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Research Article
Dietary Microbial Phytase Improves Growth, Phosphorus Digestibility and Serum Phosphorus, but not Bone Mineralization, in Juvenile *Clarias gariepinus* Fed Roasted and Oil-pressed Groundnut-Based Diet

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Abstract
The research investigated the effect of phytase in roasted groundnut meal diet on nutrient digestibility, serum parameters and bone mineralization. A total of 1612 *Clarias gariepinus* of average weight 378.56 g were stocked at 26 fish per tank and fed the phytase treated diets at 1.5% body weight. Each diet was fed to duplicate group of fish for 84 days. Significant effect of phytase (ANOVA, $p<0.05$) and interaction with groundnut meal was observed for all growth parameters (factorial, $p<0.05$). Increasing fish substitution by groundnut showed significant decline in growth performance (Duncan, $p<0.05$) with significant decline in weight gain of fish ($r^1 = 0.436$). However, regardless of fish meal substitution, dietary phytase supplementation improved growth of fish with significantly higher weight gain, daily weight gain, specific growth rate and protein efficiency compared to diets with no phytase (Tukey, $p<0.05$). Growth assessment showed that there was significant increase in weight gain, daily weight gain and feed intake with phytase addition up to 500 FTU g^{-1} in 10, 20, 30, 40 and 50% groundnut (Duncan, $p<0.05$) with the lowest growth at 1000 FTU g^{-1} phytase. Fish fed 60% groundnut meal (roasted) with phytase at 250 FTU g^{-1} (G6P1), 750 FTU g^{-1} (G6P3) and 1000 FTU g^{-1} (G6P4) had better growth performance (Duncan, $p<0.05$) compared to basal control (G6P0). increasing fish meal substitution with groundnut meal up to 50% improved survival rate compared to fish meal diet, regardless of phytase level (Tukey, $p<0.05$). Phytase addition to diet increased phosphorus digestibility (Tukey, $p>0.05$), which correlated positively with phytase level ($r = 0.144$, $p>0.05$). There was significant increase in serum phosphorus in all diets with phytase addition (Duncan, $p<0.05$) and was significantly improved by phytase ($r = 0.418$, $p<0.01$) and analyzed phytase activity ($r = 0.469$, $p<0.01$). However, significant reduction in bone mineralization with phytase to diets was observed with reduced bone phosphorus and magnesium (Tukey, $p<0.05$).

Key words: Groundnut meal, phytase, phosphorus digestibility, serum phosphorus, bone minerlization, *Claarias gariepinus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The effect of phytase on growth and nutrient utilization, mineral availability, as well as phosphorus availability has been demonstrated in several animals and fish studies with most of them of on diet based on soyabean and canola meals. In these studies, positive effect have been reported on growth (Nwanna and Schwarz, 2007; Nwanna *et al*., 2008; Van Weerd *et al*., 1999; Cheng and Hardy, 2003; Riche and Garling Jr., 2004; Yoo *et al*., 2005) and mineral availability (Nwanna *et al*., 2005; Vielma *et al*., 2000; Van Weerd *et al*., 1999; Baruah *et al*., 2007; Debnath *et al*., 2005; Liebert and Portz, 2007), phosphorus digestibility (Van Weerd *et al*., 1999; Furuya *et al*., 2001; Cheng and Hardy, 2003; Cao *et al*., 2008), nutrient retention (Cao *et al*., 2007) and protein utilization an digestibility (Schafer *et al*., 1995; Storebakken *et al*., 1998; Sugiura *et al*., 1998; Vielma *et al*., 2002, 2004). There are few studies on the effect of phytase in groundnut meal based diet in fish on phosphorus digestibility (Riche and Brown, 1996; Yan *et al*., 2002; Debnath, 2003) and growth in poultry (Biehl and Baker, 1997; Fasuyi *et al*., 2014) with all studies using groundnut cake (solvent-extracted). There are no studies on the effect of phytase on growth, nutrient digestibility with reference to phosphorus digestibility and other physiological parameters such as serum and bone mineralization in heat-treated groundnut meal (roasted) groundnut.

Groundnut has been used sparingly to replace fish meal at low levels of between 15-25% for catfish (Robinson *et al*., 2001) and tilapia (Jackson *et al*., 1982; Yildirim *et al*., 2014) due to its low amino acid profile (Jackson *et al*., 1982; Eyo and Olatunde, 1998) presence of antinutrient, particularly phytate (Kumar *et al*., 2012). Moreover, phytate is located in different position in groundnut meal, compared with soyabean, hence, the study on the effect of phytase on nutrient digestibility, bone mineralisation and serum serum parameters with reference to phosphorus status in the fish fed groundnut-meal based diets was carried out.

MATERIALS AND METHODS

The trial assessed the effects of five phytase supplemented at graded levels of 0 FTU g^{-1} (P0), 250 FTU g^{-1} (P1), 500 FTU g^{-1} (P2), 750 FTU g^{-1} (P3) and 1000 FTU g^{-1} (P4) in graded levels of 10% (G1), 20% (G2), 30% (G3), 40% (G4), 50% (G5) and 60% (G6) groundnut meal diet in a 5×6 factorial experimental design. All phytase-treated diets were fed to duplicate group of juvenile *Clarias gariepinus*.

Experimental diet: Six isonitrogenous (40% crude protein) and iso-caloric (17.69 kJ g^{-1} GE) experimental diets with no added in both inorganic phosphorus and amino acid supplements were formulated using roasted-oil-pressed groundnut meal (dehulled) as the main plant protein source to replace fish meal at 10, 20, 30, 40, 50 and 60% of groundnut meal. Another six groups of the same dietary composition as the basal diet were formulated and supplemented with phytase at 250 FTU g^{-1} (P1), 500 FTU g^{-1} (P2), 750 FTU g^{-1} (P3) and 1000 FTU g^{-1} (P4). A fish meal control diet (G0) was included among experimental diet, but with no phytase supplementation (P0) to compare performance with all phytase diets in terms of growth and nutritional performance, nutrient utilization and bone composition of juvenile *Clarias gariepinus*. About 20 kg of raw groundnuts was purchased from a reliable market source with no trace of contamination and subjected to heat treatment by roasting at a temperature of 70°C, after which they were grinded using a manually operated grinding machine. All grinded meals were packed in a large sack, which allowed free flow of air and moisture to allow for easy passage of oil and squeezed (oil-pressed) several times before being stored in a cool, dried place in a process to further reduce the level of oil before mixing with other ingredients, which were equally grinded, packed into plastic bags and stored at ambient temperature prior to inclusion in the formulation with other feed stuff for the fish (Cao *et al*., 2008). All diets were formulated without added inorganic phosphorus and amino acid supplements to optimize phytate hydrolysis in the diets. Chromic oxide was added at 0.50% (NRC., 1993) for nutrient digestibility. Pearson's method of diet formulation was used to formulate and prepare dietary proportions, which was subsequently mixed in a large bowl with clean cold water and cold-pelleted using a sieve of mesh size 2 mm to produce a noddle-like strand of feed. Pelleted feeds were sun-dried and packed air-tight polythene bags before use. Gross and proximate composition of the compounded diets were sampled duplicate per treatment combination and analysed for proximate analysis determined by method of AOAC (2006), phytate phosphorus (Oberleas, 1973), phytase activity (BASF., 1997), total phosphorus and calcium (AOAC., 2006) to conform dietary levels of these parameters that influence the efficacy of phytase addition in the experimental diets determined before and after supplementation with graded levels of liquid phytase at 0, 250, 500, 750 and 1000 FTU g^{-1} (Natuphos 5000 L, BASF, Germany). The proximate and mineral of raw and roasted groundnut meal are shown in Table 1 and 2, while the gross and nutrient composition of the basal diets based on roasted and oil-pressed groundnut meal is shown in Table 3.

Preparation of phytase solutions: The different phytase levels were prepared from a stock solution as follows.

A stock solution per tonne of feed containing 500 FTU g^{-1} phytase is prepared using a dilution ratio of 1:10. The amount of phytase required for a tonne of feed is equivalent to: 500 FTU (final activity)×1000 (for a ton of feed)/5000 FTU (initial activity of Natuphos 5000 L) = 100 g = 100/1.2 = 83.33 mL phytase.

About 1.20 g cm^{-3} is the bulk density of Natuphos 5000 L phytase (BASF., 2014), which ranges between 1.15-1.25 g cm^{-3}. Using the above dilution, stock solution contained 83.33 phytase+ 900 mL water = 983.33 mL from which 0.4917, 0.9833, 1.475 and 1.967 mL kg^{-1} diets were taken with a 1 mL syringe for 250, 500, 750 and 1000 FTU g^{-1} per kilogram diet, respectively. The equivalent of the different phytase levels were calculated using the same dilution factor.

Experimental fish: About 1612 *Clarias gariepinus* of average weight 4.5±0.2 g acclimated to laboratory conditions for 3 weeks with water temperature, pH and oxygen maintained at optimum range between 25-32°C, 7.40-7.45, 4.80-5.0 mg L^{-1}, After acclimation, all 1612 juvenile fish of average weight (378.56 g) and length 11.79±1.02 cm were randomly allocated to each of six treatment groups (including negative controls) of phytase (0, 250, 500, 750 and 1000 FTU g^{-1}) supplemented roasted, oil-pressed diet. Fish fed roasted, oil-pressed diets were stocked at 26 fish/tank (4 fish m^{-2}) with tank dimension and volume 0.43 m×0.25 m×0.265 m and 28, 917.50 cm^3, respectively fed the phytase treated diet. All fish were fed at 3% body weight for the first four weeks and subsequently, the feeding rate was reduced to 1.5% body weight for the remaining eight weeks (84 days). Each of the feed rations allocated for both forms of groundnut meal was shared so that experimental fish were fed twice daily (morning and evening) to obtain optimum nutrient utilization with uneaten feed and faeces removed 6 h after feeding period and freeze-dried at -20°C for digestibility studies, which was carried out using 0.50% chromic oxide as indigestible marker (NRC., 1993). In order to avoid buildup of nitrogen in form of ammonia resulting from high protein feed

Table 1: Proximate composition of raw and processed groundnut meal for *Clarias gariepinus*

Ingredient	Crude protein (%)	Fat (%)	Ash (%)	Moisture (%)	Crude fibre (%)	NFE (%)	Energy (kcal/100 g)
Gnm (raw)	25.13	50.61	6.65	5.18	8.47	3.96	504.43
Gnm (roasted, oil-pressed	36.37	45.03	7.86	4.12	4.45	2.17	563.559

Table 2: Mineral composition of raw and processed groundnut meal (Gnm) for *Clarias gariepinus*

Ingredient	Phosphorus (%)	Calcium (%)	Magnesium (%)	Potassium (%)	Sodium (%)	Manganese (%)	Iron (ppm)	Copper (ppm)	Zinc (ppm)
Gnm (raw)	0.37	0.07	0.14	0.86	0.13	42.62	103.08	4.08	19.76
Gnm (roasted)	0.36	0.04	0.16	1.00	1.91	77.58	260.34	4.34	24.16

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

Ingredient	G_0 (0%)	G_1 (10%)	G_2 (20%)	G_3 (30%)	G_4 (40%)	G_5 (50%)	G_6 (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
$CaCO_3$	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
Chemical composition (%)							
Phytate	0.40	0.25	0.58	0.56	0.42	0.45	0.23
Phosphorus	1.31	0.99	0.97	0.89	0.86	0.79	0.79
Available phosphorus	0.91	0.75	0.39	0.34	0.45	0.34	0.57
Calcium	1.85	2.61	2.44	1.84	4.74	1.71	1.62
Magnesium	0.25	0.12	0.11	0.11	0.50	0.15	0.14
Potassium	0.87	1.09	1.08	1.10	1.11	1.16	1.12
Native phytase (FTU g^{-1})	<100.00	60.00	50.00	70.00	60.00	50.00	50.00

[#]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 (0.15 mg), antooxidant (80 mg), methionine (100 mg), *As carboxymethyl cellulose

in the experimental feeds, water in experimental tanks was changed every day using static water renewal method and water quality monitored for all treatment tanks at the end of the experiment.

Growth and nutrient utilization: Growth performance was monitored biweekly with the following parameters measured:

$$\text{Weight gain} = \text{Final weight-Initial weight}$$

Feed conversion ratio (FCR):

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Fish weight gain (g)}}$$

Specific growth rate (%):

$$\text{SGR} = \frac{\text{In (W2-W2)}}{\text{(t2-t1)}}$$

Where:
W2 = Final weight
W1 = Initial weight
t2 = Time at the end of experiment
t1 = Time at start of experiment

Protein Efficiency Ratio (PER):

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein Intake (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Initial number of fish-Mortality}}{\text{Initial number of fish}} \times 100$$

Digestibility studies

Faecal collection: Indirect method of determination of digestibility was used in this experiment with the addition of 0.5% chromic oxide as indigestible marker (NRC., 1993). During the feeding experiment and two 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), all uneaten dissolved feed remains were removed from the tanks using rubber hose and faeces collected using filter paper. Then the following morning from 08:00 h, faeces were collected from each of the tanks before the next feeding (Nwanna *et al*., 2008). Faecal samples collected from all experimental diets were freeze-dried at -20°C (Nwanna, 2007).

After freeze drying, faeces were be analysed for chromic oxide, protein, lipid, energy (Model 6200 microprocessor-controlled isoperibol oxygen bomb calorimeter) and phosphorus (AOAC., 2006).

Apparent digestibility coefficient: Apparent Digestibility Coefficient (ADC) for protein, energy, lipid and phosphorus was determined using indirect method with diets containing chromic oxide Cr_2O_3 described by NRC (1993) and given as:

$$\text{ADC nutient} = 100 - 100\left\{\left(\frac{Cr_2O_3\,(\%)\text{ in diet}}{Cr_2O_3\,(\%)\text{ in feaces}}\right)\times\left(\frac{\text{Nutrient in feaces}}{\text{Nutrient in diet}}\right)\right\}$$

Proximate and mineral analysis: Duplicate samples of each diet used in both experiments were determined for proximate composition by method of AOAC (2006). For moisture, sample was mixed the sample thoroughly and water content determined by weighing out 2.5 g into silica dish, which has been previously dried and weighed. The dish including the sample inside it was placed in hot air oven for 24 h at 60-70°C (drying at high temperature may result in losses of heat labile or volatile component). Finally, the sample was dried to constant weight, cooled for 10 min in a desiccator each time before weighing. Dried portion was subsequently used for the determinations of protein, ash, fat and crude fibre. Nitrogen determination for crude protein estimation was done by micro Kjeldahl method, while fat was estimated using soxhlet extraction. Crude fibre was measured by trichoriacetic acid method of Zarrow and Shay (1945). The residue from the moisture determination in the muffle furnace was charred between 500-600°C for 12 h until the ash is grey or nearly white. Sample was allowed to cool and weight taken. Model 6200 microprocessor-controlled isoperibol oxygen bomb calorimeter was used for the calorific tests (AOAC., 2006). Mineral content of feed was determined using atomic absorption spectrophotometry (Model: Buck 205, Buck Scientific, USA). Phosphorus was determined by method of AOAC (1990).

