohydrates with flour of sprouted sorghum is widely practiced in Africa to improve palatability, taste and shelf life. This implies that multiple enzymes activated during sprouting of plant material are involved in breakdown of complex molecules such as sugar maltose, starch and phytic acid in cereals such sorghum into simple easily absorbed materials (e.g. glucose) (Sokrab *et al.*, 2012).

Although sprouted grain contains activated enzymes, its application in commercial production of fish feeds in Africa is limited by lack of appropriate drying facilities. This hinders timely drying exposing the feeds to mould contamination especially during rainy seasons. Moulds produce toxins that induce growth of liver cancer (tumors), reducing its function and culminating in poor growth and death (Kumar *et al.*, 2017). However, it may be an economical pre-treatment option.

2.6.3.3 Use of enzyme supplements

Historically, enzymes activated during sprouting have been found to be produced in large quantities by microbes especially fungi and bacteria (Dalsgaard *et al.*, 2012). They have therefore been extracted and packaged as commercial products for a number of industrial

processes including manufacture of animal feed. In fish feeds, use of enzymes is more convenient especially in commercial feed production than roasting and sprouting but are not yet readily available thus expensive. Enzyme supplements are reported to be effective in reducing heat stable anti-nutrients such as phytic acid (Kumar *et al.*, 2011). Although enzyme use has been based on single pure enzymes, commercial enzyme mixtures /multiple enzymes are also becoming common especially among poultry and piggery because their price is lower than for single enzymes. This re-echoes use of enzyme cocktails like those in sprouted grains and the principle of ethno botanists that separating the active ingredient from its natural cocktail distorts its effectiveness (brings in side effects compared to the cocktail). Commonly used enzymes are enlisted in (Table 2.8).

Table 2.8 Some of the dietary enzymes developed to reduce anti-nutritional factors in plants

Enzyme	Function
Amylase	Utilisation of starch
Arabinase	Degradation of anti-nutritional factors , improves energy levels
Mannanase	Degradation of mannans, a non-starch polysaccharide (NSP)
Cellulase	Degradation of cellulose in plant feedstuffs
Keratinase	Degradation of feather keratin for production of feather meal
Lipase	Emulsification and utilisation of oils and fats
Phytase	Increasing bio-availability of phosphorus, trace elements and protein in feed stuffs
Protease	Increase utilisation of animal and plant protein
Tannase	Elimination of tannin
Xylanase	Hydrolysis of non-starch polysaccharides like pentosans
Galactosidase	Acts on galactosides to reduce anti-nutritional factors
Glucosidase	Acts on glucans (NSP) to unlock nutrients
Invertase	Converts sucrose to glucose and fructose to improve body's energy level

Source: Kumar *et al.* (2012).

Phytase enzyme are famous for breaking down phytic acid, an anti-nutrient found in almost all plant ingredients to release bond phosphorus, trace elements and crude protein.

On the other hand, protease is used to increase digestibility of proteins, the most essential and expensive nutrient required for fish growth (Yan *et al.*, 2012). Incorporation of diets with either phytase or protease enzymes may thus have the same counteracting effects on protein digestion (personal opinion).

2.6.3.3.1 Use of protease enzymes

Proteases are used in feed to reduce costs through maximizing digestibility of protein, the most expensive nutrient. This enables reduction of dietary crude protein concentration and or use of low cost protein resources without compromising the health and growth performance.

Corn and soybean, the main used ingredients in fish feeds, contain a range of anti-nutritional factors that are not completely inactivated by heat treatment even during oil extraction and thermal conditioning. They include phytic acid, antigenic proteins such as trypsin inhibitors and lectins, which irritate the gut mucosa, and activate the immune system (inflammation). However, according to Ghazi *et al.* (2002), inclusion of protease reduced antigenic proteins specifically in corn and soybean meal but with varied results. In broiler chicken, inclusion of protease in soybean meal degraded the antigenic protein (β -conglycin) which causes hypersensitive immune response. This was evidenced by reduction of interleukin-8 (IL-8), anti-inflammatory cytokines in the jejunum of broiler chicken fed on soybean-based diets incorporated with proteases. Since there exists a strong linkage between the diet and immune response, suppression of immune response increases digestibility and absorption of amino acids. Cowieson and Roos (2014) noted that inclusion of protease in diets was also correlated with reduction of mucin which reduces energy loss in the gut of pigs and poultry thereby improving digestibility of amino acids. Benefits of protease in plant protein diets are based on reducing allergenicity of antigenic proteins thus intestinal inflammation and intestinal mucin which after improve amino acid digestibility.

However some ingredients such as canola meal did not responded to protease suggesting it s anti-nutrients and/or inhibiting factors interfered with its effectiveness (Khajali and Slominski, 2012). Other factors that influence effectiveness of protease were reported to include the inherent digestibility of nutrients, feed composition, species, age as well as environmental and nutritional factors (Simbaya *et al.*, 1996). This suggests that incorporation of protease may confer benefits similar to those of phytase and differences may only lie in the costs involved since one of them may be cheaper. Evaluating the cost benefits of incorporation phytase and protease enzymes will therefore guide decision making/choice of enzyme.

2.6.3.3.2 Phytase enzyme

Phytic acid is naturally broken down by phytase enzyme to release the bound nutrients in ruminants but this enzyme occurs in insufficient amounts in the gut of monogastric animals including fish (Kumar *et al.*, 2012). For this reason, incorporation of exogenous phytase has been adopted to minimize effects of phytic acid in plant-based diets. Phytase occur naturally among various life forms with larger amounts produced in micro-organisms than in higher plants while the contribution from animals is insignificant (Kumar *et al.*, 2011). Filamentous fungi such as *Aspergillus ficium*, *Mucor piriformis* and *Cladosporium* species are ranked as the major sources of microbial phytase (Haefner *et al.*, 2005).

Phytase also occurs in grains, seeds and pollen of higher plants where it is responsible for degrading phytates during germination to avail bond phosphorus and other mineral elements for plant growth and development. Phytase enzyme was also detected in

digestive tracts of Nile tilapia, common carp *Cyprinus carpio* and European bass but in limited quantities that require extraction and concentration (Ellestad *et al.*, 2003).

Incorporation of phytase enzyme in feeds was first used to safe guard the environmental against phosphorus pollution mainly in the Netherlands. Dietary phytase releases bond phosphorus in feed material increasing its absorption by monogastric animals. Phytase reduced the need for mineral supplementation, especially inorganic phosphorus thereby decreasing the cost of feeds (Baruah, 2004). However other than phosphorus, incorporation of phytase in feeds has been associated with enhancing bioavailability of crude protein, lipids and other bivalent mineral elements , that provide health benefits and feed efficiency.

2.6.4 Classification of phytases

Classification of phytases [myo-inositol (1, 2, 3, 4, 5, and 6) hexakisphosphate phosphohydrolase (EC 3.1.3.8, EC 3.1.3.26)] is of practical importance in guiding choice of appropriate kind for inclusion in diets. Major categories are based on site of action, pH tolerance and organism used in production. International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB) suggest two categories based on the position of phosphorus release (Cao *et al.*, 2007); 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). Microbial phytase contain 3-phytases whereas plant phytase contain 6-phytases. Bohn *et al.* (2008) suggests that in addition to 3-phytase, 6-phytases also contains 4 phytase denoted as 4/6 phytase (EC 3.1.3.26) and also there exists 5-phytase (EC 3.1.3.72). It is suggested that 3-phytase is homologous to histidine

acid phosphates, and is generally found in fungi and bacteria. According to the site of action, most fungi and some yeast such as *Schwanniomyces cerevisiae* and *Schwanniomyces castellii* exhibit both intracellular and extracellular phytate degrading activity (Nakamura *et al.*, 2000) while some bacteria (*Pseudomonas* sp) are mainly intracellular. Exclusive extracellular activity was however observed only in filamentous fungi with *Aspergillus niger* strains as best producers followed by *Bacillus* and *Enterobacter* bacteria genera whereas *Escherichia coli* phytase was observed to be periplasmatic.

Based on optimum pH requirements, phytase enzymes with optimal pH at 5.0 are known as acid phytase while those at near 8.0 are alkaline (Baruah *et al.*, 2007). Microbial phytase vary considerably in pH optima but most plant phytase tend to have a pH optimum at 5.0.

2.6.4.1 Major factors that affect phytase enzyme activity

A number of factors reduce efficiency of phytase including processing temperature, pH of the digestive tract and feeds, protease enzymes and metal ions (Kumar *et al.*, 2011). Phytase is thermo labile with optimum temperature at 45-60 which makes it prone to destruction by pelleting temperatures (70-80°C) (Wilkinson, 2013). Due to this, its inclusion in feeds is preferably done before pelleting and extrusion or by spraying extruded diets with liquid phytase concentrate after mixing it with stabilizers such as magnesium sulphate (MgSO₄).

The most thermo resistant phytase enzymes reported so far was isolated from *Aspergillus fumigatus* and *Schwanniomyces castellii* (Kumar *et al.*, 2011). Microbial phytase are considered more thermo tolerant than their plant counterparts which loose activity even in steam pelleted diets rendering them of little relevance in fish feed manufacture where temperatures can exceed 70°C (Kumar *et al.*, 2011).

The optimal pH of most phytase enzymes is at about 5.5 with microbial phytase being more pH stable even at pH 8.0 and below 3.0 especially phytase from *Escherichia coli*. Unlike microbial phytase, stability of plant phytase decrease dramatically at pH below 4 and above 7.5. In practice, acidification of feeds has been done by addition of organic acids such as citric acid at a rate of 3% to facilitate phytase activity (Baruah *et al.*, 2007; Phromkunthong *et al.*, 2010). Acidification also inhibits growth of intestinal bacterial that compete for nutrients thereby reducing toxic metabolites especially ammonia and amine. Protease enzymes (pepsin and trypsin) degrade phytase enzyme, reducing its efficacy. However, *Aspergillus niger*, *Escherichia coli* and *Bacillus* were found more stable and resistant to protease degradation (Kumar *et al.*, 2011).

Metal ions hinder phytase activity by formation of insoluble complexes with Cu^{2+} and Zn^{2+} except with *Aspergillus fumigatus* phytase. Ethylene diaminetetraacetic acid (EDTA) showed no major effects on acid phytase but stimulated *Aspergillus fumigates* by 50% and inhibited alkaline phytase. High Ca: P ratio (>2.1:1) reduced phytase activity while fluoride promoted several acid bacterial, fungal and plant phytases. Molybdate, wolfram and vanadate are competitive inhibitors of phytase activity as well as

phytate/phytic acid itself at as low levels as 300 mg for maize root phytase and 20 mg for soybean. This makes high specific activity, broad substrate specificity, a broad pH optimum, good storage, pelleting and digestive stability the most important attributes of a good phytase enzyme. However, the challenge is that phytase enzymes that meet all such requirements do not naturally exist. Production of phytases with high specific activity, broad substrate specificity and large pH profile has been achieved through site directed mutagenesis such as in *Aspergillus fumigatus* which makes it expensive (Lehmann *et al.*, 2000; Tomschy *et al.*, 2000).

2.7 Current trends on performance of phytase-incorporated diets

Phytase enzyme is so far the most used enzyme in the feed industry mainly because of its environmental advantage in reducing aquatic phosphorus pollution (Kumar *et al.*, 2011). Its inclusion in both ruminant and non-ruminant feed improves bio-availability of phytate-bound phosphorus and nitrogen reducing their discharges into the aquatic environment (Cao *et al.*, 2007; Debnath, 2005). However, although phytase is considered as environmentally friendly, its inclusion in fish diets has not always increased digestibility of nutrients thus requiring more research including evaluating alternative strategies.

Increased phosphorus and protein digestibility were observed in phytase supplemented diets but with no corresponding improvement in growth by Qiu (2015) in the Pacific White Shrimp (*Litopenaeus vannamei*) juveniles fed on soybean and wheat based diets. The same was observed by Goda (2007) for Nile tilapia (*Oreochromis niloticus*) fingerlings fed on soybean -based diets with phytase at 1000 units, Baruah *et al.* (2007)

when Rohu *Labeo rohita* fingerlings were fed soybean-based diets (soy 40%) supplemented with 750 FTUs of phytase, Tudkaew *et al.* (2008) recorded increased phosphorus digestibility and growth with Nile tilapia fed on soybean –based phytase supplemented diets. Supplementation of protease in low fish meal plant diets also increased growth of white shrimp tilapia *Litopenaeus vannamei*, and tilapia, *Oreochromis niloticus* × *O. aureus* in diets with high fish meal inclusion (Li *et al.*, 2015). Improved growth was also recorded in 378.56 g catfish (*Clarias gariepinus*) fed on roasted and oil pressed ground nuts by Akpoilih *et al.* (2016) although this could be attributed to easy digestibility of ground nuts compared to soybean and the big size of the catfish that is less sensitive to antinutrients.

On the contrally Yigit *et al.* (2016) found no effect on either digestibility or growth of 88g rainbow trout juveniles fed on soybean bean diets incorporated with protease and phytase at 1 - 2 gkg⁻¹ feed while Vielma *et al.* (2004) noted improved growth in 20g rainbow trout.fed on soy-based diets supplemented with lysine than in those fed on diets incorporated with 200FTUkg⁻¹. Only marginal weight gain was observed in 115g carp (*cyprinus carpio*) juveniles fed on diets with 1000 and 2000 FTUkg⁻¹ phytase by Nwanna (2007). On the contrally, Dechavez and Serrano (2012) did not find differences in protein digestibility and growth of *Oreochromis mozambicus* fed on cassava leaf, corn and soybean meal incorporated with bacillus phytase (*B. pumilus*, *B.megaterium*, *B. coagulans*, and *B. licheniformis*). Also no effect was observed in Atlantic salmon fed on canola meal incorporated with phytase by Sajjadi and Carter(2004), Japanese flounder and Korean rockfish, *Sebastes schlegeli* by Masumoto *et al.*, (2001) and Yoo *et al.* (2005)

respectively. Incorporating phytase in isolated soybean protein, soybean meal, corn gluten and wheat middlings improved phosphorus digestibility by 23% but had no effect on protein digestibility in 3 year striped bass (Papatryphon and Soares, 2001). Depression of growth and feed retention was recorded in African catfish (*Clarias gariepinus*) fed on phytase soy meal-based diets by Van Weerd *et al.* (1999). From these cases, it can be noted that performance of fish fed on diets incorporated with phytase is both inconsistent and contradicting thus making its usefulness ambiguous. The discrepancy in these results may however be attributed to differences in diet composition, age and species of the fish, amount of enzyme used and the mode of application which influence diet fibre and palatability (Baruah *et al.*, 2007).

2.7.1 Phytase and phosphorus uptake in fish diets

The effectiveness in phosphorus digestibility and absorption has been recorded at all levels of dietary phytase (≥ 200 FTUkg⁻¹). For instance, 250–500FTUkg⁻¹ improved growth and phytate phosphorus in Channel catfish and Nile tilapia (Liebert and Portz, 2005). Improved protein digestibility and growth was recorded with ranges of 500–2000 FTUkg⁻¹ but more especially with 1000 FTUkg⁻¹. In soy-based diets, (>50%), increased growth was observed with phytase levels of 2000 FTUkg⁻¹ (Cao *et al.*, 2007; Vielma *et al.*, 2002). Dietary phytase increased growth and health than inorganic phosphorus in striped bass (Papatryphon *et al.*, 1999). Phytase inclusion at 500 FTUkg⁻¹ in Crucian carp diet improved growth and mineral utilization including phosphorus. Baruah *et al.*, (2007) reported maximum apparent digestibility of phosphorus, crude protein and growth when phytase-supplemented diets with 750 FTUkg⁻¹ were fed to the carp, *Rohu*. Phytase supplementation of 500 FTUkg⁻¹ diet also improved growth in Pangus fingerlings

(Debnath *et al.*, 2005) while that of 1000 FTUkg⁻¹ diets increased growth of Korean rockfish (Yoo *et al.*, 2005). These results showed that success with phytase enzymes was achieved within 500-1500 FTUkg⁻¹ of feed in most studies. Other than phytase, there are a number of other dietary enzymes used in aqua diets to increase nutrient bioavailability. Of relevance to protein enhancement is protease (Table 2.8).

2.7.2 Other important aspects of feed that affect catfish growth

Other than the nutritional aspects of fish feed quality, the physical aspects of fish feed such as size, colour, palatability etc also directly affect fish growth (Cocker, 2014). Pellet integrity and feeding the right amount of feed the fish is able to consume is also required. Fish feed need to be pelleted to a pellet size that can be ingested by the fish otherwise it will not be eaten. The pellet size should not be too big or too small but should be appropriate for the feeding fish. The quality of feed deteriorates steadily after manufacture and the rate and magnitude depends on how it is handled and stored (Nvanna, 2003).

Feeds prepared within 4 weeks often meet the nutritional and physical standards stated on the label but gradually deteriorates in quality due to loss of vitamins, especially vitamin C, and mold attach if stored in damp places (FAO, 2010a). This implies that only feed for use within 4 to 6 weeks should be made / purchased since longer storage reduces nutritional quality. In most experiments, feed is refrigerated during the trials and this makes results not comparable to those under field conditions that are kept at room temperatures (24°C). It is however recommended that fish feed is protected from moisture, heat and direct sunlight. In practice white, cool shaded, dry and well ventilated

wooden buildings with reflective metal roofs are suggested as excellent feed stores as opposed to warm, moist and stagnant air conditions that enhance mold growth and insect infestation. Bags of feed need not be stacked leaning directly against walls or on concrete floors but rather on pallets off the walls to allow air circulation. Entry of rodents, bats and other animals is undesired and need to be controlled.

Inappropriate storage and feed handling raise feed costs the same way as selection of poor feed type (pellet and formulae), quality and feeding strategy and should therefore be given considerable attention. It has been noted that most farmers in Africa have inclined to use extruded floating pellet, without attempting management options to best utilize the sinking pellet or farm-made aquafeeds to improve their cost effectiveness (FAO, 2010). A case study from Thailand however indicated that farmers use low protein feeds (20% CP) and locally available feed by-products to minimize production costs thereby maximizing profits. In grow out systems, hand feeding three times a day provided food conversion ratio (FCR) between 1.4–1.8 and growth rates of 2–3 g/d which is above average of 0.05g/day most farms in Uganda (FISH, 2009). High feed costs suggest that efficient utilisation of low cost feed resources such as agro-by products will have a significant impact on the economic viability of aquaculture.

2.8 Evaluation of fish feeds

According to France *et al.* (2000), evaluation of feed is conducted to optimize its utilisation for growth and economic returns. The effectiveness of diets is biologically influenced by digestibility of the energy components; protein, lipids and carbohydrates

(Jobling, 1994) while the economic criteria is based on the benefit-cost ratios. Both criteria are applied for a feed to be declared appropriate.

2.8.1 Biological evaluation of fish feeds

Biological evaluation establishes the potential of major feedstuffs and the appropriate supplements in order to overcome nutritional deficiencies and raise the level of performance (France *et al.*, 2000). Feed is often evaluated by observation of survival rates, weight gain, feed efficiency and body composition of retained nutrients in the carcass (De Silva and Anderson, 1995). The term growth is embraced to refer to changes in body weight, length or composition as a result of nutrient deposition. Other criteria such as the protein efficiency ratio (PER) are also used to describe the utilisation of proteins although it only considers change in biomass. In almost all cases energy retention and deposition of new tissue result in weight gain although which is not a measure of energy retention because fat deposition reduces the water content thus changing the energy value per unit live weight (Jobling, 1994). More energy from fat (31kJ/g) is lost in heat production since it is deposited in adipose associated with little water than for protein (6kJ/g) which is deposited in the visceral and muscle where a lot of water exists (Guillaume *et al.*, 2001).

2.8.1.1 Digestibility as a measure of feed quality

Digestibility is one of the most important indicators of nutritional quality for feed ingredients or diets. It is a measure of nutrient absorption from ingested feed not eliminated as faeces and is used to determine the nutritive value of any feed (Guillaume *et al.*, 2001). Digestibility sometimes referred to as absorption efficiency indicates the

potential of nutrient utilization to guide improvement in feed quality and waste treatment for preservation of water quality and the environment. The apparent digestibility coefficient (ADC) values are used as guidelines for diet formulation to meet the fish's nutritional requirements as determined by Cho and Slinger (1979). The in-vivo protein digestibility provides an estimation of protein quality in different fish feeds.

Digestibility of a feed/diet can be studied using either the direct or indirect method. The direct method requires quantification of all ingested feed and all faeces produced within identifiable meals (De Silva and Anderson, 1995). A metabolic chamber or other faecal collection methods that can allow quantitative measurement are required for this purpose. However, in the face of difficulties in total feed and faecal collection, the indirect method has become important especially in nutrition of fish. The indirect method is based on inclusion of an inert, indigestible and non absorbable marker in the feed/diet. External markers (e.g. chromium oxide, silicone oxide and iron oxide) and internal markers (crude fiber, cellulose, lignin, hydrolysis resistant organic matter (HROM), chitin and hydrolysis resistant ash) are used but chromium oxide is the commonly used at 1-2% of feed (Guillaume *et al.*, 2001; Jobling, 1994).

During digestibility studies, feed intake and faecal output are recorded at least seven (7 days). For comparable results, chemical analysis of dry matter for both feed and faeces is first determined calculated first. For faecal collection in fish techniques including intestinal dissection, trough netting, metabolic chambers, filtration columns, settling columns such as the Guelph model and mechanically rotating screens (Cho and Slinger,

1979) are recommended for reliable results. Manual striping, squeezing of rectal contents, suction through the anus and fish dissection stress the fish, increasing enzymes, body fluids and intestinal epithelium to the faecal content (Windell *et al.*, 1978). With the indirect method, apparent digestibility coefficients (%) are calculated following the equation by (Cho *et al.*, 1982) as follows:

$$ADC(\%) = 100 - [100 * (\% \text{marker in diet} / \% \text{marker in faeces})].$$

The apparent digestibility coefficient (ADC) of nutrient(s) is however calculated according to De Silva and Anderson(1995), Guillaume *et al.* (2001) and Jobling(1994) as follows:

$$ADC (\%) = 100 - \left[100 * \left(\frac{\% \text{ Marker in diet}}{\% \text{ Marker in the faeces}} \right) * \left(\frac{\% \text{ Nutrient in faeces}}{\% \text{ Nutrient in the diet}} \right) \right]$$

Digestibility of a diet can be summed (additive) from the digestibility coefficients of its ingredients, but cases where this was not true have also been reported. Generally protein of animal origin especially fish are more digestible than those of plants sources while lipids of low melting points are more digestible (ADC > 90%) irrespective of the source (Table 2.9). For saturated fatty acids, digestibility reduces as the chain increases. Also carbohydrates are significantly lost as faeces especially if the content of lignin, cellulose and crude fiber is high because they are less digestible by fish gut bacteria/ microbes. Digestibility of starch usually incorporated in feeds to reduce costs or for other economic reasons ranges from 40-70% while that of simple sugars like glucose and sucrose, is almost 100%. Digestibility of water or ash is rarely talked of because the major interest is of dry matter / organic nutrients other than vitamins/minerals.

Table 2.9 Digestibilities of common feed ingredients in catfish

Ingredient	Dry matter (%)	Protein (%)	Lipid (%)	Energy (%)	Average ADC
Fish meal	85	92	97	91	91.25
Soy seed cake	74	96	94	85	87.25
Cooked soy	78	96	-	75	83
Maize bran	-	95	-	39	67

Source: Guillaume *et al.* (2001). N.B Dashes represent no information available.

