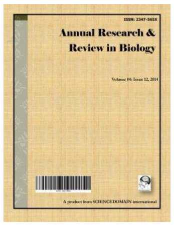

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Effect of Microbial Phytase on Nutrient Availability and Growth of Juvenile *Clarias gariepinus* Fed Soyabean and Groundnut-based Diets

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Several studies have shown the positive effect of phytase on phosphorus utilization by fish and animals, with the use of phytase sources determined for different fish species. Few studies have tested phytase response to different diets, which may affect nutrient availability for optimum growth due to differences in phytate location. The research, therefore, studied the effect of phytase to diets based on soya bean and groundnut meal for *Clarias gariepinus* on nutrient availability and growth. In trial 1, four groups of soya bean S1, S2, S3 and S4 replaced fish meal at 25%, 50%, 75% and 100% and supplemented with 250, 500, 750 and 1000 FTU/g phytase, respectively. In trial 2, four groups of groundnut meal diet G1, G2, G3, G4, G5 and G6, were similarly supplemented with the same phytase levels used in experiment 1. Fish meal control (S0=G0) was not supplemented with phytase. Result showed that 250 FTU/g phytase showed the highest mean weight gain for both plants. In conclusion, the research has shown that the chemical nature of phytate, rather than its

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concentration and location, may influence the utilization of phosphorus for optimum growth in the fish by supplementing 250 FTU/g, with a range of available phosphorus requirement of between 0.75% (Y = 0.363 + 4.155X - 2.772X^2, R^2 = 0.759) and 0.80% (Y = 0.307 + 3.303X - 2.059X^2, R^2= 0.210)

Keywords: Phytase; plant proteins; nutrient availability; growth; Clarias gariepinus.

1. INTRODUCTION

Phytate has been reported to bind protein and amino acid through the binary protein-phytate complexes formed below the isoelectric point of protein (pH < 5-6) [1,2]. At low pH, phytic acid interacts with α-NH2 groups and with the side groups of basic amino acids, which include arginine, histidine, and lysine [2,3]. Phytate also binds with crystalline amino acids in the gut, and it has been suggested that the de novo formation of binary protein–phytate complexes in the gastrointestinal tract, which are refractory to pepsin activity, may be responsible for the reduction of amino acid digestibility [4] and hence reduces the effective utilization of amino acid in fish and animals [5]. Phytase has been variously used to improve amino acid utilization in animals [2,6,7,8] through an improvement in phosphorus availability, which reported to range between 60%-80% by supplementing 500-1000 FTU/g [9]. Improvement in amino acid corresponds to improvement in growth [10]. The extent to which phytase generates improvements in protein digestibility in animal studies is variable and the topic remains controversial [1]. According to Selle and Ravindran [1], factors influencing response of nutrient availability to phytase include: differences between ingredient types, dietary levels of Ca and non-phytate-P, age of animal, the inherent digestibility of dietary amino acids, the sources and concentrations of phytate in the diet, and the inclusion level and type of added phytase. The positive effects of phytase on amino acid availability have been observed in several animal studies in low phosphorus and amino acid-deficient diets [6,7,11,12,13,14]. Huynh and Nugegoda [15] reported that inclusion of phytase and/ or amino acids did not improve amino acid profile of canola meal diet supplemented with limiting amino acid in Australian catfish (*Tandanus tandanus*), which showed no improvement in growth of the fish. Similarly, supplementation of phytase in amino acid-adequate diet of young chicks fed diet based on groundnut cake had no effect on growth [12].

Cao et al. [16] reported that Nile tilapia fed diet with and without phytase showed no difference in protein utilization, with a reduction in dietary methionine and lysine as the level of phytase-pretreated diet increased. The phosphorus requirement of juvenile African catfish has been reported to range from 0.67% to 0.82% using inorganic phosphorus [16]. However, Van Weerd et al. [17] reported that the available phosphorus of juvenile *Clarias gariepinus* fed phytase-supplemented diet could be higher than 0.40%; hence, the phosphorus requirement could still be higher than reported value. Moreover, matrix value of phytase supplementation, with particular reference to digestible protein, energy as well as lysine and methionine availability and the available phosphorus requirement by phytase supplementation of plant-based diets of African catfish has not been reported. Data on this aspect of phytase effect has been described severally for pigs and poultry (BASF and Quantum, personal communications). Nwanna et al. [18] reported an optimum growth requirement of 8000 FTU/g for juvenile *Clarias gariepinus*. In another study, the optimum level for growth was reported at 750 FTU/g, with both studies evaluated using different phytase sources supplemented in soya bean. There is no report of the optimum level determined for different plant sources, with the aim of evaluating available phosphorus response for the fish. An evaluation of available phosphorus requirement would not only improve the nutritional need of the fish, which is mainly omnivorous in feeding habit, and hence, utilize plant proteins effectively [19], but would allow a least cost formulation on an available phosphorus basis, and reduce pollution from oversupply of phosphorus in meeting the phosphorus requirement of the fish resulting from phosphorus deficiency due to phytate [5]. Additionally, the use of phytase would allow for a quantification of an estimate of the lysine and methionine requirement of the fish, which could also be used in improving the nutritional value of catfish feed. Phytate in soya bean and groundnut meal are located differently [20,21], which could show varying response [12]. Hence, the research was carried out to evaluate amino acid profile, availability of nutrient and growth performance of juvenile *Clarias gariepinus* fed soya bean and groundnut meal based diets.

2. MATERIALS AND METHODS

2.1 Diet Preparation

A growth and digestibility trial was carried out to assess the effect of phytase supplementation in roasted soya bean and groundnut meal-based diets of juvenile *Clarias gariepinus*. In both trials, about 15 and 20 kg of raw soya bean and groundnuts were purchased from a cereal and grain market in Ibadan, Oyo State, Nigeria. Soya bean and groundnut seeds were subjected to dry heat treatment [22] with slight modifications [23] by roasting on a metal plate at 120°C for 30-45 minutes. Roasted soybeans were grinded, sundried, and packed into plastic bags, and stored at ambient temperature prior to inclusion in the formulation of with other feeding stuff. Groundnut seeds were milled into meals and packed in a large sack having minute pore spaces, which allowed free flow of air and moisture to allow for easy passage of oil. The bag was squeezed (oil-pressed) several times before being stored in a cool, dried place. The bag containing milled groundnut was placed on a plain surface, with heavy wooden blocks placed on it to further reduce the level of oil, which was allowed to drain for about 3-4 days before mixing with other ingredients feedstuffs for the fish [24]. All diets were formulated using Pearson's method of diet formulation [25] without added inorganic phosphorus and amino acid supplements to optimize phytate hydrolysis in the diets. In trial one, five graded levels of soya bean were formulated to replace fish meal at 0% (S0), 25% (S1), 50% (S2), 75% and 100% (S4), using similar procedure by [26]. The phytate contents of the respective diets were 0.40%, 0.52%, 0.57%, 0.43% and 0.40%, respectively. Another four basal diets (S1, S2, S3 and S4) were formulated with similar composition and supplemented with post-pelleting liquid phytase (Natuphos 5000L, BASF Corporation, Ludwigshafen, Germany), which was produced from submerged fermentation of Aspergillus niger strain FTU-11 (CBS 491.94), a genetically modified derivative of a parental strain GAM-53, at 250 FTU/g (P1), 500 FTU/g (P2), 750 FTU/g (P3) and 1000 FTU/g (P4). One FTU (phytase unit) is defined as the amount of phytase that liberates 1 µmol of inorganic phosphorus from 0.0051 mol/L of sodium phytate per minute at pH 5.5 and 37°C [8]. In trial two, similar levels of phytase were supplemented in increasing levels of roasted and oil-pressed groundnut meal diet at 10% (G1), 20% (G2), 30% (G3), 40% (G4), 50% (G5) and 60% (G6), with basal phytate levels of 0.25%, 0.58%, 0.56%, 0.42%, 0.45% and 0.23%. All basal diets contained 0.20% calcium. S0 served as control without dietary phytase, and used to compare growth for both diets. Chromic oxide was added at 0.50% [27] for nutrient digestibility. Prior to phytase supplementation in both trials, each formulated diets was mixed using a large bowl with clean cold water and cold-pelleted using a sieve of mesh size 2mm to produce a noddle-like strand of feed. Pelleted feeds were sun-dried and packed air-tight polythene bags before use. The control diet G0 was similar to S0 (fish meal) and was also included to compare growth performance. The proximate compositions of feed ingredients (Table 1) and gross composition of experimental diets (Tables 3 and 4) a below. Chemical analysis of phytate, phytase activity, proximate, and amino acids were done according to standard methods.

