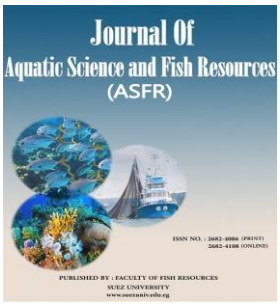
Aquatic Science and Fish Resources

http://asfr.journals.ekb.eg

Print ISSN: 2682-4086 Online ISSN: 2682-4108



Effect of Phytase-Supplemented Diets on Growth Indices, Nutrient Digestibility and Utilisation, Carcass Composition, and Sustainability of African Catfish (*Clarias gariepinus*)

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ARTICLE INFO

Article history:
Received: Aug.14, 2024
Received in revised form: Nov.21, 2024
Accepted: Dec.09, 2024
Available online: Dec.18, 2024

Keywords
Phytate
Thermostable-phytase
Cost-effective
growth

ABSTRACT

Plant protein ingredients are utilised for fish feed, but they contain phytate. The thermostable phytase-added diet will have no cost for preservation. Therefore, it will be deployed to resolve the problems of the phytate. This study aimed to assess the effect of phytase additives on growth indices, digestibility and utilisation, carcass composition, and sustainability of African Catfish fed a combination of diets. Five diets were formulated Phytase (Ronozyme hiphos GT) (500; 1000, 1500, 2000, 2500) FYT.kg^{-1} diet and a control 0 FYT.kg^{-1} diet. Comprise of 18 tanks (200 litres each), stocked with 20 fish of mean weight 20.98 ± 0.01 g, and fish were manually fed thrice for 84 days. Fish showed a significant (p < 0.05) inclusion level-base improvement of mean final weight from 81.60 ± 1.49 g in 0 FYT.kg^{-1} to 113.19 ± 3.18 g in 2000 FYT.kg^{-1}, and feed conversion ratio from (2.11 ± 0.06 to 1.56 ± 0.06), protein digestibility from (93.43 ± 0.03 to 98.19 ± 0.03) %, and carcass crude protein from (36.11 ± 0.11 to 44.03 ± 0.06) % in 0 FYT.kg^{-1} and 2000 FYT.kg^{-1} respectively. Intestine villi height improved from (405.49 ± 0.49 to 611.03 ± 0.98) µm in (0 to 2000) FYT.kg^{-1}. The net profit was increased from (954.18 ± 53.64 to 1995.42 ± 114.48) ₦ in (0 to 2000) FYT.kg^{-1}. The 2000 FYT.kg^{-1} inclusion level reflected the best growth, digestibility, carcass composition, and sustainability in African catfish, while the optimal inclusion level was 800 FYT.kg^{-1} diet.

1. INTRODUCTION

The world aquaculture production in 2020 was 122.6 million tonnes with a farm sale value of USD 352.7 billion, but in Africa, Nigeria was the second largest aquaculture producer with 261.7 thousand tonnes. The catfish alone accounted for over 70 % of Nigeria's USD 753 million aquaculture sector. Nigeria has a huge growth potential in aquaculture, in terms of its present and outlook. The increase in aquaculture production in Nigeria from 2000 to 2020 ranged from 25.718 to261.711 tonnes, representing Africa's second highest **(FAO, 2022)**. The rapid growth of fish farming in Nigeria has motivated research redirection on reducing production expenses and limiting environmental impact. Feed accounted for the bulk of the cost in intensive fish production, especially the protein ingredient of the feeds **(Kim *et al*., 2019)**. Thus, the feed producers have no choice but to produce balanced diets at a lower cost, yet not compromising on quality to satisfy the fish farmers **(Hardy, 2010; NRC, 2011; Tacon and Metian, 2015; Sanchez *et al*., 2016)**. Most ingredients for fish diet production are faced with the forces of demand and supply with attendant exorbitant prices in the global market. However, the cheap plant protein source contains many anti-nutritional

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factors among these is phytic acid which reduces its effective utilisation in fish diet. It has inherent anti-nutrient factors such as phytate which compromise the normal functioning of the fish gut, reduce nutrient biomolecules, and cause stunted growth **(Fontes *et al*., 2019; Samtiya *et al*., 2020)**. Phytate causes a shortage of nutrients, such as minerals, protein, and carbohydrates in fish-fed plant protein sources **(Cantas and Yildirim, 2020)**. The phytic acid and its compounds perform other significant functions aside from the role as a major source of organic phosphorus, these include the involvement of phytic acid in deoxyribose nucleic acid (DNA) repair, regulation of neurotransmission and clathrin-coated vesicular recycling **(Kumar *et al*., 2017)**.

Phytate suppresses nutrient efficiency in fish and can be linked to the effect of anti-nutritional factors and phytic acids inherent in most plant protein sources. The phytic acids form insoluble chelates with organic proteins and minerals, thus depriving the group of nutrients needed for proper growth and development **(Rodrigues *et al*., 2023)**. Monogastric animals including fish do not possess the innate capacity to produce endogenous enzymes for instance phytase, therefore, cannot break down insoluble nutrients in their body **(Pontes *et al*., 2021)**.