Determination of phytate and phosphorus availability: Duplicate sample of each diet was determined for phytate measured by alkaline picrate method of Oberleas (1973). Sample was extracted with 0.2 N HCl to give (3-30 μg mL^{-1} phytate solution). There was 0.5 mL of extract pipetted into a test tube fitted with a ground glass stopper. One milliliter of ferric solution was added to the tube, which was covered with the stopper and fixed with a clip. The tube was heated in a

boiling water bath for 30 min. Care was taken to ensure that for the first 5 min, the tube remained well stoppered. After cooling in ice water for 15 min, 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 min at 3000 g. One milliliter of the supernatant was transferred to another test tube and 1.5 mL of 2,2'-Bipyridine solution added. The absorbance was measured at 519 nm against distilled water. Phosphorus availability from phytase was determined by Cao *et al*. (2008) as follows:

$$\text{Phosphorus availability/released} = \frac{\text{Initial phytate-Final phytate}}{\text{Initial phytate}} \times 100$$

Phytase activity analysis: Phytase activity in feed samples were determined by Engelen *et al*. (1994) with slight modifications by BASF (1997).

Acetic buffer: There were 0.100 g Tween 20, 10.148 g calcium chloride and 30.02 g sodium acetate weighed into a glass beaker and rinsed with distilled water quantitatively into a 1 L graduated flask. The volume was made up almost to the mark and after dissolution was complete, the pH was adjusted to 5.50-0.05 with acetic acid and the volume was made up to the mark with distilled water. The acetate buffer was kept prior to use.

Ammonium heptamolybdate solution: There were 100.00 g ammonium heptamolybdate weighed into a glass beaker and transferred with about 900 mL distilled water into a 1 L graduated flask. After dissolving in an ultrasonic bath, 10 mL ammonia are pipetted in and the volume made up to the mark with distilled water.

Ammonium vanadate solution: There were 2.35 g ammonium vanadate weighed into a 1 L graduated flask and dissolved completely in distilled water by stirring at about 60°C. Twenty milliliters dilute nitric acid was pipetted into the stirred solution. After cooling to room temperature, the volume was made up to the mark with distilled water.

Stop reagent: There were 250 mL ammonium heptamolybdate solution and 250 mL ammonium vanadate solution measured out in a measuring cylinder and rinsed with distilled water into a 1 L graduated flask. Then 165 mL nitric acid was added and mixed. After cooling to room temperature, the volume was made up to the mark with distilled water. The amount of stop reagent required each day was prepared freshly.

Sodium phytate solution: An appropriate amount of sodium phytate was weighed with an accuracy of 0.1 mg into a tared Erlenmeyer flask and dissolved in acetate buffer using an ultra-sonic bath and the same buffer was used to make up almost to the required total weight. While stirring, the pH was adjusted to 5.50±0.03 with acetic acid. Acetate buffer was then used to make up to the total weight with an accuracy of 10 mg. The solution was prepared freshly each day for every analysis.

Procedure

Sample preparation: 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. There were 50.00 mL acetate buffer 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 min. The centrifugate was then diluted with buffer using the dilutor to a content of about 0.02 FTU mL^{-1}. Two milliliters of each of the two solutions was pipetted as sample and sample blank into a 10 mL centrifuge tube.

Reagent blank: For the blank, 2.00 mL portions of acetate buffer are pipetted into two 10 mL centrifuge tubes. One centrifuge tube was incubated and the other centrifuge tube was treated in analogy to the blanks.

Enzyme and sample blank: The blanks were mixed successively with 4.00 mL stop reagent and sodium phytate solution (equilibrated at 37.0±0.1°C). After waiting for at least 10 min, the solutions were centrifuged at 4000 rpm (equivalent to about 2500 g) for 20 min and then the absorbance at a wavelength of 415 nm was measured in a spectrophotometer against air.

Incubation: 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 min and then the absorbance at a wavelength of 415 nm was measured in a spectrophotometer against air. The enzyme phytase liberates inorganic phosphate from the substrate sodium phytate during incubation and the intensity of the yellow color of the vanadomolybdo-phosphorus complex is a measure of the amount of phosphate liberated.

Serum analysis: At the beginning (initial blood sampling) and end of each experiment, blood samples was taken from sampled fish from each treatment: 2 fish per tank per treatment combination in all experiments were sampled at 0 and 84 days. Blood was sampled ventrally from fish kidney using a 2 mL hypidermal syringe. After centrifugation, serum component of blood was investigated for analysis. Serum calcium, phosphorus, total protein, albumin and glucose were analysed by using Randox® kits (Randox® Laboratories, County Altrim, UK). Sodium was analysed using Teco Diagnostic kits (Teco Diagnostics, Anaheim, USA).

Bone and body mineral analysis: Duplicate samples of spinal, skull, pectoral, dorsal and caudal bones from each fish were stripping from flesh and pooled together for each fish, oven-dried, pulverized, weighed, ashed at 550°C for 6 h and digested in a boiling nitric acid and perchloric acid mixture (AOAC., 1990). Samples of whole body (carcass) and bones were analysed for calcium, magnesium, potassium, zinc content by atomic absorption spectrophotometer using flame atomic absorption spectrophotometer model Buck 205, Buck Scientific, USA, while phosphorus was estimated spectrophotometrically using molybdovanadate method (AOAC., 1990).

Statistical analysis: All data were subjected to one-way analysis of variance at significance level of $p<0.05$. Individual differences in treatment means were determined by Duncan new multiple range test (Duncan, 1955). Interactions between dietary levels of phytase and experimentals based on roasted groundnut meal-based diets were determined using factorial analysis). Tukey HSD test of comparison was used to detect mean differences between mean pair for growth, nutrient digestibility and bone mineralization. Relationship between phytase, nutrient digestibility and serum parameters was determined by correlation analysis. All data were treated as Mean±SD. A dose-response data or growth model (Belal, 2005) of graded phytase inclusion was determined by a linear equation:

$$Y = a0+a1X$$

RESULTS

The result of the experiment showed significant effect of phytase on growth (Table 4), phosphorus digestibility (Table 5), bone mineralization and serum phosphorus (Table 6 and 7).

Growth and nutrient utilization: A significant effect of experimental diet (ANOVA, $p<0.05$) and interaction on growth parameters of experimental fish was observed in this study (factorial, $p<0.05$). Growth data (Table 8 and 4) showed there was significant decline in weight gain in experimental diet with increasing fish meal substitution by groundnut meal with or without phytase. However, significant improvement in weight gain, feed intake, feed conversion ratio, specific growth rate, protein efficiency ratio and daily weight gain was observed in fish, regardless of groundnut meal level (Tukey, $p<0.05$). Weight gain and feed intake increased significantly in G1P1, G1P2, G1P4, G2P1, G2P2, G2P3, G3P1, G3P2 and G3P3 (Duncan, $p<0.05$). In 40% groundnut with phytase, fish fed G4P1 had the highest weight gain compared to G4P0, G4P2, G4P3 and G4P4 (Duncan, $p<0.05$). In fish fed 60% groundnut meal with phytase, weight gain was higher in G6P3 compared to basal control (G6P0) and other phytase diet (Duncan, $p<0.05$). Resluts in Table 8 indicate that fish fed G6P2 had the lowest weight gain (-39.81±4.70 g), while G6P3 had the highest (644.815±3.42 g). In fish fed 50% groundnut meal with phytase, G5P2 had the highest weight gain and lowest feed conversion ratio (Duncan, $p<0.05$) compared to G5P0, G5P1, G5P3, G5P4 and fish meal control (G0P0). The highest weight gain in experimental fish was in G2P1 (1533.46±10.36 g). The G2P1 had the lowest (0.87±0.01), compared to G2P0 (1.95±0.01), G2P2 (0.96+0.01), G2P3 (0.96±0.01) and G2P4 (1.34±0.01). The G3P1 had the highest compared to G3P0, G3P2, G3P3 and G3P4 (Duncan, $p<0.05$). Values of FCR declined in fish fed 40% groundnut with phytase. Protein efficiency and specific growth rate showed values increased in G1P0-G5P0 with phytase except G6P0, which showed the lowest PER and SGR compared to G6P0, G6P1, G6P2 and G6P4 (Table 8). Figure 1-6 growth performance of fish fed experimental diet, while the

Table 4: Effect of phytase and groundnut meal on growth performance of juvenile *Clarias gariepinus*

Sources of variation p-value	Final weight (g)	Weight gain (g)	Feed intake (g)	FCR	Specific growth rate (%)	Protein efficiency ratio	Daily weight gain (g/fish/day)	Survival rate (%)
Phytase	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Groundnut	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phy*Grnut	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pooled SE	1.722	1.199	2.127	0.052	0.010	0.012	0.001	0.271
Tukey HSD (mean, p-value)								
Phytase								
P0 vs P1	-376.55*, 0.000	-388.57*, 0.000	-60.49*, 0.000	0.94*, 0.000	-0.42*, 0.000	-0.69*, 0.000	-0.19*, 0.000	-2.01, 0.142
P0 vs P2	-214.52*, 0.000	-226.40*, 0.000	35.19*, 0.000	4.60*, 0.000	-0.18*, 0.000	0.40*, 0.000	-0.10*, 0.000	1.51, 0.389
P0 vs P3	-191.34*, 0.000	-199.44*, 0.000	32.67*, 0.000	0.89*, 0.000	-0.27*, 0.000	-0.50*, 0.000	-0.10*, 0.000	0.55, 0.964
P0 vs P4	-4.76, 0.897	-12.84*, 0.013	87.75*, 0.000	0.15*, 0.881	-0.04*, 0.000	-0.12*, 0.019	-0.01, 0.540	0.87, 0.835
P1 vs P2	162.03*, 0.000	162.17*, 0.000	95.68*, 0.000	3.66*, 0.000	0.24*, 0.000	0.29*, 0.000	0.08*, 0.000	3.53*, 0.003
P1 vs P3	185.21*, 0.000	189.13*, 0.000	93.16*, 0.000	-0.66*, 0.995	0.15*, 0.000	0.20*, 0.000	0.08*, 0.000	2.56*, 0.044
P1 vs P4	371.79*, 0.000	375.73*, 0.000	148.23*, 0.000	-0.80*, 0.000	0.38*, 0.000	0.57*, 0.000	0.18*, 0.000	2.88*, 0.018
P2 vs P3	23.18*, 0.002	26.96*, 0.000	-2.52, 0.996	-3.72*, 0.000	-0.09*, 0.000	-0.09*, 0.126	0.00, 1.000	-0.96, 0.802
P2 vs P4	209.76*, 0.000	213.56*, 0.000	52.55*, 0.000	-4.45*, 0.000	0.14*, 0.000	-0.28*, 0.000	0.10*, 0.000	-0.64, 0.946
P3 vs P4	186.58*, 0.000	186.60*, 0.000	55.07*, 0.000	-0.74*, 0.001	0.23*, 0.000	0.38*, 0.000	0.09*, 0.000	0.32, 0.996
Groundnut								
G0 vs G1	6.02*, 0.997	95.84*, 0.000	401.55*, 0.000	0.23, 0.990	0.30*, 0.000	-0.40*, 0.000	0.08*, 0.000	-4.61, 0.109
G0 vs G2	73.53*, 0.000	152.89*, 0.000	422.76*, 0.000	0.08, 1.000	0.36*, 0.000	-0.45*, 0.000	0.09*, 0.000	-2.69, 0.665
G0 vs G3	213.25*, 0.000	269.95*, 0.000	494.28*, 0.000	0.04, 1.000	0.41*, 0.000	-0.31*, 0.003	0.16*, 0.000	-4.61, 0.109
G0 vs G4	419.86*, 0.000	487.89*, 0.000	618.87*, 0.000	-0.07, 1.000	0.62*, 0.000	-0.12, 0.646	0.26*, 0.000	-3.07, 0.519
G0 vs G5	371.57*, 0.000	437.62*, 0.000	606.51*,0.000	-0.04, 1.000	0.59*, 0.000	-0.19, 0.157	0.24*, 0.000	-4.22, 0.173
G0 vs G6	797.47*, 0.000	903.54*, 0.000	575.90*,0.000	2.48*, 0.000	1.30*, 0.000	0.96*, 0.000	0.45*, 0.000	5.00, 0.065
G1 vs G2	67.50*, 0.000	57.05*, 0.000	21.21*,0.101	-0.15, 0.980	0.06*, 0.000	-0.05, 0.891	0.01, 0.095	1.92, 0.423
G1 vs G3	207.22*, 0.000	174.11*, 0.000	92.72*,0.000	-0.19, 0.946	0.11*, 0.000	0.09, 0.315	0.08*, 0.000	0.00, 1.000
G1 VS G4	413.84*, 0.000	392.04*, 0.000	217.32*,0.000	-0.30, 0.645	0.32*, 0.000	0.28*, 0.000	0.18*, 0.000	1.54, 0.673
G1 vs G5	365.54*, 0.000	341.78*, 0.000	204.95*,0.000	-0.27, 0.753	0.29*, 0.000	0.21*, 0.000	0.16*, 0.000	0.39, 1.000
G1 vs G6	791.45*, 0.000	807.70*, 0.000	174.35*,0.000	2.24*, 0.000	1.00*, 0.000	1.36*, 0.000	0.37*, 0.000	9.62, 0.000
G3 vs G4	206.62*, 0.000	217.93*, 0.000	124.60*,0.000	-0.12, 0.995	0.21*, 0.000	0.19*, 0.002	0.100*, 0.000	1.54, 0.674
G3 vs G5	158.32*, 0.000	167.67*, 0.000	112.23*,0.000	-0.09, 0.999	0.18*, 0.000	0.12, 0.099	0.08*, 0.000	0.38, 1.000
G3 vs G6	584.23*, 0.000	633.59*, 0.000	81.62*,0.000	2.24*, 0.000	0.90*, 0.000	1.27*, 0.000	0.29*, 0.000	9.62*, 0.000
G4 vs G5	-48.29*, 0.000	-50.26*, 0.000	-12.37*,0.651	0.03, 1.000	-0.04*, 0.000	-0.07, 0.655	-0.02, 0.063	-1.16, 0.884
G4 vs G6	377.61*, 0.000	415.66*, 0.000	-42.97*,0.000	2.55*, 0.000	0.68*, 0.000	1.08*, 0.000	0.20*, 0.000	8.08*, 0.000
G5 vs G6	425.91*, 0.000	465.91*, 0.000	-30.61*,0.005	2.52*, 0.000	0.72*, 0.000	1.15*, 0.000	0.21*, 0.000	9.23*, 0.000

*Mean differences are significant at p<0.05

relationship between growth and phytase supplemented-groundnut meal (roasted, oil-pressed) diet, is shown in Fig. 7. Survival rate showed a significant reduction in fish fed 20 and 40% groundnut meal with phytase (Duncan, p<0.05), while there were improvement in fish survival when phytase supplemented in 50% groundnut was given (p<0.05). Additionally, increasing fish meal substitution with groundnut meal up to 50% improved survival rate compared to fish meal diet, regardless of phytase level (Tukey, p<0.05). In 60% groundnut meal with phytase, the lowest survival was in fish fed G6P2, which showed the lowest weight gain (Table 8), available phosphorus and phytase activity (Table 9). Fish fed G6P1 and G6P3 showed the highest fish survival compared to G6P0 (Duncan, p<0.05).