2.8.1.2 Factors that influence digestibility

Like any metabolic process, digestibility is affected by both biological and environmental factors but in a less defined and understood manner (De Silva and Anderson, 1995). Fish age, size, sex, stocking density, feeding time and frequency, feed intake/ration, size/quantity and quality, water temperature and salinity are among variables that may affect digestibility (NRC, 2011). In catfish, feeding level and meal size, dietary protein, lipid, fiber, protein-energy ratio, types of nutrient (e.g. protein), temperature and salinity were pointed out as major factors that affect feed digestibility (Henken *et al.*, 1985). Digestibility is generally expected to increase linearly with increase in water temperature and is highly reduced with high dietary starch (lignin and cellulose). Fish meal is the most efficiently digested (over 90%) ingredient universally unlike plant products whose digestibility varies with fish species fed.

2.8.2 Profitability indices of a fish feed

The aim of every investor is to make profit and therefore profit ultimately determines the success of any business venture and aquaculture/fish farming is no exception (Sogbesan

et al., 2005). Profitability of fish farming is enhanced by efficient utilization of feed by fish since feed is the most costly input. For a feed to be efficient and cost effective, Nwanna (2003) recommended that it has to provide the highest weight gain per unit of feed consumed. Determining profitability and cost effectiveness of fish feeds has often been based on feed costs holding other parameters constant. However, a better view of feed cost effectiveness based on unit cost (incidence of costs) was provided by Vincke (1969) as adopted by Bob-Manuel and Erundu (2010) and Sogbesan *et al.* (2005) while that of cost effectiveness based on returns per unit produced (profit index) was provided by Miller (1976). In both cases, the market price of ingredients is the main determinants of the cost effectiveness thus implying that formulation of fish diets are critical for profitability of fish farming. The specific equations used in determination of profitability indices are as follows:

$$a) \text{ Profit index} = \frac{\text{Number of fish produced}}{\text{Cost of feed fed}}$$

$$b) \text{ Incidence of cost (R)} = \frac{\text{Cost of feed}}{\text{Biomass of fish produced(kg)}}$$

$$c) \text{ Net profit} = \text{Total sales} - \text{Total expenses}$$

$$d) \text{ Benefit cost ration(Bcr)} = \text{Toatlfish sales} - \text{Total feed expenses}$$

Important to note is that total cost of feeds also include transport and labour costs in its delivery if applicable. The unit costs of feed may also be determined based on nutrients supplied (protein or energy) on dry matter or as- fed feed conversions (i.e. a kilogram priced as-fed) in which case food conversion ratio (FCR) is used to estimate feed cost effectiveness.

2.8.2.1 Feed conversion ratio (FCR)

Feed utilisation has over time been described using an ingested feed to body gain ratio referred to as the feed conversion ratio (FCR) (Guillaume *et al.*, 2001). FCR is a function of food nature, feeding animal and quantity of feed ingested. Unlike weight gain which is (linearly) proportional to the amount of food ingested above maintenance requirements, FCR represents the amount of dry feed fed per unit live weight gain. The more suitable the diet is for growth, the lesser the food required to produce a unit weight gain, i.e. the lower the FCR and the reverse is true (De Silva and Anderson, 1995; Rice *et al.*, 1994). The FCR is determined from the following equation:

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

When evaluating the cost-effectiveness of a particular feed, its FCR and unit cost are simultaneously considered based on actual market prices. It was noted that using the cheapest feed available, more often than not, does not result into the lowest cost of production (FISH, 2009). The lower the FCR, the lower the amount of feed used to produce a kilogram of fish. Therefore, the feed which gives the lowest FCR, even though often gives the lowest cost of production with FCR of less than 2 often considered economical (FISH, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Four cascading experiments were undertaken to achieve the for set objectives whose final target was to reduce effects of phytic acid in feeds so as to improve digestibility and utilization for faster growth of catfish under field conditions.

3.2 Study area

3.2.1 The main experimental station

The main study was generally conducted in South Western Agro Ecological Zone, in Mbarara district at a regional research institute of the National Agricultural Research Organisation (NARO) known as Mbarara Zonal Agricultural Research and Development Institute (Mbarara ZARDI). This station is located 4 miles from the center of Mbarara municipality, on Mbarara-Kasese Road. Mbarara ZARDI and serves an ecological zone of 13 districts. This institute is located at -0.60 latitude and 30.65 longitude at elevation of 1449 meters above the sea level. Mbarara district (in which this istitute is located is bordered by districts; Ibanda to the north, Kiruhura to the east, Isingiro to the southeast, Ntungamo to the southwest, Sheema to the west and Buhweju to the northwest (Figure 3.1).



Figure 3.1 Map of Uganda with a red star showing the location of MBAZARDI

Source: One world nations online (2016).

This district/location receives an average annual rainfall of 1,200 millimetres (47 in). Temperatures range between 17 °C (63 °F) and 30 °C (86 °F). It has a bimodal type of climate with the first long rain period running from September to December with a peak in November and the the short rainy season from Febuary to April with a peak in March. The region is dominated by dairy cattle keepers and banana growers but has the least supply of wild fish (UBOS, 2014). Despite plenty of milk, the region is characterised by high human malnutrition levels (47.8%) (Kikafunda *et al.*, 2014). Limited supply of fish is implicated among factors that excalate human malnutrition and micro nutrient defficiency, in the region. In responce to this, a number of fish farmers (about 1600) have

come up but are mainly constrained by poor access to quality fish diets, poor fish survival and productivity (Mwanja *et al.*, 2015).

3.2.2 Other experimental sub stations

The National Crop Resources Research Institute (NaCRRRI), Namulonge and Department of Animal Science, School of Agriculture and Natural Resources of Makerere University Kampala were other sub-stations used for laboratory analyses. In particular, analysis of proximate composition of the feed (and any other sample) was conducted at the Department of Animal Science, College of Agriculture and Natural Resources, Makerere University, Kampala. In-vitro digestibility was conducted at the Cassava Biotechnology Laboratory of the National Crop Resources Research Institute (NaCCRI), Namulonge in Wakiso District. In-vivo digestibility, the hatchery and pond-based evaluation experiments were conducted at NARO-Mbarara ZARDI (Figure 3.1).

3.3 Study design

3.3.1 Study structure

The study structure comprised of four cascading experiments in which quantitative data was collected. The description of the studies carried out is as follows:

- i) The first study was a single factorial experiment in which major feed ingredients (comprising of about 80%) of a 37% crude protein diets were roasted before grinding and compounding into diets analysed for phytic acid, crude protein content and pH.

- ii) The second study consisted of two subset studies; a 4 by 4 factorial experiment with four dietary crude protein levels (33%, 37%, 50% and 65% CP) as factors and four enzyme types/sources as treatments (the negative control, sprouted sorghum, 750 units of phytase and 750 units of protease). This set of study determined the crude protein and phosphorus digestibility coefficient in diets incorporated with enzyme extracts in - vitro and in vitro. The second subset was a 4 by 6 factorial design with four dietary crude protein(33% ,37%, 50% and 65% CP) as factors and phytase and protease enzymes at three levels (750, 1000 and 1250 unitskg⁻¹) as the 6 (six) treatments. These two experiments were used to determine the dietary enzyme type and the enzyme level associated with the most rewarding protein and phosphorus digestibility, retention and cost effectiveness of catfish production.
- iii) The third study was of a 1 by 5 factorial experiment in which 65% CP diets as a factor and five levels of dietary protease (0,750, 1000, 1250 and brine shrimp *Artemia*; positive control) as treatments. The study was conducted to determine performance of catfish hatchlings (two-day old) fed on dry diets incorporated with protease enzymes against *Artemia*; and
- iv) Lastly, the fourth study was of a 2 by 4 factorial nature with two dietary crude protein levels (33% and 37%) as factors and four phytase levels as treatments (0, 750, 1000 and 1250unitskg⁻¹). It determined the effect of feeding diets with four (4) phytase inclusion levels on performance of catfish fingerlings raised in ponds.

3.4 Preparation of experimental diets

For the diets used in the study, proximate nutrient composition of the commonly used and locally available feed ingredients were compiled from the data base at the Department of Animal Science, School of Agriculture and Natural Sciences, Makerere University. This data was then entered into Win Feed Computer feed formulation software developed by the Practical Training center (PTC⁺), Netherlands and used to formulate all the experimental diets. The feed formulae developed based on the least cost basis to produce affordable diets while meeting the minimum nutritional requirements of catfish as described by NRC (2011). Feed ingredients (cassava flour, wheat pollard, maize flour, fish meal (silver fish), soybean, bush beans, L-lysine Hcl, DL-methionine, cotton seed cake, fish oil, fish mineral and vitamin premix, table salt and sodium benzoate) were procured from primary producers to minimize adulterations by inclusion of sand.

Almost all ingredients (except inorganic supplements and cotton seed cake) were roasted prior to grinding at 90-110°C on open-air heated aluminum Sauce pans for about 1 hour using firewood. The roasting temperature was monitored using an alcohol thermometer (0-110°C) and alternating reduction and increase of firewood. They were later air-cooled under a shed and ground separately into fine powder (0.02mm) using a locally fabricated commercial milling machine (MUSA body). The fine powder from different ingredients was mixed thoroughly according to proportions pre-determined during formulation conducted by linear programming using Feed win feed formulation soft ware.. Nile perch oil and distilled water worth 15% of total feed weight were added last together with dietary enzymes. All ingredients, were mixed, left to stand for 40 minutes and later

pelleted through a 2mm die as sinking pellets at 56-65°C by locally fabricated pelletizer (MUSA body, Kampala, Uganda) (Tables 3.1-3.5).

Table 3.1 Formulae of 33% CP diets supplemented with phytase and protease

Ingredient	33% CP diets						
	Cont rol	750 Phytase	1000 Phytase	1250 Phytase	750 Protease	1000 Protease	1250 Protease
Cassava flour	4.2	4.05	4	4	4.05	4	4
Wheat pollard	10	10	10	9.95	10.15	10.20	10.20
Maize bran	10	10	10	10	10	10	10
Soy bean	42	42	42	42	42	42	42
Bush beans	10	10	10	10	10	10	10
Fish meal	10	10	10	10	10	10	10
L-lysine	3	3	3	3	3	3	3
DL	2	2	2	2	2	2	2
Methionine							
Cotton seed cake	2	2	2	2	2	2	2
Nile perch oil	2	2	2	2	2	2	2
Vitamin and mineral premix	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Citric acid	3	3	3	3	3	3	3
Table salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lime	1	1	1	1	1	1	1
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Protease	0	0	0	0	0.00125	0.00167	0.00208
Phytase	0	0.15	0.2	0.25	0	0	0
Total	100	100	100	100	100	100	100

Table 3.2 Formulae of 37% CP diets supplemented with phytase and protease

Ingredient	Phytase				Protease		
	Control	750	1000	1250	750	1000	1250
Cassava flour	3	3	3	3	3	3	3
Wheat pollard	10	10	10	10	10.2	10.20	10.20
					0		
Maize bran	8.2	8.05	8	7.95	8.05	8	7.95
Soy bean	41	41	41	41	41	41	41
Bush beans	6	6	6	6	6	6	6
Fish meal	20	20	20	20	20	20	20
L-lysine	4	4	4	4	4	4	4
DL Methionine	2	2	2	2	2	2	2
Cotton seed cake	2	2	2	2	2	2	2
Nile perch oil	1	1	1	1	1	1	1
Fish vitamin & mineral premix	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Citric acid	1	1	1	1	1	1	1
Table salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lime	1	1	1	1	1	1	1
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Protease	0	0	0	0	0.00	0.0017	0.0021
					13		
Phytase	0	0.15	0.2	0.25	0	0	0
Total	100	100	100	100	100	100	100

Table 3.3 Formulae of 50% CP diets supplemented with phytase and protease

Ingredient	50%CP diets						
	Contro	750	1000	1250	750	1000	1250
	l	phytas e	phytas e	phytas e	proteas e	proteas e	proteas e
Wheat pollard	4	4	4	4	4	4	4
Soy bean	13.85	13.7	13.65	13.6	13.6	13.6	13.6
Bush beans	0	0	0	0	0	0	0
Fish meal	80	80	80	80	80	80	80
L-lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL-	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Methionine							
Cotton	0.15	0.15	0.15	0.15	0.4	0.4	0.4
seedcake							
Fish mineral &	0.2	0.2	0.2	0.2	0.2	0.2	0.2
vitamin premix							
Table salt	0.1	0.1	0.1	0.1	0.099	0.099	0.098
Lime	0	0	0	0	0	0	0
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Protease	0	0	0	0	0.0013	0.0017	0.0021
Phytase	0	0.15	0.2	0.25	0	0	0
Total	100	100	100	100	100	100	100

Table 3.4 Formulae of 65% CP diets supplemented with phytase and protease

Ingredient	65% CP diets						
	Control	750 Phytase	1000 Phytase	1250 Phytase	750 Protease	1000 Protease	1250 Protease
Wheat	0.45	0.5	0.5	0.45	0.45	0.45	0.45
pollard							
Soy bean	0.4	0.45	0.4	0.4	0.4	0.4	0.4
Fish meal	95	95	95	95	95	95	95
L-lysine	2	2	2	2	2	2	2
DL	1	1	1	1	1	1	1
Methionine							
Cotton	0.35	0.1	0.1	0.1	0.35	0.35	0.35
seedcake							
Fish mineral	0.2	0.2	0.2	0.2	0.2	0.2	0.2
& vitamin							
premix							
Table salt	0.1	0.1	0.1	0.1	0.09875	0.09833	0.09792
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Protease	0	0	0	0	0.00125	0.00167	0.00208
Phytase	0	0.15	0.2	0.25	0	0	0
Total	100	100	100	100	100	100	100

Table 3.5 The 65%, 50%, 37% and 33%CP diets supplemented with 1% sprouted sorghum

Sorghum diets				
Ingredient	65%CP	50%CP	37%CP	33% CP
Cassava flour	0	0	3	3.2
Wheat pollard	0	4	10	10
Maize bran	0	0	7	10
Soy bean	0.3	12	41	42
Bush beans	0	0.5	7	10
Fish meal	95	80	20	10
L-lysine	2	0.2	3.2	3
DL Methionine	1	1	2	2
Cotton seedcake	0	0.5	2	2
Nile perch oil	0	0	1	2
Fish mineral & vitamin premix	0.1	0.2	0.2	0.2
Citric acid	0	0	1	3
Table salt	0.1	0.1	0.1	0.1
Lime	0	0	1	1
Toxin binder	0.5	0.5	0.5	0.5
Protease	0	0	0	0
Phytase	0	0	0	0
Sorghum	1	1	1	1
Total	100	100	100	100

The pelleted diets were air dried on raised racks under a well ventilated iron-roofed shade for two days. The dry diets were packed in water proof laminated plastic bags and kept in wooden pallets at room conditions until used.

For determination of in-vitro digestibility, a kilogram of each diet sample was packed into air tight plastic zip lock bags and kept at 4°C until used while for in-vivo digestibility, five kilograms of diet samples were packed in water proof laminated plastic bags and kept in wooden pallets at room conditions until used (usually within a weeks' time).

For determination of in-vitro digestibility, a kilogram of each diet sample was packed into air tight plastic zip lock bags and kept at 4°C until used while for in-vivo digestibility, five kilograms of diet samples were packed in water proof laminated plastic bags and kept in wooden pallets at room conditions until used (usually within a weeks' time).

3.4.1 Analyzing proximate composition of diets (chemical analysis)

Nutrient composition of all diets was analysed in the laboratory at the Department of Animal Science, School of Agriculture and Natural Sciences, Makerere University. The amino acid profiles were thereafter determined using the High Performing Light Chromatography (HPLC) by Abingdon Health Laboratory Services, University of Birmingham, UK (Table 3.6).

Table 3.6 Amino acid profile of the 37% CP diet used in the study

Major essential amino acids	Catfish requirement	Free amino acids	Hydrolysable amino acid
Arginine	4.3	0.004	4.488
Histidine	1.5	0	2.541
Iso Leusine	2.6	0	2.731
Leusine	3.5	0.004	4.846
Lysine	5.1	0.088	5.039
Threorenine	2.1	0.004	2.555
Tryptophan	0.5	0	-
Valine	3	0.004	3.029
Methionine + Cystine	2.3	0	1.234
Phenylamine +Tyrosine	5	0.004	3.029
Total	29.9	0.105	29.492

A number of parameters including dry matter, crude protein, crude fiber, crude lipid/fatty acids, ash / phosphorus, calcium, phytic acid and pH were determined in diets fed to catfish. The same parameters were also later determined in faecal matter and whole-fish body in triplicates following standard methods by AOAC (2002) as follows:

- a) Dry matter (DM) was determined by drying samples using an electric oven (Wagtech, GPS/75/SS/250/DIG, UK and Gallencamp.SANYO OMT Oven) at 105 °C until they attained a constant weight.
- b) For determination of pH, feed (and any other samples) were first made into slurry with distilled water at a ratio of 1:10 w/v (Namulawa *et al.*, 2014). A digital Pen-type micro pH meter with a temperature reading (Model ROHS) was then inserted and used to determine the pH to the nearest 0.01.

- c) Crude protein (CP) was determined following the Micro-Kjeldahl method after sample digestion using the digester (2040 Digestor, Hoganas, Sweden) and back titration of excess sodium hydroxide (NaOH) with standard HCl and bromocresole green as an end point indicator. One atom of nitrogen was assumed to be equivalent to 6.25 crude protein i.e. $N = 6.25$.
- d) Crude fat (lipid) was determined by the Soxhlet extraction with petroleum ether BP 40-60°C from the dried samples obtained after the determination of the dry matter content in extraction thimbles.
- e) Ash content (total inorganic matter) was determined after incineration of samples in a muffle furnace (Winkler –Heinrich Funchs Strable 99, 6900 Heidelberg. West Gardian) at 550 °C for 12 hours.
- f) The chromium content of feed and faecal matters was determined using a flame ionization atomic absorption spectrophotometer (Perkin Elmer, Ueberlingen, Germany).
- g) Total phosphorus was analysed after ashing using the atomic absorption spectrophotometer according to AOAC (2002).
- h) Amino acids were analysed by reverse phase high performance liquid chromatography (HPLC)/photometric method according to (Vázquez-Ortiz *et al.*, 1995). Tryptophan and tyrosine were not analysed due to the high cost of the method involved.
- i) Chromium oxide was determined according to the method of (Furukawa and Tsukahara, 1999) after digestion of feed samples by concentrated nitric acid and

oxidising chromic oxide with 70% perchloric acid. Chromic III oxide was then calculated using the following equation:

$$\text{Chromium Oxide(\%)} = \left(\frac{\text{Absorbance} - 0.0032}{0.2089} * 100 \right) / \text{Sample weight}$$

g). Phytic acid was determined by the complex- metric method that measured residual iron (iii) after precipitation of phytic acid according to Garc  a-Esteva *et al.* (1999) assuming a 4:6 ratio of Fe:P as used by Wheeler and Ferrel (1971). The same procedures were followed for proximate analysis of whole fish samples and faecal matter samples.

Important to note is that the material and method part described above applied to all experiments but the following part describes additional materials and methods that are specific to each objective:

3.5 Experiment 1: Determining the effect of roasting on phytic acid, crude protein content and pH in plant-based diets

3.5.1 Study area

The study was conducted at Mbarara Zonal Agricultural Research and Development Institute (MBAZARDI) one of the public National Agricultural Research Organization (NARO) institutes (Figure 3.1).

3.5.2 Experimental design

This was a single factor design experiment in which diet ingredients (of 37% CP diets) as factors were subjected to roasting as the treatment to determine its effect on the proportion of phytic acid, crude protein and pH of plant-based catfish diets. Fifty five (55) adult catfish weighing $222 \pm 9.14\text{g}$ were selected from on-station ponds and stocked

into two concrete tanks (3 by 2 by 1.5 m³) of 400 litres capacity each. Fish in one tank were fed on 37% CP pelleted diets formulated from roasted ingredients (soy bean, silver fish, beans, wheat pollard) while those in the other were fed on diet formulated from unroasted ingredients. Both sets of fish were fed at a rate of 4% of body weight between 10:00 to 11:00 am daily depending on the feeding response/appetite. Three (3) fish samples were randomly seined from each of the tanks daily an hour after feeding for five consecutive hours (up to 4:00 pm). Each batch was placed separately into 20 liter basins where they were humanly killed with excess clove oil (2.5ml per litre of water) following guidelines to death as end point by Homeoffice (2014). The catfish samples in each batch were dissected and gut sections removed according to Montgomery and Pollak (1988), (Figure 3.2).



Figure 3.2 Catfish gut showing the different sections that were cut and used in the study.

3.5.3 Preparation of experimental diets from roasted and un roasted ingredients

Diets with 37% CP were formulated and manufactured as described in Section 3.4, Table 3.2 using locally available ingredients (Table 3.7). The proximate composition of the diets (Tables 3.1-3.5) was determined according to AOAC (2002) .

Table 3.7 Proximate composition of major ingredients used in formulation of experimental diets

IFN	Name	DM	ME	CP	Lysine	Methio	Fat	CF	Ca	P
	Fish oil	0					100			
4-05-190	Wheat pollard		215							
		87	3	15	0.6	0.2	0	7	0.1	0.7
5-02-009	Fish meal		330				10.			
		91	0	55	5	1.85	3	0	3.5	2.6
5-04-597	Soybean heat		331	35.			18.		0.2	0.5
	treated	89	0	5	2.27	0.51	8	5.8	3	2
	Kidney		252						0.1	0.4
	beans	86	0	23	1.52	0.28	1.6	4.3	6	6
4-02-935	Maize bran		334				0.3		0.0	
		79	0	8.7	0.22	0.2	5	3.6	3	28
4-01-152	Cassava		227						0.2	0.0
		86	0	3.3	0.08	0.03	0	4.5	2	2
5-10-680	Cotton seed cake		184	37.				16.	0.2	1.0
		92	0	1	1.41	0.59	6.9	8	4	9
4-20-893	Sorghum grain		334						0.0	0.2
		85	5	9.2					3	9

Important to note:

The four diets were formulated to contain 30% 35%, 50 and 55% CP. However analysis proved that they were actually 33%, 37%, 50% and 65% CP respectively. The observed difference could have risen from under estimation of crude protein in the used ingredients including fish meal and digestive enzymes.

Table 3.8 Basic nutrient composition of the 37%CP plant-based diet (% of dry matter and energy in kJ/g)

Parameter	Content
Dry matter	87.08
Ash	8.92
Crude protein	35.22
Crude fibre	3.53
Fat	10.44
Gross energy (kJ/g)	30.00
Calcium	1.07
Phosphorus	1.27
pH	6.72- 6.79

3.5.4 Determination of phytic, crude protein and pH in diets and ingesta

Total phytic acid in the dry feed and ingesta samples was determined using the complexometric method according to Garc  a-Esteva *et al.* (1999). The ground samples (0.5±1.40 g) were extracted under magnetic agitation with 40.0 ml of extraction solution (10 g/100 g Na₂SO₄ in 0.4 mol/l HCl) for 3 hours at room temperature. The suspension was centrifuged at 5000 rpm for 30 min and the supernatant was filtered. One (1) ml of supernatant was pipetted into a 10 ml centrifuge tube together with 1.0 ml of 0.4 moles l⁻¹ hydrochloric acid (HCl), 1.0 ml of 0.02 moles l⁻¹ iron chloride (FeCl₃) and 1.0 ml of 2 g/10 g sulphosalicylic acid. The mixture was gently shaken and the tube used was sealed with a rubber cork through a narrow 30-cm long glass tube to prevent evaporation. The tube was placed in a boiling water bath for 15 minutes and allowed to cool. The sample

was centrifuged at 5000 rpm for 10 min, decanted, filtered and the residue washed several times with distilled water. The supernatant and washed fractions were diluted to 10.0 ml. Samples of 2.0 ml were made, their pH adjusted to $\text{pH } 2.5 \pm 0.5$ by addition of glycine solution and diluted to 20 ml. The solution was heated at 70-80 °C and, whilst still warm, titrated with 50 mil moles l^{-1} of ethylenediaminetetraacetic acid (EDTA) solution. The 4:6 Iron to phytate phosphorus (Fe/P) atomic ratio was used to calculate phytic acid percentage as earlier on described by Wheeler and Ferrel (1971).

