

**Identifying conditions for phytase enzyme adaption in African catfish feeds**

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**Abstract**

This research was conducted to establish and streamline conditions and optimum amount of phytase enzyme required for its adoption for improved digestibility of plant based catfish feeds. This started with analysis of pH along African catfish (*Claria gariepinus*) gastrointestinal tract (GIT), feed pH, phytic acid content and proximate nutrient composition. Results indicated that phytase enzyme from the fungus *Aspergillus niger* could be appropriate for adoption. A feeding experiment was conducted for one month and consisted factorial combination of four phytase enzyme activity levels (0, 750, 1000 and 1250 phytase units (FTUs) in feeds at four crude protein percentages (30%, 35%, 40% and 45%) fed to 10g catfish fingerlings reared in 5L indoor glass tanks. Faecal samples were collected from each tank by siphoning one hour after every feeding, frozen and later pooled. They were finally dried and apparent digestibility coefficients (% ADC) calculated by the indirect method for protein, dry matter and energy after proximate composition analysis. Data was analysed by multiple- regression to find the right model of factors influencing digestibility followed by ANOVA to identify differences among treatments and family wise differences determined by contrasts using R console statistical software version 3.0.2. The highest digestibility was observed at the activity unit of 1000 in all protein levels followed by 750FTUs, 1250FTUs and 0 FTUs (control). It was concluded that 1000FTUs is a suitable activity unit for increased digestibility of plant based fish feeds Uganda's African catfish.

Key words: *Clarias gariepinus*, Phytase and aquafeeds

**Résumé**

Cette recherche a été menée pour établir et rationaliser les conditions et le montant optimal de l'enzyme phytase requise pour son adoption pour améliorer la digestibilité des aliments à base de plantes de poisson-chat. Ceci a commencé avec l'analyse de pH le long du tractus gastro-intestinal (GIT) de poisson-chat africain (*Clariagariepinus*), l'alimentation du pH, la teneur en acide phytique et de la composition en contenus et en nutriments approximatifs. Les résultats indiquent que l'enzyme phytase du champignon *Aspergillusniger* devrait être approprié pour l'adoption. Une expérience d'alimentation a été effectuée pendant un mois et consistait en une combinaison factorielle de quatre niveaux d'activité enzymatique de la phytase (0, 750, 1 000 et 1 250 unités de phytase (UFTs) dans les aliments à quatre pourcentages bruts de protéines (30%, 35%, 40% et 45% ) nourris à des alevins de poisson-

chat de 10g élevés dans 5L réservoirs de verre à l'intérieur. Les échantillons fécaux ont été prélevés dans chaque réservoir en siphonnant une heure après chaque repas congelés et plus tard mis en commun. Ils ont finalement été séchés et les coefficients de digestibilité apparente (% ADC) calculés par la méthode indirecte de protéines, de matière sèche et de l'énergie après l'analyse de la composition immédiate. Les données ont été analysées par régression multiple pour trouver le bon modèle de facteurs qui influent sur la digestibilité suivie par ANOVA, pour identifier les différences entre les traitements et les différences des astuces familiales visées par les contrastes en utilisant la console de R version du logiciel statistique 3.0 0,2. La digestibilité maximale a été observée à l'unité d'activité de 1000 dans tous les niveaux de la protéine suivie par 750FTUs, 1250FTUs et 0 UFP (contrôle). On a conclu que 1000FTUs est une unité d'activité approprié pour l'accroissance de la digestibilité de la nourriture à base de plantes pour le poisson à base de plantes pour le poisson-chat africain de l'Ouganda.

Mots clés: *Clarias gariepinus*, phytase et Nourritures aquatiques

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## Background

Increasing food and land shortages in Uganda demand intensive farming systems to sustain the ever growing population (MBAZARDI, 2011). Fish farming (with catfish as a major species) has been prioritized among other livestock enterprises like poultry and piggery due to their potential to provide animal protein under small land holdings. However, its development is challenged by low fish survival and growth due to inadequate quality and cost-effective feed sources such as fish meal and other animal protein. They are of high biological value nutritionally due to well balanced essential amino and fatty acids but are gradually getting replaced by plant based protein sources that are relatively cheaper and easier to obtain. However, most possess indigestible constituents and ant nutritional metabolites including phytates since fish lack phytase enzymes (FAO, 2012). Phytates form heat stable complexes with protein, phosphorus and other minerals depriving plant protein sources their full nutritional value to fish. Adaption of feed bioconversion aided by digestive enzyme technology has been poor due to inconsistencies in growth promotion. This study was thus designed to identify conditions especially in catfish GIT and feeds to guide feed treatment for efficient enzyme action.