Nutrient digestibility: Result of statistical analysis showed there was no interaction for energy, lipid and phosphorus digestibility (factorial, p>0.05). However, phytase supplementation significantly improved crude protein, energy and lipid digestibility (Tukey, p<0.05). Improvement in phosphorus digestibility and reduction in faecal phosphorus was also observed (Tukey, p>0.05). Crude protein digestibility showed significant improvement in all diets compared to G0P0 (Duncan, p<0.05). The highest value of protein digestibility was observed in at G6P1 (Duncan, p<0.05). Energy digestibility improved significantly in all diets with phytase addition, except in 10% groundnut meal (Duncan, p>0.05), while lipid digestibility declined in diets based on 50 and 60% groundnut meal when phytase was supplemented in the diets (Duncan, p<0.05). Phosphorus digestibility showed improvement with phytase addition (p>0.05), but values were lower at 1000 FTU g^{-1} compared to other phytase levels in diets based on 20, 30, 40, 50 and 60% groundnut meal (Duncan, p>0.05).

Table 5: Nutrient digestibility of groundnut meal diet (roasted, oil-pressed) with phytase

Treatment	Crude protein (%)	Energy (%)	Lipid (%)	Phosphorus (%)	Phosphorus released (%)
G0P0	89.91±0.01^{a}	86.51±0.01^{a}	76.24±0.01^{e}	89.91±0.01^{b}	
G1P0	91.80±0.01^{l}	87.70±0.01^{m}	75.97±0.01^{c}	91.08±0.01^{b}	
G1P1	91.63±0.01^{g}	87.45±0.01^{g}	76.55±0.01^{f}	90.27±0.01^{b}	16.00
G1P2	91.70±0.01^{k}	87.54±0.01^{jk}	75.08±0.01^{a}	90.77±0.01^{b}	
G1P3	91.70±0.01^{k}	87.46±0.01^{g}	87.74±0.01^{o}	90.62±0.01^{b}	
G1P4	91.41±0.01^{d}	87.13±0.01^{e}	85.80±0.01^{k}	90.47±0.01^{b}	
G2P0	91.33±0.01^{b}	86.97±0.01^{c}	79.08±0.01^{g}	65.49±0.01^{a}	
G2P1	91.69±0.01^{jk}	87.51±0.01^{hi}	82.60±0.02^{h}	91.13±0.01^{b}	10.34
G2P2	91.70±0.01^{k}	87.55±0.01^{k}	92.66±0.01^{1}	90.75±0.01^{b}	21.15
G2P3	91.44±0.01^{e}	87.14±0.01^{e}	84.12±0.01^{i}	90.23±0.01^{b}	7.69
G2P4	91.44±0.01^{e}	87.19±0.01^{f}	75.25±0.01^{b}	89.94±0.01^{b}	10.34
G3P0	91.59±0.01^{f}	86.90±0.01^{b}	86.46±0.00^{m}	90.01±0.01^{b}	
G3P1	92.06±0.01^{t}	88.02±0.01^{t}	85.49±0.01^{j}	90.72±0.01^{b}	3.57
G3P2	92.01±0.00^{s}	88.01±0.01^{t}	94.78±0.01^{2}	90.45±0.01^{b}	33.93
G3P3	91.89±0.01^{o}	87.82±0.01^{p}	95.01±0.01^{3}	90.45±0.01^{b}	
G3P4	91.98±0.01^{r}	87.94±0.01^{s}	85.82±0.01^{k}	90.36±0.01^{b}	
G4P0	91.62±0.01^{g}	87.46±0.01^{g}	86.81±0.01^{n}	89.98±0.01^{b}	
G4P1	91.83±0.00^{mn}	87.75±0.01^{no}	89.72±0.01^{s}	90.49±0.01^{b}	40.47
G4P2	91.84±0.01^{n}	87.76±0.01^{o}	86.41±0.01^{l}	90.30±0.01^{b}	14.28
G4P3	91.68±0.01^{ij}	87.54±0.01^{jk}	88.95±0.01^{p}	90.30±0.01^{b}	
G4P4	91.96±0.01^{q}	87.93±0.01^{s}	90.70±0.01^{x}	89.89±0.01^{b}	
G5P0	91.37±0.01^{c}	86.99±0.01^{c}	89.77±0.01^{t}	88.56±0.01^{b}	
G5P1	91.67±0.01^{i}	87.49±0.01^{h}	89.84±0.01^{u}	89.89±0.01^{b}	40.00
G5P2	91.82±0.01^{m}	87.73±0.01^{n}	89.05±0.01^{q}	90.09±0.01^{b}	
G5P3	91.79±0.01^{l}	87.53±0.01^{ijk}	76.07±0.01^{d}	90.38±0.01^{b}	
G5P4	91.83±0.01^{mn}	87.67±0.01^{l}	89.11±0.01^{r}	89.07±0.44^{b}	
G6P0	91.41±0.01^{d}	87.06±0.01^{d}	90.61±0.01^{w}	88.76±0.01^{b}	
G6P1	92.10±0.01^{u}	88.15±0.01^{u}	91.60±0.01^{y}	88.94±0.01^{b}	
G6P2	92.01±0.01^{s}	87.89±0.01^{r}	91.67±0.01^{z}	88.89±0.01^{b}	
G6P3	91.92±0.01^{p}	87.86±0.01^{q}	90.69±0.01^{x}	89.06±0.01^{b}	
G6P4	91.65±0.01^{h}	87.52±0.01^{ij}	90.43±0.01^{v}	85.38±0.01^{b}	
Total	91.67±0.39	87.52±0.38	86.13±6.02	89.11±6.37^{b}	

Mean values (n = 2) with the same alphabet superscript in the same column are not significantly different at the 0.05 level (2-tailed)

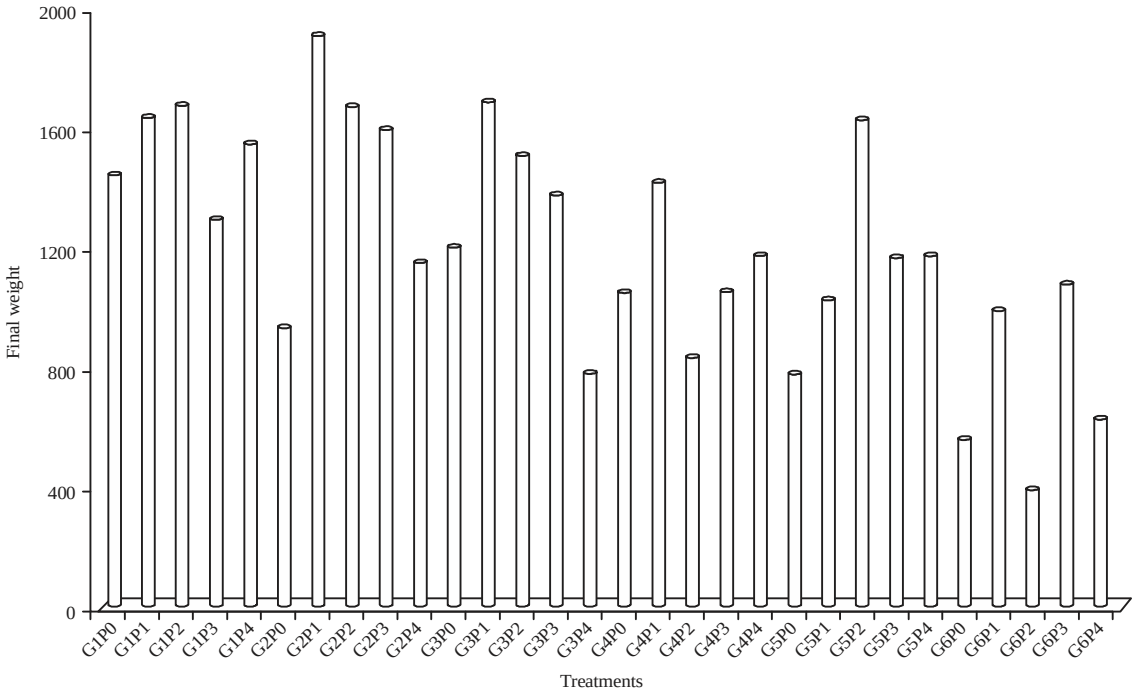

Fig. 1: Final weight performance of fish fed groundnut meal diet (roasted, oil pressed) with phytase

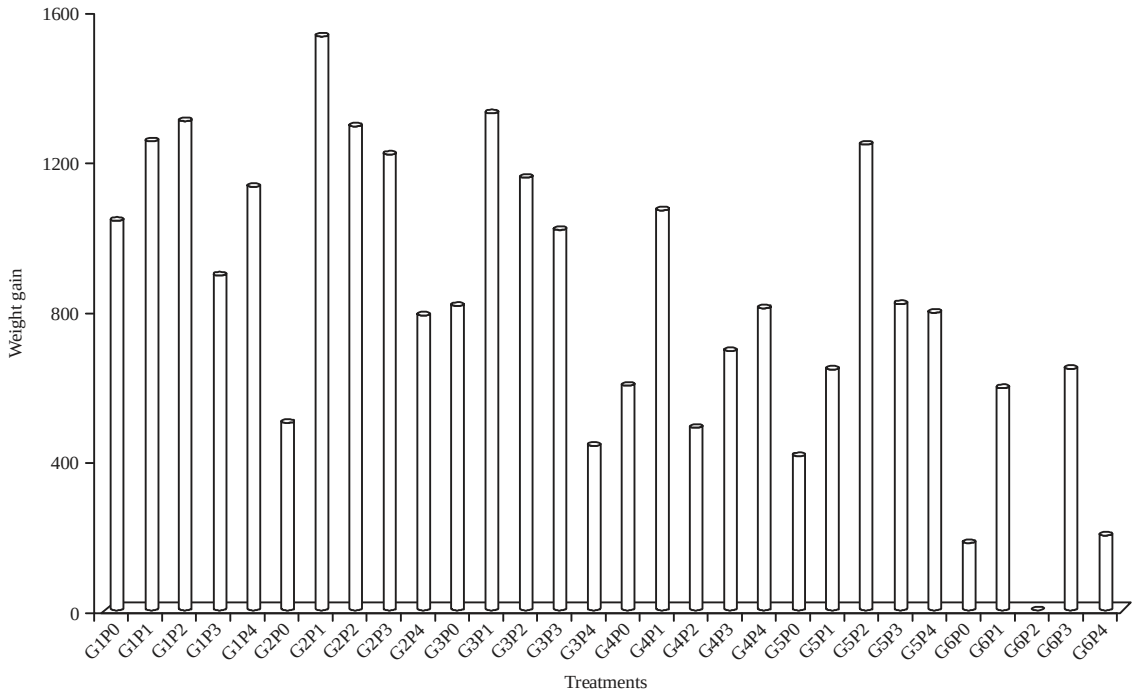

Fig. 2: Weight gain performance of fish fed groundnut meal diet (roasted, oil pressed) with phytase

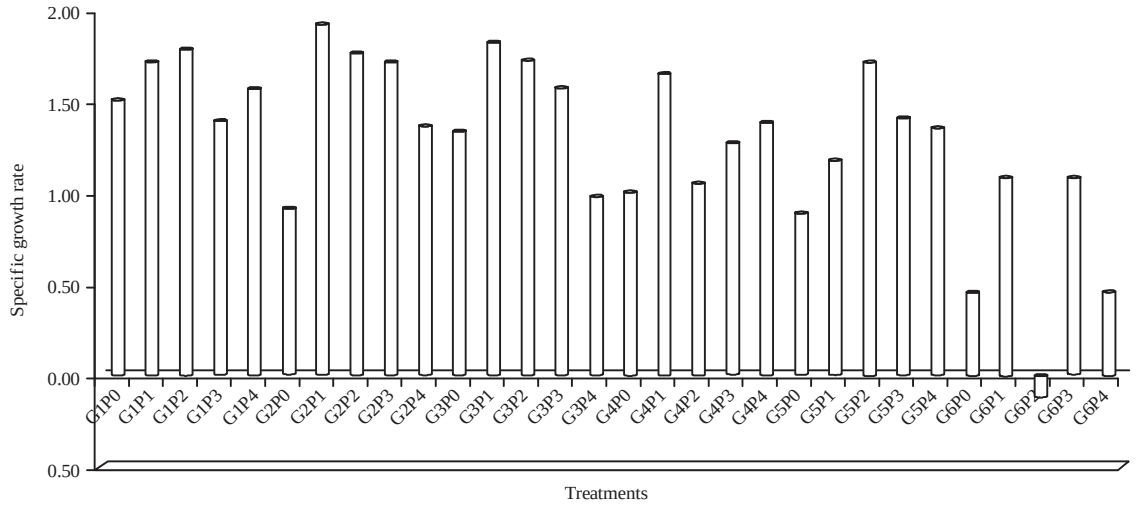

Fig. 3: Specific growth rate performance of fish fed groundnut meal diet (roasted, oil pressed) with phytase

Improvement in phosphorus digestibility (DPHOSP) showed a positive correlation (Table 7) with treatment (r = 0.0162, p>0.05), phytase level (r = 0.144, p>0.05), analyzed phytase activity, APA (r = 0.143, p>0.05), protein digestibility, DCP (r = 0.122, p>0.05), energy digestibility, DENG (r = 0.197, p>0.05) and fat digestibility, DFAT (r = 0.094, p>0.05) with a corresponding reduction in faecal phosphorus as seen the negative relationship with treatment (r = -0.0532, p>0.05), phytase (r = -0.220, p>0.05), analyzed phytase activity (r = -0.206, p>0.05), protein digestibility (r = -0.103, p>0.05), energy digestibility (r = -0.099, p>0.05) and fat digestibility. Table 10 shows the relationship between phosphorus digestibility with other parameters.