For determination of pH, feed and ingesta from the different gut sections were first made into slurry with distilled water at a ratio of 1:10 w/v (Namulawa *et al.*, 2014). A digital Pen-type micro pH meter with a temperature reading (Model ROHS) was then inserted and used to determine the pH to the nearest 0.01.

Crude protein (CP) was also determined in the diets and ingesta following the Micro-Kjeldahl method after sample digestion using the digester (2040 Digester, Hoganas, Sweden) and back titration of excess sodium hydroxide (NaOH) with standard HCl and bromocresole green as an end point indicator. One atom of nitrogen was assumed to be equivalent to 6.25 crude protein i.e. $\text{N} = 6.25$.

3.5.5 Data handling and statistical analysis

Data was analysed for ANOVA with post hoc pair wise comparisons conducted using Turkeys test using Paleontological statistics (PAST) soft ware ver. 2.17c (Hammer *et al.*, 2001). In all cases significance was declared at 95% confidence level ($p \leq 0.05$).

3.6 Experiment 2: Assessing the effect of dietary phytase, protease and sprouted sorghum on digestibility of protein and phosphorus in dry diets by catfish.

3.6.1 Experimental design for determining digestibility of protein and phosphorus in dry diets by catfish.

The study was conducted in a completely randomized experimental design at two levels. The first experiment was conducted in vitro and determined the apparent digestibility of crude protein in 33%, 37%, 50% and 65% CP diets reacted with catfish gut enzyme extract, sprouted sorghum, phytase, protease and their mixture of 500 units each using the pH drop method as described by Sultana *et al.* (2010). The second experiment was conducted in-vivo (within catfish) and assessed the effect of incorporating enzyme supplements on apparent digestibility of phosphorus and protein 33%, 37%, 50% and 65% CP. This was a 4 by 4 randomized design in which 33%, 37%, 50% and 65% CP diets incorporated with no enzyme (negative control), sprouted sorghum equivalent to 1% of the diet weight, 750 unitskg⁻¹ of phytase and 750 unitskg⁻¹ protease enzymes were fed to catfish fry. It consisted of 16 experimental diets that were randomly assigned to 48 glass tanks in triplicates.

After results of the in-vitro experiments showed that phytase and protease enzymes significantly increased apparent digestibility of the diets than sorghum and the control, the in vivo experiment was conducted with catfish fingerlings in glass tanks. It determined phytase and protease inclusion level that gives the highest digestibility among

750, 1000 and 1250 units kg⁻¹. It was a 4 by 8 randomized factorial design in which four diets of 33%, 37%, 50% and 65% CP were each incorporated with 0, 750, 1000 and 1250 units kg⁻¹ of phytase and protease enzymes and fed to catfish fry. This gave rise to 32 enzyme incorporated diets which were randomly assigned in triplicates to 96 glass tanks.

3.6.2 Preparation of enzyme extracts

3.6.2.1 Catfish digestive enzyme extracts

Fish in four Mbazardi ponds were fed on four graded protein diets (33%, 35, 50 and 65% crude protein diets) for four months. Five catfish (weight 284 ± 4.5 g and length 35.2 ± 1.20 cm) were randomly captured from each of the four pond treatments 30 minutes after feeding using a seine on the same category of experimental diets for a week. The caught fish were humanly killed by anesthetizing them with excess clove oil (2.5 ml/litre of water) according to guidelines of death as end point by Home office (2014). They were dissected over blocks of ice; guts removed together with its contents and kept in a refrigerator at -20°C for two days. On the third day, the whole gut was thawed to 4°C and the crude gut enzyme extracted according to Ali *et al.* (2009) and Sultana *et al.* (2010) as follows (Figure 3.3):

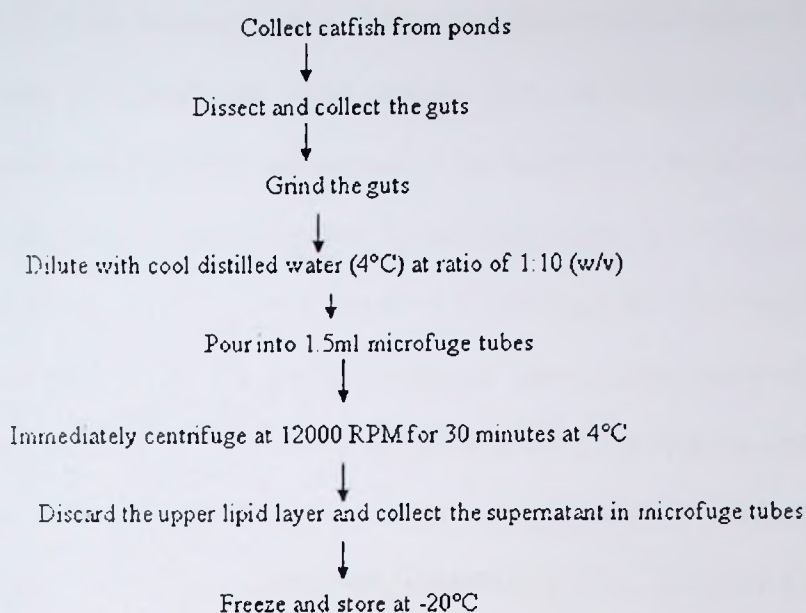


Figure 3.3 Flow chart that was used to extract catfish gut enzyme suspension

3.6.2.2 Preparation of sprouted sorghum solution

Dry sorghum grains (*Sorghum.bicolor*) were placed in a gunny bag, soaked and kept under the sun for seven days when all grain had sprouted (germinated). The sprouted grains were dried in the sun and later ground into fine flour (about 0.02mm). Sorghum flour equivalent to 1% of the feed used in each case was weighed using a digital scale (Denver Instruments, Germany Model TP-3002), made into a suspension with distilled water and mixed with the feeds.

3.6.2.3 Preparation of phytase and protease enzyme extracts

Phytase enzyme (bacteria *Bacillus lincheniformis*, trade name Koffozyme, Israel) and protease enzyme; (fungus *Trichoderma reesei*, trade name Cibenza, Novus international, USA) whose 1g was equivalent to 5000 and 600,000 activity units were procured from

Nutri-Nova international, a commercial feed additive dealer in Uganda. The amount of worth 750, 1000 and 1250 activity units per kilogram of feed was calculated and incorporated into the amount of feed at hand. For The amount of feed worth 160mg of crude protein was determined for each diet category ; 0.53g for 33%CP, 0.46g for 37% CP, 0.32 g for 50% CP, 0.29g for 65% CP diets and 0.0178g for casein). For such small feed quantities, the amount of phytase and protease required could not be accurately measured using the available weighing scale. Therefore measurable amounts were made into stock solutions of phytase and protease upon serial dilutions Tocris molality /dilution calculator provided reliable quantities worth 750, 1000 and 1250 unitsg⁻¹. For protease enzyme, 1000ml of stock solution was made with distilled water at 4°C from which 10ml worth 750, 1000 and 1250 activity units were withdrawn (Table 3.9). For phytase enzyme, stock solutions of 50ml and 5ml were made with chilled distilled water from which 2ml worth corresponding activity units was drawn (Table 3.10).

Table 3.9 Amount of protease enzyme dissolved in 1000ml of distilled water to make a stock solution from which 10ml worth corresponding activity units was withdrawn into 20ml of feed substrate suspension

Protease	Enzyme quantity (g) worth 750 activity units	Enzyme quantity (g) worth 1000 activity units	Enzyme quantity (g) worth 1250 activity units
33% CP diet	0.0066	0.009	0.011
37% CP diet	0.0058	0.0078	0.0097
50% CP diet	0.004	0.0054	0.0067
50% CP diet	0.0036	0.0049	0.0061

Table 3.10 Amount of phytase enzyme dissolved in 50 and 5 ml of distilled water to make a stock solution from which 2ml worth corresponding activity units was withdrawn into 20ml of feed substrate suspension

Phytase	Enzyme quantity (g) worth 750 activity units	Enzyme quantity (g) worth 1000 activity units	Enzyme quantity (g) worth 1250 activity units
33% CP diet	0.008	0.011*	0.013 *
37% CP diet	0.0069	0.0092	0.012*
50% CP diet	0.0048	0.0064	0.008
50% CP diet	0.0044	0.0058	0.0073

*Dissolved in 5ml from which 2 ml were withdrawn

3.6.3 Preparation of diets used in the digestibility experiments

Preparation of experimental diets and analyzing their proximate composition was conducted as described in section 3.4 and 3.4.1 respectively. To one set each diet samples (33%, 37%, 50% and 65% CP) 0.15, 0.20 and 0.25gkg⁻¹ phytase enzyme (*Bacillus lincheniformis*) and 0.00125, 0.00167 and 0.00208gkg⁻¹ of protease enzyme (*Trichoderma reesei*) worth 750, 1000 and 1250 units/kg⁻¹, respectively was incorporated (Tables 3.1-3.4). To another set of diet samples, sprouted sorghum was incorporated at a rate of 1% per kilogram of feed (Table 3.5). In each diet category, a sample not incorporated with any of the enzymes served as a negative control in determination of digestibility coefficients.

3.6.4 Experiment 3: Determination of digestibility coefficients

3.6.4.1 Determination of in-vitro digestibility coefficients

To determine the in-vitro digestibility, the experiment was conducted in 250 ml beakers incubated in a water bath at 26°C under laboratory conditions (Figure 3.4). The amount of the four diets (33%, 37%, 50% and 65% CP) equivalent to 160mg of crude protein in each case was measured to the nearest 0.01g, using a digital scale (Sartorius AG Germany, CPA52025 DS) (i.e. 0.53g for 33%, 0.46g for 37% CP, and 0.32 g for 50% CP and 0.29g for 65% CP diets. Casein (90% crude protein) from bovine milk (C7078, Sigma-Aldrich, St. Louis, MO, USA) was used as a standard protein. The amount of casein worth 60mg of crude protein (1.77g) of bovine casein (90% CP) was used as standard protein. It was measured and along with samples from formulated diets put into 200ml of distilled water cooled to 4°C in they were soaked for 12 hours. The 160mg

protein from each diet category including casein was after incubated at 26°C in a water bath (Grant TXF 200) for 3 minutes. In each case, the pH of the suspension was first adjusted to pH 8 (optimal pH of protease and phytase enzymes used) using either sodium hydroxide or hydrochloric acid as would be appropriate. Two (2) ml of catfish gut enzyme extract and sprouted sorghum suspension (1% of the feed), 750, 1000 and 1250 units/kg⁻¹ of protease were added to each diet category. Then the pH was read and recorded at an interval of one minute for 10 minutes using a desktop digital pH meter with a protected tip (pH 21; Labor-pH/mV/°C- Meter unit Microprocessor, HANNA instruments), (Table 3.11).

Graphs of pH values for enzyme-casein and enzyme – diet mixtures were plotted against time (10 minutes). The slopes of the graphs were determined and used as the rate of pH change (Figure 3.5). The rapid protein digestibility (RPD %) was calculated as the ratio of pH change (-Δ pH) in the enzyme-diet substrate: pH change in enzyme-casein substrate using a formula adapted from Lazo (1994) as follows:

$$RPD(\%) = (((-\Delta pH \text{ diet} / -\Delta pH \text{ casein}) \times 10) / 160) \times 100$$

Where 10 = number of incubation minutes; 160 = amount of protein



Figure 3.4 Research facilities at the Cassava Biotechnology Laboratory of the National Crop Resources Research Institute (NaCCRI), Namulonge where the vitro digestibility was determined

Table 3.11 pH values recorded after every one minute interval during incubation of 50%CP diets with 1250 protease activity units in triplicate samples

Protease 1250					
Casein rep	50%CP rep	Casein rep	50%CP rep	Casein rep	50%CP rep
1	1	2	2	3	3
8.09	8.08	8.07	8	8	8.07
8.02	8.05	8.01	7.98	7.93	8.06
8	8.03	8	7.96	7.93	8.05
8	8.01	7.99	7.95	7.92	8.03
8	8	7.98	7.87	7.92	8.02
8	7.97	7.97	7.82	7.92	8.01
8	7.94	7.97	7.71	7.92	7.81
8	7.93	7.97	7.64	7.92	7.81
8	7.79	7.97	7.62	7.92	7.8
8	7.73	7.97	7.62	7.92	7.8
8	7.73	7.97	7.62	7.92	7.8

A graph of pH values for casein was plotted against pH values of the diet- enzyme mixture. The slope of the graph got represented the rate of pH change within the 10 minutes (Figure 3.5).

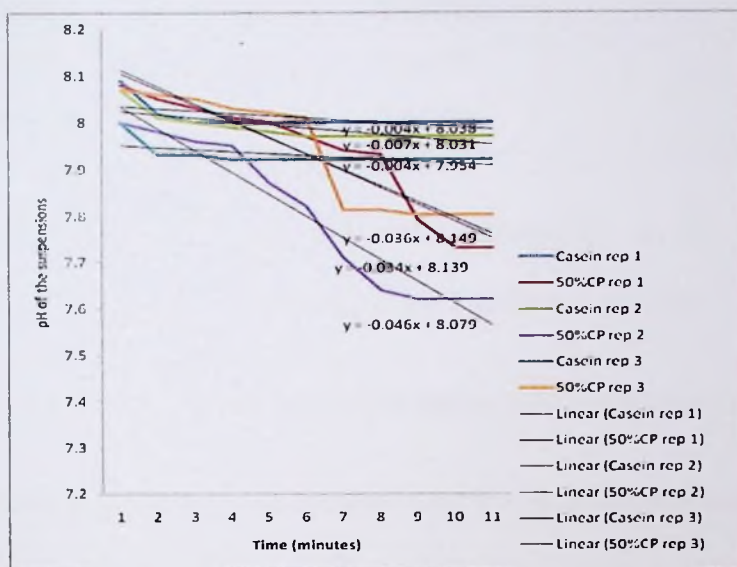


Figure 3.5 Rate of pH change in casein and diet suspensions per minute (for 10 minutes).

The pH change within the period of 10 minutes was determined for both the diet and the Casein (standard protein). The ratio of the pH change in the diet to the pH change in the casein was then determined and used to calculate the rapid protein digestibility (RPD) using the following equation:

$$RPD(\%) = [((- \Delta pH_{Diet} / - \Delta pH_{Casein}) \times 10) / 160 \times 100]$$

Where 10 =Number of minute's diet digested; 160 =Amount of protein (mg) digested.

Table 3.12 Determination of protein digestibility from pH changes of casein and the 50% CP diets incubated with 1250 units of protease enzyme in triplicates

Change in casein pH	Change in diet pH	Ratio of change in diet pH to change in Casein pH	Estimated digestibility (%)
0.0048	0.0361	7.520833	47.00521
0.0073	0.0467	6.39726	39.98288
0.0048	0.0345	7.1875	44.92188

For only determination of in-vivo digestibility, all the diets were incorporated with Chromium (III) oxide (Cr_2O_3) as inert marker at a rate of 0.05% (Ayadi *et al.*, 2012).

3.6.4.2 Determination of in-vivo digestibility coefficients

3.6.4.2.1 Stocking, management and faecal collection from experimental tanks

Fifteen (15) catfish fingerlings weighing 10.55 ± 2.06 gm (total length 8.25 ± 1.34 cm) were stocked in indoor glass tanks filled to 15litre capacity, which were constantly aerated and kept under dark conditions to minimize fish stress. The fry was fed on 33% CP, 2mm diameter pelleted diet for a week to acclimatize to glass tank feeding and faecal collection by siphoning. Each of the diets was randomly assigned to three stocked glass tanks where it was fed to fry daily at 5% of their total body weight. The daily's ration was divided into two portions; one fed in the morning between 10: 00 -11:00hrs and the other in the evening between 05:00-06:00hrs for 60 days (2 months). Leftover feed was cleaned from the tanks an hour after every feeding and faeces collected by siphoning with a flexible rubber tubing daily. About 50% of the water was replaced daily after cleaning to reduce accumulation of toxic wastes.

Experimental diets (33%, 37%, 50% and 65% crude proteins) without enzymes (controls) and incorporated with flour of sprouted sorghum (at 1%), phytase and protease enzymes (at 750, 1000 and 1250 AUkg⁻¹) respectively were randomly assigned in triplicates. Feeding and collection of faecal matter were conducted for 60 days. Water was replenished daily at 14:00hrs by removing and replacing 50% of the water volume to maintain dissolved oxygen concentration between 3-4mg l⁻¹. Tanks were gently cleaned twice daily one hour after every feeding to remove uneaten feed (at 10:00hrs and 05:00hrs). Faecal matter was collected from each tank by siphoning onto plastic strainers from where it was then put into plastic vials that were kept frozen at -20°C (HISENSE RT710N4ACUV) (Figure 3.6).

At the end of the study, the faecal matter collected from all triplicate trial tanks were thawed, pooled together into porcelain bowls and oven dried at 60°C for 48 hours to a constant weight. Fish was monitored for twenty four hours daily for deaths and any other unusual condition. Dead fish was removed from each tank during cleaning and counted and the sum subtracted from the original stock and used to calculate survival rate as described in Section 3.6.5.1.



Figure 3.6 Collecting faecal matter by siphoning it from glass tank where catfish fingerlings were reared

3.6.4.2.2 Water quality management and monitoring

The state of physical and chemical water parameters was monitored following principles of the standard methods (APHA, 2005). Water temperature and dissolved oxygen were measured in situ with a digital oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Total phosphorus, nitrates, nitrites, ammonia and pH were determined using a Sera Aqua-test kit with reagents (Sera GmbH, Heinsberg, Germany). About 4 - 6 drops of the appropriate reagents in the kit were mixed with 5 ml of experimental water samples. The mixture was shaken until it was evenly distributed and the color developed matched to the colour chart immediately for pH and after five minutes for phosphates, nitrates, nitrite and ammonia. The digital value corresponding to the colour on the chart was then read and recorded under natural light as directed on the instruction manual.

3.6.5 Data collection for digestibility experiment

3.6.5.1 Survival and growth parameters

The growth performance of catfish in-vivo was calculated according to Vielma *et al.* (2002) as follows:

a) $Survival\ rate\ (\%) = [Total\ fish\ harvest / Total\ fish\ stocked] \times 100$

b) $Weight\ gain\ (g) = [Final\ weight - Initial\ weight\ (g)] \times 100$

c) $Weight\ gain\ (\%) = [Final\ weight(g) - Initial\ weight\ (g)] \times 100$

d) Specific growth rate was determined as follows:

$$SGR\ (\% \ g/day) = 100 \times \left(\frac{[Ln\ (Final\ weight(g)) - Ln\ (Initial\ weight(g))]}{Rearing\ period(days)} \right)$$

Where $\ln$ = natural log.

e) Feed utilisation was determined using the Feed conversion ratio (FCR) which was calculated as follows:

$$FCR = Total\ feed\ given / Total\ weight\ gained.$$

3.6.5.2 Feed intake

Experimental catfish were fed to satiation twice daily from 0900-10:00hrs and 1700 - 1800hours at a rate of 5% of their body weight. Satiation was judged to have been reached when uneaten pellets were retained at the tank bottom up to an hour after feeding (at 10: 00hrs and 18:00hrs). Whole uneaten pellets were carefully counted and their weight approximated by weighing a similar number of unfed dry pellets according to Ali

and Jauncey (2004). This weight was then subtracted from the weight of total administered feed and the difference taken as the estimated feed intake (kg).

3.6.5.3 Determination of protein digestibility coefficients (in-vivo)

The indirect method as described by De Silva and Anderson, (1995), Guillaume *et al.* (2001) and Jobling, (1994) was used with Chromium (III) oxide as an inert marker incorporated in diets at a rate of 0.5%. Apparent digestibility coefficients (ADC) of dry matter were calculated according to Cho and Slinger, (1979) and Guillaume *et al.* (2001) using the following equation;

$$ADC(\% \text{ dry matter}) = 100 - \left[100 \times \left(\frac{\text{Chromium in the feed}}{\text{Chromium in the faeces}} \right) \right]$$

Apparent digestibility coefficients (ADC) of crude protein and phosphorus were calculated using Cho *et al.* (1982) and De Silva and Anderson (1995) as follows:

$$ADC \% (\text{Protein or phosphorus}) = 100 - \left[100 \times \left(\frac{Fp}{Dp} \times \frac{Dcr}{Fcr} \right) \right]$$

Where Fp = % protein or phosphorus in faeces, Dp = % protein or phosphorus in the diet.

Dcr = % Cr₂O₃ in diet; Fcr = % Cr₂O₃ in faeces. Meanwhile, phosphorus in the diet, faeces and whole-body fish was analysed by the molybdovanadate method according to Ueda and Wada (1970).

3.6.5.4 Statistical analysis

Raw data was organized in Microsoft Excel (USA) and later imported into STRATA statistical soft ware (Version 14) for the in-vitro experiment and into Genstat Windows

18th Edition (AVSNI product) for in-vivo digestibility. ANOVA was conducted and Tukey's test used for pair wise comparisons.

3.7 Experiment 3: Determining the effect of varying dietary protease from 750 , 1000 and 1250 units/kg of dry starter diets (65%CP) on growth performance and cost effectiveness of catfish hatchlings.

3.7.1 Source of experimental hatchlings

The hatchlings of African catfish used in this study were artificially produced at MBAZARDI fish hatchery using the catfish pituitary gland extract. After hatching the hatchlings were counted and transferred to the rearing tanks. On the morning of the third day, when the yolk sac was nearly completely absorbed, feeding of the hatchlings was initiated. The hatchlings were approximately 3.5 mg mean body weight each. The hatchlings were fed on diets of 65% CP incorporated with 0, 750, 1000- and 1250 units per kilogram of feed (Table 3.4).

All brood stock fish and the artificially fertilized eggs were disinfected with potassium permanganate (0.2g in 25litres of water). The hatchlings for in-vivo determination were fed on decysted *Artemia* (Packed by INVE Thailand Ltd and engineered by INVE Technologies NV,Belgium) for the first two days after absorption of the yolk sack (two days) and weaned onto a catfish starter feed (Raanan Cs 7994CO) from Israel purchased from Aqua farm Consults Ltd in Kampala ,Uganda, until they were 30 days old. After this, the fry were taken to nursing tanks and raised in a green house up to 3 months when they has attained weight $10 \pm 0.42\text{g}$ and used for tank and pond experiments. This helped

in acclimatizing all the catfish fry used in the study to feeding on dry feeds. The experimental fish were selected randomly, weighed and then transferred to experimental unit one week before the data collection commenced to allow adaptation to experimental conditions.

3.7.2 Formulation and manufacture of experimental diets

Dry diets containing 65% CP were formulated and manufactured with 0, 750, 1000- and 1250 units of phytase per kilogram of feed (Table 3.4) according to section 3.4. After pelleting, the diets were crumbled into 100-200 μm diameter micro-diets, which were further ground into fine powder and fed along with pre-decysted *Artemia* nauplii in the first week of the study and then crumbles fed after middle of the second week. Crumbles as made five diets.

The five diets were randomly assigned to fifteen hatchery tanks each of 45-L of aerated water and 8000 catfish hatchlings. Each tank was constantly aerated using rubber air diffusers connected to an air compressor (HAILEA ACO-328). Water was allowed to continuously flow through the tanks at rates of 85 ml per minute (altogether 2 liters per minute). The tanks were cleaned twice daily from 07:00 - 08:00 and 19:00 - 20:00 hours. Fish hatchlings were fed on one of the experimental diets up to satiation every two hours over 24 hours daily for 30 days. Dead fish was recorded daily during cleaning of tanks.

3.7.3 Monitoring water quality in hatchery tanks

The water quality parameters within the rearing tanks in which hatchlings were raised was maintained within acceptable ranges for catfish hatchlings (Boyd and Tucker, 1998) and monitored as described in Section 3.6.4.2.2.

