EFFECT OF PHYTASE SUPPLEMENTATION ON THE GROWTH, MINERAL COMPOSITION AND PHOSPHORUS DIGESTIBILITY OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILES

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ABSTRACT

This study investigates the effect of phytase supplemented diets on growth, bone mineralization and phosphorus digestibility of African catfish Clarias gariepinus. A 40 % crude protein diet was produced using fishmeal, soybean meal, lima bean meal, groundnut cake and yellow maize as ingredients. Diets E0, E1, E2 and E3 were supplemented with phytase (Natuphos microbial phytase, 5000 FTU/g; BASF) at 0, 1000, 2000 and 4000 FTUkg^{-1} feed respectively. The diets were fed to C. gariepinus (25.28 ± 0.03g) at 3 % body weight at two equal instalments daily. After 98 days of feeding, results indicated that mean weight gain (MWG) were significantly higher ($p<0.05$) in fish fed E1 (63.82 g), E2 (66.71 g) and E3 (64.98 g) than E0 (55.76 g). Feed consumed was marginally highest in E2 (110.91g) and least in E0 (103.83g). Phosphorus digestibility were significantly lower ($p>0.05$) in E0, with phytase resulting in over 28 % improvement. Calcium, phosphorus and zinc were highest in the carcass of E3 (30.23 mg/g, 31.96 mg/g and 52.78 µg/g respectively) and least in E0 (18.81 mg/g, 24.83mg/g and 35.55 µg/g respectively). Results also indicate significant increase ($p<0.05$) in bone minerals of fish fed diets E1, E2 and E3. Faecal phosphorus decreased with increase in phytase supplement from 15.98 mg/g in E0 to 11.01 mg/g in E3. Value of fish produced and profit index were significantly higher ($p<0.05$) in fish fed diets supplemented with phytase.

Keywords: *Clarias gariepinus*, Growth, Digestibility, Calcium, Phosphorus, Zinc

INTRODUCTION

Currently most fish like *Clarias gariepinus*, are fed practical feeds which rely heavily on animal protein feedstuffs, especially fish meal. However, major disadvantages associated with the use of animal feedstuffs are their high cost, the substantial excess of phosphorus, the increasing shortage of fish meal supplies and increasing ecological consideration over the use of wild caught fish for aquaculture feeds and phytoplankton bloom that gives rise to eutrophication (Papatryphon and Soares, 2001; Nwanna *et al.*, 2005). Therefore, the use of plant protein sources might become an alternative. However, when plants protein sources are high in a feed, there is a concern on the availability of phosphorus and some other nutrients. This is due to the presence of phytate; a major anti-nutritional factor present in all legumes.

One of the nutritional importance of phytate is its ability to chelate several mineral elements, especially, potassium, calcium, magnesium, iron, zinc, manganese forming poorly soluble complexes (Sardar *et al.*, 2007), thereby reducing their availability in the intestinal tract and retention (Laining *et al.*, 2012). Low phytate-phosphorus bioavailability in legumes to fish and to virtually all monogastric animals have been reported (Riche and Brown, 1996; Sugiura *et al.*, 1998; 2001). Unavailability of phytate-phosphorus results in phosphorus deficiency, thus necessitating the addition of inorganic phosphorus in manufactured feed. Phytate-bound phosphorus is eventually excreted and decomposed by microbes resulting in excessive nutrient loading of aquatic environment. This is in addition to the increase in cost of feed production.

However, availability of phytate-phosphorus and other chelated minerals to fish may be enhanced through the use of phytase either through pre-treatment or supplementation. Although many studies have been carried out to evaluate the effects of phytase on nutrient digestibility, nutrient retention and fish growth performance in many fish species (Forster *et al*, 1999; Van Weerd *et al.*, 1999; Storebakken *et al.*, 2000; Papatryphon and Soares, 2001; Baruah *et al.*, 2004; Liebert and Portz, 2005; Nwanna *et al.*, 2007), there is still paucity of information on the efficacy of phytase on *C. gariepinus* juveniles with adequate considerations given to the economic viability of supplementation. The present study was therefore undertaken to investigate the efficacy of phytase supplementation on growth performances and bone mineralization of *C. gariepinus* juveniles and economic benefits of this using profitability index and incidence of cost.