Serum biochemistry: Serum analysis (Table 6) showed phytase addition to groundnut meal based diets increased significantly values of glucose, sodium and phosphorus (ANOVA, p<0.05). However, serum glucose showed significant reduction in fish fed 30 and 60% groundnut meal with increasing phytase addition (Duncan, p<0.05). In G5P2 had the

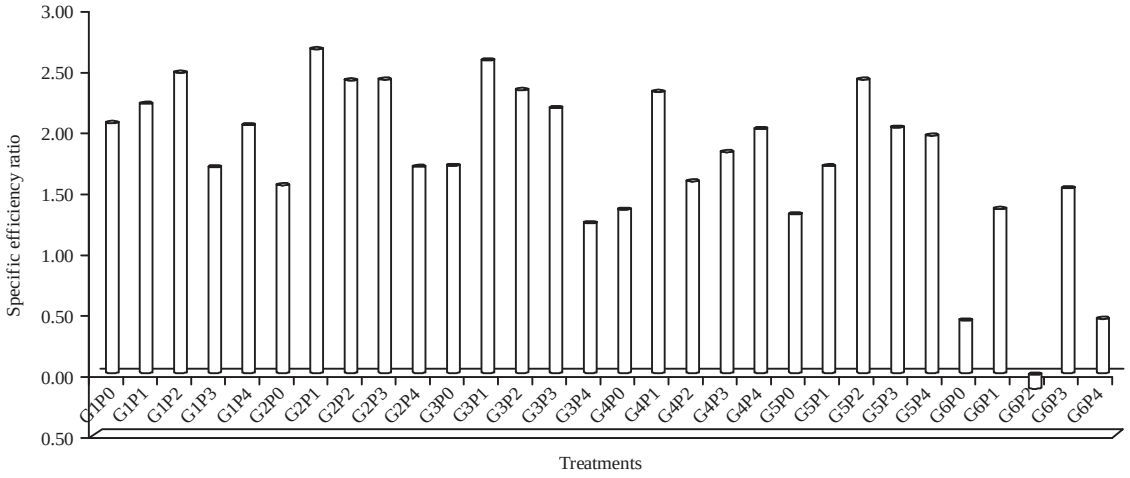

Fig. 4: Protein efficiency ratio performance of fish fed groundnut meal diet (roasted, oil pressed) with phytase

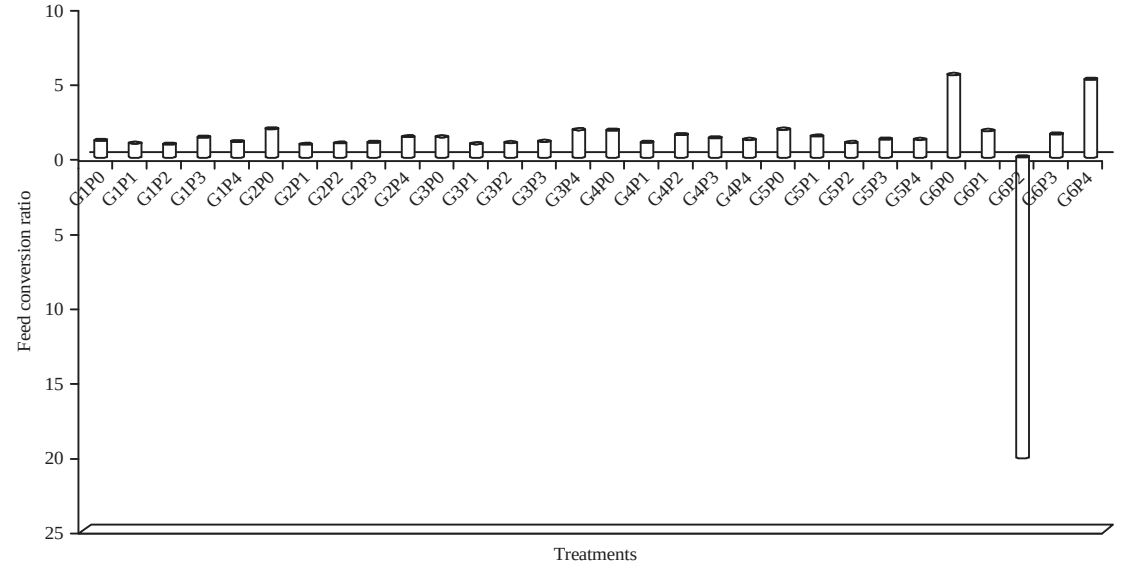

Fig. 5: Feed conversion ratio performance of fish fed groundnut meal diet (roasted, oil pressed) with phytase

highest serum glucose (335.0±1.41mg dL^{-1}) compared to G5P0 (241.5±0.71 mg dL^{-1}), G5P1 (163.5±0.71 mg dL^{-1}), G5P3 (109.5±0.71 mg dL^{-1}) and G5P4 (198.5±0.71 mg dL^{-1}). Values of serum phosphorus showed significant increase in fish fed all groundnut meal diet (Duncan, $p<0.05$), however levels of phytase at 1000 FTU g^{-1} were lower in 30% (G3P0), 50% (G5P0) and 60% (G6P0) groundnut compared to basal controls (Duncan, $p<0.05$). However, serum phosphorus is strongly and positively correlated (Table 7) with phytase level ($r = 0.418$, $p<0.01$) and analyzed phytase activity, APA ($r = 0.469$, $p<0.01$). There is a significant reduction in serum calcium of fish all groundnut meal diet with phytase addition ($p<0.05$). Table 6 shows values of serum parameters in fish fed groundnut-based diet with phytase supplementation.

Bone mineralization: A significant effect of phytase (ANOVA, $p<0.05$) and interaction with groundnut meal was detected for bone phosphorus, calcium, magnesium, iron and zinc (factorial, $p<0.05$). There were significant reduction in bone phosphorus with phytase addition to groundnut meal diet (ANOVA, $p<0.05$), except for diet based on 60% groundnut meal (Duncan, $p<0.05$). Values reduced significantly with phytase at 1000 FTU g^{-1} compared to diets without phytase in fish fed 10, 20, 30, 40 and 50% groundnut meal (Duncan, $p<0.05$). In fish fed 50% groundnut meal, G5P1 fish had the highest bone phosphorus (5.97±0.01%) compared with G5P2 (4.13±0.01%), G5P3 (4.63±0.01%) and G5P4 (2.99±0.01%) (Duncan, $p<0.05$). Phosphorus released/availability (Table 5) and increased serum phosphorus (Table 10) did not translate to increased bone phosphorus in fish fed G1P1 (5.36±0.01%)

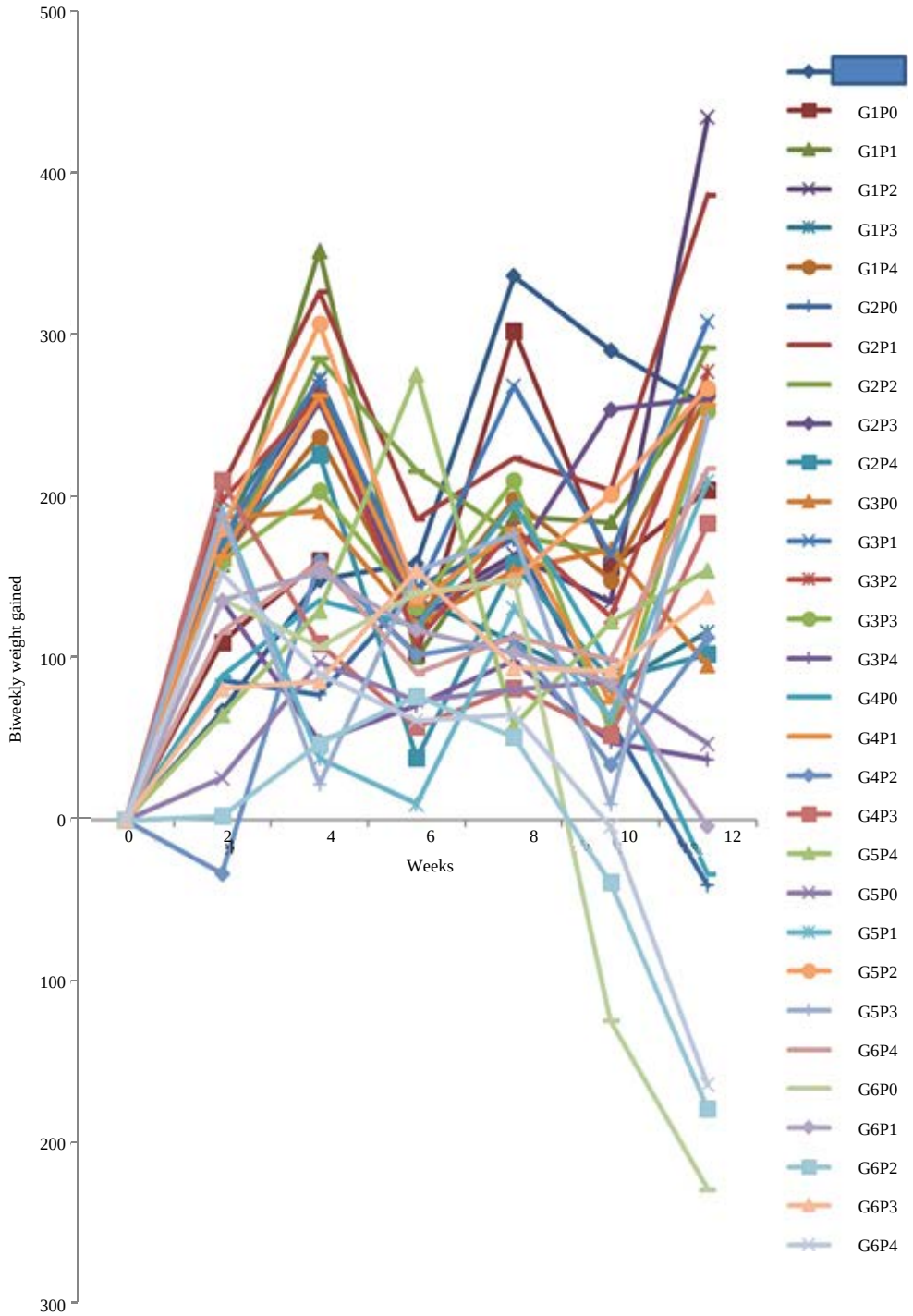

Fig. 6: Weight gain performance (biweekly) of fish fed groundnut meal diet (roasted, oil-pressed) with phytase

vs G1P0 (5.61±0.01%), G2P1 (4.21±0.01%) vs G2P0 (6.58±0.01%), G2P2 (4.99±0.01%) vs G2P0 (6.58±0.01%), G2P3 (4.90±0.01%) vs G2P0 (6.58±0.01%), G2P4 (5.19±0.01%) vs G2P0 (6.58±0.01%), G3P1 (4.66±0.01%) vs G3P0 (5.19±0.01%), G3P2 (4.66±0.01%) vs G3P0 (5.19±0.01%), G4P1 (4.96±0.01%) vs G4P0 (5.64±0.01%) and G4P2 (4.49±0.01%) vs G4P0 (5.64±0.01%). Regardless of groundnut meal level, bone phosphorus and magnesium declined significantly with increasing phytase supplementation (Tukey, $p<0.05$). Values of bone calcium showed significant reduction (Duncan, $p<0.05$) with phytase (250 and 500 FTU g^{-1}), G1P0 (250 FTU g^{-1}),

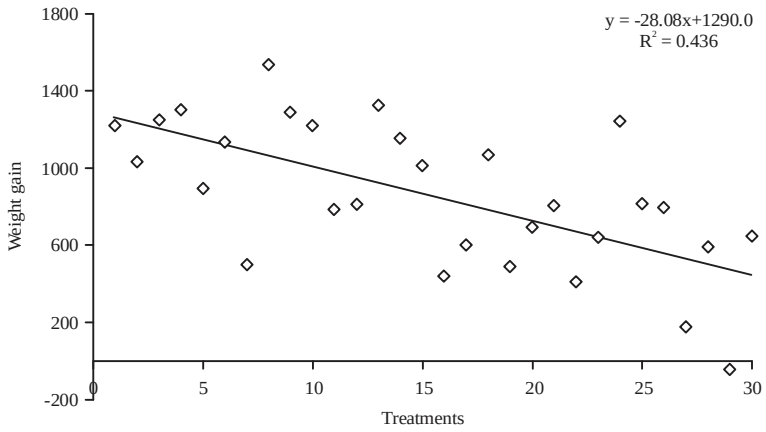

Fig. 7: Relationship between treatment and weight gain of fish fed roasted and oil pressed groundnut meal diet supplemented with phytase

Table 6: Serum biochemistry of fish fed groundnut meal diet (roasted, oil-pressed) with phytase