2.2 Experimental Fish

A total of 3978 Clarias gariepinus fish of average weight 4.5±0.2 g was procured from Jotmot Farms Alakia, in Egbeda LGA of Oyo State, Nigeria. In trial 1, A total of 1638 African sharptooth catfish of mean weight 4.5±0.2 g were acclimated to laboratory conditions for 3 weeks with water temperature, pH and oxygen maintained at optimum range between 25°C-32°C, 7.40-7.45, 4.80-5.0 mg/l. Water was sourced from a borehole through an overhead tank with a pipe, which supplied clean water to the Aquaculture and Fisheries Management laboratory, University of Ibadan, Oyo State, Nigeria. Healthy fish for the experiments were sourced from private fish farm in Ibadan, Nigeria. All phytase-supplemented diets were fed at 3% body weight to fish of mean weight of 11.55± 0.20 g, which were stocked in triplicate groups at 26 fish per tank (0.43 m x 0.25 m x 0.265 m). In trial 2, after acclimation (3 weeks), 2340 juvenile C gariepinus fish of mean weight 11.55± 0.20 g were randomly allocated to the phytase-supplemented groundnut diet. Fish were stocked at 26 fish per tank in triplicate and fed the phytase treated diet at 3% body weight for the first four weeks, which was reduced to 1.5% until the end of the experiment, which lasted for 84 days. All fish were weighed biweekly throughout the experiments using an electronic compact balance S. Mettler scale correct to 0.01 g (Model: K-BH). About 80% of water in experimental tanks were renewed every day, using a static renewal method, and water quality monitored for all treatment tanks at the end of each experiment, which lasted for 84 days.

Table 1. Proximate composition of feed ingredients

Ingredient	Crude protein (%)	Fat (%)	Ash (%)	Fibre (%)	Moisture (%)	CHO (%)	Energy (kcal/100 g)
Fish meal	66.46	5.32	4.33	0.69	9.28	13.79	380
SBM (Full-fat)	42.93	18.41	5.34	7.86	8.47	17.00	420
SBM (Raw)	35.5	19.48	5.06	5.59	12.13	22.25	420
Groundnut (roasted)	36.37	45.03	7.86	4.45	4.12	2.17	564
Groundnut (raw)	25.13	50.61	6.65	8.47	5.18	8.47	574
Maize	10.24	3.32	1.28	1.15	10.35	73.67	370

Table 2. Gross composition of roasted, full-fat soyabean-basal diets for juvenile *Clarias gariepinus*

Ingredient	S0 (0%)	S1 (25%)	S2 (50%)	S3 (75%)	S4 (100%)
Fish meal	54.29	45.38	34.16	19.62	-
Soyabean meal (full fat)	-	15.13	34.16	58.85	92.15
Maize	41.71	35.49	27.68	17.53	3.85
Vit Min Mix#	0.50	0.50	0.50	0.50	0.50
Fish oil	1.00	1.00	1.00	1.00	1.00
CaCO3	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Cellulose*	0.20	0.20	0.20	0.20	0.20
Starch	0.80	0.80	0.80	0.80	0.80

*# Micro mineral mix contains per kilogram: Vit A (20, 000 IU), Vit. D3 (5, 000 IU), Vit. E (300 mg), Vit K3 (10 mg), Vit B1 (20 mg), Vit. B2 (25 mg), Vit. C (300 mg), Niacin (120 mg), Ca. Pantothenate (60 mg), Vit B6 (10 mg), Vit B12 (0.05), Folic acid (5 mg), Biotin (1 mg), Choline chloride (5 mg), Inositol (50 mg), Manganese (30 mg), Iron (35 mg), Zinc (45 mg), Copper (3 mg), Iodine (5 mg), Cobalt (2 mg), Lysine (85 mg), Selenium (0.15mg), Antioxidant (80 mg), Methionine (100mg). * as carboxymethyl cellulose*

Table 3. Gross composition of roasted, oil-pressed groundnut meal-basal diets for juvenile *Clarias gariepinus*

Ingredient	G0 (0%)	G1 (10%)	G2 (20%)	G3 (30%)	G4 (40%)	G5 (50%)	G6 (60%)
Fish meal (66.46%)	54.29	51.19	48.22	44.87	41.07	36.70	31.66
Groundnut meal (36.37%)	-	5.67	12.06	19.23	27.38	36.70	47.49
Maize (10.24%)	41.71	39.14	35.72	31.90	27.55	22.60	16.85
Vit Min Mix#	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Fish oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CaCO3	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cellulose*	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Starch	0.80	0.80	0.80	0.80	0.80	0.80	0.80

*# Micro mineral mix contains per kilogram: Vit A (20, 000 IU), Vit. D3 (5, 000 IU), Vit. E (300 mg), Vit K3 (10mg), Vit B1 (20 mg), Vit. B2 (25 mg), Vit. C (300 mg), Niacin (120 mg), Ca. Pantothenate (60 mg), Vit B6 (10 mg), Vit B12 (0.05), Folic acid (5 mg), Biotin (1 mg), Choline chloride (5 mg), Inositol (50 mg), Manganese (30 mg), Iron (35 mg), Zinc (45 mg), Copper (3 mg), Iodine (5 mg), Cobalt (2 mg), Lysine (85 mg), Selenium (0.15mg), Antioxidant (80 mg), Methionine (100 mg). * as carboxymethyl cellulose*