An additive such as phytase in the fish diet effectively minimises or eradicates the adverse effects of phytate inherent in the fish feed of plant ingredients. It has enhanced nutrient utilisation thus increasing the availability of requisite nutrients for fish growth **(Maas *et al*., 2020; Dias and Santigosa, 2023)**. Contemporary studies that used different phytases in diets include **Adeshina *et al*. (2023)** who recorded improved growth performance of *Clarias gariepinus* fed with a phytase enzyme-supplemented diet at the sustainable inclusion level of 900 FTU.kg^{-1} of diet. Phytase supplementation at the inclusion level from 1500 to 2000 UF.kg^{-1} diet enhanced the growth performance and nutrient bioavailability of *Oreochromis niloticus* under an intensive system of production **(Rodrigues *et al*., 2023)**. The phytase inclusion level of 500 to 2000 FYT.kg^{-1} of diet enhanced final body weight, specific growth rate, protein efficiency ratio, and reduction of feed conversion ratio for Nile tilapia, European seabass, and gilthead seabream **(Dias and Santigosa, 2023)**. There was an account of phytase supplementation at 1500 FTU.kg^{-1} of diet, which optimised energy and nutritional utilisation in Nile tilapia **(Pontes *et al*., 2021)**.

The current work assessed the effect of phytase-supplemented diets on growth indices, nutrient utilisation and digestibility, carcass composition, and sustainability of African catfish using varied inclusion levels in a basal feed to ascertain the optimum levels for best growth and development.

MATERIALS AND METHODS

2.1. Experimental condition

A total number of 360 African catfish post-juvenile of average weight 20.98 ± 0.01 g, were grown at the Centre for Sustainable Aquaculture Federal University Dustin-ma Makera, 20 fish were stocked in 18 conical polyvinyl chloride tanks 200 litres attached with an aerator, extreme side drain valve, and supply water inlet from borehole each. Six treatments (crude protein 39.96) and energy of 3295 kcal.kg^{-1}, comprising 25.33 % (fish meal, soybean meal, ground nut cake meal) and phytase which is in granule and thermostable (Ronozyme hiphos GT), was purchased from DSM-NOVOZYME Switzerland. Treatments include P1-P5 (0.05, 0.10, 0.15, 0.20, 0.25) % with phytase equivalent inclusion level (500, 1000, 1500, 2000, 2500) FYT.kg^{-1} diet, an FYT is the activity that releases 1 µmol of inorganic phosphate from 5.0 mM sodium phytate per minute at pH 5.5 and 37 ^{O}C **(Engelen *et al*., 2001)** in Table 1. Comprising control C (99.49 + 0 % phytase), P1 (99.49 + 0.05 % phytase), P2 (99.49 + 0.10 % phytase), P3 (99.49 + 0.15 % phytase), P4 (99.49 + 0.20 % phytase), P5 (99.49 + 0.25 % phytase) treatments. These inclusion levels were modified from previous works **(Yigiy *et al.,* 2018; Siqwepu *et al*., 2020)**, the experimental design was a completely randomised design (CRD) with 5 treatments, triplicates, and the control, the chromium oxide (Cr_2O_3) served as an inert marker to determine apparent faecal digestibility. The experimental fishes were fed for 84 days thrice daily at 3 % body weight, starting from 8.00 to 9.00 hours in the morning, 13.00 to 14.00 hours in the afternoon and 18.00 to 19.00 hours in the evening. At the onset and end of the feeding trial, fish were anaesthetised using benzocaine at a rate of 100 mg.L^{-1}, then grouped weight and length were measured

using a digital weight scale and a calibrated meter rule respectively to establish a new feeding regime. All throughout the feeding trial average water temperature was 27.18 ± 0.02 ^{O}C, others include, dissolved oxygen of 7.07 ± 0.01 mg.l^{-1}, total ammonia of 0.20 ± 0.03 mg.l^{-1}, pH of 7.06 ± 0.01 and alkalinity of 30.06 ± 0.01 mg.l^{-1} respectively

Table 1. Feed ingredients composition of experimental diets

Ingredients %	**C**	**P1**	**P2**	**P3**	**P4**	**P5**
Fish meal	25.33	25.33	25.33	25.33	25.33	25.33
Soybean meal	25.33	25.33	25.33	25.33	25.33	25.33
Groundnut cake	25.33	25.33	25.33	25.33	25.33	25.33
Millet	3.00	3.00	3.00	3.00	3.00	3.00
Yellow corn	3.00	3.00	3.00	3.00	3.00	3.00
vegetable oil	4.00	4.00	4.00	4.00	4.00	4.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Vitamins and minerals premix^{+}	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	4.00	4.00	4.00	4.00	4.00	4.00
Methionine	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	2.00	2.00	2.00	2.00	2.00	2.00
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50
Ronozyme Hiphos	0	0.05	0.10	0.15	0.20	0.25
Total	99.49	99.54	99.59	99.64	99.69	99.74
Composition/analyses (% dry matter)						
Protein %	39.96 ± 0.05	39.96 ± 0.03	39.96 ± 0.01	39.96 ± 0.01	39.96 ± 0.01	39.96 ± 0.01
Lipid %	13.61 ± 0.09	13.30 ± 0.10	14.17 ± 0.67	13.93 ± 0.57	14.37 ± 0.14	13.64 ± 0.60
Fibre %	4.20 ± 0.17	4.48 ± 0.14	4.42 ± 0.19	4.34 ± 0.09	4.64 ± 0.17	4.45 ± 0.11
Ash %	18.57 ± 0.15	19.96 ± 0.08	18.11 ± 0..11	18.82 ± 0.69	18.27 ± 0.06	19.61 ± 0.42
Moisture %	4.35 ± 0.25	4.08 ± 0.03	4.49 ± 0.29	4.16 ± 0.05	4.45 ± 0.10	4.19 ± 0.12
Gross energy kcal.kg^{-1}	3267.6 ±11.0	3204.3 ± 5.7	3297.8 ± 27.5	3275.3 ± 45.6	3294.9 ± 9.7	3229.8 ± 47.9
Phytase activity fyt.kg^{-1} diet	0	459.7 ± 0.6	970.3 ± 0.6	1408.0 ± 2.7	1899.3 ± 2.5	2309.0 ± 2.0