Treatment	Glucose (mg dL^{-1})	Sodium (mEq L^{-1})	Phosphorus (mg dL^{-1})	Calcium (mg dL^{-1})
Initial	256.00±12.29	106.00±5.08	3.40±0.15	7.90±0.63
G0P0	143.00±0.00^{ef}	108.50±0.71^{a}	0.75±0.07^{a}	5.70±0.00^{o}
G1P0	113.50±0.71^{c}	355.50±0.71^{ef}	1.70±0.01^{de}	1.90±0.14^{ef}
G1P1	115.50±0.71^{c}	481.50±0.71^{n}	1.70±0.01^{de}	6.55±0.07^{q}
G1P2	203.50±0.71^{m}	462.00±1.41^{m}	2.00±0.14^{fgh}	1.65±0.07^{cd}
G1P3	236.00±1.41^{r}	441.50±0.71^{l}	1.75±0.07^{de}	7.05±0.07^{r}
G1P4	223.00±1.41^{no}	590.00±1.41^{s}	2.55±0.07^{jk}	1.25±0.07^{a}
G2P0	216.50±0.71^{n}	352.00±1.41^{e}	1.70±0.01^{de}	6.15±0.07^{p}
G2P1	164.50±0.71^{h}	357.50±0.71^{f}	1.50±0.01^{c}	5.25±0.07^{n}
G2P2	144.00±1.41^{f}	372.00±1.41^{h}	2.05±0.07^{gh}	5.25±0.07^{n}
G2P3	359.00±1.41^{u}	394.50±0.71^{i}	1.85±0.07^{ef}	1.75±0.07^{de}
G2P4	225.00±1.41^{op}	590.00±1.41^{s}	4.45±0.07^{o}	1.40±0.14^{b}
G3P0	180.50±0.71^{i}	493.50±3.54^{o}	1.75±0.07^{de}	3.35±0.07^{j}
G3P1	160.50±0.71^{g}	366.50±2.12^{g}	1.75±0.07^{de}	2.00±0.14^{fg}
G3P2	184.00±1.41^{j}	292.50±4.95^{c}	1.95±0.07^{fg}	2.90±0.14^{i}
G3P3	142.50±0.71^{ef}	394.50±0.71^{i}	1.95±0.07^{fg}	1.95±0.07^{ef}
G3P4	105.50±0.71^{a}	412.00±2.83^{j}	1.15±0.07^{b}	2.15±0.07^{g}
G4P0	141.50±0.71^{e}	461.50±2.12^{m}	1.71±0.07^{de}	3.35±0.07^{j}
G4P1	164.50±0.71^{h}	412.50±3.54^{j}	1.85±0.07^{ef}	7.35±0.07^{s}
G4P2	135.00±1.41^{d}	342.50±0.71^{d}	2.15±0.07^{hi}	4.15±0.07^{k}
G4P3	226.50±2.12^{q}	518.00±1.41^{p}	3.95±0.07^{n}	2.45±0.07^{h}
G4P4	243.50±0.71^{s}	572.00±1.41^{r}	3.00±0.14^{l}	2.55±0.07^{h}
G5P0	241.50±0.71^{s}	370.50±0.71^{gh}	2.05±0.07^{gh}	4.45±0.07^{l}
G5P1	163.50±0.71^{h}	412.50±3.54^{j}	1.75±0.07^{de}	7.20±0.14^{rs}
G5P2	335.00±1.41^{t}	463.50±0.71^{m}	2.05±0.07^{gh}	1.75±0.07^{de}
G5P3	109.50±0.71^{b}	491.50±0.71^{o}	3.55±0.07^{m}	1.85±0.07^{def}
G5P4	198.50±0.71^{l}	419.50±0.71^{k}	1.75±0.07^{de}	1.85±0.07^{def}
G6P0	241.50±0.71^{s}	199.50±0.71^{b}	2.65±0.07^{k}	4.80±0.14^{m}
G6P1	192.00±1.41^{k}	524.50±3.54^{q}	2.25±0.07^{i}	2.05±0.07^{fg}
G6P2	222.00±1.41^{o}	414.00±1.41^{j}	1.25±0.07^{b}	1.95±0.07^{ef}
G6P3	197.50±0.71^{l}	420.00±1.41^{k}	1.65±0.07^{d}	1.85±0.07^{def}
G6P4	163.50±0.71^{h}	463.50±3.54^{m}	2.45±0.07^{j}	1.55±0.07^{bc}
Total	190.06±59.02	417.73±101.32	2.08±0.77	3.40±1.97
Sig.	p<0.05	p<0.05	p<0.05	p<0.05

Mean values with the same alphabet superscript in the same column are not significantly different at the 0.05 level (2-tailed)

G2P0 (500 FTU g^{-1}), G3P0 (250 and 500 FTU g^{-1}) and G4P0 (250-750 FTU g^{-1}). However, there was significant increase in bone calcium of fish fed 50 and 60% groundnut meal with phytase addition (Duncan, p<0.05). Value bone calcium was higher with phytase at 750 and 1000 FTU g^{-1} compared to diet without phytase (Tukey, p<0.05, Table 11).

Table 7: Correlation between treatments, phytase level, Analysed Phytase Activity (APA), nutrient digestibility and serum parameters (phosphorus and glucose) of fish fed groundnut meal diet (oil-pressed) with phytase

Correlation	Treatments	Phytase level	APA	DCP	DENG	DFAT	Faecal phosphorus	DPHOSP	Fe(diet)	Serum phosphorus	Serum glucose
Treatments	1										
Phytase level	0.226134	1									
	0.077176										
APA	0.188582	0.968**	1								
	0.142136	7.46E-38									
DCP	0.421**	0.309*	0.259*	1							
	0.000658	0.014669	0.041967								
DENG	0.399**	0.337**	0.290*	0.868**	1						
	0.001327	0.007343	0.02216	6.59E-20							
DFAT	0.597**	0.163081	0.098618	0.437**	0.432**	1					
	3.07E-07	0.205347	0.445715	0.000387	0.000459						
Faecal Phosphorus	-0.0532	-0.220	-0.206	-0.103	-0.099	-0.050	1				
	0.681388	0.085517	0.108389	0.427594	0.442615	0.69873					
DPHOSPH	0.0162	0.144	0.143	0.122	0.197	0.094	0.0263	1			
	0.9006	0.264	0.269	0.345	0.125	0.469	0.8391				
Fe(diet)	0.355**	0.048	0.022	0.199	0.242	0.347**	-0.0322	0.071	1		
	0.004598	0.712569	0.862421	0.120493	0.058273	0.005787	0.803391	0.583499			
Serum phosphorus	0.219925	0.418**	0.469**	0.138423	-0.001	-0.085	-0.1013	0.050	-0.055	1	
	0.08588	0.000726	0.000119	0.2833	0.994842	0.51369	0.433544	0.702155	0.66843		
Serum glucose	0.1563	0.174	0.132	-0.051	-0.184	0.204	-0.1013	-0.077	-0.051	0.2007	1
	0.224938	0.176846	0.306375	0.69356	0.152639	0.111536	0.433302	0.549882	0.694661	0.117812	

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed)

Table 8: Growth performance of *Clarias gariepinus* fed groundnut meal diet (roasted, oil-pressed) with phytase

Treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	FCR	Specific growth rate (%)	Protein efficiency ratio	Survival rate (%)
G0P0	309.09±14.38	1526.56±64.78^{op}	1219.47±47.57^{r}	1574.02±84.88^{r}	1.29±0.01^{bc}	1.90±0.01^{v}	1.70±0.02^{ef}	92.31±0.00^{cd}
G1P0	407.44±0.08	1443.72±0.13^{n}	1036.275±0.05^{n}	1096.92±0.26^{jkl}	1.06±0.00^{bc}	1.51±0.00^{p}	2.07±0.01^{hi}	98.08±2.72^{ef}
G1P1	389.75±0.57	1640.69±1.45^{r}	1250.94±0.88^{s}	1247.15±10.42^{q}	1.00±0.01^{bc}	1.72±0.01^{s}	2.21±0.01^{ijk}	98.08±2.72^{ef}
G1P2	372.92±0.05	1677.48±8.46^{s}	1303.57±7.09^{t}	1158.01±2.99^{mn}	0.89±0.01^{b}	1.79±0.01^{t}	2.47±0.01^{lmn}	96.15±0.00^{def}
G1P3	399.09±0.75	1293.95±0.70^{l}	894.855±1.45^{l}	1165.42±6.41^{mn}	1.31±0.01^{bc}	1.40±0.01^{no}	1.69±0.01^{ef}	92.31±0.00^{cd}
G1P4	414.35±1.09	1546.835±1.72^{p}	1132.49±2.81^{p}	1194.86±0.42^{no}	1.06±0.01^{bc}	1.57±0.01^{q}	2.04±0.01^{hi}	100.00±0.00^{f}
G2P0	428.56±2.28	930.96±0.84^{f}	502.395±1.44^{f}	977.82±7.44^{ef}	1.95±0.01^{c}	0.92±0.01^{d}	1.55±0.52^{de}	96.15±0.00^{def}
G2P1	379.39±1.08	1912.83±9.26^{t}	1533.46±10.36^{v}	1327.10±0.21^{r}	0.87±0.01^{b}	1.93±0.01^{w}	2.66±0.01^{n}	98.08±2.72^{ef}
G2P2	380.00±2.30	1672.94±3.75^{s}	1292.94±1.46^{t}	1233.39±3.42^{pq}	0.96±0.01^{bc}	1.77±0.01^{t}	2.41±0.01^{klm}	94.23±2.72^{cde}
G2P3	378.81±4.00	1597.05±3.03^{q}	1218.25±7.02^{r}	1169.40±2.06^{n}	0.96±0.01^{bc}	1.72±0.01^{s}	2.41±0.01^{klm}	96.15±0.00^{def}
G2P4	365.54±1.53	1151.39±1.46^{j}	785.85±0.07^{j}	1048.59±5.71^{hi}	1.34±0.01^{bc}	1.37±0.01^{lm}	1.70±0.01^{ef}	90.39±2.72^{ab}
G3P0	388.78±1.46	1203.96±7.35^{k}	815.18±5.90^{k}	1130.49±1.41^{lm}	1.39±0.01^{bc}	1.34±0.01^{k}	1.70±0.01^{ef}	98.08±2.72^{ef}
G3P1	364.83±0.37	1690.54±6.46^{s}	1325.74±6.04^{u}	1208.92±1.83^{op}	0.92±0.01^{b}	1.83±0.01^{u}	2.57±0.01^{mn}	94.23±2.72^{cde}
G3P2	355.10±0.68	1509.49±0.81^{o}	1154.375±0.13^{q}	1157.06±10.55^{mn}	1.01±0.01^{bc}	1.73±0.01^{s}	2.33±0.02^{jkl}	98.08±2.72^{ef}
G3P3	369.19±0.74	1381.98±0.28^{m}	1012.795±1.03^{m}	1089.27±3.59^{jk}	1.08±0.01^{bc}	1.58±0.01^{q}	2.18±0.01^{hij}	96.15±0.00^{def}
G3P4	341.12±1.63	780.60±1.94^{d}	439.49±3.57^{e}	813.00±12.05^{b}	1.85±0.01^{bc}	0.98±0.01^{ef}	1.24±0.01^{c}	98.08±2.72^{ef}
G4P0	450.20±1.82	1050.75±1.58^{hi}	600.56±0.23^{g}	1077.00±6.55^{ijk}	1.80±0.01^{bc}	1.01±0.01^{ef}	1.35±0.01^{cd}	100.00±0.00^{f}
G4P1	353.13±4.33	1422.32±4.68^{n}	1069.20±0.35^{o}	1110.38±8.68^{kl}	1.04±0.01^{bc}	1.66±0.01^{r}	2.31±0.01^{jkl}	96.15±0.00^{def}
G4P2	343.84±2.18	831.95±0.97^{e}	488.11±1.22^{f}	738.90±5.53^{a}	1.51±0.01^{bc}	1.06±0.01^{g}	1.58±0.01^{e}	96.15±0.00^{def}
G4P3	361.00±1.54	1055.24±7.93^{hi}	694.24±6.39^{i}	910.865±2.90^{c}	1.32±0.01^{bc}	1.28±0.01^{j}	1.82±0.01^{fg}	86.54±2.72^{b}
G4P4	367.42±2.68	1173.23±0.59^{j}	805.81±2.09^{jk}	938.61±0.76^{cd}	1.17±0.01^{bc}	1.39±0.01^{mn}	2.01±0.01^{ghi}	98.08±2.72^{ef}
G5P0	366.60±2.71	777.13±3.46^{d}	410.53±6.17^{d}	759.80±1.77^{a}	1.85±0.03^{bc}	0.89±0.01^{c}	1.31±0.01^{c}	92.31±0.00^{cd}
G5P1	382.47±1.35	1026.98±0.19^{h}	643.51±0.13^{h}	916.02±3.62^{c}	1.43±0.01^{bc}	1.18±0.01^{i}	1.71±0.01^{ef}	98.08±2.72^{ef}
G5P2	386.50±0.55	1629.84±1.03^{r}	1243.34±0.48^{s}	1237.62±7.83^{pq}	1.00±0.01^{bc}	1.72±0.01^{s}	2.42±0.02^{klm}	98.08±2.72^{ef}
G5P3	351.28±2.41	1167.10±1.63^{j}	815.83±0.78^{k}	970.40±16.14^{de}	1.19±0.01^{bc}	1.42±0.02^{o}	2.02±0.03^{ghi}	98.08±2.72^{ef}
G5P4	377.89±4.97	1173.91±0.47^{j}	796.03±5.44^{jk}	953.76±0.56^{de}	1.20±0.01^{bc}	1.36±0.02^{kl}	1.96±0.01^{gh}	96.15±0.00^{def}
G6P0	381.42±7.02	561.83±9.36^{b}	180.42±2.34^{b}	1008.57±18.05^{fg}	5.57±0.01^{d}	0.46±0.01^{b}	0.44±0.00^{b}	86.54±2.72^{b}
G6P1	397.52±2.77	990.18±3.49^{g}	592.71±6.19^{g}	1088.74±9.75^{jk}	1.84±0.01^{bc}	1.09±0.02^{h}	1.36±0.01^{cd}	96.15±0.00^{def}
G6P2	429.45±3.10	389.645±7.80^{a}	-39.81±4.70^{a}	799.25±4.65^{b}	-20.21±2.28^{a}	-0.12±0.01^{a}	-0.12±0.01^{a}	76.92±0.00^{a}
G6P3	432.14±0.78	1076.95±2.63^{i}	644.815±3.42^{h}	1033.98±2.31^{gh}	1.61±0.01^{bc}	1.09±0.01^{h}	1.53±0.01^{de}	96.15±0.00^{def}
G6P4	447.82±0.35	626.82±30.64^{c}	201.50±1.53^{c}	1060.08±2.16^{hij}	5.26±0.03^{d}	0.46±0.01^{b}	0.46±0.01^{b}	80.77±5.44^{a}
Total	382.99±31.94	1223.06±375.00	840.80±383.61	1070.82±175.15	0.85±4.03	1.32±0.47	1.77±0.64	94.60±5.55

Mean values (n = 2) with the same alphabet superscript in the same column are not significantly different at the 0.05 level (2-tailed)

Table 9: Mineral composition, phytate, Ca/P ratio and phytase activity of groundnut diet (roasted, oil-pressed) with phytase