2.3 Proximate and Chemical Analysis

Feed samples per diet were analysed for proximate and mineral composition (Analytical Lab., International Institute of Tropical Agriculture, Moniya, Ibadan, Nigeria); phytate and amino acid profile (Multi Environmental Management Consultants Limited, Ikorodu, Lagos, Nigeria); while phytase activity was performed at the analytical lab., BASF SE, Lampertheim, Germany. Sample of feeds were analyzed for phosphorus, calcium, sodium, copper, and manganese and determined using flame atomic absorption spectrophotometer model Buck 205, Buck Scientific, USA. Phosphorus was determined spectrophotometrically by molybdovanadate method [28]. Proximate composition of the diets were determined by AOAC [29]. Phytate was measured according to method used in previous work by Akpoilih et al. [30]. The sample was extracted with 0.2N HCl. Test tube containing 0.50ml of extract was sealed with a ground glass stopper. A tube containing 1 millilitre of frric solution was heated in a boiling water bath for 30 minutes. Care was taken to ensure that for the first 5 minutes, the tube remained well stoppered. After cooling in ice water for 15 minutes, the tube was allowed to adjust to room temperature. Once the tube reached room temperature, the content of the tube was mixed and centrifuged for 30 minutes at 3000 g. 1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2, 2'- Bipyridine solution added. The absorbance was measured at 519nm against distilled water. Phytase activity in feed samples were determined (Analytical Lab., BASF SE, Lampertheim, Germany) by methods of [31]. About 100 g feed was milled to a particle size less than 0.5 mm. Two 5.0 g portions of each sample of feed was weighed with an accuracy of 10 mg into an Erlenmeyer flask. 50.00 ml acetate buffer was metered by a dispenser into each sample, and the mixture was then stirred on a magnetic stirrer for 60 min. The stirring was followed by decantation into 10 ml centrifuge tubes and centrifugation at 4000 rpm (equivalent to about 2500 g) for 20 minutes. The centrifugate was then diluted with buffer using the dilutor to a content of about 0.02 FTU/ml. 2.00 ml of each of the two solutions was pipetted as sample and sample blank into a 10 ml centrifuge tube. For the blank, 2.00 ml portions of acetate buffer were pipetted into two 10 ml centrifuge tubes. One centrifuge tube was incubated, and the other centrifuge tube was treated in analogy to the blanks as enzyme standard. The centrifuge tubes with the enzyme, sample blank and control solutions were each placed at a defined time interval (e.g. every 10 sec) in a water bath at 37.0 +/- 0.1°C and equilibrated for exactly 5 min. Then, at the same time intervals (every 10 sec), 4.00 ml sodium phytate solution (equilibrated at 37.0 +/- 0.1°C) was added by a dispenser and mixed. After incubation for exactly 60 min, the reaction was stopped, again at the same time intervals (every 10 sec), with 4.00 ml stop reagent and mixed to produce a colored complex with the phosphate formed. After waiting for at least 10 min, the solutions were centrifuged at 4000 rpm (equivalent to about 2500 g) for 20 minutes and then the absorbance at a wavelength of 415 nm was measured in a spectrophotometer. The enzyme phytase liberates inorganic phosphate from the substrate sodium phytate during incubation and the intensity of the yellow color of the vanadomolybdophosphorus complex is a measure of the amount of phosphate liberated.

The Amino Acid profile of feed (n=2) was determined using methods described by Benitez [32], which involved defatting, determination of nitrogen, hydrolysis, evaporation and analysis.

2.4 Growth Evaluation

Growth performance was monitored biweekly with the following parameters measure. Mean weigh gain was determined from the difference between mean final weight and initial weight [33]. Daily weight gain was derived from dividing mean weight gain by period of feeding [34]. Feed Conversion Ratio (FCR) was estimated from the ratio of feed Intake and fish weight gain at the end of feeding [35]. Specific Growth Rate was determined from In (W2-W1)/ (t2-t1), where W2 and W1 are weights on day t2 and t1, respectively [36]. Survival rate was determined from initial number of fish –mortality/ initial number of fish X 100 [37].

2.5 Digestibility Studies

The indirect method of determination of digestibility was used in this experiment with the addition of 0.5% chromic oxide as indigestible marker [28]. During the feeding experiment and two (2) weeks before the end of each experiment, faecal collection was done as follows. After feeding the fish for a second time each day (18:00 h), faeces was drained out along with the water into a plastic containers. Water was gently drained out of the containers and faces collected using filter paper. Based on the maximum duration of activity of 2 hours of phytase, that is the interval between ingestion of feed and 80% of stomach evacuation, and an assumed cessation of phytase activity in the intestine [18], faeces were collected from each of the tanks 2-3 hours after each feeding. Faecal samples collected from all tanks were freeze-dried at -20°C [38]. After freeze drying, faeces were analyzed (n=3) for chromic oxide [30], protein [30], lipid [30], energy (Model 6200 microprocessor-controlled isoperibol oxygen bomb calorimeter) and phosphorus [30]. Apparent digestibility coefficient (ADC) was calculated for protein, energy, lipid, and phosphorus using the formula:

> ADC nutrient = 100- 100[(% Cr2O3 diet / % Cr2O3 faeces X Nutrient faeces / Nutrient diet)]. Digestible nutrients were determined by multiplying digestibility values by dietary nutrients [18].

2.6 Statistical Analysis

Interactions between phytase and diets based on soya bean and groundnut meal were determined for mineral, amino acid profile, growth and nutrient digestibility using factorial analysis. Tukey HSD test of comparison was used to detect mean differences between mean pair for amino acid, mineral, growth and nutrient digestibility. Quadratic regression determined optimum available phosphorus level. All data were presented as means $\pm$ S.D with a significant level of P=0.05.

3. RESULTS

3.1 Nutrient Digestibility

The effects of experimental diets and phytase on proximate composition are presented in Table 4 and 5. In full fat soya bean, significant interaction was observed for crude protein, energy, fat and protein digestibility (P<0.05). Digestibility values for crude protein increased significantly with phytase supplementation, with the highest value recorded for 1000 FTU/g (Table 6). Energy digestibility decreased significantly for fish for all diets with phytase addition (P<0.05) compared to diets without phytase. Phytase at 250 FTU/g showed the highest fat digestibility compared to other diets, including control (P<0.05). Increasing substitution of fish meal with soya bean resulted in a decline in phosphorus digestibility (P<0.05). However, phytase at 1000 FTU/g showed the highest phosphorus digestibility compared to 250, 500 and 750 FTU/g. Phytase at 1000 FTU/g showed the lowest phytate phosphorus and highest available phosphorus compared with control, 250, 500 and 750 FTU/g (Table 7).

In diet based on groundnut meal, no interaction between phytase and groundnut meal was observed for energy, lipid and phosphorus digestibility (P>0.05). However, phytase supplementation significantly improved crude protein, energy and lipid digestibility (Table 8). The highest crude protein digestibility was recorded for 500 FTU/g, while 250 FTU/g showed the highest phosphorus digestibility compared to control, 500, 750 and 1000 FTU/g (P>0.05). The lowest phytate phosphorus and highest available phosphorus were also recorded for 250 FTU/g compared to 500, 750 and 1000 FTU/g (Table 9).

3.2 Growth

Growth performance of fish showed a significant reduction (P<0.05) in mean weight gain, daily weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio with increasing level of soya bean (Table 10) and groundnut meal (Table 11), regardless of phytase level. However, phytase addition significantly improved the parameters of growth for both diets, with the highest growth performance recorded for 250 FTU/g (Figs. 1, 2). Survival rate declined significantly, regardless of soya bean level (P<0.05). However, in groundnut meal, there was no significant effect of phytase on fish survival, which was highest in 250 FTU/g (P>0.05).

4. DISCUSSION

Research conducted by several studies have demonstrated improvements in growth performance following phytase supplementation of inadequate phosphorus diets may be consistent with enhanced protein availability [2]. Many studies have demonstrated that phytase supplementation makes the chelated phytate-phosphorus available to fish [8], and increase phosphorus digestibility compared to diets without phytase [39]. Yu and Wang [9] found that in soybean meal based diet for Crucian carp (*Carassius carassius*), about 60% and 80% of phosphorus can be released from phytate by the addition of 500 and 1000 FTU/g phytase, respectively. This is in line with this study (Table 7), which showed that the higher available phosphorus in soya bean diet supplemented with

250 FTU/g (0.53%) compared to control (0.47%) translates to an increase of 0.13% [40], which is 60% phosphorus availability [9]. Protein digestibility improved significantly in soya bean with the highest value observed with 1000 FTU/g phytase, suggesting that protein digestibility may benefit significantly from phytase in fish diet, regardless of dietary composition and fish species [41,42,43]. Hussain et al. [42] reported the highest protein digestibility with addition of 1000 FTU/g phytase to corn-gluten based diet of *Labeo rohita* fingerlings compared to control, 250, 500, 750, 1250 and 1500 FTU/g diet. Pham et al. [44] also reported that supplementation of 1000 FTU/g improved significantly protein digestibility in cotton seed and soya bean meal based diet of juvenile Olive flounder (*Paralichthys olivaceus*). Phytate-phosphorus is converted to available phosphorus by phytase which can be utilized directly by animals [45].