3.7.4 Growth monitoring among catfish hatchlings

During the experiment, fish weight was determined weekly by weighing a batch of hatchlings into a beaker with pre-weighed water and counting the number of hatchlings in the batch to get the average weight of each hatchling. The feed utilisation indices were determined as described in Section 3.6.5.1. However, unlike catfish fingerlings whose weights were recorded in grams (g), all data for catfish hatchlings was in mg due to its small size as follows;

$$i) \text{ Average individual weight (mg)} = \frac{\text{Total batch weight (mg)}}{\text{Total number of fish in the batch}}$$

$$ii) \text{ Weight gain (mg)} = \text{Final weight (mg)} - \text{Initial weight (mg)}$$

$$iii) \text{ Weight gain (\%mg)} = 100 \times [\text{Final weight (mg)} - \text{Initial weight (mg)}]$$

$$iv) \text{ SGR (\%mg/day)} = 100 \times \left[\frac{\text{Ln Final weight (mg)} - \text{Ln Initial weight (mg)}}{\text{Rearing period (days)}} \right]$$

$$v) \text{ Feed intake (Kg/tank)} = \text{Total feed given during the experiment}$$

3.7.5 The cost effectiveness of the diets

For cost effective analysis, simple economic analysis based on incidence of costs and profit index were used to assess the cost effectiveness of producing catfish fry by feeding catfish

hatchlings using dry diets incorporated with protease. Feed costs using real time market prices were considered as the main expense since it accounts for about 70% of operational costs and other operational costs were assumed constant. The Incidence Cost (IC), which is governed by the unit cost of the feed and its apparent FCR was employed for cost analysis of hatchery and catfish fry fattening (grow out) diets along with the net profit and cost benefit ratio (US \$) as follows:

i) *Profit index = Number of fish produced / Total cost of feed fed*

ii) *Incidence of cost (R) = Cost of feeds / Biomass of fish produced (Kg)*

iii) *Net profit = Total fish sales – Total feed costs*

iv) *Gross profit = Total fish sales – Total feed costs*

v) *Benefit cost ratio (Bcr) = Total fish sales – Total feed expenses*

3.7.6 Data handling and statistical analysis

The obtained data were subjected to two-way ANOVA performed using SPSS program version 20 (SPSS, Richmond, VA, USA) as described by Dytham (2011). In all cases ANOVA tests were performed and multiple comparisons conducted using Turkey's post hoc tests. Significance was declared at 95% confidence level ($p \leq 0.05$) for all parameters.

3.8 Experiment 4: Determining the effect of varying dietary protein from 33% to 37%CP and phytase enzyme from 750, 1000 to 1250FTUs in grow-out diets on productivity of catfish fry in earthen ponds

3.8.1 Preparation of diets for grow out trials in ponds

Two (2) grow out diets were formulated one containing 33% and the other 37% crude protein (Table 3.1 and 3.2 respectively). For each batch four samples were incorporated with 0.0, 750, 1000 and 1250 FTU kg⁻¹ of phytase enzyme. This made eight experimental diets/treatments (Table 3.13).

Table 3.13a Ingredients composition of the formulated diets

Dietary CP	33% CP				37% CP			
Phytase level (FTU/kg diet)	0.0	750	1000	1250	0.0	750	1000	1250
Ingredients								
Fish meal	10	10	10	10	20	20	20	20
Soybean meal	42	42	42	42	41	41	41	41
Wheat pollard	10	10	10	10	10	10	10	10
Maize bran	10.2	10.05	10	9.95	8.2	8.05	8	7.95
Bush beans	10	10	10	10	6	6	6	6
Cotton seed cake	2	2	2	2	2	2	2	2
Cassava flour	4	4	4	4	3	3	3	3
L-lysine	3	3	3	3	4	4	4	4
DL Methionine	2	2	2	2	2	2	2	2
Fish oil	2	2	2	2	1	1	1	1
Vitamin and mineral premix*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Citric acid	3	3	3	3	1	1	1	1
Table salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lime	1	1	1	1	1	1	1	1
Binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phytase	0	0.15	0.2	0.25	0	0.15	0.2	0.25
Total	100	100	100	100	100	100	100	100
Cost/kg (US\$)	0.691	0.702	0.705	0.709	0.776	0.789	0.790	0.793

* Vitamins and minerals premix: each 2.5 kg contains vitamins A (12 MIU), D3 (2 MIU), E (10 g), K (2 g), B1 (1 g), B2 (4 g), B6 (1.5 g), B12 (10 mg), pantothenic acid (10 g), nicotinic acid (20 g), folic acid (1 g), biotin (50 mg), choline chloride (500 mg).

copper (10 g), iodine (1 g), iron (30 g), manganese (55 g), zinc (55 g), and selenium(0.1 g).

Table 3.13b Proximate composition (% dry matter) of the formulated diets

Dietary CP	33% CP				37% CP			
FTU/kg	0	750	1000	1250	0	750	1000	1250
Organic matter	89.7 ± 0.15	87.8 ± 0.02	85.7 ± 0.71	88.9 ± 0.32	88.6 ± 0.33	88.9 ± 0.05	86.6 ± 0.1	87.9 ± 0.13
Crude protein	33.6 ± 0.29	32.3 ± 0.48	33.7 ± 0.44	34.4 ± 1.49	36.2 ± 0.19	37.7 ± 0.97	37.7 ± 0.97	36.2 ± 1.32
Lipids	11.6 ± 0.18	12.4 ± 0.54	11.3 ± 1.64	11.5 ± 1.11	11.6 ± 0.15	12.4 ± 2.0	11.2 ± 2.42	12.3 ± 0.26
Phosphorus	2.2 ± 0.1	2.0 ± 0.02	2.3 ± 0.07	2.6 ± 0.32	2.0 ± 0.14	2.1 ± 0.01	2.4 ± 0.15	2.2 ± 0.04

The diets were pelleted as sinking pellets, dried under a ventilated iron roofed shade and packed into laminated polyethene bags until they were used. Twenty four (24) fish ponds (of 100m² surface area) with clay soils were purposively selected out of 42 ponds available on-farm (Figure 3.7) to reduce light penetration and growth of phytoplankton that would present confounding effects on diets/feed.



Figure 3.7 The fish farm from which 24 ponds experimental ponds were selected

Each pond was stocked with 440 catfish fingerlings of 11.65 ± 2.19 g body weight basing on 2kgm^{-2} as the estimated pond catfish carrying capacity (FISH, 2009) and 500g as the desired size at harvest using the following equation:

$$\begin{aligned} \text{Number of fish to stock (m2)} \\ = [\text{Carrying capacity (Kg)}/\text{Desired weight (kg)}] \div 10\% \end{aligned}$$

3.8.2 Pond stocking and management

The 24 ponds with clay soils were purposively chosen from the farm (Figure 3.7) to minimize development of plankton as reported by Boyd and Tucker (1998). Each was drained, desilted and disinfected with 20 kg of builders lime (calcium hydroxide). Two weeks later, the ponds were filled with water from a natural wetland up to 1.0 m depth and left to stand for three weeks. Each pond was then stocked with 440 catfish fingerlings of weight 10.7 ± 0.4 g and randomly assigned a test diet.

3.8.2.1 Management of experimental ponds

The stocked fish ponds were covered with a 3 inch mesh sized netting to prevent predation and stress from birds (Figure 3.8). The stocked fish were fed at a rate of 5% of their total biomass daily, with the daily ration divided into three portions that were fed manually by hand from 10:00-11:00 hours, 13: 00-14:00 and 17:00-18:00 hours daily according to the fish's feeding response (appetite). Fish was graded monthly to remove shooters that would eat up their smaller counter parts and negatively influence data on weight gain due to cannibalism. Only final weights were however determined after rearing the fish for three months when pond drainage total was possible to enable total counting of the harvest. Catfish harvesting required total pond drainage which is stressing to the fish that it can reduce growth. During partial grading, half of the pond water volume was drained and replenished by fresh water from a clayish water reservoir to minimize plankton growth and accumulation of toxic wastes.

The whole feeding trial was repeated for two consecutive three months- rearing cycles. Growth performance, nutrient deposition, retention and cost effectiveness were determined as described in Section 3.7.4 and 3.7.5.



Figure 3.8 How experimental ponds were covered by netting to guard against disturbances and predators especially birds

3.8 3 Data collection in grow out pond trials

3.8.3.1 Determination of catfish performance in ponds

3.8.3.2 Growth parameters and cost benefit of catfish production in ponds

The growth performance and cost effectiveness of producing catfish in ponds was calculated according to Section 3.6.5.1 and 3.6.5.2.

3.8.3.3 Determination of protein and phosphorus deposition and retention

Feed utilisation by catfish was judged based on the feed conversion ratio (FCR), the protein and phosphorus efficiency (NRE), deposition and retention (ND) that were determined according to Storebakkena *et al.* (1998) as follows:

$$i) \text{ FCR} = \text{Total feed given (g)} / \text{Total weight gained (g)}$$

$$\text{Protein retention ratio (PRE)} = \text{ii) } \text{Weight gain(g)} / \text{Total protein intake(g)}$$

$$\text{iii) Phosphorus retention(g/kg)} = \text{Weight gain (g)} / \text{Phosphorus intake(kg)}$$

$$\text{iv) } NRE(g/kg - 1) = 100 \times \left(\left[\frac{FSW}{N_f} \right] - \left[\frac{ISW}{N_i} \right] \right) / \text{Feed intake(kg)} / N \text{ diet}$$

Where FBW is the final body weight (g), IBW is the initial weight (g); Nf is the concentration of phosphorus or crude protein in the fish at the end and Ni at the start of the experiment.

Nutrient deposition (ND gkg⁻¹) for protein and phosphorus was determined using the following equations.

$$\text{v) } ND(g/kg - 1) = [\text{Final nutrient in the body}/100] \times \text{Feed intake(kg)}$$

3.8.3.4 The cost effectiveness of the diets as in experiment 3

For cost effective analysis, simple economic analysis based on incidence of costs and profit index were used to assess the cost effectiveness of using enzyme incorporated diets in catfish production as described in Section 3.7.5.

3.8.3.5 Water quality monitoring (already described above)

The state of physical and chemical water parameters was monitored based on principles of standard methods (APHA, 2005) but using water testing kits as described in Section 3.6.4.2.2.

3.8.3.6 Data handling and statistical analysis for pond productivity

Data on survival, weight, and feed dispensed body nutrient composition was collected and used to determine survival rate, FCR, weight gain, Protein and phosphorus efficiency and retention. The raw data was organized in Microsoft Excel (USA) and later imported into SPSS program version 20 (SPSS, Richmond, VA, USA) as described by Dytham (2011). It was then analysed for two-way ANOVA using SPSS program version 20 (SPSS, Richmond, VA, USA) as in Section 3.10. ANOVA tests with multiple comparisons conducted using Turkey's post hoc tests. Significance was declared at 95% confidence level ($p \leq 0.05$) for all parameters.

3.9 General data handling and statistical analysis

Data for all parameters was first analysed for normal distribution using the Shapiro-Wilk test and homogeneity of variance using the Bartlett's test ($P < 0.001$). All data was first analysed for normal distribution using the Shapiro-Wilk test and homogeneity of variance using the Bartlett's test. Data in percentages was transformed into arcsine as ($\text{ASIN}(\text{SQRT}(\text{number}))$). It was later back-transformed by squaring the sine of the arcsine after re-testing for normality and homogeneity of variance. Data that wasn't normally distributed even after transformations was analysed using the non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA). Data with normal distribution and homogeneity of variance was analysed using the parametric two way (ANOVA) while considering interactions. Data with significant variance was subjected to pair wise comparisons using Tukey's test.

The proportion of phytic acid, protein, pH was recorded and summarized in Microsoft excel (USA) and imported into Paleontological statistics (PAST) software ver. 2.17c developed by Hammer *et al.* (2001). Data on in-vivo protein digestibility (section 3.6.4.2) was analysed using STATA software version 14. Data on in-vivo protein and phosphorus digestibility coefficients, growth parameters (weight gain, specific growth), protein and phosphorus retention, was analysed using Genstat version 18, VSN International, UK. To determine the effect of varying dietary protease and phytase on performance of catfish hatchlings and fry respectively, statistical analyses were performed using SPSS program version 20 (SPSS, Richmond, VA, USA) as described by Dytham (2011). In all cases ANOVA tests were performed and multiple comparisons conducted using Turkey's post hoc tests. Significance was declared at 95% confidence level ($p \leq 0.05$) for all parameters. The information derived from this study was shared with stakeholders through national and international conferences. Five manuscripts were prepared of which four are already published (Appendices I-IV) while one is still in place at the Journal of Ichthyology.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Experiment 1: Determining effect of roasting on phytic acid, crude protein content and pH in plant-based diets

4.1.1 Phytic acid ,CP and pH in diets

The phytic acid percentage in diets formulated from unroasted ingredients statistically differed significantly from that of the diet made from roasted ingredients ($p \leq 0.002$) while their crude protein percentages did not differ ($p = 0.894$; Table 4.1).

Table 4.1 Phytic acid, protein and dry matter content in roasted and unroasted diets ingredients (Means $\pm$ SE)

Diet ingredients	Dry matter (%)	Crude protein (%)	Phytic acid (%)	pH
Unroasted	88.59 $\pm$ 0.81	35.27 $\pm$ 1.78	5.89 $\pm$ 0.021 ^a	6.77-6.89
Roasted	87.07 $\pm$ 2.76	35.22 $\pm$ 0.29	5.67 $\pm$ 0.022 ^b	6.72-7.00

4.1.2 The pH values in diets made from roasted and unroasted ingredients

The pH values in the diets made from roasted and unroasted ingredients did not statistically differ ($p > 0.05$). The pH in the diet made from roasted ingredients ranged from 6.72 -7.08 while the pH of the diet made from unroasted ingredients ranged from 6.84 to 6.77.

4.1.3 Variation of phytic acid and crude protein in diets from roasted ingredients and its ingesta along the gut

The variation of phytic acid and crude protein was determined in the diet from unroasted ingredients but not in its ingesta along the gut. The diet made from unroasted ingredients was not ingested by the catfish thus no ingesta were obtained. This was attributed to the offensive odour of raw soybean and kidney beans and implied that roasting enhanced flavor. This limited analysis of phytic acid and crude proteins to the ingesta of catfish fed diets with roasted ingredients only (Table 4.2).

Table 4.2 Dry matter, phytic acid and crude protein content (%) in the diet and ingesta of diets from roasted ingredients

	Dry matter (%)	Crude protein (%)	Phytic acid (%)
Diet (before ingestion)	87.07 ± 4.37	35.14 ± 3.01	5.67 ± 0.72 ^a
Ingesta in stomach	8.96 ± 3.51	28.85 ± 2.40	8.77 ± 1.38 ^b
Ingesta in anterior intestine	13.68 ± 3.85	21.52 ± 0.79	9.27 ± 0.31 ^c
Ingesta in posterior intestine	9.79 ± 1.54	20.18 ± 1.80	9.33 ± 1.04 ^c
Ingesta in the rectum	14.11 ± 2.06	17.50 ± 2.03	9.65 ± 1.09 ^c

Phytic acid content in the ingesta along the gut (stomach, anterior intestine, posterior intestine and rectum) was significantly higher than that of the diet. ($p < 0.05$).

The phytic acid concentration was lowest in un-ingested diet and increased along the gut reaching the highest concentration in the rectum. Meanwhile the dietary crude protein decreased as it passed along the gut remaining only 50% in the rectum (Table 4.2).

4.1.4 Relationship between CP and phytic acid content in ingesta

There was negative linear correlation ($p < 0.0001$, r^2 adjusted = 0.662) recorded between variations in protein and phytic acid percentage in the diet and ingesta of the diet from roasted ingredients (Figure 4.1 and Figure 4.2).

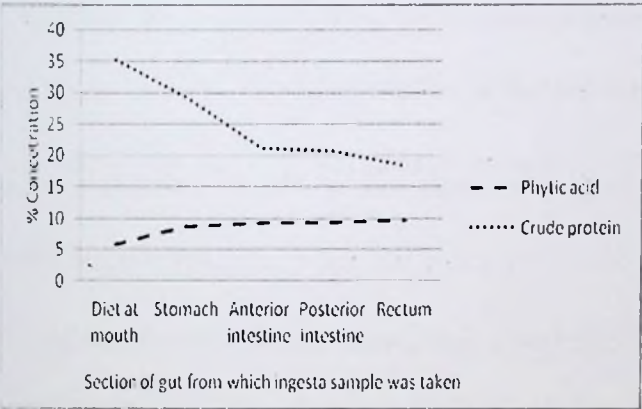


Figure 4.1 Phytic acid and crude protein variations in ingesta of diets from roasted ingredients along the digestive tract

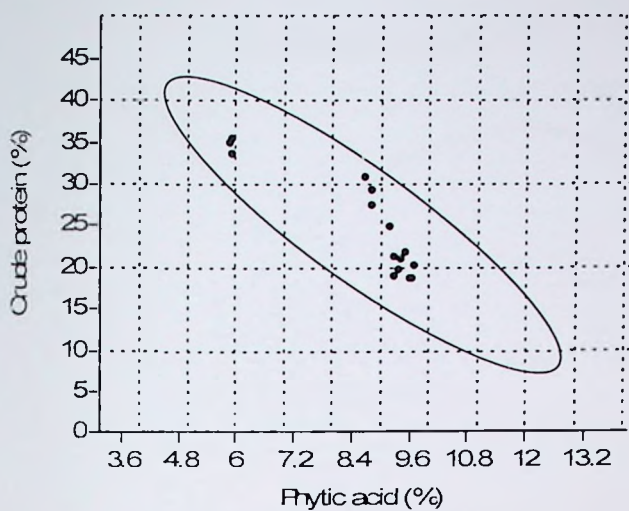


Figure 4.2 A correlation plot of crude protein and phytic acid percentage in the ingesta of the diet from roasted ingredients

4.1.5 Variation of pH in the diet and ingesta along the different sections of the catfish gut tract

The pH in the diet from roasted ingredients (6.72 - 7.08) did not differ from that of the diet from unroasted ingredients (6.77 - 6.89; $p > 0.05$). There was however pH variation along the gut for the diet the diet from roasted ingredients that was ingested. The diet pH (6.79) reduced after it was ingested in the stomach to about 5.69, increased in the anterior and posterior intestine (6.43-6.72) and again reduced in the rectum (6.43) (Figure 4.3).

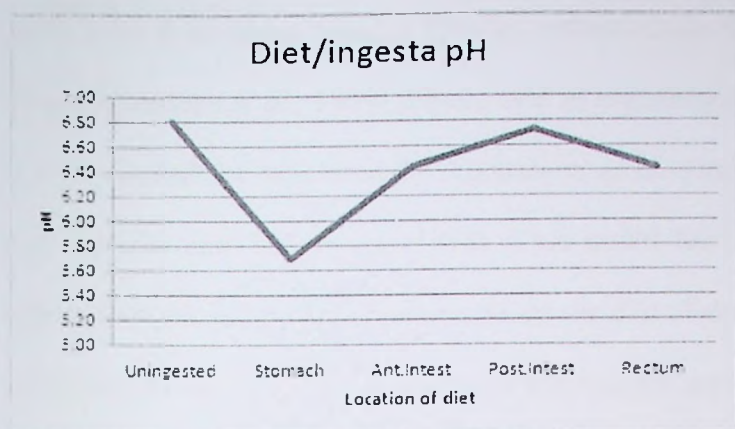


Figure 4.3 pH values in the different sections of the gut

The pH of the ingesta in small and large intestine differed from that of the ingesta in the stomach ($p < 0.05$) while the pH of the ingesta from the rectum did not differ from that from the stomach ($p > 0.05$).

4.1.6 Discussion of results on effects of roasting

The significantly lower phytic acid in the diet with roasted ingredients (5.65-5.76%) than in the diet with unroasted ingredients (5.85-5.92%) was attributed to possible break down of phytic acid by innate phytase in plant seeds activated during roasting (Abdoulaye *et al.*, 2011). Activation of phytase enzyme in plant material was reported to be activated at low roasting temperatures of about (60-70°C) to foster breakdown of phytic acid (Azeke *et al.*, 2011). As the heat increases, the phytase enzyme is inactivated thereby preventing further breakdown of phytic acid. The reduction in phytic acid although significant was however small (about 0.27%), similar to findings reported by Omoruyi (2007) after roasting potatoes and Ndidi *et al.* (2014) after roasting Bambara nuts (*V. subterranean*). The content of phytic acid in both diets was however higher than 0.7-2.5% reported for most individual plant ingredients and some compound diets (e.g. 1-1.5% in soy, 1% in

corn, 2.5-5.8 in wheat bran, 0.75% in Indian whole bread made up of mixed cereals (García-Estépa *et al.*, 1999). Phytic acid in experimental diets was comparable to the 5-7.5 % reported for rape seed by Abdoulaye *et al.* (2011), García-Estépa *et al.* (1999) and Serraino and Thompson (1984). This could have been attributed to the additive effects from the ingredients that composed the diet (Table 3.13). Variation in the growing conditions of cereals and the unrefined ingredients used could have also contributed to these variations as reported by Abdoulaye *et al.* (2011).

The increase in phytic acid (%) along the digestive tract of catfish fed on the diet with roasted ingredients (from 5.67 ± 0.04 in the diet to 9.65 ± 0.04 in the rectum) suggests that catfish has no mechanisms of breaking down phytic acid in feed along its gut. This resulted into its accumulation which can trigger chelating with nutrients including mineral elements and protein (Mugendi *et al.*, 2010; Reddy *et al.*, 1989) thus compromising nutrient bioavailability.

Crude protein in the diet from roasted ingredients (35.22 ± 0.51) did not differ from that of the diet made from unroasted ingredients (35.27 ± 2.30). Lack of difference in crude protein was also observed by Masoero *et al.* (2005) and Ndidi *et al.* (2014) after roasting peas and bambara nuts (*Vigna subterranean*) respectively. However Balogun (2013) reported a significant reduction in crude protein of Bauhinia seed roasted at 300°C. The difference in the observations was attributed to differences in roasting temperatures and feed ingredients material used. The reduction of crude protein in the ingesta of catfish fed on the diet with roasted ingredients was attributed to digestion and absorption of protein.

However absorption of about 50% of the protein was attributed to build up of phytic acid which might have chelated with protein to form insoluble complexes thus limiting its digestion as reported earlier by Abdoulaye *et al.*(2011) and Kumar *et al.*(2011). This implied that crude protein in such diets could not be fully utilized by catfish. This was confirmed by the negative correlation between phytic acid and crude protein (i.e. as phytic acid increased, protein bioavailability decreased).

The more or less neutral pH range in the two diets ;(both from roasted (6.72 - 7.08) and unroasted (6.77 - 6.89) ingredients) and one from unroasted ingredients at a temperatures of 24⁰C was attributed to dominance of plant proteins in the diet (Table 3.2). Plant protein contains lower quantities of essential amino acids particularly the sulphur-containing Cystine and Methionine than animal proteins (Campbell and Campbell, 2006). Limited sulphur-containing amino acids reduce accumulation of acid producing metabolites, maintaining a moreless neutral pH even after the diet was incorporated with supplemental Methionine.

However, the significantly higher pH in the small and large intestines than in the stomach and rectum was attributed neutralization of stomach contents by hydrochloric acid produced during the acid digestion phase in most teleost fishes. Hydrochloric acid is produced to activate zymogens and pepsinogen fluids in the endo-gastric cells to activate pepsin and trypsin-like enzymes for digestion of proteins (Solovyev *et al.*, 2015).