C control; P Phytase; P1 (0.05P) %; P2 (0.10P) %; P3 (0.15P) %; P4 (0.20P) %; P5 (0.25P) %.

Vitamins: represented (mg.kg^{-1} diet) vitamin C, 100; vitamin E, 100 IU; A vitamin, vitamin B1, 10; vitamin B2, 10; vitamin B6, 10; vitamin B5, 10; vitamin B3, 20; inositol, 400; choline, 1500; Antioxidant BHT A palmitate, 3000 IU; D-Rovimix D3–500, 2400 IU; K3 K-menadionesodium bisulphite (51%), 10; (E300 –321), 100; calcium propionate, 1000.

Minerals are shown (mg.kg^{-1} diet): ferric sulphate, 50; zinc sulphate, 30; cobalt sulphate, 0.1; copper sulphate, 10; Sodium selenite, 0.5; manganese sulphate, 20; magnesium sulphate, 500; chromic chloride, 1; cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; dicalcium phosphate, 8.02 (g.kg^{-1} diet).

2.2. Diet procedure

A mildly heated fry pan on fire was used to toast the soybean seed until it slightly turned brownish then milled it into less than 200 µm powdery form using a grinding machine (Shanghai Shenji Company. china). Likewise, the yellow corn, millet, and groundnut cake were ground. This is followed by mixing the milled grains with salts; vitamins; minerals; calcium carbonate; methionine; lysine; chromium oxide and phytase then an interrupted addition of palm and groundnut oils during the mixing for up to 10 minutes using a mixer (Astar Company China). After-which the

mixture was used to prepare a thickener made up of 2 kg of the mix in 1.5 litre of hot water at 80 ^{O}C, which was then pelleted using a 2 mm dice in a pelletiser. The resultant pellets were sundried until 8.0 % dried matter under ambient temperature then packed in an air-tight polythene bag and stored at room temperature until feeding commenced. The phytase activity of the diet was determined using a procedure stipulated by **Engelen *et al*. (2001)**.

2.3. Growth performance and nutrient utilisation

Following the 12 weeks of the feeding trial, the fish were starved for at least 24 hours and their grouped weight was taken to measure the following parameters: mean weight gain = w2 – w1, $\text{percentage mean weight gain} = 100 \times \left(\frac{w2-w1}{w2}\right)$, where w1 = mean initial weight, w2 = mean final weight, $\text{specific growth rate} = 100 \times \left(\frac{\text{Log e w2} - \text{Log e w1}}{t}\right)$, where t = duration of the feeding trial in days, $\text{percentage survival} = 100 \times \frac{\text{final number of fish}}{\text{initial number of fish}}$ and $\text{feed intake} = 100 \times \frac{fc}{\left(\frac{w2+w1}{2}\right)\frac{t}{1}}$, where fc = feed consumption, $\text{protein efficiency ratio} = \frac{\text{weight gain of fish}}{\text{protein fed}}$, $\text{apparent net protein utilization} = \frac{fcp-icp}{pf}$, where fcp = final carcass protein, icp = initial carcass protein, pf = protein fed, $\text{protein production value} = \frac{\text{carcass protein gain}}{\text{protein intake}}$ and $\text{feed conversion ratio} = \frac{\text{feed consumed}}{\text{weight gain}}$.

2.4. Apparent digestibility coefficient and carcass composition

In the last two weeks to the close of the feeding trial, faeces were siphoned from individual rearing tanks at about an hour post-feeding of fishes and kept at – 4 ℃ until analysis. The crude protein, ash, lipid, and carbohydrate were analysed according to **AOAC (2006)**, while, phosphorus and marker (Cr_2O_3) compositions in diets and faeces were analysed by an Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES, VISTA-MPX, Varian, USA) following perchloric acid digestion to compute the $\text{apparent digestibility coefficient of nutrient} = 100 \times \frac{(\text{faecal } Cr_2O_3 \times \text{feed Y}) - (\text{feed } Cr_2O_3 \times \text{faecal Y})}{\text{faecal } Cr_2O_3 \times \text{feed Y}}$, where: Y represents a crude protein, carbohydrate, ash, lipid, or phosphorus, and before the commencement of the feeding trial, three fish were randomly picked from the pool of fish under each treatment including the control diet to measure the initial carcass proximate composition and at the close of the feeding trial, another three fish were collected from a pool of fish under each treatment, then the fish carcass was frozen to assess the proximate composition in line with the procedure by **AOAC (2006)**.