Improvement in fat digestibility with phytase (250 FTU/g) is indicative of the release of small amount of amino acids [46] that may lead to an overall growth improvement [5]. Phytate has been reported to negatively influence energy utilization in broilers [1]. Enhanced availability of amino acids would increase the utilization of energy derived from protein [1,8] and improve growth performance [2]. In this study, however, improved growth of fish (Fig.1) and fat digestibility did not translate to an overall improvement in energy digestibility compared to groundnut meal. This is contrary to the report that the positive impact of phytase on energy utilization stems from an accumulation of increases in fat digestibility [47], which needs further investigation. The reduction in energy digestibility with phytase may be due to a reduced amino acid profile in soya bean, with only an improvement in lysine, threonine, cysteine and isoleucine (Table 12) compared to a much improved amino acid (histidine, arginine, threonine, cysteine, isoleucine, leucine, and phenylalanine) with phytase in groundnut meal (Table 13). Improvement of histidine and arginine in groundnut by phytase may suggest the participation of the essential amino acids in the formation of phytate-protein complex through their alpha amino groups [2,3]. Reduction in arginine in soya bean by phytase may explain reduced energy digestibility. The reduction in lysine and methionine profiles in groundnut meal, which are the first limiting in most plants for optimal growth of fish [48] may suggest the need for dietary supplementation of the essential amino acids in groundnut meal diets for optimal growth of fish in future research, however, interactions with phytase should be taken into consideration. The significant improvement of amino acids in groundnut meal by phytase may possibly explain higher growth performance of fish (Fig. 2) compared to fish fed soya bean (Fig.1). Additionally, a reduction in energy digestibility reflects reduction in arginine profile of soya bean diet with increasing phytase. Similar response pattern was observed in soya bean diet, regardless of phytase level, which showed an improvement in arginine and a corresponding improvement in energy digestibility. This findings confirm reports in literature on the role of arginine in energy metabolism [48,49] and amino-induced increase in energy release by phytase [46]. Reduction in energy digestibility may also result from the reduction in lipase activity in phytase supplemented diets [50] and high dietary fat in in diet compared to an improved energy digestibility in phytase-supplemented diet based on solvent-extracted soya bean [51].

The significant reduction in phosphorus digestibility in soya bean meal may be due to the high levels of saturated fat in soya bean compared to a much higher levels of monounsaturated and polysaturated fatty acids than saturated fat in groundnut meal [52]. High fat has also been reported to inhibit phosphorus absorption [53,54]. The improvement in phosphorus digestibility in solvent-extracted soya bean with 500, 750 and 1000 FTU/g compared to a diet with no phytase [51] suggest that fat in soya bean may inhibit phosphorus absorption in roasted soya bean, which also showed slight increase in faecal phosphorus (Table 6). This was also reported in *Clarias gariepinus* fed phytase supplemented soya bean diet, in another study, which suggest slight increase in faecal phosphorus by phytase is possible [17]. However, the higher availability of phosphorus and growth improvement in roasted soya bean compared to solvent-extracted soya bean [51] may suggest the reabsorption of phosphorus from the kidney through the sodium transporters NaPiIIa,c and III due to a reduced intestinal absorption [55]. The increase in phosphorus digestibility in groundnut meal supplemented with 250 FTU/g compared to control, 500, 750 and 1000 FTU/g may be related to its high phytate contents compared to soya bean [5], which enhanced the magnitude amino acid response to phytase [1,56], resulting overall growth improvement. Reduction in phytate phosphorus in groundnut meal with phytase at 250 FTU/g compared to control, 500, 750 and 1000 FTU/g

(Table 9) may explain improved phosphorus digestibility and reduction in faecal phosphorus (Table 8). Improvement in nutrient digestibility may also result from the high levels of digestible energy and polyunsaturated fatty acids in groundnut meal [57].

Improvement in growth performance (Fig. 1) of fish fed soya bean by phytase at 250 FTU/g could be due to increased available phosphorus by phytase (Table 7), which reflected the requirement for catfish [28]. Phytase at 250 FTU/g in soya bean also showed improved threonine and methionine compared to other phytase diets (Table 12). The lysine level in 250 FTU/g (5.14%) is higher than the reported lysine requirement of catfish, 5.10% [28].The highest methionine content in 250 FTU/g diet (2.14%) is, however, lower than the requirement for catfish, 2.30% [28]. This may be due to the lack of significant response of methionine to phytase [1], which showed a reduction in roasted groundnut meal diet (Table 12). Selle and Ravindran [1] reported that, methionine is less responsive amino acid to phytase compared to ther amino acids. Only few studies have seen positive responses in animals [7,12]. Others did not find any improvement by phytase [13]. Richie et al. [58] observed decreased methionine with increasing phytase in soya bean-based diet of Nile tilapia. Improvement in growth with phytase at 250 FTU/g could possibly be explained by a corresponding improvement in available phosphorus from phytate [46]. This is in line with the report by (Cao et al. [8] who reported that channel catfish fed the diets containing 250 FTU/g phytase showed higher growth performance compared to diet without phytase. Improved growth performance by phytase in fish diet have also be reported for juvenile *Clarias gariepinus* fed diet based on full fat soya bean [18]. Haghbayan and Mehrgan, [59] reported growth improvement in rainbow trout fed soya bean diet supplemented with enzyme complex. Although a possibly higher than 0.40% available phosphorus by phytase was reported [17], the highest growth achieved at 250 FTU/g, with a corresponding available phosphorus of 0.53% compared to 0.54% recorded for 1000 FTU/g, which recorded the lowest growth performance compared to control, 250, 500 and 750 FTU/g phytase, showed that the available phosphorus of 250 FTU/g (0.53%) and phosphorus of 0.81% may be required for optimum growth performance of juvenile C. gariepinus. Similarly, the high available phosphorus and phosphorus of 250 FTU/g (0.51%) supplemented in groundnut meal compared to 500, 750 and 1000 FTU/g phytase (Table 9) may suggest requirement of the fish may not depend on plant source of protein, as the values of available phosphorus for both diets with 250 FTU/g values are almost similar. The available phosphorus requirement determined in this study may not be significantly different from that of walking catfish (Clarias leather) fed 0.55% available phosphorus based on growth data [60]. Quadratic regression of specific growth rate on phytase level showed an available phosphorus ranging from 0.75% ($Y = 0.363 + 4.155X - 2.772X^2$, $R^2 = 0.759$) to 0.80% ($Y = 0.307 + 3.303X - 2.059X^2$, $R^2 = 0.210$), which is line with the requirement of omnivorous fish species [20].