MATERIALS AND METHODS

Diets: A 40 % crude protein diet containing 71 % plant feedstuffs was formulated. Available phosphorus requirement of approximately 0.4 % as determined for channel catfish (Wilson *et al.*, 1982) was used in feed formulation (Table 1). The whole ingredients were thoroughly mixed in Hobart A200 (Troy-Ohio USA) pelleting machine to obtain homogeneous mass. The homogeneous mass was extruded through a 2 mm die and pelleted. The diets were divided into four parts (E0, E1, E2 and E3) and supplemented with phytase (Natuphos$^{@}$ 5000 L, 5000 FTUkg^{-1}BASF) at concentrations of 0, 1000, 2000 and 4000 FTU kg^{-1} respectively, post-pelleting. Measured phytase for each treatment were dissolved in 40 ml of distilled water and sprayed on the diets. The control diet (E0) without phytase, was sprayed with 40 ml distilled water to ensure uniform moisture content. Diets were sundried and packed into cellophane bags, labelled and stored in refrigerators until used.

Table 1: Ingredient and proximate compositions of experimental diets supplemented with varied levels of phytase

<u>Ingredients Composition</u> (%)	**E0**	**E1**	**E2**	**E3**
Fish meal	23.50	23.50	23.50	23.50
Soybean meal	5.87	5.87	5.87	5.87
Lima bean meal	17.63	17.63	17.63	17.63
Groundnut cake	23.50	23.50	23.50	23.50
Yellow maize	24.00	24.00	24.00	24.00
Vitamin premix	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Oyster shell	0.50	0.50	0.50	0.50
Palm oil	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Starch	1.00	1.00	1.00	1.00
Phytase FTUkg^{-1}	0	1000	2000	4000
<u>Proximate Composition</u>				
Crude protein (% DM)	39.98	39.98	39.98	39.98
Crude fat (% DM)	6.93	6.98	6.98	6.90
Crude fibre (% DM)	7.26	7.20	7.26	7.21
Ash (%)	7.25	7.23	7.18	7.28
NFE (% DM)	32.78	32.77	32.97	32.77
Total-Phosphorus (%)	8.38	8.33	8.50	8.58
Zinc (µg g^{-1} DM)	39.69	39.69	39.62	39.65
Manganese (µg g^{-1} DM)	27.19	27.18	27.15	27.19
Copper (µg g^{-1} DM)	9.39	9.59	9.38	9.59
Phytate-P (%)	5.52	4.71	4.56	4.54
Phytase activity FTUkg^{-1}	108	1239	2264	4409

Key: E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet

Catfish: This experiment was conducted at the Research Laboratory, Zartech Limited Ibadan, Nigeria. After acclimation for seven days, fifteen *C. gariepinus* juveniles (25.28 ± 0.03 g) each were stocked into 12 aerated 70 litre capacity rectangular aquaria in a completely randomized design. Diets E0, E1, E2 and E4 were fed to triplicate groups of fish at 3 % body weight daily; feeding was done twice daily at 8.00 – 9.00 hour and 16.00 – 17.00 hours throughout the 94 days experimental period.

Digestibility: Apparent digestibility coefficient (ADC) of protein, energy, fat and phosphorus were determined according to the acid insoluble ash (AIA) methods as described by Jimoh *et al.* (2010). Twenty one days to the end of the feeding trial, uneaten feed were cleaned 30 minutes after feeding to prevent excess feed contaminating the faecal wastes. Faeces were gently siphoned from the tanks using 2 mm diameter hose, every 8 hours after each feeding. The siphoned faeces were drained using filter papers. The faeces were pooled for each treatment and freeze-dried at -80°C until required for further processing.