Treatment	Phytate (%)	Phosphorus (%)	Available phosphorus (%)	Calcium (%)	Magnesium (%)	Potassium (%)	Iron (ppm)	Ca/P	Analysed phytase activity (FTU g^{-1})
G0P0	0.40±0.02^{f}	1.31±0.01^{t}	0.91±0.01^{r}	1.85±0.01^{l}	0.25±0.01^{j}	0.87±0.01^{a}	45.02±0.01^{b}	1.41±0.00^{a}	<100
G1P0	0.25±0.02^{bc}	0.99±0.00^{r}	0.75±0.01^{q}	2.61±0.00^{y}	0.12±0.00^{c}	1.09±0.02^{f}	76.17±0.01^{j}	2.64±0.00^{w}	60
G1P1	0.21±0.01^{a}	0.95±0.00^{o}	0.74±0.01^{q}	2.27±0.00^{q}	0.11±0.00^{b}	1.13±0.00^{j}	70.17±0.00^{d}	2.63±0.00^{v}	330
G1P2	0.66±0.01^{p}	1.00±0.01^{s}	0.34±0.01^{de}	2.12±0.01^{p}	0.11±0.00^{b}	1.25±0.01^{p}	90.60±0.00^{q}	2.12±0.00^{l}	740
G1P3	0.45±0.00^{g}	0.99±0.01^{r}	0.55±0.01^{mn}	2.39±0.01^{t}	0.12±0.00^{c}	1.27±0.00^{q}	71.80±0.00^{f}	2.41±0.00^{r}	960
G1P4	0.59±0.01^{no}	0.98±0.00^{q}	0.39±0.01^{gh}	2.35±0.00^{s}	0.12±0.00^{c}	1.11±0.00^{h}	89.62±0.01^{n}	2.40±0.00^{q}	1310
G2P0	0.58±0.01^{mno}	0.97±0.00^{p}	0.39±0.01^{gh}	2.44±0.00^{w}	0.11±0.02^{b}	1.08±0.01^{e}	87.70±0.00^{m}	2.52±0.00^{u}	50
G2P1	0.52±0.01^{i}	1.00±0.01^{s}	0.50±0.01^{l}	2.06±0.01^{n}	0.12±0.00^{c}	1.19±0.01^{n}	84.38±0.00^{l}	2.06±0.00^{i}	330
G2P2	0.41±0.01^{f}	0.97±0.00^{p}	0.56±0.01^{n}	1.95±0.00^{m}	0.12±0.01^{c}	1.11±0.00^{h}	90.28±0.02^{p}	2.01±0.00^{g}	600
G2P3	0.48±0.01^{h}	0.94±0.00^{n}	0.47±0.01^{k}	2.29±0.00^{r}	0.12±0.01^{c}	1.21±0.01^{o}	76.31±0.01^{k}	2.44±0.00^{s}	730
G2P4	0.52±0.01^{i}	0.94±0.01^{n}	0.43±0.01^{ij}	2.35±0.00^{s}	0.15±0.01^{f}	1.17±0.01^{l}	90.98±0.00^{r}	2.50±0.00^{t}	1420
G3P0	0.56±0.01^{klm}	0.89±0.02^{k}	0.34±0.01^{de}	1.84±0.00^{l}	0.11±0.00^{b}	1.10±0.00^{g}	104.51±0.00^{w}	2.07±0.00^{j}	70
G3P1	0.54±0.00^{jk}	0.91±0.00^{m}	0.38±0.01^{fg}	2.52±0.01^{x}	0.16±0.01^{g}	1.17±0.01^{l}	74.49±0.00^{h}	2.77±0.00^{y}	390
G3P2	0.37±0.01^{e}	0.89±0.01^{k}	0.53±0.01^{m}	2.07±0.00^{o}	0.13±0.01^{d}	1.16±0.00^{k}	75.17±0.00^{i}	2.33±0.00^{p}	460
G3P3	0.57±0.01^{klmn}	0.90±0.00^{l}	0.34±0.01^{de}	1.41±0.01^{b}	0.13±0.00^{d}	1.12±0.01^{i}	159.74±0.01^{5}	1.57±0.00^{b}	1030
G3P4	0.59±0.01^{no}	0.91±0.00^{m}	0.32±0.01^{d}	2.40±0.00^{u}	0.09±0.01^{a}	1.11±0.00^{h}	47.83±0.00^{c}	2.64±0.00^{w}	1290
G4P0	0.42±0.01^{f}	0.86±0.01^{h}	0.45±0.01^{jk}	4.74±0.00^{z}	0.50±0.01^{k}	1.11±0.01^{h}	74.11±0.00^{g}	5.51±0.00^{z}	60
G4P1	0.25±0.01^{bc}	0.90±0.00^{l}	0.66±0.01^{p}	1.81±0.01^{j}	0.15±0.00^{f}	1.19±0.00^{n}	39.16±0.00^{a}	2.01±0.00^{g}	280
G4P2	0.36±0.01^{e}	0.89±0.00^{k}	0.54±0.01^{m}	1.76±0.00^{i}	0.21±0.00^{i}	1.38±0.01^{r}	99.99±0.01^{u}	1.98±0.00^{f}	540
G4P3	0.52±0.00^{ij}	0.88±0.01^{j}	0.37±0.01^{f}	2.41±0.01^{v}	0.14±0.01^{e}	1.02±0.00^{c}	104.53±0.01^{x}	2.74±0.00^{x}	1090
G4P4	0.40±0.01^{f}	0.86±0.01^{h}	0.47±0.01^{k}	1.95±0.00^{m}	0.13±0.00^{d}	1.01±0.02^{b}	101.82±0.00^{v}	2.27±0.00^{o}	1110
G5P0	0.45^{g}±0.01	0.79±0.00^{e}	0.34±0.01^{de}	1.71±0.00^{g}	0.15±0.01^{f}	1.16±0.01^{k}	97.40±0.01^{t}	2.16±0.00^{m}	50
G5P1	0.27^{d}±0.01	0.88±0.01^{j}	0.61±0.01^{o}	1.72±0.00^{h}	0.14±0.00^{e}	1.04±0.00^{d}	93.43±0.00^{s}	1.95±0.00^{e}	420
G5P2	0.52±0.02^{i}	0.85±0.01^{g}	0.34±0.01^{de}	1.62±0.00^{f}	0.13±0.00^{d}	1.09±0.00^{f}	113.58±0.01^{1}	1.91±0.00^{d}	700
G5P3	0.56±0.01^{klm}	0.87±0.00^{i}	0.32±0.01^{d}	1.39±0.00^{a}	0.16±0.00^{g}	1.18±0.00^{m}	133.86±0.00^{4}	1.60±0.00^{c}	920
G5P4	0.55±0.01^{kl}	0.82±0.00^{f}	0.28±0.01^{c}	1.82±0.00^{k}	0.17±0.00^{h}	1.16±0.00^{k}	110.42±0.02^{z}	2.22±0.00^{n}	1360
G6P0	0.23±0.01^{ab}	0.79±0.00^{e}	0.57±0.01^{n}	1.62±0.00^{f}	0.14±0.00^{e}	1.27±0.00^{q}	107.37±0.00^{y}	2.05±0.00^{h}	50
G6P1	0.57^{mn}±0.00^{l}	0.75±0.00^{d}	0.19±0.01^{b}	1.54±0.00^{d}	0.13±0.00^{d}	1.04±0.00^{d}	90.12±0.02^{o}	2.05±0.00^{h}	490
G6P2	0.60±0.00^{o}	0.74±0.00^{c}	0.14±0.00^{a}	1.53±0.00^{c}	0.14±0.00^{e}	1.18±0.00^{m}	125.9±0.00^{3}	2.07±0.00^{j}	280
G6P3	0.35±0.01^{e}	0.75±0.00^{d}	0.41±0.01^{hi}	1.58±0.00^{e}	0.16±0.00^{g}	1.08±0.00^{e}	118.38±0.00^{2}	2.11±0.00^{k}	1010
G6P4	0.25±0.01^{cd}	0.61±0.00^{b}	0.36±0.01^{ef}	1.54±0.00^{d}	0.15±0.00^{f}	1.02±0.00^{c}	70.25±0.00^{e}	2.52±0.00^{u}	1170

Mean values (n=2) with the same alphabet superscript in the same column are not significantly different at the 0.05 level (2-tailed)

Bone magnesium were lower in phytase diets compared to diets without phytase (Duncan, p<0.05). Bone iron showed a significant increase in all diets with phytase addition (Duncan, p<0.05). However, there was significant reduction in bone zinc in fish fed increasing phytase addition to 10, 20 and 50% groundnut meal (Duncan, p<0.05). Values of bone zinc were increased with phytase supplementation to 60% groundnut meal (Duncan, p<0.05). Values of zinc declined significantly in diet with 250 FTU g^{-1} compared to diet without phytase, regardless of groundnut meal level (Tukey, p<0.05). Body mineral composition is shown in Table 12, which showed significant increase in body phosphorus, calcium and magnesium with phytase addition (Duncan, p<0.05).

DISCUSSION

Growth and nutrient utilization: Phytase supplementation in groundnut meal (roasted, oil-pressed) diet of *Clarias gariepinus* resulted in growth improvement compared to diets without phytase (Table 8 and 4), which was also observed for other fish fed soyabean diets by Debnath *et al*. (2005), Li and Robinson (1997), Van Weerd *et al*. (1999) and Sajjadi and Carter (2004). The improvement in weight gain observed in fish fed phytase supplemented groundnut meal diets (roasted, oil-pressed) may be due to increase feed consumption and the release of nutrient from the phytase-mineral complex (Qian *et al*., 1996; Sebastian *et al*., 1998). The decline in weight gain and feed intake of fish fed G2P4, G3P4, G4P2, G4P3, G4P4, G5P3 and G5P4 and G6P2, could be due to the inability of phytase to break the zinc-phytate complex (Kumar *et al*., 2012; Harland and Oberleas, 2010), which may be due, in part, to high calcium and calcium/phosphorus ratio (Table 9), which facilitate calcium-zinc complex (Singh, 2008; Kumar *et al*., 2012), thereby making it less assessable to phytase (Maenz *et al*., 1999) resulting in reduced phytase activity for phytase activity and available phosphorus for the diet (Table 9). The participation of calcium in facilitation of the complex stem from the underlying negative effect of calcium in a diet sufficient in available phosphorus (Cowieson *et al*., 2012) as

Table 10: Effect of phytase and groundnut meal on nutrient digestibility of juvenile *Clarias gariepinus*

Sources of variation, p-value	Crude protein (%)	Energy (%)	Lipids (%)	Phosphorus (%)	Faecal phosphorus (%)
Phytase	0.000	0.000	0.000	0.346	0.243
Groundnut	0.000	0.000	0.000	0.492	0.575
Phytase* Groundnut	0.000	0.095	0.183	0.446	0.552
Pooled SE	0.001	0.001	0.001	0.807	0.042
Tukey HSD (means, p-values)					
Phytase					
P0 vs P1	-0.54*, 0.000	-0.64*, 0.000	-2.40*, 0.000	-3.99, 0.511	0.24, 0.372
P0 vs P2	-0.56*, 0.032	-0.66*, 0.000	-4.71*, 0.000	-3.95, 0.520	0.24, 0.367
P0 vs P3	-0.45*, 0.000	-0.48*, 0.000	-3.53*, 0.000	-3.92, 0.528	0.24, 0.363
P0 vs P4	-0.42*, 0.000	-0.48*, 0.000	-2.62*, 0.000	-2.92, 0.767	0.23, 0.392
P1 vs P2	-0.02*, 0.000	-0.02*, 0.000	-2.31*, 0.000	0.03, 1.000	0.00, 1.000
P1 vs P3	0.09*, 0.000	0.17*, 0.000	-1.13*, 0.000	0.07, 1.000	0.00, 1.000
P1 vs P4	0.12*, 0.000	0.16*, 0.000	-0.22*, 0.000	1.06, 0.994	0.00, 1.000
P2 vs P3	0.11*, 0.000	0.19*, 0.000	1.18*, 0.000	0.325, 1.000	0.00, 1.000
P2 vs P4	0.14*, 0.000	0.18*, 0.000	2.09*, 0.000	1.02, 0.995	-0.01, 1.000
P3 vs P4	0.02*, 0.000	-0.01*, 0.000	0.91*, 0.000	0.99, 0.995	-0.01, 1.000
Groundnut					
G0 vs G1	-1.73*, 0.000	-0.95*, 0.000	-3.98*, 0.000	-0.72, 1.000	0.12, 0.999
G0 vs G2	-1.61*, 0.000	-0.76*, 0.00	-6.50*, 0.000	4.41, 0.971	0.07, 1.000
G0 vs G3	-1.99*, 0.000	-1.23*, 0.000	-13.27*, 0.000	-0.49, 1.000	0.13, 0.999
G0 vs G4	-1.87*, 0.000	-1.17*, 0.00	-12.28*, 0.000	-0.28, 1.000	-0.12, 0.999
G0 vs G5	-1.78*, 0.000	-0.97, 0.000	-10.52*, 0.000	0.31, 1.000	0.14, 0.998
G0 vs G6	-1.90*, 0.000	-1.19*, 0.001	-14.76*, 0.000	1.71, 1.000	0.14, 0.998
G1 vs G2	0.13*, 0.000	0.18*, 0.000	-2.51*, 0.000	5.13, 0.553	-0.05, 1.000
G1 vs G3	-0.26*, 0.000	-0.28*, 0.000	-9.28*, 0.000	0.24, 1.000	0.01, 1.000
G1 vs G4	-0.14*, 0.000	-0.23*, 0.000	-8.29*, 0.000	0.49, 1.000	-2.37, 0.674
G1vs G5	-0.05*,0.000	-0.03*, 0.000	-6.54*, 0.000	1.04, 1.000	0.02, 1.000
G1 vs G6	-0.17*, 0.000	-0.24*, 0.000	-10.77*, 0.000	2.44, 0.976	0.02, 1.000
G3 vs G4	0.12*, 0.000	0.05*, 0.000	0.99*, 0.000	0.21, 1.000	-0.25, 0.638
G3 vs G5	0.21*, 0.000	0.26*, 0.000	2.74*, 0.000	0.80, 1.000	0.01, 1.000
G3 vs G6	0.09*, 0.000	0.04*, 0.000	-1.49*, 0.000	2.19, 0.986	0.01, 1.000
G4 vs G5	0.09*, 0.000	0.21*, 0.000	1.75*, 0.000	0.59, 1.000	0.25, 0.605
G4 vs G6	-0.03*, 0000	-0.01, 0.418	-2.48*, 0.000	1.99, 0.992	0.25, 0.605
G5 vs G6	-0.12*, 0.000	-0.21*, 0.000	-4.23*, 0.000	1.39, 0.999	0.000, 1.000