The average phosphorus of 250 FTU/g (0.86%) determined for both plants sources may suggest a higher value of phosphorus requirement for juvenile C. gariepinus compared to an average value of 0.75% (0.67-0.82%) reported for the fish [16]. The improvement in mean weight gain observed in fish fed 250 FTU/g phytase in both plants may be due to increase in feed consumption and the release of nutrient from the phytase-mineral complex [61,62,63].The optimum growth performance at 250 FTU/g, regardless of plant protein used may suggest that the location and concentration of phytate in different plants may not be as necessary as the chemical nature of phytate, feed processing, fish species, stomach pH and phytase dose [42]. The report of this study may be corroborated by similar observations of an optimum dose of 750 FTU/g for nutrient availability and growth of *Labeo rohita* fingerlings fed different diets based on cotton seed meal [43], Canola meal [64] and sunflower meal [63]. The optimum growth performance by supplementing 250 FTU/g in this study compared to 750 FTU/g in *Labeo roriha* [64] may be due to an enhanced phytate hydrolysis at low stomach pH of *Clarias gariepinus* [17] compared to stomachless fish species such as *Labeo rohita* and *Cyprinus carpio* [42]. This may be similar to that of Channel catfish, which showed optimum growth performance with 250 FTU/g. Robinson et al. [65] also reported similar findings for the fish, which also corroborated the report that a minimum of 250 FTU/g phytase is required in the diet of most fish species [8]. However, the result of this study contradicts the report of an optimum phytase of 750 FTU/g phytase (Natuphos 5000) for *Clarias gariepinus* fed soya bean diet, which may be due to formulation of phytase and the use of wheat [18]. The activity of phytase in wheat is high enough to compromise optimum phytate hydrolysis [66]. In a similar study, which also showed a different optimum phytase level of 8000 FTU/g (Ronozyme 5000) reported for the fish, the difference in the outcome may be due differences in phytase source, which could differ in phytate hydrolysis and thus show varying response in terms of phosphorus availability and growth performance [8,42]. Specie differences in optimum phytase showed different dietary requirement of 500 FTU/g for *Pangasius pangasius* [67], 750 FTU/g for *Labeo rohita* [43,65] and 1000 FTU/g for Nile tilapia [25]. The decline in growth performance with phytase at 1000 FTU/g compared to 250, 500 and 750 FTU/g in groundnut meal may be due to the inability of phytase to efficiently release phosphorus and other minerals from zinc-phytate complex [5,68], which may be due, in part, to high dietary fibre (Table 1) and calcium/phosphorus ratio (Table 11), which may facilitate calcium-zinc complex [2,5], thereby making it less assessable to phytase [69], resulting in reduced phytase activity and available phosphorus for the diet. The low growth performance in the fish fed 1000 FTU/g compared to 250 and 500 FTU/g phytase was also reported *Labeo rohita* fingerlings fed sunflower meal [63] and in *Pangasius pangasius* fingerlings [67]. The reduction of growth performance in both diets as the inclusion of soya bean and groundnut increased (Figs.1,2) may be due to the low amino acid profile [70] and presence of antinutrients [60].

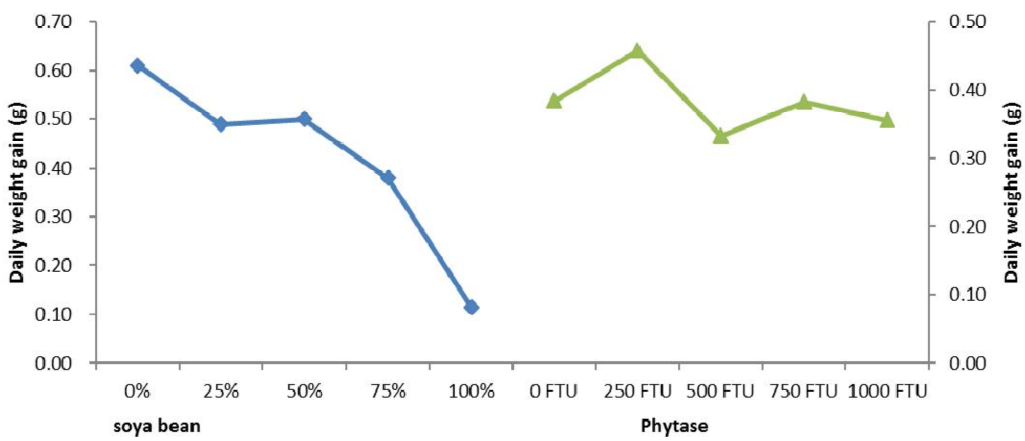

Fig. 1. Effect of soyabean and phytase on daily weight gai of juvenile *Clarias gariepinus*

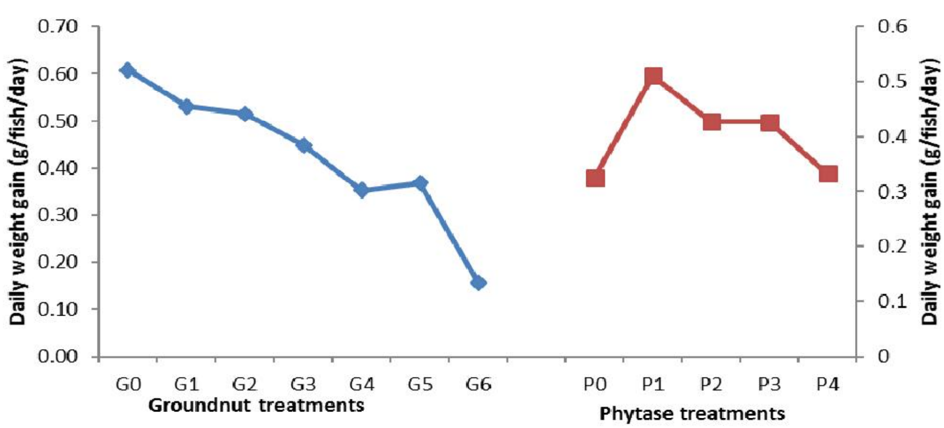

Fig. 2. Effect of groundnut meal and phytase on daily weight gain of juvenile *Clarias gariepinus*

Table 4. Effect of soya bean (full fat) and phytase on proximate composition of juvenile *Clarias gariepinus*

Sources of variation, P value	Crude protein (%)	Ash (%)	Moisture (%)	Fat (%)	CF (%)	Energy (Kcal/g)	NFE (%)
Phytase	0.01	0.00	0.77	0.00	0.00 .	0.00	0.00
Soya bean	0.000	0.00	0.00	0.00	0.00	0.00	0.00
Phytase* Soya bean	0.001	0.00	0.00	0.00	0.00	0.00	0.00
Pooled SE	0.48	0.00	0.00	0.00	0.00	0.00	0.01
Main effects							
Phytase							
P0	42.30±1.99d	10.25±1.56e	9.04±1.67a	15.74±5.34a	7.38±2.78c	4.30±0.30b	15.29±2.71d
P1	41.59±0.9a	8.71±1.59a	10.88±1.37d	20.09±7.27e	8.45±2.50d	4.40±0.02d	10.28±7.52a
P2	41.80±0.86b	9.86±2.38d	10.34±2.75c	15.93±7.25b	6.84±1.57a	4.40±0.02c	15.23±3.89d
P3	42.10±0.68c	8.83±0.18b	10.30±1.19b	17.73±4.23c	7.33±1.89c	4.30±0.02b	14.72±2.16c
P4	42.52± 0.63e	9.62±2.16c	9.35±1.16b	17.87±4.44d	7.09±1.96b	4.20±0.01a	13.55±5.51b
Soya bean							
S0	45.75±0.01e	12.88±0.15e	10.83±0.04e	11.18±0.00a	3.92±0.11a	3.90±0.00a	15.45±0.31d
S1	42.67±0.32d	11.13±0.85d	10.20±0.99d	12.64±4.69b	7.79±1.59d	4.20±0.00b	15.99±5.09e
S2	42.39±0.27c	10.18±1.00c	10.63±1.98c	15.64±3.89c	5.95±1.52b	4.20±0.01c	15.24±4.01c
S3	41.80±0.44b	9.14±0.25b	0.61±0.61b	18.15±2.90d	6.73±1.51c	4.40±0.00d	14.57±3.84b
S4	40.72±0.59a	6.84±1.02a	8.33±2.40a	24.79±1.70e	9.19±1.36e	4.60±0.00e	9.42±4.42a