The AIA was determined by ashing the samples of faeces and feeds in muffle furnace (Gallenkamp) at 550 °C for 5 hours, cooled and weighed. Then 25 ml of 10 % HCl was added to the samples separately (feeds and fish faeces) in conical flasks. This was covered with a watch glass and boiled gently over a low flame for 5 minutes after which it is filtered through ashless filter and watched with hot distilled water; the residue from the filter is returned into the crucible and ignited until it is carbon free and then cooled and weighed. The values obtained are used as indicator in the calculation of apparent digestibility:

$$\% \text{ AIA} = \frac{\text{Weight of AIA}}{\text{Weight of Ash}} \text{ x } 100$$

$$\text{ADC} = 100 - 10^2 \left(\frac{AIA\ in\ Feeds}{AIA\ in\ Feaces}\right) x \left(\frac{Nutrient\ in\ Feaces}{Nutrient\ in\ Feeds}\right)$$

Measurements and Analysis

Fish weight: Fish were weighed in grams using electronic top-loading balance (OHAUS corporation model: V21PW15) at the beginning of the experiment, bi-weekly during the experimental period and at the end of the experiment. During the experiment, bi-weekly weights were used to adjust for feed requirement.

Water quality parameters: The water quality parameters (temperature, dissolved oxygen and pH) were measured weekly and average taken at the end of the feeding trial (APHA, 1992).

Nutrient utilization and growth: The nutrient utilization and growth performance of the fish were assessed following Castell and Tiews (1980). At the end of the feeding trial, the vertebral bones were collected from three fish randomly selected per treatment. Bones were prepared according to method described by Nwanna and Schwarz (2007) with little adjustment. Slaughtered fish were soaked in hot water (80°C) for 5 minutes and bones thoroughly cleared of flesh and soft tissue before freeze drying. Mineral composition of bones was determined according to AOAC (2005). Feed ingredients, diets and fish carcasses were analysed for proximate and mineral compositions using AOAC (2005).

Phytase activity: Phytase activities were determined using methods described by Engelen *et al.* (2001). The enzyme phytase is incubated with a known concentration of the sodium salt of phytic acid under specified experimental conditions. During incubation, phytase liberates inorganic phosphate from the phytate. Incubation is terminated by the addition of a molybdate/vanadate reagent. This reagent reacts with the phytase-liberated phosphate and produces a yellow colored vanadomolybdo-phosphor complex. The concentration of this colored complex is determined spectrophotometrically at a wavelength of 415 nm and is directly proportional to the phytase concentration in the sample.

Economic Analysis: Economic analysis of feeding catfish with enzyme supplemented diets was carried out with emphasis on profit index and incidence of cost determination (Nwanna, 2003; Orisasona *et al.*, 2016).

Profit Index (PI) and Incidence of Cost (IC): This was done by relating value of fish produced to the cost of feed consumed by fish. PI = value of fish produced (₦/kg)/ cost of feed used in production (₦/kg), IC = cost of feed used in production (₦/kg) / total weight of fish produced (kg), where ₦ = Naira.

Feed Cost, Fish Value and Total Fish Weight: (i) The cost of feeds formulated was based on the prevailing market prices at the feed mills, while phytase cost of 10 Euro/kg was used, (ii) the value of fish was based on the selling price of fish/kg (₦ 450/kg) in fish markets around Ibadan at the end of the experiment and (iii) total weight of fish produced was got from the total weight of fish recovered at the end of the experiment.

Statistical Analysis: Data resulting from the experiment were subjected to one-way analysis of variance at $p<0.05$ using SPSS (Statistical Package for Social Sciences, Version 15, IBM Corporation, New York, USA). Duncan multiple range test was used to compare differences between means (Duncan, 1955).