2.5. Histology of intestine

The intestine from three (3) fish samples of different diets were dissected after the fish euthanisation. The tissue preparation for histological study followed the procedure of **Stoskopf (1993)**. Micrographs of the histological settings were taken using a camera DP70 Olympus® linked to a computer. The histological evaluation was through the method by **Allen (1992)**.

2.6. Economic analysis

The sustainability of the tested diets was performed under the following indices: Net Profit; Incident of Cost; Profit Index and Benefit-Cost Ratio by procedures stipulated in **(Orisasona *et al*., 2017; Jimoh *et al*., 2020)**. $\text{net profit (₦)} = \text{sales} - \text{total cost}$, the Incident of Cost represents the cost of feed consumed that produced 1 kg of weight in fish. A lower Incident of Cost indicates better returns on investment with the feed. $\text{incident of cost (₦. kg}^{-1}) = \frac{\text{cost of feed}}{\text{weight of fish produced}}$, $\text{profit index (₦)} = \frac{\text{value of fish}}{\text{cost of feed}}$, $\text{benefit cost ratio (₦)} = \frac{\text{total cost}}{\text{total sales}}$.

2.7. Statistics analysis

The mean and standard deviation values of data were presented in tables and charts, and one-way Analysis of Variance (ANOVA) was used to test for equality of means at ($p < 0.05$) level of significance. The means with the significant relationships were separated using Tukey's test. Quadratic polynomial regression was used to estimate the optimal level of inclusion of treatments. The International Business Machines (IBM) Statistics Package for Social Sciences (SPSS) Statistics software of 21.0 version was used for the analysis.

RESULTS AND DISCUSSION

3.1. Growth performance, survival rate, and nutrient utilisation

The growth performance, survival rate, and nutrient utilisation of *Clarias gariepinus* fed phytase treatments are shown in Table 2, the mean initial weight and survival rate in all the fish reflected no significant differences ($p > 0.05$).

Table 2. Growth performance, survival, and nutrient utilization of *Clarias gariepinus* fed phytase-added diets.

	MIW (g)	MFW (g)	PMWG (%)	SGR (%.day-1)	SR (%.)	FI (%)	PER	ANPU	PPV	FCR
C	20.98 ± 0.01^{a}	81.60 ± 1.49^{e}	74.28 ± 0.47^{e}	1.62 ± 0.02 ^{e}	83.33 ± 2.89^{a}	2.97 ± 0.05^{a}	1.52 ± 0.04^{e}	14.32 ± 0.26^{f}	0.45 ± 0.01^{f}	2.11 ± 0.06^{a}
P1	20.98 ± 0.01^{a}	90.25 ± 0.51^{d}	76.75 ± 0.13^{d}	1.74 ± 0.01^{d}	83.33 ± 2.89^{a}	2.79 ± 0.01^{b}	1.73 ± 0.02^{d}	19.72 ± 0.06^{e}	0.54 ± 0.00^{e}	1.89 ± 0.01^{b}
P2	20.98 ± 0.01^{a}	96.18 ± 0.77^{c}	78.18 ± 0.17^{c}	1.81 ± 0.01^{c}	85.00 ± 0.00^{a}	2.71 ± 0.02^{bc}	1.88 ± 0.02^{c}	21.78 ± 0.03^{d}	0.59 ± 0.01^{d}	1.77 ± 0.02^{c}
P3	20.99 ± 0.00^{a}	110.76 ± 3.08^{ab}	81.04 ± 0.53^{ab}	1.98 ± 0.03^{a}	83.33 ± 2.89^{a}	2.57 ± 0.04^{d}	2.25 ± 0.08^{a}	29.80 ± 0.17^{b}	0.72 ± 0.02^{b}	1.58 ± 0.04^{d}
P4	20.98 ± 0.01^{a}	113.19 ± 3.18^{a}	81.45 ± 0.52^{a}	2.00 ± 0.04^{a}	85.00 ± 0.00^{a}	2.56 ± 0.06^{d}	2.30 ± 0.08^{a}	34.09 ± 0.01^{a}	0.75 ± 0.03^{a}	1.56 ± 0.06^{d}
P5	20.99 ± 0.01^{a}	105.89 ± 2.52^{b}	80.17 ± 0.47^{b}	1.93 ± 0.03^{b}	83.33 ± 2.89^{a}	2.68 ± 0.06^{c}	2.12 ± 0.07^{b}	25.25 ± 0.07^{c}	0.64 ± 0.02^{c}	1.68 ± 0.06^{c}