**Means with the same superscript in the same column are not significant (P>0.05)*

Table 5. Effect of groundnut (roasted) and phytase on proximate composition of juvenile *Clarias gariepinus*

Sources of variation, P value	Crude protein (%)	Fat (%)	Ash (%)	Moisture (%)	Crude fibre (%)	Energy (Kcal/g)	NFE (%)
Phytase	0.01	0.00	0.00	0.00	0.00 .	0.00	0.00
Groundnut	0.000	0.00	0.00	0.00	0.00	0.00	0.00
Phytase* Groundnut	0.001	0.00	0.00	0.00	0.00	0.00	0.00
Pooled SE	0.48	0.00	0.00	0.00	0.00	0.00	0.00
Main effects							
Phytase							
P0	42.89±2.05d	16.92±5.73a	9.70±2.53e	7.32±2.81a	6.36±1.81a	4.23±0.02c	16.81±4.80e
P1	41.62±1.55b	18.71±5.56c	7.71±1.05b	7.99±0.90e	7.71±1.50b	4.26±0.02d	16.50±3.20c
P2	41.67±1.69c	18.18±11.65b	7.58±1.69a	7.80±0.66b	8.13±1.83c	4.22±0.02b	16.64±7.00d
P3	41.60±1.55a	18.68±12.32c	8.47±1.46d	7.81±1.24c	8.51±0.89d	4.23±0.02c	14.93±6.44b

Sources of variation, P value	Crude protein (%)	Fat (%)	Ash (%)	Moisture (%)	Crude fibre (%)	Energy (Kcal/g)	NFE (%)
P4	42.57± 1.64e	19.90±5.28d	8.40±0.86c	7.86±0.68d	8.95±1.53e	4.13±0.02a	12.32±4.96a
Groundnut							
G0	44.75±0.01f	10.83±0.04a	12.88±0.15g	11.18±0.00a	3.92±0.11a	3.94±0.01a	16.45±0.31de
G1	43.75±0.48e	14.41±4.88b	8.53±1.45e	9.04±0.75b	8.12±1.67cd	3.95±0.01b	16.15±6.75c
G2	42.53±0.33d	17.24±8.79d	9.14±0.42f	6.81±2.57c	7.95±1.81bc	4.05±0.01c	16.33±8.16d
G3	41.91±0.48c	17.09±7.65c	8.01±0.95c	8.09±0.51d	8.27±1.63cd	4.16±0.01d	16.63±5.42f
G4	41.83±0.47c	20.78±3.14f	7.94±0.85b	7.62±0.76e	8.60±1.77d	4.31±0.00e	13.23±5.48b
G5	41.65±0.51b	19.79±5.40e	8.45±2.87d	7.49±0.49	7.42±1.73b	4.36±0.01f	15.20±5.15d
G6	40.98±0.46a	25.83±0.85g	7.53±1.82a	6.72±0.83	7.43±1.31bc	4.51±0.01g	11.41±2.57a

**Means with the same superscript in the same column are significant (P>0.05)*

Table 6. Effect of soya bean and phytase on nutrient digestibility of juvenile *Clarias gariepinus*

Sources of variation, P value	Crude protein (%)	Energy (%)	Fat (%)	Phosphorus (%)	Faecal phosphorus (%)
Phytase	0.007	0.000	0.000	0.000	0.00
Soya bean	0.002	0.011	0.003	0.002	0.011
Phytase* Soya bean	0.003	0.003	0.001	0.012	0.005
Pooled SE	0.003	0.003	0.003	0.072	0.003
Main effects					
Phytase					
P0	90.86± 0.56a	86.71±0.16a	81.00±6.47b	83.21±7.07d	0.41±0.01a
P1	92.16±0.14b	86.58±0.24ab	83.08±9.28d	78.08±9.05b	0.52±0.01e
P2	92.46± 0.26c	86.60±0.30b	76.63±10.31a	77.11±8.82a	0.51±0.01d
P3	92.73± 0.12d	86.66±0.16c	82.98±4.10c	78.07±8.08b	0.50±0.01c
P4	92.89± 0.05e	86.56±0.02ab	81.51±3.36b	82.05±3.26c	0.49±0.01b
Soya bean					
S0	89.91± 0.01a	86.51±0.01a	76.24±0.01b	89.91±0.01e	0.42±0.00a
S1	92.09±7.60b	86.51±0.13a	73.81±8.98a	87.12±1.13d	0.49±0.05d
S2	92.19± 0.68c	86.49±0.13a	80.73±4.76c	81.22±1.32c	0.49±0.04d
S3	92.30± 0.66d	86.60±0.09b	83.00±3.56d	80.23±2.00b	0.48±0.05c
S4	92.49± 0.59e	86.87±0.17c	87.57±2.59e	68.90±6.23a	0.48±0.04b

**Means with the same superscript in the same column are not significant (P>0.05)*

Table 7. Effect of soya bean and phytase on total phosphorus, phytate and available phosphorus

Sources of variation, P value	Total phosphorus (%)	Phytate (%)	Available phosphorus (%)
Phytase	0.001	0.000	0.002
Soya bean	0.004	0.000	0.003
Phytase* Soya bean	0.012	0.000	0.005
Pooled SE	0.002	0.002	0.001
Main effects			
Phytase			
P0	0.93± 0.33c	0.46±0.08bc	0.47±0.31c
P1	0.81±0.28b	0.28±0.12a	0.53±0.32d
P2	0.79± 0.31a	0.44±0.14b	0.35±0.19b
P3	0.79± 0.27a	0.47±0.06c	0.32±0.25a
P4	0.81± 0.30b	0.27±0.11a	0.54±0.36d
Soya bean			
S0	1.31±0.01e	0.40±0.02b	0.91±0.01e
S1	1.18±0.03d	0.39±0.20b	0.49±0.79d
S2	0.87±0.09e	0.40±0.13b	0.47±0.11c
S3	0.75±0.02b	0.45±0.08c	0.30±0.08c
S4	0.42±0.02a	0.31±0.10a	0.11±0.09a

**Means with the same superscript in the same column are significant (P>0.05)*

Table 8. Effect of phytase and groundnut meal on nutrient digestibility of juvenile *Clarias gariepinus*

Sources of variation, P value	Crude protein (%)	Energy (%)	Fat (%)	Phosphorus (%)	Faecal phosphorus (%)
Phytase	0.007	0.000	0.000	0.346	0.243
Groundnut	0.002	0.001	0.003	0.492	0.575
Phytase* Groundnut	0.003	0.095	0.183	0.446	0.552
Pooled SE	0.001	0.001	0.001	0.807	0.000
Main effects					
Phytase					
P0	91.29± 0.16a	87.08±0.15a	83.56±5.37a	86.25±6.07a	0.53±0.01a
P1	91.83±0.14d	87.72±0.21c	85.96±9.28b	90.24±7.05a	0.29±0.01a
P2	91.84± 0.26e	87.74±0.30d	88.27±4.31e	90.20±6.82a	0.29±0.01a
P3	91.73± 0.12c	87.56±0.16b	87.09±4.13d	90.17±8.06a	0.29±0.01a
P4	91.71± 0.05b	87.56±0.02b	86.18±3.31c	89.18±3.26a	0.29±0.01a
Groundnut					