*Mean differences are significant at $p<0.05$

noticed in these basal diets (Table 3 and 9). Evidence of this zinc-phytate complex (Cao *et al*., 2007; Singh, 2008) in the diet (G6P2), which produced poor growth response is the reduction of zinc levels in these diets with concomitant decrease in their available phosphorus levels compared with diets without phytase or other phytase diets based on 60% groundnut meal (Table 9). The negative effect of this zinc-phytate complex was more prominent in G4P2 and G6P2, which had the poorest weight gain and feed intake. Zinc is required for normal growth, development and function in all animal species (NRC., 1980). Fish fed 50% groundnut meal with 500 FTU g^{-1} (G5P2) showed growth and nutrient performance that is comparable to phytase supplemented 10 and 20% groundnut meal diets as well as the fish meal-based diet (G0P0), unlike optimum phytase of 750 FTU g^{-1} reported for *Clarias gariepinus* fed soyabean meal diet (Kumar *et al*., 2012). The difference in the response of groundnut in terms of optimum levels could be explained by the differences in the location of the phytate or phytic acid in their seed (Biehl and Baker, 1997). In oilseeds such as soybeans, phytate is associated with the protein bodies. These protein bodies have no specific site of localization, so they are widely distributed throughout the seed (Maga, 1982). In contrast, the phytate in groundnut seeds is concentrated in substructures called crystalloids or globoids, which are located within the protein body membrane (Erdman, 1979). Phytase level at 500 FTU g^{-1} has been reported to be optimum for Common carp (Kumar *et al*., 2012), Rainbow trout (Forster *et al*., 1999), *Pangasius pangasius* (Debnath *et al*., 2005). Feed conversion, specific growth rate and protein efficiency ratio were also consistent with improved growth performance observed in other studies with fish (Li and Robinson, 1997; Debnath *et al*., 2005) and animals (Rao *et al*., 1999; Lim *et al*., 2001). Fish fed 60% groundnut meal (roasted) with phytase at 250 FTU g^{-1} (G6P1), 750 FTU g^{-1} (G6P3) and 1000 FTU g^{-1} (G6P4) had better growth performance compared to basal control (G6P0), which could be due to a low phosphorus in the diet, which enhanced phytase efficacy (Lim *et al*., 2001; Ravindran *et al*., 2000, 2001). However, fish fed the diet with

Table 11: Effect of phytase and groundnut meal on bone mineralization of juvenile *Clarias gariepinus*

Sources of variation, p-value	Phosphorus (%)	Calcium (%)	Magnesium (ppm)	Iron (ppm)	Zinc (ppm)
Phytase	0.000	0.000	0.000	0.000	0.000
Groundnut	0.000	0.000	0.000	0.000	0.000
Phytase* Groundnut	0.000	0.000	0.000	0.000	0.000
Pooled SE	0.001	0.001	0.001	0.532	0.001
Tukey HSD (means, p-values)					
Phytase					
P0 vs P1	0.57*, 0.000	0.52*, 0.000	1.13*, 0.000	-74.37*, 0.000	0.31*, 0.000
P0 vs P2	0.84*, 0.032	0.12*, 0.000	1.12*, 0.000	-40.71*, 0.000	-5.85*, 0.00
P0 vs P3	0.26*, 0.000	-0.17*, 0.000	1.13*, 0.000	-38.59*, 0.000	-1.69*, 0.00
P0 vs P4	1.12*, 0.000	-0.84*, 0.000	1.13*, 0.000	-77.52*, 0.000	-0.05*, 0.00
P1 vs P2	0.27*, 0.000	-0.40*, 0.000	-0.01, 0.08	33.66*, 0.000	-6.17*, 0.00
P1 vs P3	-0.31*, 0.000	-0.68*, 0.000	0.00, 0.989	35.78*, 0.000	-2.00*, 0.00
P1 vs P4	0.55*, 0.000	-1.36*, 0.000	0.00, 0.752	-3.16, 0.368	-0.36*, 0.00
P2 vs P3	-0.58*, 0.000	0.29*, 0.000	0.01*, 0.030	2.11, 0.731	4.17*, 0.00
P2 vs P4	0.28*, 0.000	0.97*, 0.000	0.01*, 0.005	-36.82, 0.000	5.80*, 0.00
P3 vs P4	0.86*, 0.000	-0.68*, 0.000	0.00, 0.951	-38.93, 0.000	1.64*, 0.00
Groundnut					
G0 vs G1	0.44*, 0.000	1.26*, 0.000	7.78*, 0.000	-95.29*, 0.000	-2.70*, 0.00
G0 vs G2	0.56*, 0.000	0.91*, 0.00	7.78*, 0.000	-126.37*, 0.000	-3.34, 0.00
G0 vs G3	0.88*, 0.000	0.88*, 0.000	7.79*, 0.000	-131.31*, 0.000	-8.14*, 0.00
G0 vs G4	0.84*, 0.000	0.72*, 0.00	7.79*, 0.000	-132.95*, 0.000	-2.96*, 0.00
G0 vs G5	1.20*, 0.000	0.88, 0.000	7.80*, 0.000	-120.21*, 0.000	-4.54*, 0.00
G0 vs G6	1.38*, 0.000	0.04*, 0.000	7.80*, 0.000	-53.37*, 0.00	0.15*, 0.00
G1 vs G2	0.13*, 0.000	-0.35*, 0.000	-0.003, 0.985	-31.07*, 0.000	-0.64*, 0.00
G1 vs G3	0.45*, 0.000	-0.37*, 0.000	0.02*, 0.002	-36.02*, 0.000	-5.44*, 0.00
G1 vs G4	0.40*, 0.000	-0.53*, 0.000	0.01, 0.153	-37.66*, 0.000	-0.25*, 0.00
G1vs G5	0.76*, 0.000	-0.38*, 0.000	0.02*, 0.000	-24.92*, 0.000	-1.84*, 0.00
G1 vs G6	0.95*, 0.000	-1.22*, 0.000	0.01, 0.248	41.92*, 0.000	2.85*, 0.00
G3 vs G4	-0.05*, 0.000	-0.16*, 0.000	-0.01, 0.534	-1.64, 0.974	5.18*, 0.00
G3 vs G5	0.32*, 0.000	-0.01*, 0.719	0.01, 0.534	11.11*, 0.000	3.60*, 0.00
G3 vs G6	0.50*, 0.000	-0.85*, 0.000	-0.01, 0.378	77.94*, 0.000	8.29*, 0.00
G4 vs G5	0.36*, 0.000	0.15*, 0.000	0.01*, 0.014	12.74*, 0.000	-1.59*, 0.00
G4 vs G6	0.54*, 0.000	-0.69*, 0.00	-0.001, 1.000	79.58*, 0.000	3.11*, 0.00
G5 vs G6	0.18*, 0.000	-0.84*, 0.000	-0.02*, 0.007	66.83*, 0.000	4.69*, 1.000

*Mean differences are significant at $p<0.05$

500 FTU g^{-1} (G6P2) showed the poorest growth performance and nutrient utilization, including the lowest survival rate of fish compared to other diets (Table 8), which may be due to high fibre (Settaluri *et al*., 2012), which may modify phytate-mineral interaction and affect phytase efficacy (Wise, 1983), low available phosphorus (Table 3 and 9), which is less than the requirement for the fish (NRC., 1993; Robinson *et al*., 2001), low phytase activity, which may be impacted by fibre-phytate-mineral complex and high calcium (Wise, 1983), phytate (Table 9) and other antinutrients. According to Lim and Dominy (1991), reduced growth response and feed utilization in various warm-water aquaculture species fed diets in which fish meal was replaced with oilseed meals have been explained by sub-optimal amino acid balance, inadequate levels of phosphorus, inadequate levels of energy, low feed intake caused by poor palatability, presence of endogenous antinutrients or dietary level of fish oil.

Phytate degradation, phosphorus availability and phosphorus digestibility: There has not been any study on phosphorus digestibility by phytase in groundnut meal diet in any fish. In this experiment, an improvement in phosphorus digestibility was observed in all diet with phytase, except G1P0, which is consistent with decreased protein and energy digestibility (Forster *et al*., 1999; Singh, 2008). Increase in phosphorus digestibility in groundnut meal (roasted), particularly for 20-50% diets, could be related to the high phytate contents, which enhanced the magnitude of phytase response (Ravindran *et al*., 1999; Selle and Ravindran, 2007) resulting in an improvement in phosphorus and available phosphorus in the diets (Table 9). Phosphorus digestibility correlated positively with protein, energy, lipid, dietary fat and phytase (Table 7) in groundnut meal diet (roasted), which could be explained by the low dietary level of phosphorus in groundnut meal, which enhanced the efficacy of phytase (Selle and Ravindran, 2007). Improvement in nutrient

Table 12: Body mineral composition of fish fed groundnut meal diet (roasted, oil-pressed) with phytase

Treatment	Phosphorus (%)	Calcium (%)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Initial fish	1.39±0.01	0.93±0.01	0.16±0.01	209.74±0.01	54.36±0.01
G0P0	1.12±0.01^{f}	0.74±0.01^{o}	0.14±0.01^{f}	190.98±0.01^{o}	41.47±0.01^{m}
G1P0	1.04±0.01^{e}	0.55±0.01^{i}	0.08±0.01^{ab}	75.60±0.01^{b}	51.02±0.01^{r}
G1P1	1.06±0.01^{e}	0.62±0.01^{l}	0.07±0.01^{a}	91.24±0.01^{d}	54.75±0.01^{x}
G1P2	1.04±0.01^{e}	0.85±0.01^{q}	0.08±0.01^{ab}	100.05±0.02^{e}	53.34±0.01^{u}
G1P3	0.90±0.01^{ab}	0.63±0.01^{l}	0.09±0.01^{bc}	114.35±0.01^{ijk}	60.82±0.01^{1}
G1P4	1.14±0.01^{fgh}	1.18±0.01^{r}	0.12±0.01^{e}	120.85±0.01^{lm}	50.97±0.01^{q}
G2P0	0.98±0.01^{d}	0.27±0.01^{b}	0.09±0.01^{bc}	70.04±0.01^{a}	89.50±0.01^{5}
G2P1	0.98±0.01^{d}	0.45±0.01^{gh}	0.08±0.01^{ab}	102.70±0.01^{ef}	53.60±0.01^{v}
G2P2	0.91±0.01^{b}	0.31±0.01^{c}	0.07±0.01^{a}	116.43±10.5^{jkl}	41.22±0.01^{l}
G2P3	1.25±0.01^{jk}	0.60±0.01^{k}	0.09±0.01^{bc}	107.42±0.01^{fgh}	63.61±0.01^{2}
G2P4	1.27±0.01^{k}	0.77±0.01^{p}	0.12±0.01^{e}	116.39±0.01^{jkl}	58.38±0.01^{y}
G3P0	1.17±0.01^{hi}	0.73±0.01^{o}	0.09±0.01^{bc}	77.05±0.01^{b}	43.92±0.01^{o}
G3P1	1.25±0.01^{jk}	0.64±0.01^{l}	0.11±0.01^{de}	110.0±0.02^{ghi}	60.04±0.01^{z}
G3P2	1.32±0.01^{l}	0.44±0.00^{fgh}	0.08±0.01^{ab}	84.29±0.01^{c}	44.59±0.01^{p}
G3P3	1.19±0.01^{i}	0.77±0.01^{p}	0.11±0.01^{de}	111.34±0.02^{hij}	51.77±0.01^{t}
G3P4	1.25±0.01^{jk}	0.68±0.01^{n}	0.12±0.01^{e}	109.76±0.01^{ghi}	42.74±0.01^{n}
G4P0	1.05±0.01^{e}	0.66±0.01^{m}	0.09±0.01^{bc}	77.74±0.01^{b}	73.19±0.01^{4}
G4P1	1.69±0.01^{m}	0.58±0.01^{j}	0.07±0.01^{a}	88.64±0.01^{cd}	64.65±0.01^{3}
G4P2	1.05±0.01^{e}	0.37±0.01^{d}	0.08±0.01^{ab}	104.46±0.01^{efg}	37.8±0.01^{e}
G4P3	1.06±0.01^{e}	0.44±0.01^{fg}	0.12±0.01^{e}	118.11±0.01^{kl}	38.56±0.01^{g}
G4P4	0.94±0.01^{c}	0.37±0.01^{d}	0.08±0.01^{ab}	178.49±0.01^{n}	37.84±0.01^{f}
G5P0	0.90±0.01^{ab}	0.56±0.01^{ij}	0.06±0.01^{a}	87.62±0.01^{cd}	40.05±0.01^{j}
G5P1	1.13±0.01^{fg}	0.78±0.01^{p}	0.09±0.01^{bc}	107.74±0.01^{fgh}	54.42±0.01^{w}
G5P2	0.99±0.01^{d}	0.43±0.01^{f}	0.07±0.01^{a}	107.98±0.01^{fgh}	39.50±0.01^{h}
G5P3	1.11±0.01^{f}	0.46±0.01^{h}	0.10±0.01^{cd}	124.52±0.01^{m}	33.00±0.01^{c}
G5P4	1.16±0.01^{ghi}	0.41±0.01^{e}	0.11±0.01^{de}	177.75±0.01^{n}	36.82±0.01^{d}
G6P0	1.19±0.01^{i}	0.31±0.01^{c}	0.12±0.01^{e}	193.59±0.01^{o}	51.42±0.01^{s}
G6P1	0.87±0.01^{a}	0.24±0.01^{a}	0.11±0.01^{de}	211.34±0.01^{p}	30.29±0.01^{b}
G6P2	1.04±0.01^{e}	0.37±0.01^{d}	0.10±0.01^{cd}	210.23±0.01^{p}	39.70±0.01^{i}
G6P3	1.23±0.01^{j}	0.68±0.01^{n}	0.11±0.01^{de}	192.06±0.01^{o}	29.47±0.01^{a}
G6P4	1.18±0.01^{i}	0.56±0.01^{i}	0.12±0.01^{e}	222.18±10.6^{q}	40.18±0.01^{k}
Total	1.11±0.16	0.56±0.20	0.09±0.02	125.83±45.2	48.66±13.04

Mean values (n = 2) with the same alphabet superscript in the same column are not significantly different (p>0.05) (2-tailed)

digestibility could also result from the high levels of digestible energy and polyunsaturated fatty acids in groundnut meal (Agbo., 2008). The positive relationship between phytase, phosphorus, energy, crude protein and lipid digestibility (Table 7) and decline in faecal phosphorus (r = 0.206) showed that phosphorus was well utilized by the fish (Cao *et al*., 2007; Kumar *et al*., 2012) and faecal phosphorus effectively reduced (Table 10) with phytase supplementation in groundnut meal diet. Thus, the negative effect of phytate plant-based diet and phosphorus loading in aquatic environment could be mitigated by phytase supplementation (Nwanna *et al*., 2008).

From the experiment, phytate is generally less assessable by phytase supplementation in roasted groundnut meal which may be due to its localized nature, with range of phosphorus released from phytate between 3.57-40.47%, but highly digestible by phytase, with digestibility values ranging from 65.49-91.13% (Table 5). Apparent phosphorus digestibility is one of the most sensitive criteria for assessing the influence of phytase on phosphorus utilization in fish (Sajjadi and Carter, 2004). The low level of phosphorus released (availability) from phytate in roasted groundnut with phytase could be due to the location of phytate, which exist in crystalloids or globoid bodies within the seed (Erdman, 1979), which is unlike the phytate in soyabean that is located in protein bodies, which have no specific site of localization and so they are widely distributed throughout the seed (Maga, 1982), which could increase the surface area for phytase function and hydrolysis of phytate.