P = phytase, statistical analysis: each value is a mean ± SD (n = 3) values in a column with different superscripts are significantly different ($p < 0.05$) Tukey's test. C = control 0 FYT.kg-1, P1- P5 = (500, 1000, 1500, 2000, 2500) FYT.kg-1 feed, MIW = mean initial weight, MFW = mean final weight, SGR = specific growth rate, SR = survival rate, FI = feed intake, PER = protein efficiency ratio, ANPU = apparent net protein utilization, PPV = protein productive value, FCR = feed conversion ratio

The mean final weight was significantly ($p < 0.05$) increased from (81.60 to 113.19) g in treatment (0 and 2000) FYT.kg-1 respectively. Other, significant improvements include a specific growth rate from (1.62 to 2.00) %.day-1, a protein efficiency ratio was elevated from (1.52 to 2.30), and a feed conversion ratio improved from (2.11 to 1.56) in treatment (0 and 2000) FYT.kg-1 respectively. At every higher inclusion level of different treatments in this experiment, there was a commiserate growth of fish, and nutrient utilisation until its highest point at the 2000 FYT.kg-1 treatment, then plummeted. The second-order polynomial regression analysis of different treatments estimated an optimum inclusion level of 800 FYT.kg-1 of diet, which reflected an average weight gain of 88.65 ± 0.01 g at the end of the experiment in Figure 1. Although, phytase has been deployed in different fish diets to serially break down complex components of nutrients in the form of phytate, information on the use of thermostable granule phytase in fish diets is scanty. It improved the bioavailability of nutrients in the form of inorganic phosphorus, protein, and minerals. Phosphorus with calcium contributes to the growth of bones and muscles in the body, therefore, leading to total body growth in fish. Adding phytase to the diet may have increased starch and protein digestibility, improving energy and protein bioavailability as demonstrated in Tilapia fish **(Pontes *et al*., 2021)**. The phytase-added diet probably reduces non-retained phosphorus effluent from the culture facilities. Thus, ameliorating the environmental impact of the culture system and ensuring sustainable fish production.

The enhanced growth performance and nutrient utilisation can be attributed to the phytase treatment, which successfully breaks down the complex molecules of phytate, protein, and minerals. Therefore, setting loose the optimal quantity of bioavailable nutrients for body utilisation **(Maas *et al*., 2020; Rodrigues *et al*., 2023)**. The growth improvement can also be linked to the increase of endogenous enzyme activity for instance, trypsin and amylase which were boosted for effectiveness, digestibility, and the assimilation of requisite protein and energy for growth and development. Contemporary studies that applied a delicate phytase to other fish diets include **Pontes *et al*. (2021)** who studied nutrient utilisation in Nile tilapia.

Each nutrient that probably contributes to fish's growth and development is protein, lipids, energy, minerals, and vitamins constituting the nutrient quality or utilisation. It involves nutrient-growth parameters that evaluate specific nutrient quality based on their contributions to the growth and development of fish, thus, the growth response in the experimental fish was a measure of the nutrient efficiency. Previous works on European seabass and gilthead seabream-fed phytase-supplemented diets at (500 to 2000) FYT.kg^{-1} showed the best protein efficiency ratio and feed conversion ratio **(Dias and Santigosa, 2023)**.

However, the fish treated with the control group 0 FYT.kg^{-1} recorded the poorest feed conversion ratio, and the lowest protein efficiency ratio, apparent net protein utilisation, and protein productive value. The high acceptability of diets suggests that basal feed contains a good source of protein (isonitrogenous). However, only an insignificant portion of the digestible proteins may be efficiently converted to the growth and development in the group, this was evident in the lowest protein efficiency ratio, apparent net protein utilisation, and poorest feed conversion ratio. The suppression of nutrient efficiency in the 0 FYT.kg^{-1} group can be linked to the effect of anti-nutritional factors such as phytic acids inherent in the plant protein sources. The phytic acids usually form insoluble chelates with organic proteins and minerals, thus depriving the fish of the nutrients needed for proper growth and development. Moreover, monogastric animals including fish do not possess the innate capacity to produce the required phytase and, therefore, cannot break down insoluble nutrients in their body.

3.2. Digestibility and carcass composition

The apparent protein digestibility was significantly ($p < 0.05$) elevated from (93.43 ± 0.03 to 98.19 ± 0.03) % in different treatments (0 and 2000) FYT.kg^{-1} in Figure 2, similarly, apparent phosphorus digestibility was increased from (93.90 ± 0.02 to 98.63 ± 0.04) % respectively. The digestibility of nutrients in fish administered with different treatments exhibited the same patterns as seen in the growth, and nutrient utilisation ($p < 0.05$) as the highest digestibility was recorded in the 2000 FYT.kg^{-1}. The carcass crude protein of the tested experimental fish was improved ($p < 0.05$) from (36.11 ± 0.11 to 44.03 ± 0.01) % in different treatments (0 and 2000) FYT.kg^{-1} respectively in Figure 3, and the ether extract from (2.17 ± 0.03 to 2.52 ± 0.01) % respectively.