Sources of variation, P value	Crude protein (%)	Energy (%)	Fat (%)	Phosphorus (%)	Faecal phosphorus (%)
G0	89.91± 0.01a	86.51±0.01a	76.24±0.01a	89.91±0.01a	0.42±0.00a
G1	91.52±0.60c	87.45±0.13c	80.22±4.98b	90.64±1.13a	0.30±0.05a
G2	91.51± 0.63b	87.27±0.13b	82.74±4.76c	85.50±1.32a	0.35±0.04a
G3	91.90± 0.66g	87.74±0.09b	89.51±3.51f	90.40±2.06a	0.29±0.05a
G4	91.78± 0.59e	87.68±0.17e	88.52±2.19e	90.19±6.23a	0.54±0.04a
G5	91.69±0.45d	87.48±0.20d	86.76±0.34d	89.60±4.55a	0.28±0.05a
G6	91.81±0.44f	87.69±0.44e	91.00±0.56g	88.20±0.32a	0.28±0.02a

Means with the same superscript in the same column are not significant (P>0.05)

Table 9. Effect of phytase and groundnut meal on total phosphorus, phytate and available phosphorus composition

Sources of variation, P value	Total phosphorus (%)	Phytate (%)	Available phosphorus (%)
Phytase	0.001	0.001	0.002
Groundnut	0.000	0.000	0.000
Phytase* Groundnut	0.000	0.000	0.000
Pooled SE	0.004	0.003	0.002
Main effects			
Phytase			
P0	0.94±0.17d	0.40±0.13b	0.54±0.20d
P1	0.90±0.08c	0.39±0.16a	0.51±0.18c
P2	0.88±0.08b	0.48±0.12c	0.40±0.12b
P3	0.89±0.07b	0.49±0.08c	0.40±0.13b
P4	0.85±0.12a	0.48±0.13c	0.37±0.21a
Groundnut			
G0	1.31±0.01g	0.40±0.02a	0.91±0.01g
G1	0.98±0.02f	0.43±0.19b	0.55±0.20f
G2	0.96±0.02e	0.50±0.06d	0.46±0.04d
G3	0.90±0.01d	0.52±0.09e	0.38±0.13c
G4	0.88±0.02c	0.39±0.09a	0.49±0.11e
G5	0.84±0.03b	0.47±0.11c	0.37±0.16b
G6	0.73±0.06a	0.40±0.17a	0.33±0.18a

Means with the same superscript in the same column are not significant (P>0.05)

Table 10. Effect of soya bean and phytase on growth performance of juvenile *Clarias gariepinus*

Sources of variation, P value	Mean initial weight (g/fish)	Mean final weight (g/fish)	Mean weight gain (g/fish)	Daily weight gain (g/fish/day)	Mean feed intake (g/fish)	FCR	Protein efficiency ratio	Ca/P	Survival rate (%)
Phytase	0.24	0.02	0.007	0.000	0.016	0.000	0.007	0.000	0.079
Soya bean	0.27	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phytase* Soya bean	0.24	0.02	0.001	0.095	0.078	0.000	0.001	0.000	0.094
Pooled SE	0.17	0.53	0.483	0.008	0.637	0.058	0.483	0.058	0.031
Main effects Phytase									
P0	11.92±6.90	42.94±16.46	31.07± 16.49bc	0.38±0.21a	50.73±7.91ab	4.48±6.47c	1.36± 0.65bc	1.40±0.12a	97.31±3.65c
P1	11.25±1.78	43.38±12.34	32.13±13.26c	0.46±0.19b	49.57±8.50ab	1.85±0.91a	1.59±0.47c	1.46±0.04d	91.35±0.42b
P2	11.82±1.09	38.15±12.41	26.33± 12.18a	0.33±0.14a	46.13±9.60a	2.28±1.34ab	1.35± 0.55a	1.71±0.35ab	91.35±12.29b
P3	12.56±1.22	42.19±15.92	29.82±15.40abc	0.38±0.18a	53.98±11.35b	2.41±1.38b	1.24± 0.54abc	1.59±0.14c	89.90±9.41ab
P4	11.82±1.16	39.09±14.30	15.40± 27.27ab	0.36±0.17a	50.16±10.33ab	2.51±1.60b	1.22± 0.62ab	1.54±0.17c	84.47±18.29a
Soya bean									
S0	11.89±0.55	58.71±2.49	46.90±1.83d	0.61±0.03d	60.54±3.26c	1.29±0.01a	1.70± 0.0c	1.41±0.00ab	92.31±0.01b
S1	12.45±1.30	52.21±3.24	39.96±3.03c	0.49±0.05c	57.09±3.77c	1.43±0.13a	1.65±0.13c	1.39±0.07a	97.31±3.17b
S2	11.30±0.90	47.89±6.45	36.59±5.25c	0.50±0.09c	49.08±5.51b	1.35±0.08a	1.80± 0.16c	1.62±0.27a	93.85±4.87b
S3	11.89±1.64	42.24±6.31	30.35±7.23b	0.38±0.09a	54.99±7.07bc	1.89±0.46a	1.34± 0.35ab	1.45±0.10a	96.15±3.14b
S4	11.87±0.84	19.10±3.71	7.23±3.53a	0.12±0.05b	37.34±4.38a	6.78±5.29b	0.48± 0.22b	1.70±0.22b	77.19±15.39a

**Means with the same superscript in the same column are not significant (P>0.05)*

Table 11. Effect of groundnut and phytase on growth performance of juvenile *Clarias gariepinus*

Sources of variation, P value	Mean initial weight (g/fish)	Mean final weight (g/fish)	Mean weight gain (g/fish)	Mean feed intake (g/fish/day)	FCR	Specific growth rate (%)	Daily weight gain (g/fish)	Ca/P ratio	Survival rate (%)
Phytase	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Groundnut	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00
Phytase* Groundnut	0.00	0.00	0.001	0.00	0.00	0.00	0.00	0.00	0.00
Pooled SE	0.01	0.08	0.05	0.01	0.05	0.00	0.00	0.058	0.27
Main effects									
Phytase									
P0	12.01±1.70	38.18±12.29	26.17±13.47a	41.85±9.15c	2.13±1.49d	1.14±0.45a	0.33±0.17a	2.62±1.3e	94.68±4.68ab
P1	11.53±0.60	52.66±13.72	41.13±13.94e	44.22±5.30d	1.18±0.36a	1.56±0.33e	0.51±0.18c	2.25±0.34c	96.79±2.22b
P2	11.54±1.09	46.43±19.96	34.89±20.43d	40.54±8.23b	2.48±8.31e	1.32±0.72c	0.43±0.26b	2.07±0.14a	93.27±7.89a
P3	11.69±1.09	45.54±7.56	33.85±7.80c	40.64±3.85b	1.24±0.21b	1.41±0.21d	0.43±0.09b	2.15±0.46b	94.23±4.18a
P4	12.83±1.92	39.36±12.01	26.53± 11.95b	38.52±4.78a	1.98±1.56c	1.19±0.39b	0.33±0.14a	2.43±0.15d	93.91±7.24a
Groundnut									
G0	11.89±0.55	58.71±2.49	46.82±1.83g	60.54±3.26e	1.29±0.01d	1.90±0.01g	0.61±0.02a	1.41±0.00a	92.31±0.00b
G1	12.26±0.59	55.48±5.65	43.22±5.98f	45.10±2.00d	1.06±1.15a	1.60±0.15f	0.53±0.07d	2.44±0.02d	96.92±3.03c
G2	11.86±0.88	52.89±14.55	41.03±15.05e	44.28±5.09d	1.21±0.42b	1.54±0.38e	0.52±0.19d	2.31±0.24c	95.00±3.17bc
G3	11.99±0.64	48.51±12.58	36.52±12.37d	41.53±5.64c	1.25±0.36c	1.49±0.32d	0.45±0.16c	2.28±0.45c	96.92±2.43c
G4	11.43±15.57	42.57±7.81	28.14±8.05b	36.74±5.38a	1.36±0.28f	1.28±0.25b	0.35±0.11b	2.90±1.40e	95.38±5.06bc
G5	12.34±0.57	42.42±11.25	30.08±11.05c	37.21±6.26a	1.33±0.31e	1.31±0.29c	0.37±0.13b	1.97±0.23b	96.54±2.84c
G6	12.06±1.00	24.04±10.62	11.98±10.62a	38.39±4.18b	2.06±0.35g	0.59±0.48a	0.16±0.13a	2.16±0.19bc	87.30±8.51a