RESULTS

Growth performance and feed utilization parameters of fish fed diets supplemented with varying phytase levels indicated that the mean weight gained and SGR were significantly higher ($p<0.05$) in fish fed phytase supplemented diets (Table 2). Weight gained plateaued in group E2 catfish. Feed consumption was however statistically similar ($p>0.05$) in all experimental treatments. ADC protein was highest in E3 diets and closely followed by E2 diet. Values for ADC protein were significantly higher ($p<0.05$) in fish fed phytase supplemented diets. This same trend was observed in values recorded for ADC phosphorus.

The results of proximate and mineral composition of experimental fish fed varying levels of phytase showed that the ash content significantly increased ($p<0.05$) with increased phytase supplementation (Table 3). Phytase supplementation caused 10 % increase in carcass phosphorus from diet E0 to E1 and E1 to E2, while between diet E2 and E3 there was an increase of 5 %. Phytase supplementation caused significant variation ($p<0.05$) in the phosphorus content of experimental fish. The highest value of manganese occurred in E3, followed by E2 group and the least in E0 diet.

Zinc contents also showed significant ($p<0.05$) variation among treatments with the higher values recorded in E2 and E3 groups. The mineral composition of fish bone fed varying levels of phytase supplement showed that the calcium contents of the fish bones were significantly higher ($p<0.05$) in fish fed E2 and E3 diets with the least value occurring in the control. The phosphorus content varied significantly ($p<0.05$) with the highest value recorded in fish fed diet E3 and the least in fish fed diet E0. Phosphorus content was improved between 7.6 – 19.0 % with phytase supplement (Table 4).

Faecal calcium and phosphorus were reduced between 5.9 – 21 % and 9.7 – 31 % respectively with phytase supplements (Figure 1).

Calculated cost of feed, cost of feed consumed, value of fish, incidence of cost (IC) and profit index (PI) indicated that the cost of feed production per kilogram was least in E0 and highest in E3. Cost of feed used in fish production was not significantly affected ($p<0.05$) by phytase supplementation, but there was significant variation ($p<0.05$) between fish fed phytase and those without. Value of fish produced increased from E0 to E3 (Table 5).

PI in E0 did not show significant ($p>0.05$) variation with E1, but varied significantly with other treatments. Calculated incidence of cost was least in E2 and highest in E0. Phytase supplementation significantly reduced ($p<0.05$) the IC.

The result of mean water quality parameters shows that temperature ranged from 27.6°C to 27.9°C in experimental units, while DO ranged from 5.45 to 5.80 mg/l (Table 6).

DISCUSSION

Supplemental phytase resulted in significant growth variation ($p<0.05$) in experimental fish, confirming improved protein utilisation. Within groups of phytase-supplemented diets, weight gain plateaued in the E2 group, suggesting that supplementation in excess of 2000 FTUkg^{-1}may not be beneficial to growth. The increase in growth could be attributed to effective phytate hydrolysis (breaking down the phosphate group in the IP6 molecule), causing the release and effective utilisation of phosphorus and other nutrients, resulting in more flesh conversion.

Table 2: Growth and nutrient utilization of *Clarias gariepinus* juveniles fed diets supplemented with varied levels of phytase