Digestibility of feed determines the amount that is digested and assimilated by fish out of the entire feed consumed and is a formidable tool for the assessment of the nutrient quality of fish diets **(Deng *et al*., 2020)**. The improvement of nutrient digestibility in the current work can be linked to the successful breakdown and bioavailability of nutrients that were naturally present in the form of insoluble phytate complexes attached to proteins and minerals. The phytase-supplemented diets cause the dephosphorisation of phytate, thereby releasing phosphorus a significant part of cell metabolites such as phospholipids, ribose, and deoxyribose nucleic acids. Since the most important minerals required for the building of bone in fish are phosphorus and calcium, any nutritional limitations of these minerals are detrimental to its growth. Other works that corroborate this study include **Cantas and Yildirim (2020)**, who reported enhancement of total phosphorus digestibility in Rainbow trout fed a phytase-supplemented diet. A multienzymes mixture supplemented diet also improved the digestibility of protein, fibre, and lipid in Siberian sturgeon **(Shekarabi *et al*., 2022)**. The similarities in nutrient digestibility indicate the efficacy of the phytase-added diets. The feed additive may have facilitated enough bioavailability of nutrients for physiological and metabolic functions and storage in the fish body.

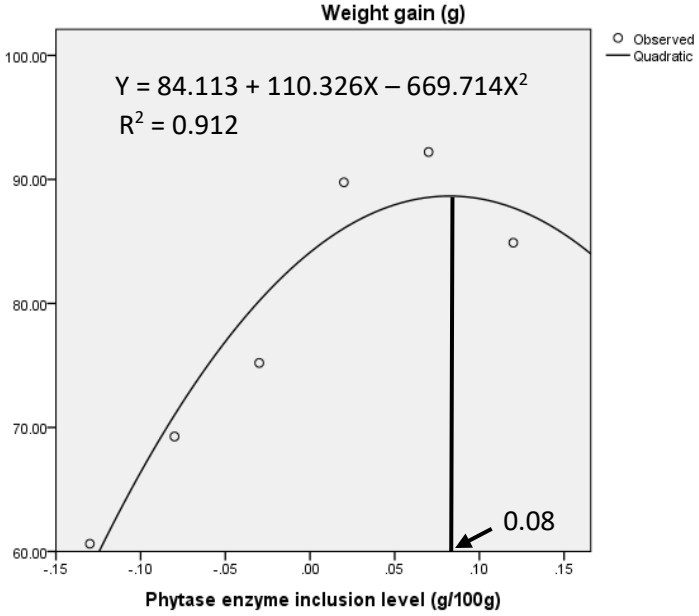

Figure 1. Relationship between weight gain of Clarias gariepinus and phytase inclusion level of diets

The nutrient capacity of a fish determines to some extent its carcass composition and the nutrient constituents of the body must first be at equilibrium in anticipation for growth. The effect of phytase-added diets that probably hydrolysed phytate into biomolecules, thereby, easing the provision of protein, phosphorus, and minerals. Also, the phytase-added diet may have provided enough requisite nutrients thus enhancing the nutrient composition of the group, since fish possess an innate ability to store bioavailable nutrients in their tissues. Previous studies have attested that fish fed with basal diet + 0,02 % phytase (SOP) and 20 % soybean meal + 0.02 % phytase diets respectively recorded a higher body muscle crude protein as seen in *Salmo trutta caspius* **(Mohseni *et al*., 2023)** improved final carcass composition of the experimental fish in the current work compared to the initial carcass results can be linked to the

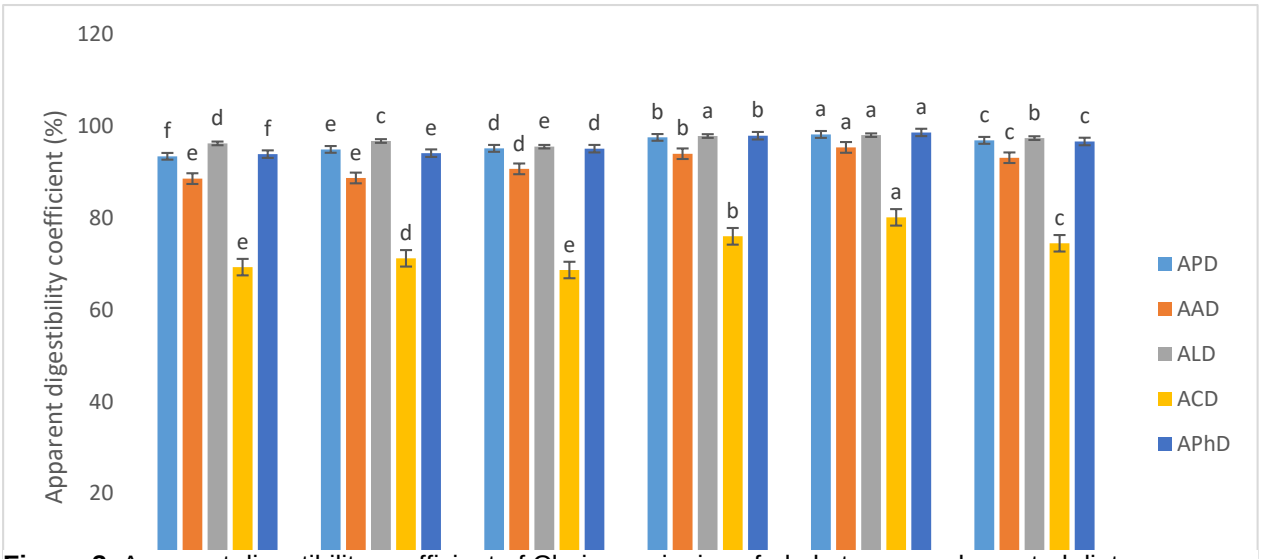

Figure 3. Apparent digestibility coefficient of Clarias gariepinus fed phytase-supplemented diets