Accumulation of free amino acids as a result of protein hydrolysis in the stomach could also have influenced the pH. The pH of the stomach ingesta (pH 5.30 – 6.75) observed in this study were however higher than pH 2 - 4 reported by (Page *et al.*, 1976) for channel catfish (*Ictalurus punctatus*), a species closely related to African catfish (*Clarias gariepinus*), pH 2.5 reported for South American catfish by (Hernandez *et al.*, 2009), pH (1-2) for Nile tilapia reported by Evans *et al.* (2005) and pH 3.5–4.5 of Nile perch (*Perca fluviatilis*) and Zander (*Sander lucioperca*) reported by Solovyev *et al.* (2015). The variation of gut pH among species was influenced by factors such as the diet composition, presence/absence of food and water temperature. Gut pH is reported among factors that form the basis for diversity in fishes (Montgomery and Pollak, 1988). The increase in ingesta pH within the intestines (pH 6.3-7.7) was attributed to presence of sodium hydroxide, bicarbonate rich mucus cells in gut walls, alkaline bile salts and cholesterol produced by the pancreas, bile and liver to emulsify lipids as reported by De Silva and Anderson (1995).

This trend of pH in the intestines is in agreement with the reported mild alkaline pH in the midgut of most teleost fishes by Smith (1980). The intestinal ingesta at pH of 6.3-7.7 was close to pH 6.5–7.2 reported by Solovyev *et al.* (2015) in the intestine of perch and zander. The reduction in ingesta pH within the rectum to more less the values in the stomach was, however, attributed to reduction in gastric enzyme production and accumulation of feed fermentation products including alkaline ammonia.

The fact that only the diet made from roasted ingredients was ingested and not the diet from unroasted ingredients implied that other than phytic acid reduction, roasting improved acceptability of diets. This could be attributed to the sweet aroma imparted by roasting and inactivation of heat labile toxic compounds such as tannins and some enzyme inhibitors like anti-trypsin as reported by Serna-Saldivar (2010). Similar results were reported by Jurkovic and Colic (1993) and Mridula *et al.* (2007) after roasting grains and cereals. The roast aroma was reported to be enhanced in seeds and grains roasted at temperatures of 80- 110°C for 30 minutes to one hour than at lower and higher temperatures (Sandhu *et al.*, 2015). It should however be noted that roasting conducted under alkaline conditions can induce formation of unnatural anti-nutrients in form of toxic amino acid derivatives, e.g. oxidized forms of sulphur containing amino acids, D-amino acids, and lysine-alanine (LAL) that reduce palatability and nutritive value of the feed (Jurkovic and Colic, 1993).

4.2 Experiment 2: Assessing the effect of dietary phytase, protease and sprouted sorghum on digestibility of protein and phosphorus in dry diets by catfish.

4.2.1 In vitro apparent digestibility of protein.

The apparent digestibility of crude protein significantly differed ($p < 0.05$) among diets incorporated with phytase and protease enzymes and diets with sprouted sorghum, a mixture of protease and phytase and catfish gut enzyme extract (Table 4.3).

Table 4.3 Apparent digestibility regression of diets incorporated with sprouted sorghum, protease, phytase and a combination of 500 units of phytase and 500units of protease with the fish gut enzymes as the explanatory variable

Enzyme Type	33%CP	37%CP	50%CP	65% CP
Fish Enzyme	3.07	3.13	11.57	21.2
Sprouted sorghum	14.23	16.93	13.97	27.37
Protease	28.76	43.61	54.44*	59.86*
Phytase	85.16*	76.43*	43.84	33.2
Protease & Phytase	13.67	17.67	7.2	20.57

* p values significant

The data in Table 4.3 was analysed using the non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA because the mean variance was not homogeneous.

Among phytase and protease levels, protein digestibility was highest in 65% CP diets incorporated with protease and 33% CP diets incorporated with phytase enzyme (Figure 4.4). Digestibility in diets incorporated with phytase reduced with increasing dietary crude protein while the reverse was true for diets incorporated with protease enzyme. Among diets with phytase, protein digestibility was highest (88.9%) in diets with 750 units kg^{-1} while in protease diets it was highest (70.1%) in diets with 1000 units kg^{-1} (Figure 4.5 and Figure 4.6, respectively).

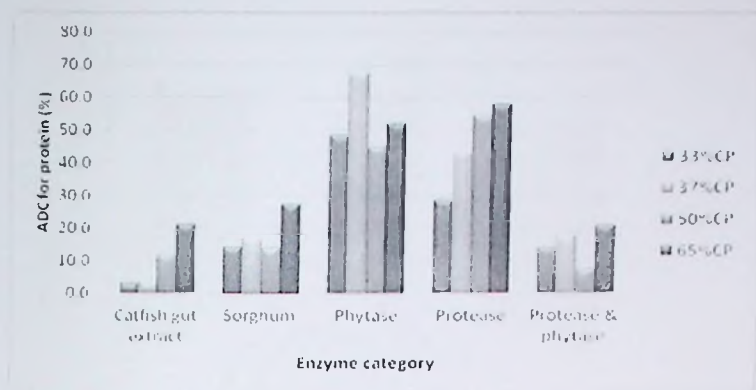


Figure 4.3 Rapid protein digestibility coefficients (%) of diets incorporated with catfish gut enzyme extract, sprouted sorghum (*S. bicolor*), fungal protease (*Trichoderma reesei*), bacteria phytase (*Bacillus licheniformis*) and a combination of 500 units of phytase and protease.

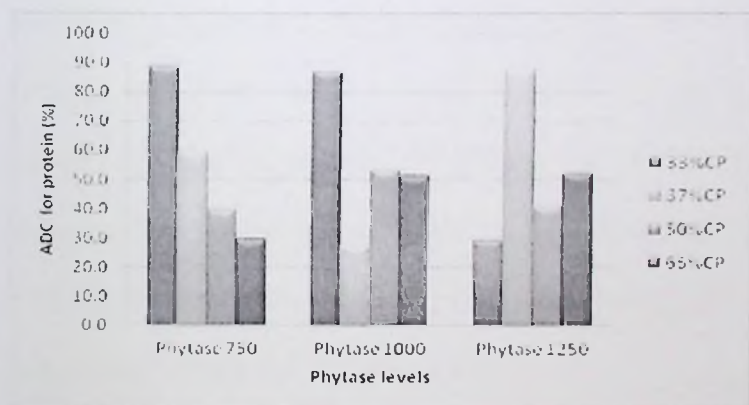


Figure 4.5 Protein digestibility coefficients for diets with 750, 1000 and 1250 units of phytase enzyme

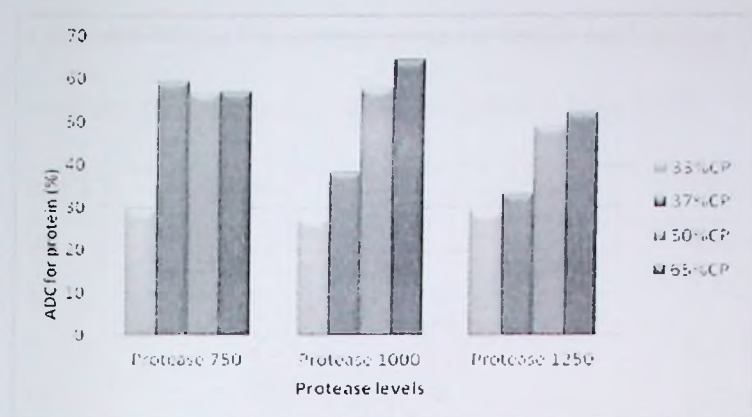


Figure 4.6 Protein digestibility for diets incorporated with 750, 1000 and 1250 activity units of protease enzyme

4.2.2 Water quality during in-vivo digestibility trials

On the basis of enzyme levels, ammonia and nitrates were significantly higher in diets incorporated with sprouted sorghum and the control than in diets with phytase and protease ($p < 0.001$ and $p = 0.010$) respectively. On the other hand, total phosphorus, dissolved oxygen and pH did not differ among diets ($P = 0.780$; $P = 0.729$ and $p = 0.727$, respectively). Un-ionised ammonia ranged from 3-12 mg/l, nitrate from 0.2-3.4mg/l⁻¹, total phosphorus, from 0.4-11.5 mg/l⁻¹, dissolved oxygen from 3 -5 mg/l⁻¹ (Figure 4.7 and 4.8) and pH from 6.5-7.5 (Table 4.4).

Table 4.4 Water pH among tanks in which catfish was fed on the control diet, diets with sorghum, phytase and protease enzymes (Mean $\pm$ SE)

Diet CP	Control	Sorghum	Phytase	Protease
30	6.81 $\pm$ 0.23	7.27 $\pm$ 0.21	6.90 $\pm$ 0.12	6.90 $\pm$ 0.12
35	7.01 $\pm$ 0.14	6.77 $\pm$ 0.14	6.95 $\pm$ 0.11	6.95 $\pm$ 0.11
50	6.97 $\pm$ 0.15	6.98 $\pm$ 0.10	6.81 $\pm$ 0.26	6.81 $\pm$ 0.26
55	6.80 $\pm$ 0.46	6.78 $\pm$ 0.11	6.70 $\pm$ 0.07	6.70 $\pm$ 0.07

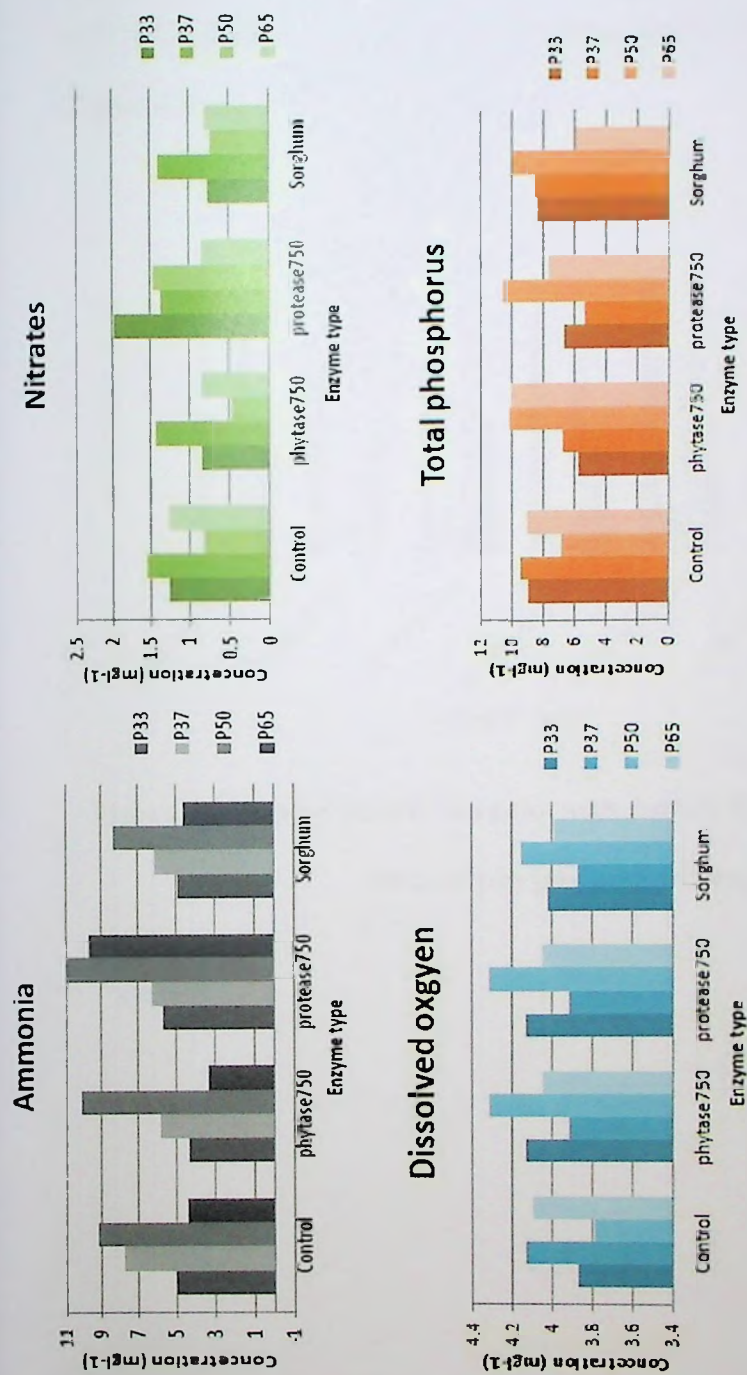


Figure 4.7 Variation of ammonia, orthophosphates, nitrates and dissolved oxygen in water tanks containing catfish fed on diets mixed with sprouted sorghum, phytase and protease enzymes.

Considering phytase and protease inclusion level, ammonia significantly differed ($p < 0.001$) among diets while nitrates, orthophosphates, dissolved oxygen and pH did not ($p > 0.05$) i.e. $p = 0.173$; $p = 0.479$; $p = 0.398$; $p = 0.87$ respectively. Ammonia was highest in 50% CP diets incorporated with protease and lowest in 65% CP with phytase enzyme (Figure 4.8 and 4.9).

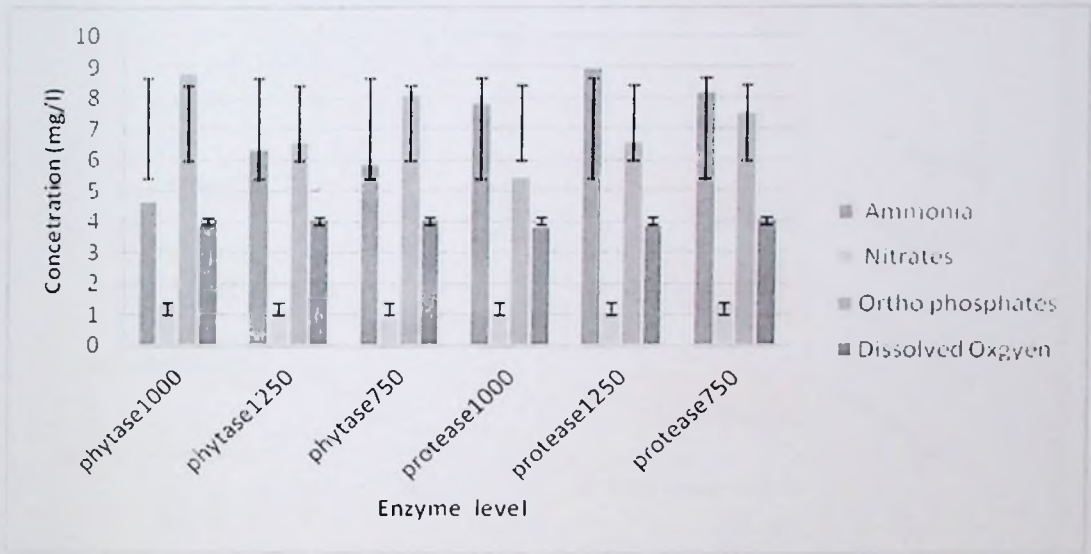
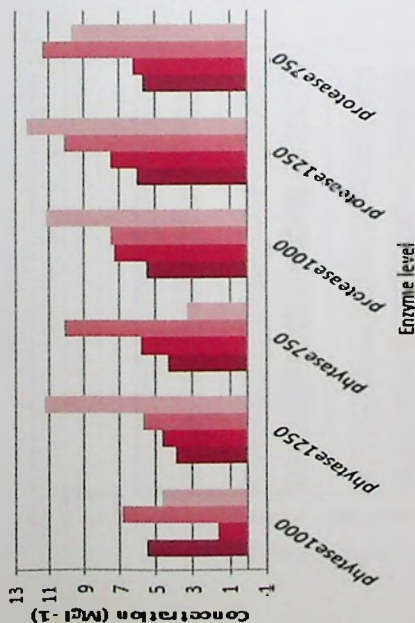
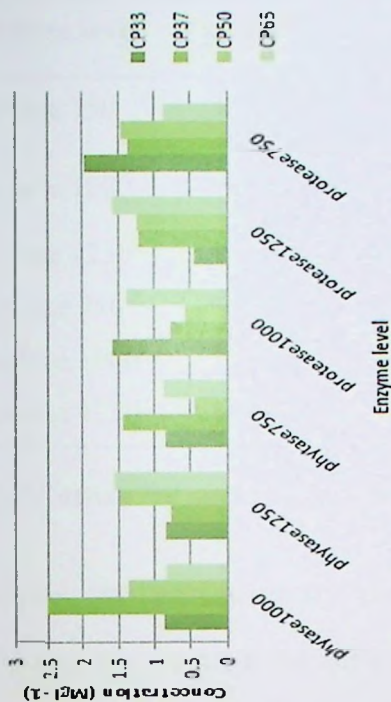


Figure 4.8 Water quality in tanks with catfish fed on diets mixed with different units of phytase and protease enzymes

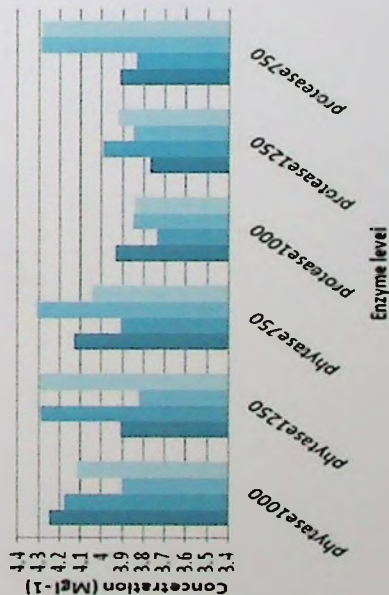
Ammonia



Nitrates



Dissolved oxygen



Total phosphates

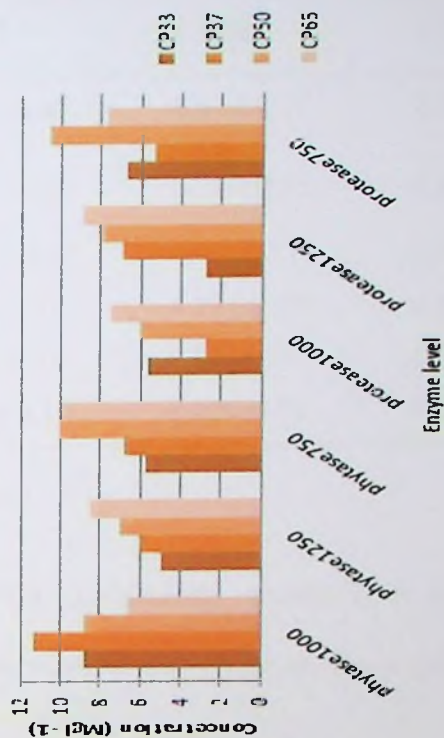


Figure 4.9 Water quality parameters recorded in phytase and protease enzyme level treatments

Table 4.5 The pH of the water in tanks with catfish fed on 750, 1000 and 1250 units of phytase and protease (Mean $\pm$ SE)

Enzyme level	CP33	CP37	CP50	CP65
Phytase 750	6.90 $\pm$ 0.07	6.95 $\pm$ 0.06	6.81 $\pm$ 0.15	6.70 $\pm$ 0.04
Phytase 1000	6.76 $\pm$ 0.10	6.90 $\pm$ 0.29	6.78 $\pm$ 0.15	6.87 $\pm$ 0.21
Phytase 1250	6.97 $\pm$ 0.06	6.91 $\pm$ 0.08	6.95 $\pm$ 0.1	6.84 $\pm$ 0.08
Protease 750	6.98 $\pm$ 0.20	6.72 $\pm$ 0.12	6.73 $\pm$ 0.07	6.82 $\pm$ 0.08
Protease 1000	6.88 $\pm$ 0.08	6.75 $\pm$ 0.16	6.86 $\pm$ 0.1	6.93 $\pm$ 0.07
Protease 1250	6.77 $\pm$ 0.15	6.95 $\pm$ 0.10	7.02 $\pm$ 0.21	6.81 $\pm$ 0.12

4.2.3 Catfish fry survival and weight gain

Survival (%) of catfish fry did not differ significantly among both enzyme types (p=0.151; Figure 4.10) but differed among phytase and protease levels (p=0.43; Figure 1.11).

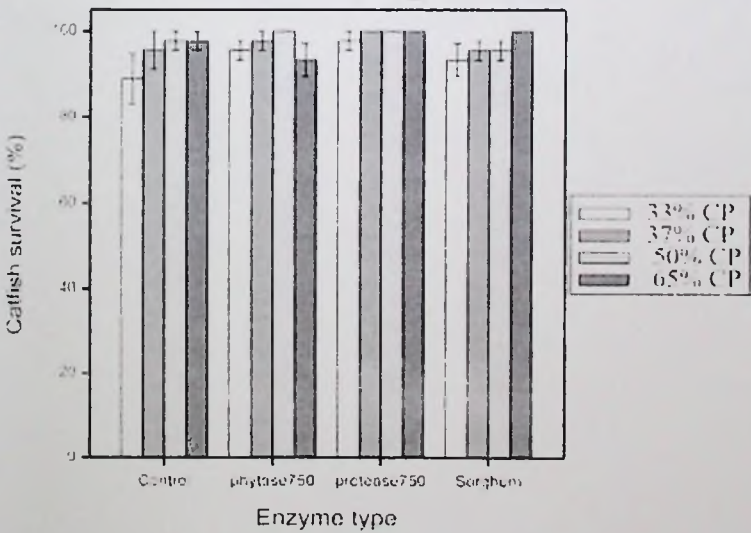


Figure 4.10 Fish survival based on enzyme type

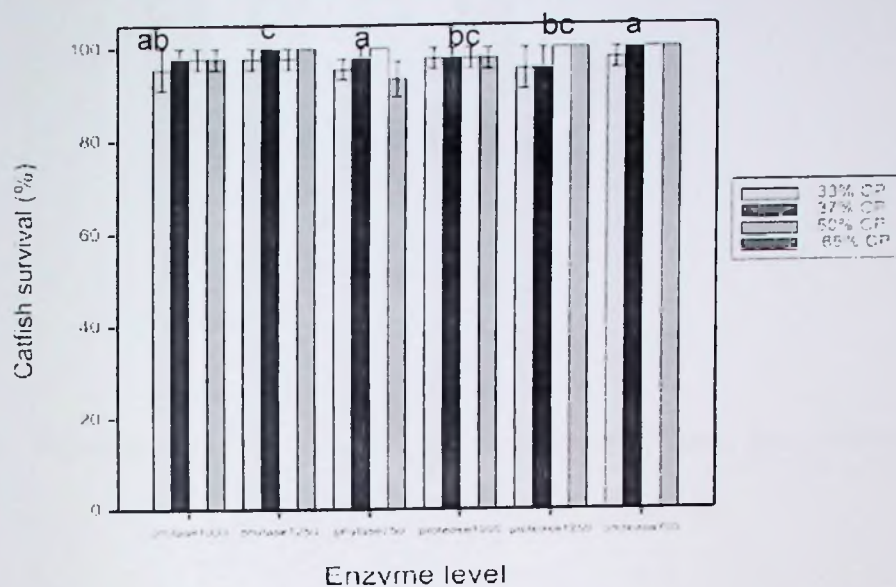


Figure 4.11 Fish survival based on dietary phytase and protease enzyme level.

The final weight (g) of catfish fry did not differ based on both dietary enzyme type ($p = 0.642$, Figure 4.12) and dietary phytase and protease levels ($p = 0.60$; Figure 4.13). Final weight however ranged from 33.12-39.02g.

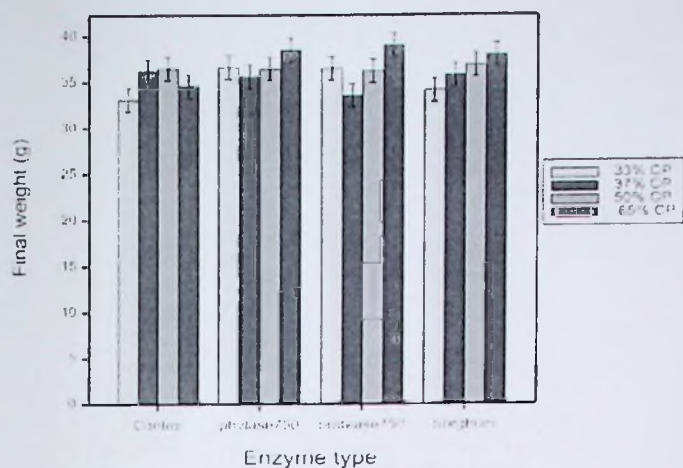


Figure 4.12 Final weight of fish fed on control diets, those with sorghum, phytase 750 and Protease 750.