## Literature summary

Most plant protein sources with potential for use in feed manufacturing especially grains and seeds contain metabolites that present ant nutritional effects in monogastric animals such as poultry, piggery and fish. Most of them are considerably reduced by heat ; roasting, drying, autoclaving, boiling soaking but phytates are heat stable thus requiring more specified treatment. Is and grains (NRC, 1993; Gabriel *et al.*, 2007; Kumar *et al.*, 2012). With adoption of plant based protein sources, incorporation of exogenous microbial phytase digestive enzymes is evolving steadily to facilitate cleaner and cost-effective fish production. It increased growth and waste quality in poultry and piggery but needs proper understanding of microbial ecology for adaption in fish farming due to uniqueness of the water media, fish physiology that

demand further processing of feed for water stability before presentation. The GIT pH, feed pH, feed phytate content, feed: enzyme ratio and stage of enzyme incorporation during processing/manufacture need considerable scrutiny before sourcing of microbial phytase can be effected ( Kumar *et al.*, 2012)

### Study description

The study was conducted at Mbarara Zonal Agricultural Research Institute (MBAZARDI) and Makerere College of Natural Sciences, Department of Chemistry. It started with analysis of catfish GIT pH and nutrient proximate composition of selected feed ingredients. Formulae for 30%, 35%, 40% and 45% were developed as in table 1. The experiment was conducted for one month in 72 indoor glass tanks(5L) Treatments were two factorial combinations of phytase enzyme activity levels (0, 750, 1000 and 1250 FTUs) in feeds at four crude protein levels (30%, 35%, 40% and 45%). The enzyme was incorporated in feeds immediately after pelleting using a hand sprayer in a closed plastic tank, air dried and fed after 10 days of storage. Twenty (20) catfish fingerlings (8-10g) were stocked in each tank and fed on experimental feed for four weeks .Faeces were collected by siphoning within the third and four week of rearing, pooled together and analysed for dry matter, protein and energy composition. Digestibility coefficients expressed as percentages (% DC) were determined based on proximate nutrient composition of the feed (diet) fed and collected faeces. Multiple regression analysis was used to find the relative importance of enzyme units, feed crude protein and water quality as confounding factors and later ANOVA was conducted to find whether or not there was significant differences among DC between and within enzyme/protein levels using R console statistical computer software version 3.02.

### Research application

PH in the gut and small intestines where most digestion occurs ranged from 5-6 (Fig. 1). The pH of the compound feed ranged from neutral to slightly alkaline (7.74-8.32) thus, citric

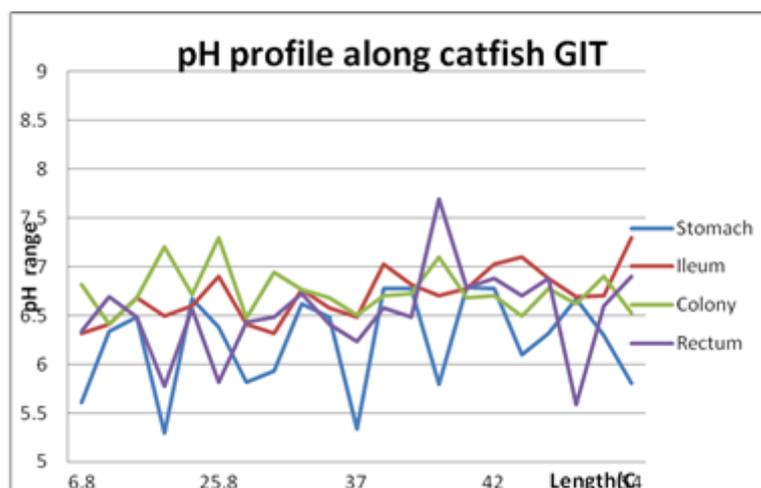


Figure 1. Stomach pH of African cat fish at 75 - 100% fullness.

**Table 1. Basal feed composition and analysed crude protein content.**

Ingredient	Feed 1	Feed 2	Feed 3	Feed 4
Cotton seed cake	5	6	6	5
Azolla powder	5	2	0	0
Farm compost	8	4	5	0
Brewers waste	5	0	0	0
Maize bran	15	10	7	4
Soybean seed cake	26	31	38	39
Wheat middlings	3	3	3	2
Fish meal	12	20	29	40
Ground beans	21	24	12	10
Protein	30.21	34.91	40.01	45.1

acid (an inorganic acid) was added to acidify it (bring it to 5.5). Phytate /phytic acid in the diet was estimated to be about 1.4mg/g. Proximate nutrient composition in the compound feed /diet was as presented Table 1. The DC ranged from 54-81% with the highest from 1000FTUs across all protein levels with the lowest from controls. Digestibility coefficients (DC) were significantly higher in the 35% crude protein diet than with 30%, 40% and 45% crude protein and in controls. This indicated that the type of phytase enzyme required for digestion of phytates in the diet was one with optima pH at 5 and that inclusion of an organic acid into feed was also necessary for increased enzyme efficiency.

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