P = phytase, C = control 0 FYT.kg-^{1}, P1- P5 = (500, 1000, 1500, 2000, 2500) FYT.kg-^{1} diet, APD-APhD = apparent digestibility of protein, ash, lipid, carbohydrate, Phosphorus. Statistical analysis: each value is a mean ± SD (n = 3) value on an error bar with different alphabets are significantly different (p < 0.05) Tukey's test.

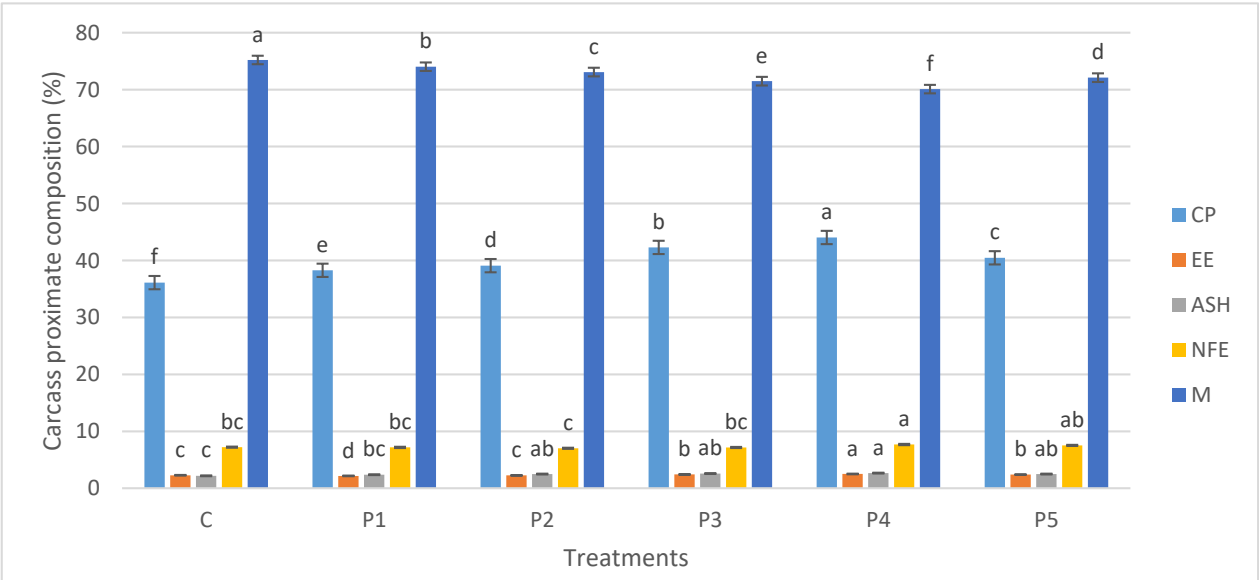

Figure 2. Carcass composition of Clarias gariepinus fed phytase-supplemented diets P = phytase,C = control 0 FYT.kg-^{1}, P1- P5 = (500, 1000, 1500, 2000, 2500) FYT.kg-^{1} diet. Statistical analysis: each value is a mean ± SD (n = 3) values on an error bar with a different alphabet are significantly different (p < 0.05) in Tukey's test. CP = crude protein, EE = ether extract, NFE = nitrogen free extract, M = moisture

3.3. Histology of intestine

The intestinal histology of *Clarias gariepinus* fed phytase-added diets showed significant variations ($p < 0.05$) among treatments in Figure 4 and Table 3, and it followed the same patterns with growth performance, digestibility, and economic viability. The villi height recorded an improvement from (405.49 ± 0.49 to 611.03 ± 0.98) µm in (0 to 2000) FYT.kg^{-1} respectively, while, goblet cell number was increased from (9 to 15).

Histological assessments of the intestine have been revealed to be reliable proof for facts on the physiology, nutrient availability, and healthiness of fish **(Caballero *et al*., 2004; Couto *et al*., 2015)**. A normal *Clarias gariepinus* intestine is characterised by hematoxylin and eosin (H and E) stained evenly, including the mucosal epithelial cells, villi, lamina propria, and goblet cells which are sight-able. In the present study the improved intestine villi height from (405.49 ± 0.49 to 611.03 ± 0.98) µm in (0 to 2000) FYT.kg^{-1} treatments, and goblet cell numbers from 9 to 15 respectively, may be an aftermath of the effective use of the digestive part of the fish intestine probably for digestibility and assimilation of nutrient for growth and development. It attested to the group's large absorptive area under enough protective mucin and mucous-producing cells. This suggests the availability of a gfiood quality diet with high feed efficiency and nutrient utilisation. A similar result with a high villi height and goblet cell number was obtained in *Rasbora lateristriata* fed- Bio-fuel algal waste diet **(Manganang *et al*., 2020)**.