**Means with the same superscript in the same column are not significant (P>0.05)*

Table 12. Effect of soya bean and phytase on dietary amino acid profile of juvenile *Clarias gariepinus*

Sources of variation, P value	**Lysine (%)**	**Histidine (%)**	**Arginine (%)**	**Threonine (%)**	**Cysteine (%)**	**Methionine (%)**	**Isoleucine (%)**	**Leucine (%)**	**Phenylalanine (%)**
Phytase	0.001	0.001	0.001	0.002	0.002	0.001	0.001	0.002	0.001
Soya bean	0.000	0.011	0.002	0.010	0.001	0.001	0.002	0.000	0.001
Phytase* Soya bean	0.003	0.005	0.001	0.003	0.001	0.000	0.001	0.003	0.001
Pooled SE	0.003	0.003	0.002	0.002	0.002	0.001	0.002	0.000	0.002
Main effects									
Phytase									
P0	4.72± 0.66a	2.26±0.02c	6.35±1.48e	2.92±0.47a	0.94±0.22a	2.04±0.21d	2.81±0.23a	7.35±0.56e	4.20±0.36d
P1	5.14±0.36d	2.25±0.12d	5.50±0.43a	3.33±0.22e	1.03±0.18c	2.14±1.04e	3.33±0.35d	6.60±0.33d	4.17±0.13b
P2	5.45± 0.65e	2.38±0.16e	6.11±0.18d	3.02±0.77b	1.11±0.27e	1.36±0.60a	3.49±0.52e	6.18±1.45b	4.19±0.38c
P3	4.99± 0.69c	2.24±0.09a	5.57±0.43b	3.04±0.13c	1.05±0.32d	1.61±0.58b	3.26±0.26b	6.34±2.01a	4.07±0.07a
P4	4.97± 0.69b	2.24±0.14a	5.61±0.60c	3.05±0.30d	1.02±0.34b	1.96±0.85c	3.27±0.50c	6.40±0.79c	4.24±0.62e
Soya bean									
S0	5.24±0.02c	2.24±0.01b	4.83±0.01a	3.26±0.01d	0.79±0.02a	2.19±0.01d	2.44±0.01a	7.32±0.01e	3.88±0.02b
S1	5.61±0.28d	2.38±0.13e	5.55±0.41c	3.39±0.25e	0.95±0.18c	2.56±0.49e	3.29±0.36d	6.97±0.78d	4.21±0.10d
S2	4.88±0.68b	2.14±0.14a	5.54±0.63b	2.70±0.50a	0.79±0.21b	2.05±0.84c	3.00±0.23b	5.68±1.13a	3.76±0.23a
S3	4.32±0.26a	2.26±0.04c	6.10±0.46d	2.97±0.36b	1.14±0.25d	1.28±0.30a	3.27±0.54c	5.90±1.99b	4.13±0.23c
S4	5.30±0.48e	2.27±0.08	6.42±1.31e	3.15±0.41c	1.27±0.12e	1.37±0.32b	3.43±0.39e	6.95±0.41c	4.65±0.38e

**Means with the same superscript in the same column are not significant (P>0.05)*

Table 13. Effect of phytase and groundnut meal on dietary amino acid composition of juvenile *Clarias gariepinus*

Sources of variation, P value	Lysine (%)	Histidine (%)	Arginine (%)	Threonine (%)	Cysteine (%)	Methionine (%)	Isoleucine (%)	Leucine (%)	Phenylalanine (%)
Phytase	0.001	0.001	0.001	0.002	0.002	0.000	0.001	0.002	0.001
Groundnut	0.000	0.011	0.002	0.010	0.001	0.000	0.000	0.001	0.001
Phytase* Groundnut	0.003	0.005	0.001	0.003	0.001	0.003	0.001	0.001	0.000
Pooled SE	0.003	0.003	0.002	0.002	0.002	0.001	0.002	0.002	0.002
Main effects									
Phytase									
P0	4.19± 0.55e	2.20±0.08e	6.59±1.00c	3.02±0.19d	1.06±0.16c	2.23±0.04e	2.86±0.25c	6.51±0.66d	4.04±0.36d
P1	4.17±0.51d	2.22±0.04d	7.14±0.90e	3.05±0.17e	1.20±0.11e	2.22±0.02d	3.13±0.26e	6.63±0.43e	4.29±0.52e
P2	3.84± 0.44c	2.21±0.06c	6.81±0.46d	2.89±0.25c	1.08±0.15d	2.18±0.17c	2.91±0.15d	6.18±0.52c	3.90±0.39c
P3	3.84± 0.44b	2.19±0.09a	6.40±1.06a	2.70±0.37a	0.97±0.17a	2.16±0.10a	2.78±0.40b	5.31±1.22a	3.56±0.43a
P4	3.74± 0.78a	2.21±0.05d	6.58±1.22b	2.83±0.31b	1.00±1.00b	2.18±0.08b	2.69±0.34a	6.07±0.55b	3.84±0.64b
Groundnut (roasted)									
G0	5.24±0.02g	2.24±0.01e	4.83±0.01a	3.26±0.24g	0.79±0.02a	2.19±0.01c	2.44±0.01a	7.32±0.00g	3.88±0.02e
G1	3.87±0.53d	2.22±0.07c	6.47±0.91d	2.87±0.24d	1.09±0.15d	2.22±0.03g	2.86±0.39d	6.56±0.46e	3.85±0.34d
G2	3.98±0.28e	2.16±0.09a	6.30±0.47c	2.99±0.27e	1.00±0.21b	2.20±0.06e	2.87±0.38e	5.99±0.63c	4.14±0.46f
G3	3.74±0.36b	2.17±0.08b	6.59±0.55e	3.16±0.16f	1.08±0.17c	2.20±0.07f	2.99±0.26f	6.14±0.59d	3.82±0.48c
G4	3.55±0.31a	2.22±0.04c	6.01±0.69b	2.73±0.31a	1.00±0.17c	2.19±0.06b	2.78±0.17b	5.59±0.61a	3.52±0.27a
G5	3.80±0.58c	2.23±0.03d	7.22±0.64f	2.82±0.21c	1.08±0.17b	2.22±0.05d	3.06±0.31g	5.78±1.51b	3.73±0.55b
G6	4.59±0.48f	2.22±0.04c	7.98±0.67g	2.81±0.36b	1.19±0.15e	2.15±0.13a	2.78±0.29c	6.62±0.34f	4.49±0.51g

**Means with the same superscript in the same column are not significant (P>0.05)*

5. CONCLUSION

The research has shown that the significant reduction in phytate phosphorus and an improvement in available phosphorus, amino acids profile and growth performance of fish were achieved at the lowest level of phytase (250 FTU/g) in both diets, with an available phosphorus of between 0.75% and 0.80% (quadratic regression), which may indicate that the chemical nature and structure of phytate, rather than the concentration and location, as well as stomach pH of *Clarias gariepinus*, may influence phosphorus utilization, and hence, optimum growth response to phytase. However, several processing methods of reducing the fat levels in fish diets without reducing phytate concentration should be explored to reduce possible impact on phosphorus utilization by phytase.

ETHICAL APPROVAL

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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