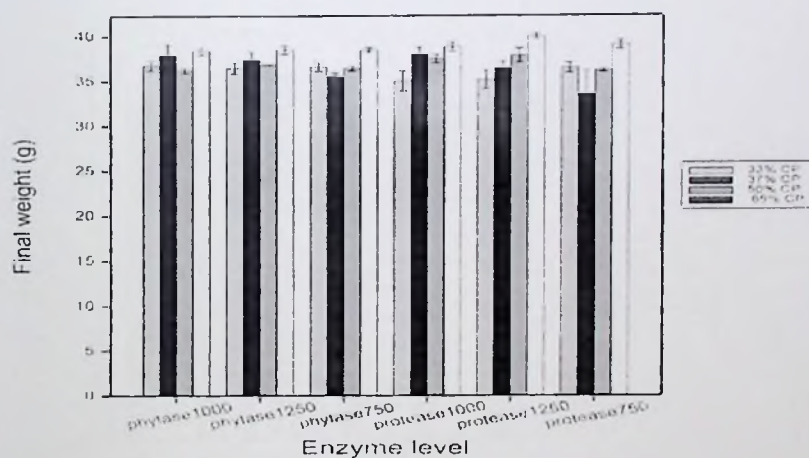


Figure 4.13 Final weight of catfish fed on diets with 750, 1000 and 1250 units of phytase and protease enzymes

The weight gain (g) of catfish fry did not also differ both among enzyme types ($p = 0.758$; Figure 4.14) and among phytase and protease levels ($p = 0.33$; Figure 4.15). The weight gain among catfish fry ranged from 22.96 - 30.02g.

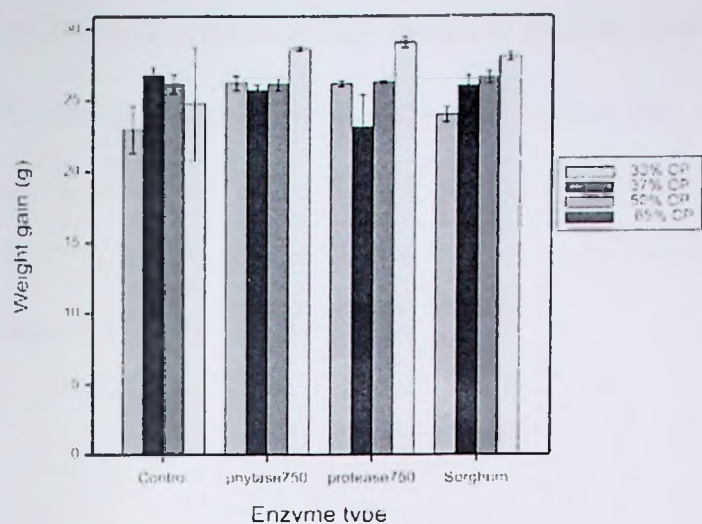


Figure 4.14 Weight gain of catfish fed on diets incorporated with sprouted sorghum, phytase and protease as enzyme sources.

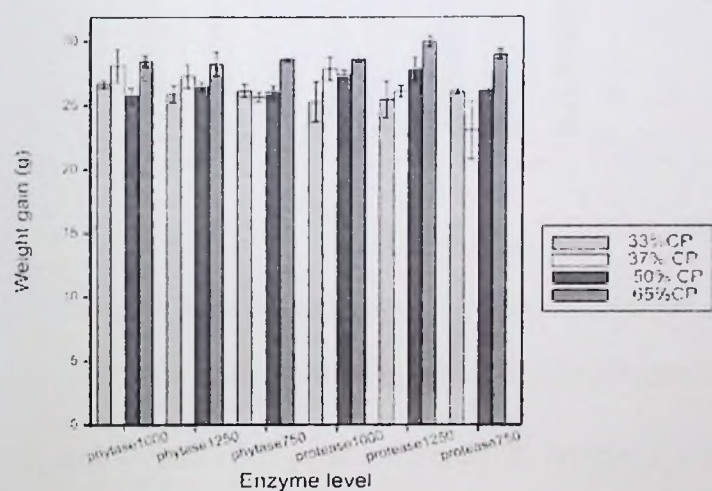


Figure 4.15 Weight gain (g) of catfish fed on diets with 750, 1000 and 1250 units of phytase and protease enzymes

4.2.4 In –vivo apparent digestibility of protein, phosphorus and organic matter

Considering enzyme types, apparent digestibility of phosphorus was significantly increased in catfish fry fed on diets mixed with protease and phytase ($p < 0.001$; 75.65-98.53 %) than in fry fed on diets with sprouted sorghum (81.94-93.42) and the control diet (74.44 - 89.91%) (Figure 4.16) .

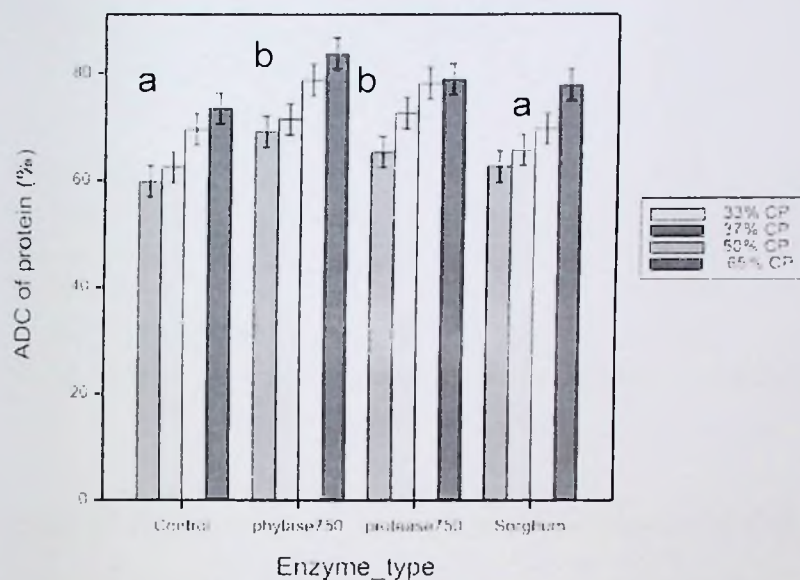


Figure 4.16 Digestibility (%) of crude protein by catfish fry fed on diets incorporated with sorghum, phytase and protease as enzyme sources.

The digestibility of organic matter, crude protein and phosphorus likewise significantly increased with increasing dietary phytase and protease level (Table 4.6 and Table 4.7).

Table 4.6 Nutrient digestibility (means) by African catfish fed on 33%,37%,50% and 65% CP diets incorporated with 750,1000 and 1250 units of phytase enzyme.

Protein level	Phytase level (FTU/kg diet)	Organic matter	Protein	Phosphorus
33% CP	0.0	24.2 ^d	59.8 ^c	74.4 ^c
	750	28.3 ^{cd}	62.5 ^{dc}	80.6 ^{dc}
	1000	34.1 ^c	69.1 ^{cd}	85.3 ^{bcd}
	1250	52.0 ^b	71.5 ^{bc}	89.2 ^{bc}
37% CP	0.0	29.2 ^{cd}	67.4 ^{cdc}	80.4 ^{dc}
	750	50.7 ^b	73.4 ^{bc}	81.5 ^{cdc}
	1000	68.9 ^a	79.4 ^{ab}	92.1 ^{ab}
	1250	67.3 ^a	86.6 ^a	98.4 ^a
Pooled SE		3.54	1.88	1.57
Two-way ANOVA		<i>P</i> value		
Protein level		0.001	0.001	0.001
Phytase level		0.001	0.001	0.001
Protein level x Phytase level		0.001	0.016	0.004

Means having the same letter in the same column are not significantly different at $P < 0.05$.

Table 4.7 Nutrient digestibility by African catfish fed on 65% CP diets incorporated with 750,1000 and 1250 units of protease enzyme (Mean $\pm$ SE)

Protease (unit/kg diet)	ADC protein
Control	63.3 $\pm$ 1.35 c
Protease 750	79.0 $\pm$ 7.82 b
Protease 1000	90.5 $\pm$ 1.75 a
Protease 1250	91.7 $\pm$ 1.42 a

Increasing dietary phytase and protease significantly increased digestibility coefficients of organic matter, crude protein and phosphorus by catfish ($p < 0.001$; $p = 0.49$ and $p < 0.001$) respectively. High protein digestibility was registered by catfish fed on diets with 1000-1250 units/kg⁻¹ protease (90.5-91.7 %; Table 4.7) and catfish fed on diets with 1250 units of phytase (Table 4.6).

Table 4.8 Growth performance of African catfish fingerlings fed on diets containing 750,1000 and 1250 units/kg⁻¹ for 2 months(Mean $\pm$ SE)

Protease (unit/kg)	Final weight (g)	Weight gain (g)	Weight gain %	SGR (%/day)	Feed intake (g /tank)	FCR	Surviv al (%)
Control	33.5 $\pm$ 0.55 ^b	23.4 $\pm$ 0.6 ^b	231.6 $\pm$ 9.7 ^{5^c}	2.00 $\pm$ 0.0 ^{5^b}	30.7 $\pm$ 1.0 ^{7^b}	1.31 $\pm$ 0.04	95.6 $\pm$ 2.2
750	38.8 $\pm$ 0.48 ^a	28.6 $\pm$ 0.2 ^{7^a}	280.4 $\pm$ 5.3 ^{0^b}	2.23 $\pm$ 0.0 ^{3^a}	37.3 $\pm$ 2.6 ^{0^a}	1.30 $\pm$ 0.09	97.8 $\pm$ 2.2
1000	39.0 $\pm$ 0.47 ^a	29.0 $\pm$ 0.3 ^{8^a}	290.0 $\pm$ 4.3 ^{2^{ab}}	2.27 $\pm$ 0.0 ^{4^a}	40.3 $\pm$ 2.3 ^{7^a}	1.39 $\pm$ 0.07	100.0 $\pm$ 0.00
1250	40.0 $\pm$ 0.29 ^a	30.0 $\pm$ 0.3 ^{8^a}	300.0 $\pm$ 6.5 ^{0^a}	2.31 $\pm$ 0.0 ^{47^a}	42.2 $\pm$ 1.4 ^{7^a}	1.41 $\pm$ 0.04	100.0 $\pm$ 0.0

Means having the same letter in the same column are not significantly different at $P < 0.05$.

The initial weight of catfish was 10.0 ± 2.2 g.

The growth indices among catfish fingerlings was significantly higher in those fed on diets with protease than in the control (Table 4.8) but survival rates did not differ ($p > 0.05$).

Table 4.9 Digestibility (mean %) of organic matter, crude protein and phosphorus in diets mixed with sorghum, phytase and protease as enzyme types fed to African catfish

Nutrient	Diet %		Enzyme type		
	CP	Control	phytase750	protease750	Sorghum
Organic matter (%)	33% CP	80.57	77.26	96.48	73.17
	37% CP	88.95	91.17	91.03	43.9
	50% CP	63.01	92.91	55.27	83.8
	65% CP	83.02	56.82	52.73	80.99
Crude protein (%)	33% CP	90.24	90.11	97.28	87.59
	37% CP	93.77	94.77	93.06	66.98
	50% CP	75.07	96.5	71.27	88.38
	65% CP	88.06	87.42	78.38	88.56
Phosphorus (%)	33% CP	74.44	93.25	98.53	84.69
	37% CP	89.24	97.55	96.02	81.94
	50% CP	87.79	98.50	82.32	93.42
	65% CP	89.91	75.65	85.65	86.3

Crude protein deposition significantly differed among both enzyme types ($p = 0.004$) and at different phytase and protease levels ($p < 0.001$) but phosphorus deposition did not in either case ($p = 0.856$) and ($p = 0.763$), respectively. Unlike phosphorus, crude protein deposition was influenced by the interaction of enzyme type and dietary crude protein ($p = 0.007$). It varied from 242.00 - 428.40 gkg^{-1} among enzyme types and increased with increase in phytase and protease units (242.00 - 705.10 gkg^{-1}) being highest in diets with 1250 units of protease. Phosphorus deposition ranged from 1.20 - 1.36 gkg^{-1} among

enzyme types and also increased (5.19-13.32 gkg⁻¹ with increasing phytase activity (Table 4.10).

Table 4.10 Whole body crude protein and phosphorus deposition (mean g/kg⁻¹) among catfish fry fed on diets incorporated with sprouted sorghum, phytase and protease enzymes

	Enzyme type	33% CP	37% CP	50% CP	65% CP
Protein	Control	362.30	402.90	308.90	266.00
	phytase750	395.00	364.50	269.40	296.60
	protease750	378.20	374.00	381.00	428.40
	Sorghum	391.80	356.70	294.50	360.90
Phosphorus	Control	1.38	2.62	2.35	2.72
	phytase750	1.80	1.24	3.16	2.68
	protease750	1.65	1.20	1.73	2.13
	Sorghum	1.52	1.88	1.69	2.20
	Phytase and protease level	33% CP	37% CP	50% CP	65% C
Protein	phytase1000	353.30	434.70	242.20	302.80
	phytase1250	399.30	460.10	271.30	365.70
	phytase750	395.00	364.50	269.40	296.60
	protease1000	607.20	632.30	519.60	442.90
	protease1250	705.10	686.90	599.80	562.10
	protease750	378.20	374.00	381.00	428.40

4.2.5 In-vivo protein and phosphorus retention

Crude protein retention differed along enzyme types (p = 0.004) as well as among phytase and protease levels (p<0.001) but phosphorus retention did not (p = 0.191. and p=0.93). Crude protein retention ranged from 4.12 - 6.48% among enzyme types and from 4.74-10.03% among diets with different phytase and protease levels. It was in all

cases higher in diets incorporated with protease than in those with phytase. Phosphorus retention varied from 1.20 -3.16 % among enzyme types and from 0.69 - 3.91% among phytase and protease levels (Table 4.11).

Table 4.11 Whole body crude protein and phosphorus retention (mean) among catfish fed on diets with sprouted sorghum, phytase and protease (i.e., by enzyme type and among phytase and protease levels)

	Enzyme type	33% CP	37% CP	50% CP	65% CP
Crude protein	Control	4.22	4.21	4.12	4.87
	Phytase750	4.93	5.16	4.92	5.34
	Protease750	4.74	4.21	5.10	6.48
	Sorghum	4.60	4.53	4.51	4.50
Phosphorus	Control	1.38	2.62	2.35	2.72
	Phytase750	1.80	1.24	3.16	2.68
	Protease750	1.65	1.20	1.73	2.13
	Sorghum	1.52	1.88	1.69	2.20
	Enzyme level	33%C	37%CP	50%CP	65%CP
Crude protein	Phytase1000	6.12	10.05	6.99	6.74
	Phytase1250	13.32	8.63	7.55	6.73
	Phytase750	7.86	5.19	8.96	7.99
	Protease1000	8.45	9.52	8.46	10.45
	Protease1250	8.57	6.66	9.43	11.74
	Protease750	6.46	5.65	7.93	12.40
Phosphorus	Phytase1000	1.45	1.09	2.77	3.78
	Phytase1250	1.41	0.69	1.80	2.89
	Phytase750	1.28	1.36	2.32	2.66
	Protease1000	1.44	0.90	2.84	3.91
	Protease1250	1.35	1.29	1.60	3.64
	Protease750	1.60	1.57	1.80	2.29

4.2.6 Discussions of results for digestibility of trial diets by catfish

4.2.6.1 In vitro Protein digestibility

Significantly high protein digestibility in diets incorporated with single phytase and protease under in-vitro condition could have been attributed to minimal antagonistic effects from other native enzymes in the crude catfish gut enzyme extracts, sprouted sorghum and the mixture of protease and phytase as anticipated by Azeke *et al.* (2011). High protein digestibility in 33% and 37% diets with phytase enzyme like in 50 and 65% CP diets was attributed to breakdown of phytic acid by phytase thus rendering the bond protein digestible. These diets were comprised of mainly dicotyledonous seeds in which Chow and Schell (1980) noted that protein is closely linked to phytic acids to form inseparable/indigestible complexes. Incorporation of phytase enzyme therefore broke down phytic acid releasing the bond protein for digestion. The digestibility of protein in diets with phytase and protease enzymes was however higher than 78.08% for fish meal, 76.08% for soy bean meal and 35.86% for rice polish obtained by Ali *et al.* (2009) with gut enzyme extract of Thai koi (*Anabas Testudineus*).

Reduced protein digestibility in diets incorporated with 500 units of phytase and 500 units of protease was attributed to antagonistic reactions of phytase and protease. Reduced efficiency of phytase in presence of protease was also reported by Ravindran (2013) and Sultana *et al.* (2010). Phytase was reported to be degraded by proteases such as pepsin and trypsin-like enzymes in the fish stomach except *Aspergillus niger*, *Escherichia coli* and some *Bacillus* species of which it is not documented whether *Bacillus licheniformis* is among these bacillus bacteria (Kumar *et al.*, 2011). This

implied that combining phytase and protease at equal activity units reduces efficiency than when used independently unless further research is conducted to guide the optimal phytase: protease ratio.

4.2.7 In-vivo apparent digestibility of protein, phosphorus and organic matter

4.2.7.1 Nutrients digestibility by African catfish fry fed on diets containing different protein and phytase levels

Protein and phosphorus were more readily available in 37% CP diet incorporated with 750 phytase units than in other diets. The supplemental phytase resulted in increased ADCs of organic matter and proteins. A similar observation was reported by Debnath *et al.* (2005) in Pangas catfish, *P. pangasius*, Baruah *et al.* (2007) in rohita carp, *L. rohita*, Vandenberg *et al.* (2012) in rainbow trout, and Liu *et al.* (2014 and 2012) in grass carp. In the present study, the supplemental phytase resulted in increased ADCs of organic matter, protein, and phosphorus. A similar observation was determined by Yoo *et al.* (2005) in Korean rockfish and Papatryphon and Soares (2001) in striped bass and Zhu *et al.* (2014). A positive relationship between phytase, phosphorus, energy, crude protein and lipid digestibility was also reported by Akpoilih *et al.* (2016). These results suggest that protein and phosphorous were well utilized by the catfish due to phytase supplementation. This was attributed to hydrolyzing effect of phytin-protein and phytin-phosphorus complexes by phytase (Akpoilih *et al.*, 2016; Cao *et al.*, 2007; Kumar *et al.*, 2011; Liu *et al.*, 2014).

4.2.7.2 Status of water quality parameters

The high ammonia (3.33-11.30 mg l⁻¹) and orthophosphates (5.29 - 11.28 mg l⁻¹) recorded in catfish tanks fed with diets incorporated with protease and phytase could have resulted from accumulation of feed and faecal waste due to poor water replenishment to enable collection of faeces. The ammonia and phosphorus levels were however beyond tolerable levels for catfish (0.2, 2 and 0.3 mg l⁻¹) recommended by Boyd and Tucker (1998) and allowable discharge quality of 10mg l⁻¹ in Uganda by NEMA (1999). This implies that poor water quality could have negatively influenced growth of fish observed.

4.2.7.3 Catfish fry survival and weight gain

The high survival (88.89 to 100%) observed in this study was associated with the ability of the feeds to support catfish growth. Although the ammonia levels were high, its toxicity did not result in significant death of fish. Survival was thought to have been supported by the high and constant dissolved oxygen (3.78 - 4.32 mg l⁻¹) that was provided by artificial aeration (Figure 4.13) and the morelense neutral water pH (6.76 - 7.01) (Table 4.4). A stable pH was attributed to the high carbonate hardness (alkalinity) of the water used. Unlike in pond systems where the soil and some phytoplankton absorb some ammonia and phosphates, such is not expected in glass tanks.

4.2.7.4 Digestibility based on enzyme types

Values for digestibility of organic matter (55.27-96.48%) and protein (71.27-96.50%) were higher in diets with phytase and protease enzymes than in diets with sorghum and the control where it was 43.9 -88.95 for organic matter and 66.98 -93.77% for crude protein (Table 4.6).

This was attributed to more hydrolysis of phytate-protein complexes in the diets by enzymes as opposed to sprouted sorghum. This implied that purified enzyme extracts have better efficiency than the enzyme mixture activated in sorghum during sprouting. Efficiency of phytase and protease mixture might have been negatively affected by antagonistic effects of the two enzymes while that of the crude enzyme cocktail in sorghum could have been reduced by proteases activated in sorghum during sprouting as reported by Azeke *et al.* (2011).

4.2.7.5 Digestibility of organic matter, crude protein and phosphorus based on phytase and protease levels (in diets with 750, 1000 and 1250 Units of phytase and protease enzymes)

The high apparent digestibility of phosphorus in 33% and 37%CP diets with phytase enzyme at 1250 activity units was attributed to up take of liberated bond phosphorus reported to constitute 80-90% of total phosphorus in plant material. On the other hand the high digestibility of crude protein in 50 and 65% CP diets with 1250 units kg^{-1} of protease was ascribed to break down of crude protein into absorbable amino acids. This is in agreement with Yan *et al.* (2012) who observed that benefits of protease were greater in starter phase (high protein) diets than to finisher (low protein) diets.

The apparent digestibility of organic matter and crude protein obtained were however higher than 32.10-35.33% and 70.44-77.92% respectively observed by Gul *et al.* (2007) when 32%CP with and without fish meal respectively were fed to *Labeo rohita*. Apparent digestibility of organic matter was also higher than 66.6 % for fish meal and 57.8% for extruded soybean fed to Nile tilapia by Guimarães *et al.* (2012). This was

attributed to low crude fibre (<4) content in the diet. However, protein digestion in 30 and 37% CP diets could have been also hindered by phytic acids. This implied that incorporation of protease increased protein digestibility at higher protein while phytase enzyme increased phosphorus digestibility at low dietary protein.

4.2.7.6 Protein and phosphorus retention

High protein and phosphorus deposition and retention recorded in diets incorporated with protease and phytase at 1250 activity units was ascribed to better apparent digestibility of these nutrients. The 3.5 fold increase in deposited crude protein than in phosphorus coincided with the high protein digestibility in diets with 1250 protease units. It is in line with FAO (2004) that as long as energy is not limiting, increasing diet protein increases protein deposition. However, retention of only 1.42-1.96% of the deposited protein (242-705.10gkg⁻¹) compared to 13.29-100% out of deposited phosphorus indicated a protein balance.

This implied that the deposited crude protein was metabolized for life maintenance with little contributing to building body tissue. The excessive protein breakdown could have been caused by stress due to poor water especially ammonia and disturbance during faecal collection. High protein breakdown was also reflected in extremely high ammonia levels, a product of protein metabolism in the culture water. Similar excessive protein deamination was observed by Mansoor *et al.* (1996) in patients with head trauma. On the other hand the high phosphorus retention (100%) at 750 phytase and protease inclusion levels than at 1250 units of enzymes (13.29-29.37% was attributed to high absorption

/uptake into the body tissue and metabolic substrates such as enzymes and co-enzymes etc).

4.3 Experiment 3: Effect of varying dietary protease from 750 , 1000 and 1250 units/kg of dry starter diets (65%CP) on growth performance and cost effectiveness of catfish hatchlings.

4.3.1 Growth and survival of catfish hatchlings fed dry diets incorporated with protease enzyme

There was statistically significant difference in survival among hatchlings with the lowest observed for hatchlings fed on the control diet (11.0%) as compared with the other diets (Table 4.12; $P < 0.05$). The highest survival (52.75 %) was obtained with catfish hatchlings fed on *Artemia* nauplii while it ranged from 39.9 – 42.3% for fish fed on diets enriched with dietary protease. Furthermore, larval growth, weight gain, weight gain %, and SGR increased significantly as protease level increased over those fed the control diet ($P < 0.05$; Table 4.12).