3.4. Economic analysis

The economic analysis exhibited the same trend among fishes fed different diets ($p < 0.05$) in Table 4, the value of fish was improved from (2937.60 ± 53.64 to 4074.84 ± 114.48) ₦ in (0 to 2000) FYT.kg^{-1}, likewise, net profit was increased from (954.18 ± 53.64 to 1995.42 ± 114.48) ₦, profit index was elevated from (1.75 ± 0.04 to 2.29 ± 0.06), while, the incident of cost declined steadily from (1146.38 ± 20.94 to 873.83 ± 24.56) ₦.kg^{-1} respectively.

The improved economic profit index from (1.75 ± 0.04 to 2.29 ± 0.06) in (0 to 2000) FYT.kg^{-1} was analogous to the growth pattern in the *Clarias gariepinus* which also improved with higher inclusion levels among treatments. However, the cost of a phytase-added diet that generated 1 kg of weight in the *Clarias gariepinus* (incident of cost) reduced with increased treatments which signifies a divergent pattern to the growth performance indices. The sustainability of aquaculture production depends on its viability in terms of profit index and benefit-cost ratio. Therefore, in the present study, the 2000 FYT.kg^{-1} treatment, may be recommended as a viable inclusion level for *Clarias gariepinus* production, since its profit index was above the controls. **Orisasona *et al*. (2017)** recorded a similar lowered 'incident of cost' and increased profitability index among treatments in African catfish fed-visceral meal **(Jimoh *et al*., 2020)**.

Table 3. Average villi height and goblet cell number of Clarias gariepinus fed phytase diets

Treatments	Average villi height (µm)	Goblet cell number
C	405.49 ± 0.49^{e}	9
P1	415.86 ± 0.26^{d}	9
P2	417.33 ± 0.26^{d}	10
P3	596.68 ± 0.68^{b}	11
P4	611.03 ± 0.98^{a}	15
P5	459.54 ± 0.47^{c}	10
P values	0.000	NA

P = phytase, NA = not available, statistical analysis: each value is a mean ± SD (n = 3) values in a column with different superscripts are significantly different ($p < 0.05$) Tukey's test. C = 0 FYT.kg^{-1} P1 = 500 FYT.kg^{-1} P2 = 1000 FYT.kg^{-1} P3 = 1500 FYT.kg^{-1} P4 = 2000 FYT.kg^{-1} P5 = 2500 FYT.kg^{-1}

Table 4. Least cost and economic assessment of Clarias gariepinus fed phytase diets

Indices	C	P1	P2	P3	P4	P5
MFW (g)	81.60 ± 1.49^{e}	90.25 ± 0.51^{d}	96.18 ± 0.77^{c}	110.76 ± 3.08^{ab}	113.19 ± 3.18^{a}	105.89 ± 2.52^{b}
VF (₦)	2937.60 ± 53.64^{e}	3249.00 ± 18.36^{d}	3462.60 ± 27.72^{c}	3987.24 ± 110.70^{ab}	4074.84 ± 114.48^{a}	3812.04 ± 90.72^{b}
NP (₦)	954.18 ± 53.64^{e}	1233.58 ± 18.36^{d}	1431.18 ± 27.72^{c}	1923.82 ± 110.70^{a}	1995.42 ± 114.48^{a}	1700.62 ± 90.72^{b}
IC (₦.kg^{-1})	1146.38 ± 20.94^{a}	1055.99 ± 5.97^{b}	1000.11 ± 8.01^{c}	884.99 ± 24.58^{e}	873.83 ± 24.56^{e}	950.73 ± 22.63^{d}
PI	1.75 ± 0.04^{e}	1.89 ± 0.01^{d}	2.00 ± 0.02^{c}	2.26 ± 0.06^{a}	2.29 ± 0.06^{a}	2.10 ± 0.05^{b}
BCR	1.48 ± 0.03^{e}	1.61 ± 0.01^{d}	1.70 ± 0.02^{c}	1.93 ± 0.06^{a}	1.96 ± 0.06^{a}	1.81 ± 0.05^{b}

MFW = mean final weight, VF = value of fish, NP = net profit, IC = incident of cost, PI = profit index, BCR = benefit cost ratio, Nigeria market price of 1 kg = ₦ 2000, 1600 Nigeria Naira = 1 US Dollar. P = phytase, statistical analysis: each value is a mean ± SD (n = 3) values in a row with different superscripts are significantly different ($p < 0.05$) Tukey's test. C = 0 FYT.kg^{-1} P1 = 500 FYT.kg^{-1}, P2 = 1000 FYT.kg^{-1}, P3 = 1500 FYT.kg^{-1}, P4 = 2000 FYT.kg^{-1}, P5 = 2500 FYT.kg^{-1}.

Conclusion

The phytase-added diets recorded significant improvements in the mean final weight, specific growth rate, protein efficiency ratio, feed conversion ratio, protein and phosphorus digestibility, carcass protein, and the best, and optimal inclusion levels in African catfish *Clarias gariepinus* were 2000 FYT.kg^{-1} and 800 FYT.kg^{-1} respectively.

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