Treatments	Initial weight (g)	Final weight (g)	Weight gained (g)	Feed consumed/fish (g)	Feed conversion ratio	Specific growth rate
E0	25.25 ± 0.00	81.01 ± 7.25	55.76 ± 6.90^{a}	103.83 ± 9.11^{a}	1.88 ± 0.26^{a}	1.38±0.09^{a}
E1	25.32 ±0.00	89.10 ± 6.24	63.82 ± 5.90 ^{b}	108.59 ± 9.24^{a}	1.70 ± 0.14^{a}	1.49±0.07^{b}
E2	25.29 ± 0.00	92.00 ± 12.47	66.71 ± 12.06^{b}	110.91 ± 15.16^{a}	1.67 ± 0.09^{a}	1.53±0.13^{b}
E3	25.26 ± 0.00	90.20 ± 12.59	64.98 ± 12.16^{b}	107.51 ± 10.86^{a}	1.68 ± 0.18^{a}	1.50±0.13^{b}
	Protein efficiency ratio	**ADC protein**	**ADC fat**	**ADC energy**	**ADC phosphorus**	**Mean survival rate (%)**
E0	1.34 ± 0.19^{a}	68.38 ± 4.83^{a}	95.21 ± 1.76^{b}	73.78 ± 8.55^{c}	58.28 ± 3.45^{a}	79.5±0.03
E1	1.46 ± 0.13^{a}	70.75 ± 5.36^{b}	94.92 ± 1.64^{b}	72.30 ± 9.10^{b}	70.43 ± 2.22^{b}	90.6±0.11
E2	1.48 ± 0.08^{a}	73.12 ± 4.63^{c}	94.99 ± 1.91^{b}	71.56 ± 7.98^{a}	71.14 ± 1.96^{c}	84.84±0.00
E3	1.49 ± 0.19^{a}	74.49 ± 3.59^{d}	94.28 ± 1.10^{a}	71.33 ± 7.10^{a}	71.33 ± 1.85^{c}	75.96±0.00

Mean values with similar superscript are not significantly different (p>0.05), E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet, ADC: Apparent digestibility coefficient

Table 3: Proximate and mineral compositions of carcass of *Clarias gariepinus* fed diets supplemented with varied levels of phytase

Treatments	Crude protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	MC (%)	Calcium (mg/g)
E0	52.94 ± 1.72^{b}	6.48 ± 0.85^{a}	0.98 ± 0.15^{a}	7.50 ± 0.69^{a}	7.83 ± 1.42^{a}	18.81±4.37^{a}
E1	51.82 ± 1.48^{a}	7.09 ± 0.99^{b}	1.04 ± 0.18^{b}	8.02 ± 0.27^{b}	8.19 ± 1.31^{b}	22.78±4.08^{b}
E2	53.90 ± 2.56^{c}	7.47 ± 0.80^{c}	1.13^{c} ± 0.28^{c}	8.43 ± 0.34^{c}	8.82 ± 1.18^{c}	26.72±5.10^{c}
E3	53.98 ± 0.72^{c}	7.73 ± 0.75^{d}	1.03 ± 0.12^{b}	8.74 ± 0.35^{d}	9.03 ± 1.19^{c}	30.23±4.38^{d}
	Phosphorous (mg/g)	**Magnesium (mg/g)**	**Zinc (µg/g)**	**Copper (µg/g)**	**Manganese (µg/g)**	
E0	24.83 ± 2.01^{a}	0.92 ± 0.57^{ab}	35.55 ± 5.45^{a}	9.67 ± 2.56^{a}	29.18 ± 2.36^{a}	
E1	27.46 ± 2.16^{b}	0.85 ± 0.11^{a}	42.66 ± 4.60^{b}	11.03 ± 1.59^{a}	34.25 ± 2.77^{b}	
E2	30.32 ± 3.33^{c}	1.13 ± 0.63^{bc}	49.69 ± 4.79^{c}	13.98 ± 8.10^{b}	39.60 ± 5.33^{c}	
E3	31.96 ± 2.79^{d}	1.27 ± 0.34^{c}	52.78 ± 4.98^{d}	11.71 ± 1.89^{a}	44.37 ± 3.01^{d}	

Means values with similar superscript are not significantly different (p>0.05), Key: E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet

Table 4: Mineral composition of vertebral bone of *Clarias gariepinus* fed diets supplemented with varied levels of phytase