Table 4.12 Growth performance of African catfish hatchlings fed on diets containing different protease levels for 30 days (Mean±SE)

Protease (unitkg ⁻¹) 1)	Final fish weight (mg)	Fish weight gain (mg)	Weight gain %	SGR (%g/day)	Fish survival (%)
Artemia	576.0±15.0 9 ^a	547.2±15.09 ^a	15199.14±419.0 6 ^a	16.92±0.09 ^a	52.8±7.8 ^a
Control	43.3±3.33 ^d	14.5±3.33 ^d	402.90±92.59 ^d	8.27±0.25 ^d	11.0±0.8 ^c
750	213.7±24.6 4 ^c	184.8±24.64 ^c	5133.94±684.36 c	13.56±0.40 ^c	39.9±4.6 ^b
1000	224.8±23.1 1 ^c	196.0±23.11 ^c	5442.98±641.89 c	13.74±0.35 ^c	42.3±5.2 ^{ab}
1250	325.0±11.2 3 ^b	296.2±11.23 ^b	8226.98±306.22 b	15.01±0.11 b	41.7±4.7 ^{ab}

* Initial fish weight was 3.6 ± 0.2 mg.
Means having the same letter in the same column are not significantly different at *P* < 0.05.

Catfish hatchlings fed on *Artemia* nauplii showed highest growth followed by those fed on diets enriched with protease 1250 unit/kg diet (Table 4.12). It is noticed that dietary protease has a positive effect on larval growth over that fed the control diet. The relationship between final larval weight and supplemental protease levels (Figure 4.17) was best expressed by the second-order polynomial regression equations as follows:

$$Y = - 30.968 X^2 + 247.17 X - 173.29 \text{ (R}^2 = 0.8309\text{)}$$

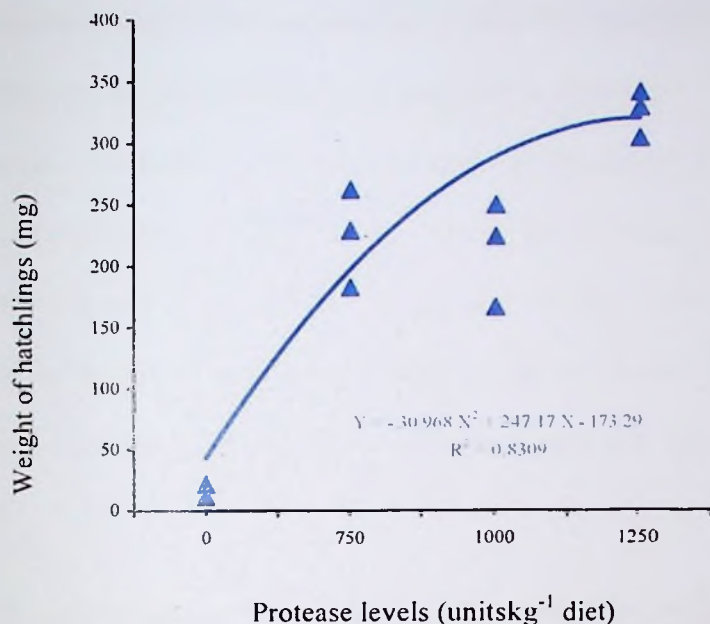


Figure 4.17 The relationship between final weight (mg) of African catfish hatchlings and different levels of dietary protease

From figure 4.17 the optimum dietary inclusion level for protease in starter diets for catfish hatchling with 65CP is 1250 unitkg⁻¹.

4.3.2. Cost effectiveness of using *Artemia* and dry diets

There was statistical significance in total feed cost used with the highest observed in hatchlings fed on *Artemia* and commercial starter crumbles (US\$ 74.76) as compared with the other diets (Table 4.13; $P < 0.05$). The total feed cost did not differ among diets with protease and the control and it ranged from US\$0.46 in for the control diet to US\$0.79 for the diet with 1250 units/kg diet. The incidence of cost (R) also differed among hatchlings fed on the control diet, diets with 1000 and 1250 units of protease kg⁻¹

diet and *Artemia* and diets with 750 units/kg⁻¹ diets ($p < 0.05$; Table 4.13). It was significantly high in the control diet (US\$0.74), intermediate for *Artemia* and diets with 750 units/kg diets (US\$0.04-0.07) and least in diets with 1000 and 1250 units of protease enzymes (~US\$0.001). Furthermore gross profit, profit index and the benefit cost ratio significantly differed ($p < 0.05$). Gross profit was lowest with the control diet, (US\$87.50) and highest for hatchlings fed on diets with 1250 protease units/kg⁻¹ while profit index was highest in for hatchlings fed on diets with protease (US\$0.34-0.41) > control > *Artemia* and the same trend was recorded for the benefit cost ration (Table 4.13).

Table 4.13 Profit indices (means) of fry production using protease incorporated diets (US\$)

Dietary protease	Total feed cost	Incidence of cost	FCR	Gross profit	Profit index	Benefit cost ratio
<i>Artemia</i>	74.76b	0.04079ab	0.37a	258.9	0.01	4.4
Control	0.46 a	0.0742b	61.27b	87.5	0.09	191.2
750	0.62a	0.00108ab	0.89a	318.7	0.36	510.8
1000	0.65a	0.00106a	0.88a	338.1	0.34	521.4
1250	0.79a	0.00067a	0.56a	421.6	0.41	534.4

4.3.3 Discussions on performance of catfish hatchlings fed dry diets with protease enzyme

4.3.3.1 Growth performance of African catfish hatchlings fed on diets containing different protease levels for 30 days

The successful rearing of fish hatchlings using *Artemia* nauplii has also been reported for many fish species (Hansen *et al.*, 2018; Harzevili Alireza *et al.*, 2012; Hogendoorn, 1980; Kerdchuen and Legendre, 1994; Lavens and Sorgeloos, 2000; Leger, 1986). Feeding African catfish hatchlings on live or frozen *Artemia* nauplii singly or in combination with a trout starter diets provided higher faster growth than other diets (Lim *et al.*, 2002 and Watanabe *et al.*, 1983). Similarly, channel catfish, *Clarias batrachus* hatchlings fed *Artemia* nauplii during the first 7 days of rearing displayed the best growth response and survival rates compared to fish fed on other feed resources such as rotifers, cladocerans and fish meal (Knud-Hansen *et al.*, 1990). Olurin *et al.* (2012) found that the growth and survival were highest in fish fed decapsulated *Artemia*, and least in fish fed a commercial starter diet.

4.3.3.2 Optimal protease inclusion

The use of exogenous digestive enzymes including protease has been one of the most widely used strategies to improve feed utilization especially, in the early stages of larval and juvenile fish with poorly developed digestive tract and low digestive enzyme activity (Drew *et al.*, 2005; Kolkovski, 2001; Kolkovski *et al.*, 1993; Liu *et al.*, 2018). Studies have found that dietary protease could improve protein utilization of fish (Li *et al.*, 2015; Liu *et al.*, 2018).

The control diet tested in the present study led to both low survival and growth rates and proved to be poorly utilized by African catfish hatchlings. These results may be related to lack of functional stomach and particularly the absence of digestive enzymes during the first days of exogenous feeding (Cahu and Zambonino Infante, 2001; Verreth *et al.*, 1993). Therefore, protease supplementation improved larval growth and survival. The present results are in agreement with previous results of Hogendoorn (1980) and Verreth and Van Tongeren, (1989) who also found that trout starter diet (65% CP) was not suitable for early feeding of African catfish.

4.3.3.3 Cost effective of using *Artemia* and dry diets

These parameters indicate that profitability of fry production is low with use of *Artemia* and the control diet and is high with use of diets supplemented with protease enzyme.

Low profitability with *Artemia* is attributed to its high cost while that of the control diet is due to poor utilization as indicated by the high FCR (61.27) and high mortalities.

High profitability of diets incorporated with protease enzymes especially 1250 units was attributed to lower cost than that of *Artemia* and higher utilisation than for the control diet.

This implied producing catfish fry using dry diets incorporated with protease enzymes could be more profitable than using *Artemia* nauplii.

4.4 Experiment 4: The effect of varying dietary protein from 33% to 37%CP and phytase enzyme from 750, 1000 to 1250FTUs in grow-out diets on productivity of catfish fry in earthen ponds

4.4.1 Growth Performance of catfish fry in ponds

Final weight (g), weight gain (g), daily weight gain (gday^{-1}) and SGR (% g/day) of the catfish fingerlings were significantly influenced by phytase level ($P > 0.05$) but not by dietary protein level or protein – phytase interaction ($P > 0.05$; Table 4.14).

Similarly, fish production showed the same trend as individual fish growth. Final weight and weight gain were significantly increased by increasing phytase level with their optimum values recorded between 750-1000 FTU kg^{-1} diet irrespective of dietary protein level ($P < 0.05$; Table 4.14).

Table 4.14 Growth performance (means) of African catfish reared in earthen ponds and fed on diets containing different protein and phytase levels for 3 months

Protein level	FTU /kg	Initial weight(g)	Final weight(g)	Weight gain (g)	Daily weight gain(g/day)	SGR (%g/day)	Survival (%)
33% CP	0.0	10.3	125.4 ^c	115.1 ^c	1.3 ^c	2.75 ^c	88.5
	750	10.0	181.8 ^a	171.8 ^a	1.9 ^a	3.21 ^a	87.4
	1000	10.0	174.4 ^{ab}	164.4 ^{ab}	1.8 ^{ab}	3.16 ^{ab}	91.9
	1250	9.9	141.6 ^{bc}	131.7 ^{bc}	1.5 ^{bc}	2.95 ^{bc}	92.0
37% CP	0.0	9.9	123.7 ^c	113.9 ^c	1.3 ^c	2.81 ^c	88.1
	750	10.1	157.0 ^b	147.0 ^b	1.6 ^b	3.05 ^{ab}	87.9
	1000	10.3	167.5 ^{ab}	157.2 ^{ab}	1.8 ^{ab}	3.08 ^{ab}	89.5
	1250	9.9	147.7 ^{bc}	137.9 ^{bc}	1.5 ^{bc}	2.99 ^{bc}	90.0
Pooled SE		0.13	4.88	4.86	0.054	0.034	0.72
Two-way ANOVA		P value					
Protein (CP) level		0.886	0.425	0.424	0.424	0.436	0.568
Phytase level		0.852	0.001	0.001	0.001	0.001	0.423
CP x Phytase level		0.676	0.615	0.602	0.602	0.456	0.242

Means having the same letter in the same column are not significantly different at $P < 0.05$.

The relationship between final weight and supplemental phytase levels (Figure 4.18) in 33% and 37% CP diets was best expressed by the second-order polynomial regression equations as follows:

$$Y = -26.723 X^2 + 127.49 X + 50.442 \text{ (33\% CP)}$$

$$Y = -17.29 X^2 + 91.712 X + 48.76 \text{ (37\% CP)}$$

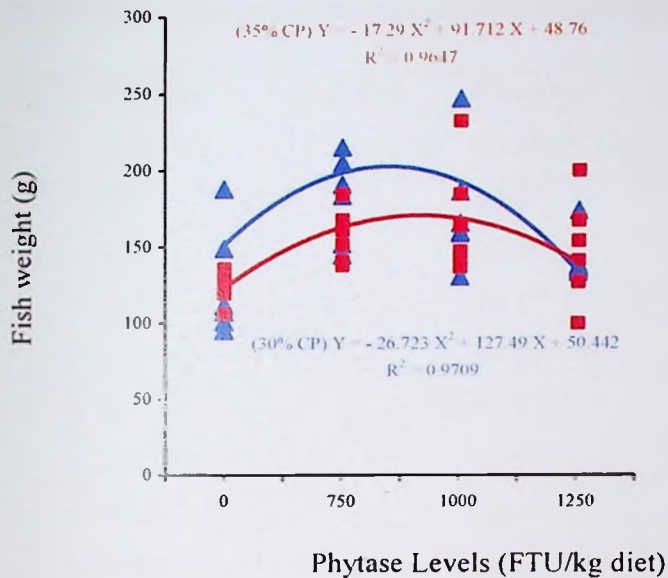


Figure 4.18 The relationship between final weight (g) of African catfish and different levels of dietary phytase at 33 and 37% CP diets

Figure 4.18 shows that the optimum phytase level for African catfish is 850 and 950 FTU/kg diet for 33 and 37% CP diets, respectively.

4.4.2 Feed utilisation

Feed intake was significantly influenced by supplemental phytase and increased significantly ($P < 0.05$) as dietary phytase increased between 750 -1000 FTUkg⁻¹ diet at which both protein levels resulted in similar FCR values, that ranged from 1.07 to 1.17 (Table 4.15).

Table 4.15 Productivity of African catfish (Means) reared in earthen ponds and fed on diets containing different protein and phytase levels for 3 months

Protein level	Phytase (FTU/kg)	Initial fish Biomass (kg)	Final fish Biomass (kg)	Biomass gain (kg)	Total feed intake (kg)	FCR
33% CP	0.0	4.7	51.3 ^b	46.6 ^b	43.5 ^b	1.07
	750	4.6	73.4 ^a	68.8 ^a	58.2 ^{ab}	1.18
	1000	4.6	74.1 ^a	69.5 ^a	59.5 ^a	1.17
	1250	4.5	60.2 ^{ab}	55.7 ^{ab}	48.8 ^b	1.14
37% CP	0.0	4.5	50.3 ^b	45.8 ^b	43.5 ^b	1.05
	750	4.7	63.8 ^{ab}	59.1 ^{ab}	52.9 ^{ab}	1.12
	1000	4.8	69.2 ^a	64.4 ^a	57.3 ^{ab}	1.12
	1250	4.6	51.3 ^b	46.7 ^b	43.6 ^b	1.07
Pooled SE		0.24	3.88	3.67	2.57	0.033
Two-way ANOVA		P value				
Protein (CP) level		0.625	0.125	0.097	0.321	0.469
Phytase level		0.990	0.003	0.002	0.009	0.765
CP x Phytase level		0.792	0.840	0.793	0.915	0.994

Means having the same letter in the same column are not significantly different at $P < 0.05$.

Likewise protein efficiency ratio (PER), protein and phosphorus retention were significantly influenced by protein level and phytase levels; while phosphorus efficiency ratio (PhER) was only affected by dietary phytase only ($P < 0.05$; Table 4.16).

Table 4.16 Nutrients utilization by African catfish (means) reared in earthen ponds and fed on diets containing different protein and phytase levels for 3 months

Protein level	Phytase level (FTU/kg)	Protein efficiency ratio	Protein Retention (%)	Phosphorus efficiency ratio	Phosphorus Retention (%)
33% CP	0.0	0.296 ^b	14.9 ^d	14.1 ^c	53.6 ^b
	750	0.307 ^a	22.0 ^a	14.5 ^a	68.1 ^a
	1000	0.305 ^a	21.6 ^{ab}	14.5 ^a	54.2 ^b
	1250	0.302 ^{ab}	20.0 ^{bc}	14.3 ^{ab}	65.2 ^a
37% CP	0.0	0.259 ^c	18.4 ^c	14.2 ^{bc}	39.8 ^d
	750	0.263 ^c	19.4 ^c	14.4 ^a	43.7 ^{cd}
	1000	0.263 ^c	19.0 ^c	14.4 ^a	34.7 ^d
	1250	0.261 ^c	20.3 ^{bc}	14.3 ^{ab}	49.6 ^{bc}
Pooled SE		0.0031	0.334	0.033	1.978
Two-way ANOVA		P value			
Protein (CP) level		0.001	0.001	0.713	0.001
Phytase level		0.035	0.001	0.001	0.001
CP x Phytase level		0.201	0.001	0.480	0.472

4.4.3 Cost effectiveness of experimental diets

The main indicators of cost effectiveness determined in this study are enlisted in Table 4.17. The amount of total feed used this study, their total cost and incidence cost (US\$) was not influenced by phytase inclusion level ($p = 0.993$, $p = 0.995$ and $p = 0.059$) nor by its interaction with the diet crude protein ($p = 0.434$, $p = 0.205$ and $p = 0.581$). The three (3) parameters were instead influenced by diet crude protein (CP) levels total feed used ($p = 0.017$), total feed cost $p = 0.003$, and for the incidence of cost ($p < 0.001$).

Table 4.17 Indicators of cost effectiveness (means) for phytase incorporated diets determined

Protein level	Phytase level (FTUkg ⁻¹)	Total feed (kg)	Total feed cost	Gross profit	Net profit	Incidence of cost	Profit index	Benefit cost ratio (Ber)
33% CP	0	26.9	18.6	46.6	28	0.93	1.25	3.57
	750	23.2	16.3	74.4	58.1	0.57	2.02	5.76
	1000	32.5	22.9	131	108	0.63	2.22	6.34
	1250	29.9	21.2	87.2	66	0.62	1.77	5.06
37% CP	0	40	31.1	68.2	37.2	1.03	1.19	3.4
	750	42.9	33.9	127	92.6	0.67	1.64	4.68
	1000	37.8	29.9	114	83.6	0.63	1.75	5.01
	1250	39.6	31.4	124	92.5	0.71	1.63	4.64

a-c Means with different letters within columns are not significantly different at P < 0.05

4.4.4 Water Quality Parameters

Water quality parameters in fish ponds did not differ among treatments due to the differences in dietary protein, phytase levels and their interaction (P > 0.05; Table 4.18).

Table 4.18 Changes of water quality parameters (means) in earthen ponds stocked with African catfish and fed on diets containing different protein and phytase levels for 3 months

Protein level	FTU/kg	Temp (°C)	Oxygen (mg/l)	pH	Total phosphorus (mg/l)	Ammonia (mg/l)	Nitrate (mg/l)	Alkalinity (mg/l)
33% CP	0.0	25.8	4.1	7.22	1.17	1.41	2.19	39.5
	750	25.9	3.9	7.19	1.25	1.58	2.16	41.8
	1000	26.5	4.5	7.15	1.34	1.78	2.18	38.6
	1250	26.1	4.1	7.18	1.26	1.44	2.25	42.2
37% CP	0.0	25.9	4.3	7.33	1.32	1.42	2.41	41.3
	750	26.4	4.3	7.12	1.27	1.45	2.29	38.3
	1000	26.1	4.6	7.22	1.56	1.35	2.13	45.7
	1250	26.4	4.6	7.17	1.32	1.25	2.45	42.7
Pooled SE		0.22	0.14	0.09	0.16	0.13	0.38	2.12
Two-way ANOVA		P value						
Protein (CP) level		0.226	0.793	0.887	0.122	0.121	0.221	0.471
Phytase level		0.352	0.816	0.794	0.617	0.327	0.356	0.189
CP x Phytase level		0.327	0.511	0.524	0.926	0.461	0.169	0.422

Also, no significant changes were observed in total phosphorus, unionized ammonia, and nitrate concentrations due to the differences in dietary protein and/or phytase levels.

4.4.5. Discussion of results for catfish performance in ponds

4.4.5.1 Water quality

There were no significant differences in water quality parameters ponds subjected to the various feed treatments. This showed that fish growth was mainly influenced by the feed type and not water quality and that the feed given had no significant effect on water quality. It is important to note that, pH, alkalinity, total phosphorus and nitrogen were within tolerable levels for catfish culture (Boyd and Tucker, 1998). Water temperature (25.8 – 26.5 °C) was also suitable for culture of African catfish (Degani *et al.*, 1889). Alkalinity of 38-45 was sufficient to maintain relatively stable pH due to its buffering capacity as recommended by Boyd and Tucker, (1998). However, ammonia levels of 1.25 - 1.78 were about 20 fold higher than 0.6mg l⁻¹ the maximum tolerable levels recommended by Bhatnagar and Devi, (2013). These high levels were attributed to loading from of fish waste and leftover feed that were constantly given to fish. Fortunately no significant fish mortalities were registered a factor that could be attributed to hardiness of African catfish (Nabulime, 2014). It was anticipated that may be limited absorption due to inadequate primary production and constant bioturbation of sediments that kept all the organic matter in suspension due to bottom dwelling behavior of catfish could have been enhancing factors. The same explanation was attributed to the considerably high phosphorus levels (>0.025mg/l) that would have stimulated algal growth in non-silty ponds. The observed poor correlation between oxygen and ammonia could not however be explained based on the current results. Determining phytoplankton concentration and chlorophyll a could have probably offered better insights.

4.4.5.2 Growth Performance by protein level

It was expected that the performance of African catfish would be improved by increasing dietary protein levels since it is essential for fish growth but it was influenced by phytase inclusion instead. This was contrarily to Abdel-Tawwab *et al.* (2010) and Degani *et al.* (1889) who found that the growth of African catfish (10 – 12 g) increased as protein level increased up to 40%. Similarly, Ali and Jauncey (2005) found that the highest growth rate of African catfish (10.9 ± 0.11 g) was in fish fed 43% CP. The insignificant effect of dietary protein observed in the present study could be because the present study was conducted in earthen ponds where it is possible that fish could graze on other natural food items, while the studies of Degani *et al.* (1889) and Ali and Jauncey (2005) were indoor.

4.4.5.3 Growth performance of catfish fed on 33% and 37% CP diets with three levels of dietary phytase

Results showed that the highest catfish performance and production were observed in fish fed diets containing 33.0 % CP enriched with phytase at levels of 850 FTU/kg⁻¹ diets. These results suggest that supplemental phytase improved protein utilization and high dietary protein content needs high phytase level to optimize fish growth. In a similar study, Baruah *et al.* (2007) investigated the effect of dietary microbial phytase, citric acid, crude protein levels and their interactions on growth performance, nutrient digestibility and body composition of rohita carp, *L. rohita*, juveniles (12.6 - 13.7 g) using plant-based ingredients containing either 25 and 37% CP levels. They found that the growth-promoting effect was higher in fish fed a 25% diet containing both 3% citric acid and 500 FTU/kg diet of microbial phytase than those fed 37% CP diets. They also

suggest that microbial phytase and citric acid in low protein diet had a synergistic effect on nutrient digestibility and growth performance of rohi carp, *L. rohita* juveniles.

In their review of the anti-nutritional factors for fish, Francis *et al* (2001) indicated that fish performance is negatively affected by phytate-containing ingredients in diets. Dietary phytate form phytate – protein complexes reducing the availability of dietary protein and amino acids (Liu *et al.*, 1998; Sugiura *et al.*, 2001). The phytate - protein complexes are insoluble and consequently less digestible by proteolytic enzymes (Ravindran *et al.*, 1995). Therefore, supplemental phytase may have protein-sparing effects in fish diets by releasing phytate-bound protein and improving its bioavailability.

Irrespective of dietary protein levels, fish performance and production increased significantly with increasing phytase showing optimum performance at 850 and 950 FTUkg⁻¹ diet for 33 and 37% CP respectively. The increase in catfish growth herein could partially be attributed to the increased bioavailability of phosphorus and other nutrients un bound by phytase. These results are concomitant with findings of Liebert and Portz.L., (2005) in Nile tilapia. Debnath *et al.* (2005) found maximum weight gain and SGR in Pangas catfish, *Pangasius pangasius* (2.0 - 2.1 g) fed on diets containing 500FTUkg⁻¹ of diet, while Goda (2007) reported optimum growth of Nile tilapia fed on soybean-based diets with 1000 FTUkg⁻¹. According to Hussain *et al.*(2011) the phytase supplementation increased on growth and mineral digestibility of rohi carp, *L. rohita* fingerlings fed corn gluten-based or cottonseed meal based diets with diet 750 FTUkg⁻¹ diet phytase. Supplementation of phytase in diets improved the growth of grass carp (Liu

et al., 2014). Dietary phytase (500Units) in roasted groundnut meal also increased final body weight and specific growth rate of yellow catfish, *Pelteobagrus fulvidraco* (Zhu *et al.*, 2014) and in 378.6 g African catfish *C. gariepinus*. (Akpoilih *et al.*, 2016). It is therefore worth noting that phytase level that enhances fish growth has been inconsistent and sometimes contradicting. This may be attributed to differences in fish species and size, phytase source, diet formulation, and rearing conditions among other factors.