Serum biochemistry: Increased level of serum sodium clearly indicates its role in nutrient absorption, particularly, phosphorus by using the active sodium transporter NaPi-IIb (Segawa *et al*., 2004; Forster *et al*., 2011). Values of serum glucose increased significantly in G1P0, G2P0 and G4P0 with phytase addition. In G5P0, values increased significantly in G5P2, which was the highest compared to G5P0, G5P1, G5P3, G5P4 and reflected increased energy digestibility (Table 5) compared to these diets. Increase in serum glucose positively correlated (Table 7) with serum phosphorus (r = 0.2007, p>0.05) and increased along with sodium (Table 6), which indicated that phytase-induced sodium uptake enhanced

Table 13: Bone mineral composition of fish fed groundnut meal diet (roasted, oil-pressed) with phytase

Treatment	Phosphorus (%)	Calcium (%)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Initial fish	2.21±0.01	2.59±0.01	0.17±0.01	7.29±0.01	28.77±0.01
G0P0	5.74±0.01^{v}	2.89±0.01^{w}	0.25±0.01^{l}	5.89±0.01^{a}	27.41±0.01^{d}
G1P0	5.61±0.01^{t}	1.23±0.01^{e}	0.24±0.01^{fg}	47.98±0.01^{b}	30.05±0.01^{m}
G1P1	5.36±0.01^{q}	0.96±0.01^{b}	0.22±0.01^{def}	154.93±0.01^{k}	30.67±0.01^{p}
G1P2	5.21±0.01^{p}	1.71±0.01^{i}	0.23±0.01^{ef}	64.22±0.01^{cd}	29.98±0.01^{l}
G1P3	5.52±0.01^{r}	1.61±0.01^{h}	0.20±0.01^{abcd}	48.30±0.01^{b}	31.09±0.01^{q}
G1P4	4.81±0.01^{k}	2.65±0.01^{t}	0.23±0.01^{ef}	171.04±0.01^{l}	28.76±0.01^{i}
G2P0	6.58±0.01^{x}	1.82±0.01^{j}	0.28±0.01^{i}	48.01±0.01^{b}	32.07±0.01^{t}
G2P1	4.21±0.01^{f}	1.88±0.01^{k}	0.26±0.01^{h}	175.90±0.01^{lm}	29.39±0.01^{k}
G2P2	4.99±0.01^{o}	1.31±0.01^{f}	0.20±0.01^{abcd}	123.66±0.01^{h}	32.94±0.01^{v}
G2P3	4.90±0.01^{m}	2.42±0.01^{p}	0.20±0.01^{abcd}	90.64±0.01^{e}	31.30±0.01^{r}
G2P4	5.19±0.01^{p}	2.46±0.01^{q}	0.19±0.01^{ab}	203.62±0.01^{n}	28.05±0.01^{g}
G3P0	5.19±0.01^{p}	2.34±0.01^{o}	0.22±0.01^{def}	69.43±0.01^{d}	31.95±0.01^{s}
G3P1	4.66±0.01^{i}	1.03±0.01^{c}	0.20±0.01^{abcd}	183.30±0.01^{m}	30.44±0.01^{n}
G3P2	4.66±0.01^{i}	1.44±0.01^{g}	0.22±0.01^{def}	107.35±0.01^{g}	40.66±0.01^{z}
G3P3	5.55±0.01^{s}	2.48±0.01^{q}	0.19±0.01^{abc}	91.20±0.01^{e}	38.88±0.01^{y}
G3P4	4.21±0.01^{f}	2.74±0.01^{u}	0.20±0.02^{abcd}	215.31±0.01^{o}	35.82±0.01^{x}
G4P0	5.64±0.01^{u}	2.47±0.01^{q}	0.18±0.01^{a}	95.43±0.01^{ef}	25.08±0.01^{b}
G4P1	4.96±0.01^{n}	0.82±0.01^{a}	0.21±0.01^{abcd}	212.73±0.01^{o}	27.80±0.01^{e}
G4P2	4.49±0.01^{g}	2.13±0.01^{l}	0.25±0.01^{gh}	127.08±0.01^{hi}	42.18±0.012
G4P3	5.52±0.01^{r}	2.18±0.01^{m}	0.22±0.01^{def}	148.63±0.01^{jk}	28.72±0.01^{h}
G4P4	3.90±0.01^{b}	3.23±0.01^{y}	0.20±0.01^{abcd}	90.89±0.01^{e}	28.05±0.01^{g}
G5P0	4.97±0.01^{n}	1.16±0.01^{d}	0.22±0.01^{def}	134.39±0.01^{i}	30.58±0.01^{o}
G5P1	5.97±0.01^{w}	1.43±0.01^{g}	0.19±0.01^{ab}	58.60±0.01^{c}	27.96±0.01^{f}
G5P2	4.13±0.01^{e}	2.23±0.01^{n}	0.19±0.01^{ab}	145.31±0.01^{j}	32.65±0.01^{u}
G5P3	4.63±0.01^{h}	2.11±0.01^{l}	0.20±0.01^{abcd}	170.70±0.01^{l}	41.47±0.01^{1}
G5P4	2.99±0.01^{a}	3.13±0.01^{x}	0.20±0.01^{abcd}	102.06±0.01^{fg}	27.10±0.01^{c}
G6P0	4.21±0.01^{f}	2.52±0.01^{r}	0.23±0.01^{ef}	48.15±0.01^{b}	29.18±0.01^{j}
G6P1	3.95±0.01^{c}	3.14±0.01^{x}	0.18±0.01^{a}	42.52±0.01^{b}	28.70±0.01^{h}
G6P2	4.02±0.01^{d}	2.82±0.01^{v}	0.22±0.01^{def}	58.39±0.01^{c}	33.56±0.01^{w}
G6P3	4.87±0.01^{l}	2.56±0.01^{s}	0.23±0.01^{ef}	63.85±0.01^{cd}	15.50±0.02^{a}
G6P4	4.72±0.01^{j}	3.22±0.01^{y}	0.21±0.01^{cde}	63.98±0.01^{cd}	29.37±0.01^{k}

Mean values with the same alphabet superscript in the same column are not significantly different at the 0.05 level (2-tailed)

intestinal uptakes of glucose, since it is involved in acid–base homeostasis (Selle and Ravindran, 2007). The high levels of serum glucose and sodium (Table 6) could also be related to the high dietary iron, which has been reported to be associated with increased glucose levels and glucose metabolism (Pushparani and Nirmala, 2014). Iron serves as a constituent in proteins such as haemoproteins: haemoglobin, myoglobin (and binds oxygen to them); non-haemo proteins: ferritin, transferrin and as a cofactor for many important iron-dependent enzymes such as cytochromes A, B, C, peroxidases, catalases (EFSA., 2013), which prevents free radical formation that can damage lipids, proteins and nucleic acids (Huang *et al*., 2013). Serum phosphorus increased significantly in all diet with increasing phytase supplementation, which indicated that phytase release inorganic phosphorus from phytate (Cao *et al*., 2007; Kumar *et al*., 2012) for absorption to maintain optimum level and requirement of the fish. Evaluation of plasma inorganic phosphorus is one of the indices used to estimate phytate-phosphorus utilization in animals (Singh, 2008). Masumoto *et al*. (2001) observed that phosphorus concentrations in plasma were higher in Japanese flounder fed a phytase supplemented diet. Perney *et al*. (1993) reported increase ($p<0.01$) in plasma inorganic phosphorus by phytase supplementation and increasing levels of available phosphorus in broilers. Broz *et al*. (1994) found significant ($p<0.05$) increase in serum inorganic phosphorus as a result of phytase supplementation at 500 FTU g^{-1} to broilers. Sebastian *et al*. (1994) reported that microbial phytase supplementation at 500 FTU g^{-1} in corn-soyabean diet increased the plasma phosphorus by 15.7% in broilers. In fish, a sign of phosphorus deficiency is reduced serum phosphorus (NRC., 1993). In this study, increase in serum phosphorus with phytase (Table 6 and 7) by phytase could be related to high phytate in the diets (Ravindran *et al*., 1999; Selle and Ravindran, 2007), which reflected increased phosphorus digestibility (Table 6). Increased serum phosphorus could also be due to high quality and quantity of polyunsaturated fatty acids in groundnut compared to soyabean (Agbo, 2008; Settaluri *et al*., 2012, which may enhance phosphorus

absorption compared to report of low phosphorus absorption in serum in mice fed soya bean oil in the diet of mice (Rezq *et al*., 2010). Evidence of this is the fact that serum phosporus is strongly, but positively correlated (Table 3) with phytase ($r = 0.418$, $p<0.01$) and analyzed phytase activity ($r = 0.469$, $p<0.01$). Serum calcium (Table 6) declined significantly with phytase addition in all diets, which may be explained by the fact that dietary calcium level exceeded the requirement (NRC., 1993), hence, the negative relationship between serum with no net intestinal absorption since calcium, unlike phosphorus, is tightly regulated (Favus *et al*., 2006). Values of serum calcium showed significant reduction with phytase addition to diet (Table 6), indicating a reduction in calcium intestinal uptake to maintain balance and suggest that dietary fat inhibit calcium absorption (Chiba, 2004). However, levels in serum is less tightly regulated (Favus *et al*., 2006) than phosphorus.

Bone mineralization: Phytase supplementation can hydrolyze phytate and increase the concentration of minerals like magnesium, calcium, manganese and zinc in plasma, bone and the whole body (Vielma *et al*., 1998). Channel catfish fed phytase-supplemented diets had higher concentrations of ash, calcium, phosphorus and manganese in their bones than the fish fed on a control diet (Yan *et al*., 2002). Nwanna *et al*. (2008) reported increased bone mineralization by phytase in common carp diet. Phytase has been used to improve dietary mineral retention in salmonids (Cain and Garling, 1995; Storebakken *et al*., 1998), common carp (Schafer *et al*., 1995), stripped bass (Papatryphon *et al*., 1999; Hughes and Soares Jr., 1998) and Nile tilapia (Liebert and Portz, 2007).

In this study, despite increased bone resorption (demineralization), body phosphorus (Table 12) and bone phosphorus (Table 13 and 11) showed a significant reduction with phytase supplementation to diet based on roasted groundnut due to phosphorus deficiency (Storebakken *et al*., 1998) arising from low dietary phosphorus in groundnut meal (Table 2). Insufficient phosphorus intake leads to the mobilization of phosphorus from the bone and transfer to soft tissues and metabolic processes (Baeverfjord *et al*., 1998). According to Storebakken *et al*. (1998), phosphorus deficiency result in increased body (carcass) phosphorus (Table 12) along with decreased body magnesium level. After phosphorus is incorporated into the blood as observed in the high serum phosphorus levels serum (Table 6), it can either be utilized by the animal or stored with calcium in a 2:1 ratio as hydroxyapatite crystal in the bones (Anderson *et al*., 2006). However, due to phosphorus deficiency in the diet, which may either result from low phosphorus intake (Table 2) or high Ca/P ratio (Table 9), there was increased resorption (demineralization) from bone (Table 11) to maintain metabolic process (Baeverfjord *et al*., 1998; Storebakken *et al*., 1998). Evidence of the low diet level of phosphorus is the fact that phosphorus released by phytase (Table 5) in G1P1, G2P1, G2P2, G2P3, G2P4, G3P1, G3P1, G3P2, G4P1 and G4P2 diet did not translate to increased bone mineralization (Table 13 and 11) for the diets, except G5P1, which showed both increased phosphorus availability (Table 5) and improved bone mineralization (Table 13 and 11). Phosphorus was more efficiently conserved in the whole body (Table 12) than calcium and zinc (Storebakken *et al*., 1998) due to limited phosphorus supply in the diet. Hence, concentrations of phosphorus in bone are reduced by bone resorption to maintain whole body phosphorus levels (Baeverfjord *et al*., 1998). Iron levels in carcass increased significantly probably due to higher levels in groundnut compared to fish meal and soyabean and also due to release by phytase from phytate-mineral complex (Selle and Ravindran, 2007). Bone ash and bone phosphorus are sensitive indicators of the phosphorus status in fish. This is because the phosphorus requirement for maximum bone mineralization is greater than maximum body weight gain (Kumar *et al*., 2012) as observed for higher bone values (Table 13) than whole body (Table 12). Bone phosphorus reduced significantly in all diets with phytase, except in 60% groundnut meal. The reduced phosphorus was aimed at maintaining phosphorus levels for metabolism through retention in the body from bone mobilization or resorption (Baeverfjord *et al*., 1998). Reduction in bone mineralization could also be due to high Ca/P level in the diet, which has been reported to impair bone development (Kumar *et al*., 2012). According to Kumar *et al*. (2012), several aberrations in bone mineral homeostasis and bone metabolism are associated with higher Ca:P ratio. However, dietary Ca levels and Ca:P ratios are also crucial to phytase efficacy as reviewed by Lei and Stahl (2000). Wise (1983) reported that calcium forms an insoluble complex with phytase which obviates phytate hydrolysis. Increase in bone phosphorus in 60% groundnut meal with phytase in the diet could be explained by improved phytase efficacy enhanced by low phosphorus than high phosphorus in the diet (Ballam *et al*., 1984; Lim *et al*., 2001; Ravindran *et al*., 2000, 2001). Wise (1983) reported that excess intake of inorganic phosphorus might inhibit the catalytic activity of microbial phytase. Increased bone calcium in fish fed diets with phytase could be due to the tight regulation of calcium (Favus *et al*., 2006) and the inverse effect of phosphorus and calcium (Kini and Nandeesh, 2012) and the critical nature of phosphorus to meet requirement than calcium

(Cowieson *et al*., 2012). Reduced bone phosphorus could also result from increased dietary iron content (Table 3 and 9) and in bone (Table 13), which has an inverse relationship with phosphorus (Naser, 2000; Chiba, 2004) and affect phosphorus absorption (NRC., 1998). Zinc levels increased in fish fed G3P0-G6P0 with phytase probably due to release of minerals from phytate complex, thereby increasing bone mineralization of the mineral (Vielma *et al*., 1998). Akhuemokhan *et al*. (2013) reported that about 90% of zinc exist in the bone.

CONCLUSION

Dietary supplementation of phytase in roasted groundnut has shown to increase nutrient digestibility and enhanced intestinal uptake of the essential nutrient for juvenile *Clarias gariepinus*; but the reduction in bone mineralization resulted in low phosphorus status in the fish, which could be due to either an absolute (low dietary intake) or relative deficiency from high fibre, Ca/P ratio or high iron levels, which competes with phosphorus for absorption. It is on the basis of this research that groundnut meal, which has a high digestibility coefficient for phosphorus compared with fish meal, in addition to its low phosphorus and substantially high dietary phytate levels, should be included with other readily available, but less digestible plant protein sources in fish diet for increased phosphorus utilization by phytase. This is necessary for not only an enhanced growth improvement, but also the retention of adequate amount of available phosphorus for other metabolic processes and bone mineralization in fish.

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