4.4.5.4 Nutrients utilization by African catfish reared in earthen ponds and fed on diets containing different protein and phytase levels for 3 months

The significant high protein efficiency ratio (PER), protein and phosphorus retention influenced by both dietary protein and phytase level and phosphorus efficiency ratio (PhER) only affected by dietary phytase shows that phytase mainly influenced bioavailability of phosphorus.

It implied that nutrients in 33% CP plant-based diets incorporated with 850 FTUkg⁻¹diet are better utilized by catfish than those in 37% CP diets. However, the PER value in the present study, decreased with increasing dietary protein from 33% to 37% CP. This suggests that more protein was broken down (deamination), as its dietary proportion in the diet increased. A similar result was obtained by Abdel-Tawwab *et al.* (2010) who found that lowest PER was obtained with the 45%-CP diet; whereas, its highest value was obtained with the 25%-CP diet. Reduced PER was also reported by Baruah *et al.* (2007) as dietary protein was increased from 25% to 37% CP diets for rohita carp, *L. rohita*. They also suggested that phytase supplementation in the plant-based diets at the 25% CP level is as effective as a 37% CP containing diet. The obtained results suggest

that supplemental phytase improves the nutritional quality of plant-based diets through breakdown of phytin–protein complexes (Kornegay and Qian, 1996) and neutralizes the negative effect of phytate on protein and other nutrients in fish diets (Mitchell *et al.*, 1997). Significant enhancement in PER has been reported for Nile tilapia (Cao *et al.*, 2007) rainbow trout (Wang *et al.*, 2009), and grass carp (Liu *et al.*, 2014) due to phytase supplementation. Similar studies on channel catfish and striped bass demonstrated that supplemental phytase significantly influences the protein utilization of experimental fish (Cain and Garling, 1995; Papatryphon and Soares, 2001). Additionally, dietary phytase liberates the complexed phytin-minerals in plant feedstuffs, thereby improving the phosphorus utilization in different species of fish at different levels (Cheng and Hardy, 2002).

4.4.5.5 Cost effectiveness of experimental diets

The cost effectiveness of using phytase incorporated diets was not influenced by phytase but rather dietary crude protein. This was attributed to the differences quantities of protein ingredients used in manufacture of 33 and 37% CP diets especially high costs associated with fish meal and soybean meal. The amount of phytase included was similar for both 33 and 37% CP diets and therefore could not influence costs and nor its interactions with crude protein. This is in agreement with the general norm that protein is the most expensive nutrient and major determinant of feed costs. This implies that effective utilisation of the two diet CP levels will eventually determine profitability.

The gross profit did not significantly differ among diets by dietary CP levels, phytase level or their interaction ($p > 0.05$). The profit index, (the cost of producing one head of catfish) and the benefit cost ratio (Bcr) were on the other hand significantly influenced by the phytase inclusion level and its interaction with diet CP. ($p < 0.001$) and not by diet crude protein ($p > 0.076$). This was attributed to the influence of phytase enzyme levels on nutrient utilisation particularly on the Food conversion ratio (FCR). Food conversion ratio (FCR) was significantly low ($P < 0.001$) in 33% CP diets incorporated with 1000 FTUkg⁻¹ (0.712-0.801). This implied that FCR is instrumental in determining profitability of using a given feed as pointed out by (FISH, 2009). Reduction in FCR was also observed by Hassan (2009), when soybean diets incorporated with 1000 FTU kg⁻¹ were fed to Juvenile Milkfish, *Chanos chanos*. Although, the FCR in diets incorporated with phytase (0.76) and the control diet (1.33) significantly differed, it was still within the economic threshold of 1.80-2.00 for viable pond fish farming in Uganda recommended by the FISH (2009).

Contrally, the gross profit was not influenced by either diet crude protein nor by phytase inclusion level or their interactions this was attributed to poor integration of details in its determination. It implies that it is not a good tool for evaluating of profitability of feeds thus consideration of other indicators is required. Meanwhile the influence of crude protein on incidence of cost implied that feed costs were more determined by crude protein and not phytase enzyme. However its range of 0.63-1.03 was less than 17.6 and 23.7 recorded by (Ansa *et al.*, 2013) when tilapia was fed on dry local and imported pelleted diets.

The profit index and benefit cost ratio (Bcr) were significantly higher in 33%CP diets incorporated with 1000 and 750 FTUkg⁻¹ ($P < 0.001$) than in other diets. This was attributed to better utilisation of these diets for growth (weight gain) by fish as indicated by the low FCR and incidence of cost (R) of these diets. These implied that feeding grow out catfish in ponds using 33% CP diets incorporated with 750 and 1000 FTUkg⁻¹ was more cost effective than using 37% diets .

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1. Background to conclusions

The fish feed industry in Uganda is still under developed and most quality diets are imported (Hecht, 2013). Culturing catfish is undertaken in intensive systems that mainly depend on agro by products as inputs (Hyuha *et al.*, 2011). Increasing fish demand and use of commercial feed is getting important to enable intensification and profitability but constrained by high prices. This has necessitated low-cost formulations using under-utilised local inputs such as agro-byproducts (Ayadi *et al.*, 2012). However, these naturally contain antinutrients including phytic acid which bind nutrients reducing their utilisation since catfish lack appropriate digestive enzymes to break them down (Kumar *et al.*, 2011). Phytic acid is found in almost all potential plant feed ingredients thus requiring strategic interventions to reduce its effects. Supplementing fish diets with phytase has shown inconsistent and sometimes contradicting results. This study thus evaluated effects of roasting, phytase, protease and sprouted sorghum as enzyme supplements on phytic acid and or its effects including digestibility and utilisation of dietary organic matter, phosphorus and crude protein on growth and cost-effectiveness of catfish production.

5.1.1 Recap of major findings of the study

The major findings of this study were as follows:

- 1) Roasting diet ingredients at 90-110°C statistically reduced phytic acid content in plant ingredients but not to levels of economic and nutritional significance for fish growth. Roasting however no effect on dietary crude protein and pH of resultant compound diets.
- 2) Supplementing diets with phytase and protease enzymes independently significantly increased digestibility of organic matter, phosphorus and protein while mixing equal activity units each of phytase and protease as well as sprouted sorghum did not.
- 3) Incorporating 1250 Units/kg⁻¹ of protease enzymes into dry diets with about 65% CP enhances their use as alternative starter diets for African catfish hatchlings to the brine shrimp *Artemia* cysts.
- 4) Increasing dietary Protein from 33% to 37% and phytase from 750 to 1250 units significantly reduces growth performance of catfish fry in earthen ponds⁻¹.

Conclusions:

Roasting feed ingredients significantly reduced dietary phytic acid and had no effect on crude protein and pH. However, the reduction in phytic acid content (0.27%) is too small to be considered of nutritive significance. This implies that in addition to roasting, evaluation of other pre-treatments that would further reduce phytic acid such as incorporation of enzyme supplements especially those that are heat stable and can enhance digestibility of protein are required. Exploration of locally available enzyme

sources such as sprouted grain was recommended to reduce feed costs. This is explored in the Chapter 5 to provide proper flow or sign posting.

In-vitro digestibility results got in this study were for most part in agreement with the in-vivo digestibility. They showed that phytase and protease enzymes significantly increased digestibility of diets than their mixture in equal enzymatic activity and sprouted sorghum. In particular, incorporating 1250 units of phytase and protease enzymes gave the most rewarding digestibility. We therefore recommend use of purified enzymes over sprouted sorghum until its phytase and protease activity essays and feasibility are determined.

5.1.2 Specific conclusions

5.2 Effects of roasting on phytic acid, crude protein and pH

Roasting feed ingredients significantly reduced dietary phytic acid and had no effect on crude protein and pH. However, the reduction in phytic acid content (4%) is considered of less nutritive significance. This implies that in addition to roasting, additional pre-treatments that can further reduce phytic acid such as incorporation of enzyme supplements especially those that remain viable at high temperatures during pelleting of fish diets was required. Exploration of additional local enzymatic pre-treatments such as sprouting/germinating grain ingredients was recommended to reduce feed costs. This is explored in the Chapter 5 to provide proper flow or sign posting.

5.2.2 Nutrient digestibility by catfish

5.3 In vitro digestibility

In-vitro digestibility results got in this study were for most part in agreement with the in-vivo digestibility. They showed that phytase and protease enzymes significantly increased digestibility of diets than their mixture in equal enzymatic activity and sprouted sorghum. In particular, incorporating 1250 units of phytase and protease enzymes gave the most rewarding digestibility. We therefore recommend use of purified enzymes over sprouted sorghum until its phytase and protease activity essays and feasibility are determined.

5.3.1 In-vivo digestibility

5.4 Effect of varying dietary protease from 750 , 1000 and 1250 units/kg of dry starter diets (65%CP) on growth performance and cost effectiveness of catfish hatchlings

Increasing dietary protease in dry diets improves growth of hatchlings and profitability of catfish fry production. In absence of protease, there is poor utilisation of dry diets which limits survival and growth of African catfish hatchlings. Varying dietary protease from 750 to 1250 units in dry starter diets significantly increases growth performance and cost effectiveness of catfish hatchlings.

5.5 The effect of varying dietary protein from 33% to 37%CP and phytase enzyme from 750 ,1000 to 1250FTUs in grow-out diets on productivity of catfish fry in earthen ponds

The study showed that feeding pond catfish with 33% CP diets incorporated with 750-1000 FTUkg⁻¹ phytase promotes faster weight gain and is more cost effective than using the 37 % CP diets. This was attributed to increased nutrient utilisation by catfish and enhanced phosphorus content. From the study we recommend adoption of feeding practices that utilize plant-based diets incorporated with phytase to boost profitable cat fish production. In particular, plant based diets with 33% CP incorporated with 850FTUs be fed to pond catfish to maximize growth and profitability.

Further research is however still required to evaluate viability of locally available phytase enzymes in diets manufactured under high temperatures such as 190-200 used during extrusion.

5.6 Implications of results

5.6.1 Roasting diet ingredients statistically reduces phytic acid content but had no effect on crude protein and pH of resultant diets

Compared to fish meal, plant ingredients commonly used to make fish feeds contain anti-nutritional factors of which phytic acid is considered the most detrimental. It binds with phosphorus, lipids and protein molecules reducing their bio-availability, rendering them nutrient poor. Pre-treatments of phytic acid are therefore important to break the bonds and render the nutrients readily available for fish nourishment. Roasting is a traditional

food pre-treatment in Uganda employed to destroy toxic metabolites thence enhancing aroma and palatability in many food stuffs. Roasting ingredients statistically reduced phytic acid content in plant – based diets (by 3.74% i.e. from 5.89-5.67 %.), had no significant effect on dietary crude protein (35.22-35.27%) and pH (6.83-6.87). The phytic acid that remained (5.67) is however still of nutritional significance, because even $0.5\mu\text{molg}^{-1}$ was reported to reduce solubility of mineral elements in fish diets. The phytic acid (%) that remained increased by 64% (from 5.87- 9.68%) as the ingested diet moved along the gut with a corresponding reduction in absorption of crude protein of 47.72% (from 35.27 to 18.44%). This implied that catfish lack appropriate endogenous enzymes to breakdown phytic acid and roasting is not sufficient to destroy phytic acid and thus alleviating its effects. It was therefore suggested that additional pre-treatments such as supplementation with dietary enzymes be explored to maximize nutrient utilisation. The insignificant effect of roasting on dietary crude protein (35.22-35.27% and pH (6.83-6.87) on the other hand indicates that the roasting temperatures used conserved nutrient quality in fish diets.

5.6.2 Supplementing diets with phytase and protease enzymes independently, significantly increases digestibility of organic matter, phosphorus and protein while incorporating a combination of 500 units of phytase and 500 units of protease as well as sprouted sorghum does not.

Increasing prices of fish feed have encouraged new approaches in formulation including introduction and increased use of feed additives, such as enzymes. Adoption of purified enzymes in animal feed manufacturing to increase nutrient bioavailability is important for increased economic and environmental benefits. Improvement of feed digestibility by

phytase and protease enzymes will enable use of low cost feed resources such as terrestrial plant material to nourish fish. This will reduce fishing pressure on the wild fisheries that are over exploited while searching for small fish species used to make quality fish feed. It will also enhance environmental health through reduced nutrient enrichment through phosphorus and nitrogen which are one of the global challenges.

5.6.2.1 Enzymes and nutrient digestibility

Incorporating 1250 units/kg⁻¹ of protease enzymes into 65% CP dry diets enhanced their utilisation by African catfish hatchlings as alternative starter diets to the brine shrimp *Artemia* cysts.

Phosphorus digestibility was significantly increased in diets incorporated with protease and phytase than in the control, sorghum and the mixture of phytase and protease while organic matter and protein digestibility did not. However, increasing phytase and protease to 1000 and 1250 units increased digestibility of phosphorus, protein and organic matter. This shows that digestibility of diets with single phytase and protease enzymes are superior to that of diets with their mixtures and sprouted sorghum. It also indicates that the beneficial effects of supplemental phytase are lost in presence of phytase, thus presenting a caution on use of brands of multiple enzymes as cheaper options.

The two peaks of protein digestibility (76.43 - 85.16%) in 30 and 37% CP diets and (54.44 - 59.86%) in 65% CP diets incorporated with protease enzyme, implied that phytate-protein moieties in plant-based diets are more responsive to phytase than

protease. Likewise there was high phosphorus digestibility (92.66%) in 33% and 37% CP diets incorporated with 1250 units/kg⁻¹ of (81.49%) in 50 and 65% CP diets with 1250 units of phytase and protease/kg diet. This suggests that enzyme effectiveness was diet dependent. The potential of protease was more pronounced when used as micro additive for starter diets used for early developmental stage as oppose to grow-out or 'fattening' diets and vice versa was for phytase.

5.6.2.2 Effect of dietary enzymes on nutrient loading and retention

Farmed fish is supposed to supply all the essential nutrients as contained in wild caught fish if aquaculture is to fulfill the nutritional needs of society. Also environmental pollution with nitrogen and phosphorus are still major challenges for the aquaculture industry that need to be minimized. Supplementing diets with phytase and protease significantly increases phosphorus deposition and retention while only protein deposition and not retention was increased. This implied that incorporation of phytase is instrumental in reducing phosphorus water pollution while application of protease would reduce nitrogen loading.

5.7 Varying dietary protease from 750 to 1250 units in dry starter diets significantly increases growth performance and cost effectiveness of catfish hatchlings

The survival and growth performance of catfish hatchlings fed on 65% CP diets incorporated with 1250 units of protease as first larval diets became second to that of hatchlings fed on *Artemia*. This indicates the potential of protease enzymes to enhance the use of locally developed dry diets in commercial catfish fry production. This will enhance availability of catfish seed required to support the growing fish farming industry

amidst climate change where fast growing and drought tolerant species of fish are mostly required.

The higher cost of production (~62 fold) of producing catfish fry using *Artemia* as live feed compared to that of using dry diets incorporated with protease implied reduced profitability of fry production associated with its use. Dry diets incorporated with protease enzymes provide a cheaper alternative to the brine shrimp *Artemia* and could enhance rural youth employment since fry production is a clean job compared to cultivation which most youth do not want to be associated with.

5.8 Incorporating 850 units of phytase per kilogram of plant –based diets with about 33% CP promotes weight gain faster and is more cost effective than incorporating phytase in 37 % CP diets.

Faster weight gain recorded in fingerlings fed with 33% CP diets incorporated with 850 FTUkg⁻¹ than in fingerlings fed with 37% CP diets indicates that it is possible to increase use of plant material to produce high value catfish protein. The high benefit- cost ratio showed that diets compounded from roasted ingredients supplemented with enzymes significantly increase cost effectiveness of fish production. To small-scale fish farmers, who fish is both a source of income and nutrients, improved production efficiencies due to dietary enzyme supplements in locally made diets improves fish production, livelihoods and food security as desired by the United Nations Sustainable Development goals (2015).

5.9 General conclusions

Roasting of major feed ingredients and supplementation with 850 phytase units/kg in grow-out diets (33% CP) and 1250 protease units/kg in dry starter diets (about 65% CP) enhances growth of African catfish due to improved efficiency and retention of protein and phosphorus.

5.10 Recommendations from the study

- For catfish diets, major ingredients need to be roasted and incorporated with phytase for grow out and protease for starter diets.
- The governments should promote use of plant-based fish diets since enabling technology based on enzyme extracts is available to spare fish turned into fish meal for direct human consumption.
- Parallel support to investment in production and analysis infrastructure for dietary enzymes in Africa is however required to enable continuous improvement of low quality feed resources for fish production.
- Further research be conducted on enzymatic assays in sprouted sorghum and catfish gut extract, the tolerable levels of phytic acid for catfish and effect of different treatments on the dietary content of phytic acid. to guide appropriate

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APPENDICES

Appendix I. Manuscript abstract: "Effect of toasting on phytic acid, protein and pH in a plant-based diet and its ingesta in fed catfish".

Citation: Kemigabo C., Kang'ombe J., Jere W.L., Sikawa D., Dhikusooka T.M., Masembe C. (2017). Effect of toasting on phytic acid, protein and pH in a plant-based diet and its ingesta in fed catfish. *African Journal of Rural Development* 2:77-28.

Abstract

Feed account for up to 70% of production costs in aquaculture thus reducing this cost is very relevant to rural development. Plant ingredients commonly used in feed contain phytic acid that forms indigestible complexes with nutrients reducing their use for fish growth. Toasting feed material is widely practiced to reduce antinutrients but its effect on phytic acid remains unclear. Phytic acid, crude protein (CP) and pH in plant-based diets from untoasted and toasted ingredients and their ingesta along the gut were analysed using complex-metric titration, Kjeldahl method and micro pH meter respectively. Phytic acid in the diet from untoasted and toasted ingredients differed ($p \leq 0.002$) while CP and pH did not ($p \geq 0.06$ and $p \leq 0.40$ respectively). The content of phytic acid in the diet double along the gut CP halved and pH was 5.30 - 7.78. Diet from untoasted ingredients was not eaten thus no ingesta was analysed. Results showed that toasting plant ingredients had no effect on phytic, CP and pH acid implying that the future of replacing animal protein with plant protein will depend on proper pre-treatment of phytic acid.

Key words: *Toasting; Phytic acid; Protein; pH. Plant diets; catfish*

Appendix II. Manuscript abstract: "Effects of protease enzyme supplementation on protein digestibility of legume and/or fish meal-based fish feeds".

Citation: Kemigabo C., Kang'ombe J., Masembe C., Jere L.W., Sikawa D. (2017). Effects of protease enzyme supplementation on protein digestibility of legume and/or fish meal-based fish feeds. *International Journal of Fisheries and Aquaculture* 9:73-80.

DOI: 10.5897/IJFA2017.0620 <http://www.academicjournals.org/IJFA>,

Abstract

Improving protein digestibility in nutrient poor fish feeds through incorporation of dietary enzymes is expected to be achieved with protease. Understanding the role of other dietary enzymes was therefore evaluated to guide appropriate use for optimal fish growth. Protein digestibility of 30, 35, 50 and 65% crude protein (CP) diets was determined with catfish gut enzyme extract, sprouted sorghum, protease and phytase both singly and in a mixture of 500 units of protease and phytase using the pH drop method in vitro. Significant ($p < 0.05$) digestibilities were recorded in 30 and 37% CP diets incorporated with phytase and in 50 and 65% CP diets incorporated with protease singly. These results showed that protein digestibility was more efficient with protease enzyme in high protein diets while phytase was efficient in low protein diets. This implied that the use of protease was more beneficial in catfish starter feeds and phytase in grower/finisher diets and provided a basis for enzyme selection for production of cost-effective catfish diets.

Key words: *In-vitro protein digestion, catfish gut enzyme extract, phytase, protease, sprouted sorghum.*

Appendix III. Manuscript abstract: "Apparent digestibility and utilisation of protein and phosphorus in diets of incorporated with sprouted sorghum, phytase and protease enzymes for African catfish (*Clarias gariepinus*)".

Citation: Kemigabo C., Jere .L.W, Sikawa D., Kang'ombe J., Masembe C. (2017) Apparent Digestibility and Utilization of Protein and Phosphorus in diets of incorporated with Sprouted Sorghum, Phytase and Protease Enzymes for African Catfish (*Clarias gariepinus*). Internal Journal of Aquaculture and Fisheries Science 3:77-87. DOI: 10.17352/2455-8400.000033. Published by <https://www.peertechz.com>

Abstract

Apparent digestibility, deposition and retention of crude protein and phosphorus were determined for 33%, 37%, 50% and 65% CP diets incorporated with sprouted sorghum, 750, 1000 and 1250 units/kg-1 phytase and protease enzymes, fed to African catfish fingerlings. All digestibility coefficients significantly differed ($p < 0.05$) among diets with the (92.66%) observed for phosphorus in 33% and 37% CP diets with 1250 units of phytase, while that of protein (81.49%) was in 50% CP and 65% CP diets with 1250 units of protease. Crude protein deposition and retention significantly differed ($p < 0.05$) with the highest (705.10 and 10.03 respectively) recorded in diets with 1250 protease while that of phosphorus did not in both cases ($p > 0.05$). This demonstrated that protease was more useful in starter (high protein) diets and phytase enzyme in grower (low protein) diets and forms a basis for efficient use of phytase and protease enzymes in catfish diet formulations for different growth stages.

Key words: Apparent digestibility, Sprouted sorghum; phytase; protease; African catfish

Appendix IV. Manuscript abstract “Combined effect of dietary protein and phytase levels on growth performance, feed utilization, and nutrients digestibility of African catfish, *Clarias gariepinus* (B.) reared in earthen ponds”

Citation: Kemigabo, C., Abdel-Tawwab,M., Jere,W.L., Sikawa,D., Masembe,C.and Kang’ombe, J. (2018). Combined effect of dietary protein and phytase levels on growth performance, feed utilization, and nutrients digestibility of African catfish, *Clarias gariepinus* (B.), reared in earthen ponds. Journal of Applied Aquaculture, 30:(3)211-226. DOI:10.1080/10454438.2018.1439425

Abstract

This study was based on a 2×4 factorial design with two levels of dietary protein (33 and 37%) and four phytase levels (0.0, 750, 1000, and 1250 FTU/kg diet). African catfish, *Clarias gariepinus* (B.), (10.7 ± 0.4 g) was distributed into earthen ponds (100 m² surface area per each) at a density of 4.5 fish per m². Fish were fed on one of the experimental diets up to satiation twice daily for 3 months. Water quality parameters in fish ponds did not differ significantly due to the difference in dietary protein, phytase levels, and their interaction. It is noticed that incorporating phytase in plant – based diets increased significantly the growth and production of African catfish reared in earthen ponds but dietary protein did not. Feed intake, was significantly affected by supplemental phytase only where it increased significantly ($P < 0.05$) as dietary phytase increased at both protein levels resulting in similar FCR values. Additionally, protein efficiency ratio, protein and phosphorus retention were significantly affected by protein level and phytase levels; while phosphorus efficiency ratio was affected by dietary phytase only. It is also noticed that organic matter, protein, and phosphorus digestibility coefficients were

significantly affected by dietary protein and phytase levels but not their interaction where they were more digestible in fish fed 37% CP enriched with 750 FTU/kg diet phytase. These results suggest that supplemental phytase improved the protein utilization and high dietary protein content needs high phytase level to optimize fish growth. The optimum performance and production of African catfish were observed when fish fed diets containing 33.0 % CP enriched with phytase at levels of 850 FTU/kg diet.

Keywords: *African catfish; Phytase enzyme; Nutrient Utilization; Digestibility; Profitability; Earthen Ponds.*