Treatments	Calcium (mg/g)	Phosphorous (mg/g)	Magnesium (mg/g)	Zinc (µg/g)	Copper (µg/g)
E0	89.71 ± 14.62^{a}	62.08 ± 8.41^{a}	1.49 ± 0.07^{a}	0.23 ± 0.06^{a}	5.34 ± 0.56^{a}
E1	97.14 ± 26.98^{b}	66.81 ± 7.97^{b}	1.63 ± 0.12^{b}	0.27 ± 0.06^{b}	6.05 ± 0.84^{b}
E2	112.15 ± 17.99^{c}	70.03 ± 7.72^{c}	1.63 ± 0.08^{b}	0.29 ± 0.07^{c}	6.86 ± 1.03^{c}
E3	120.25 ± 17.55^{c}	74.38 ± 7.67^{d}	1.69 ± 0.08^{c}	0.31 ± 0.04^{c}	7.16 ± 1.11^{c}

Mean values with same superscript along column are not significantly different (p>0.05), E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet

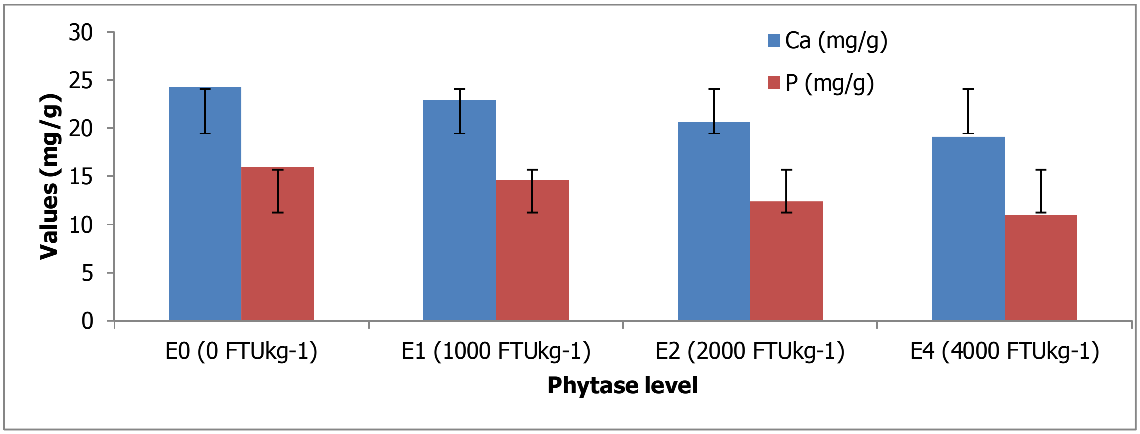

Figure 1: Phosphorus and calcium contents in faecal waste of experimental fish

Table 5: Economic evaluation of *Clarias gariepinus* fed diets supplemented with varied levels of phytase

Treatments	Total feed consumed	Mean weight gain (g)	Cost of feed (₦/Kg)	Cost of feed used (₦)	Value of fish (₦)	IC	PI
E0	103.83 ± 9.11	55.76 ± 6.90^{a}	170.98	17.75 ± 1.55	25.09 ± 3.10^{a}	0.322 ± 0.04^{b}	1.420 ± 0.19^{a}
E1	108.59 ± 9.24	63.82 ± 5.90^{b}	171.47	18.59 ± 1.50	28.72 ± 2.65^{b}	0.293 ± 0.02^{a}	1.540 ± 0.13^{ab}
E2	110.91 ± 15.16	66.71 ± 12.06^{b}	171.96	19.07 ± 2.85	29.24 ± 5.42^{b}	0.286 ± 0.01^{a}	1.571 ± 0.08^{b}
E3	107.51 ± 10.86	64.98 ± 12.16^{b}	172.66	18.56 ± 2.18	30.02 ± 5.47^{b}	0.289 ± 0.03^{a}	1.574 ± 0.19^{b}

IC = cost of feed used in production, PI = value of fish produced, Mean values with same superscript along column are not significantly different ($p>0.05$), E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet

Table 6: Water quality parameters in experimental tanks of fish fed diets supplemented with varied levels of phytase

Parameters	**Initial**	**E0**	**E1**	**E2**	**E3**
Temperature (°C)	27.9±0.00^{b}	27.8±0.11^{ab}	27.6±0.05^{a}	27.9±0.05^{b}	27.8±0.05^{ab}
pH	7.0±0.05^{a}	7.2±0.00^{b}	7.0±0.05^{a}	7.3±0.05^{b}	7.2±0.00^{b}
Dissolved oxygen (mg/l)	5.55±0.00^{b}	5.75±0.00^{d}	5.64±0.01^{c}	5.80±0.00^{e}	5.45±0.00^{a}

E0: 0FTUkg^{-1} diet, E1: 1000 FTUkg^{-1} diet, E2: 2000 FTUkg^{-1} diet, E3: 4000 FTUkg^{-1} diet,

The result of improved weight observed in this present study was in agreement with the reports of Nwanna *et al.* (2007) in *Cyprinus carpio*, Li and Robinson (1997) in Channel catfish and Laining *et al.* (2011) in Red sea bream. Phosphorus deficiency in finfish has been reported to cause reduced growth and poor feed efficiency (Lall, 2002). Baeverfjoord *et al.* (1998) reported that insufficient phosphorus intake leads to mobilisation of phosphorus from the bone and transfer to soft tissues and metabolic processes.

Feed consumption was statistically similar ($p>0.05$), but marginally higher in diets with phytase supplement. The statistical similarity in feed intake had also been reported for Atlantic salmon fed diets supplemented with phytase (Sajjadi and Carter, 2004).

In the present study, available phosphorus in the diets was about 0.40 %, which was above the lower limits in the study of Van Weerd *et al.* (1999). Thus the reduced growth in fish fed diet without phytase suggested that the phosphorus requirement of *C. gariepinus* may be above 0.5 %, was in support of the assertion by Van Weerd *et al.* (1999). Phosphorus digestibility increased with phytase supplement in the present study. This was similar to results obtained in other studies (Vielma *et al*., 1998; Sugiura *et al*., 2001; Nwanna *et al*., 2007). Papatryphon and Soares (2001) reported a 23 % increase in phosphorus digestibility in striped bass (*Morone saxatilis*), while Nwanna *et al.* (2007) reported 9 – 22 % faecal phosphorus reduction due to increased phosphorus digestibility when 1000 or 4000

FTUkg^{-1} was supplemented in the diet of common carp.

Apparent digestibility coefficient of protein varied significantly ($p<0.05$) in this study, with phytase supplementation resulted in improved protein digestibility. The efficacy of phytase to liberate protein in complexes, thus enhancing nutrient utilisation is affirmed with this result. This assertion is supported by the works of Cheng and Hardy (2003), Debnath *et al.* (2005), Vielma *et al*. (2004). This result is however, in contrast to that observed by Papatryphon *et al.* (1999) for striped bass and Storebakken *et al.* (1998) for salmonids.

Vielma and Lall (1998) noted that bone ash and bone phosphorus are sensitive indicators of the phosphorus state in fish. This is because of the variation in the phosphorus requirement for maximum bone mineralisation is greater than the requirement for maximum body weight gain (Baruah *et al.,* 2007). Bone ash associated with mineral bioavailability increased significantly ($p<0.05$) with phytase increase. This was in accordance with reports of Van Weerd *et al.* (1999) on phytase-treated soybean meal-based diets fed to African catfish, Vielma *et al.* (2002) in rainbow trout, Yan and Reigh (2002) in channel catfish, Sajjadi and Carter (2004) in Atlantic salmon, Debnath *et al.* (2005) in *Pangasius pangasius* and Baruah *et al.* (2007) on phytase supplementation in diets fed to *Labeo rohita.*

Increased phytase level reduced excreted calcium and phosphorus between 5 to 21% and 9 to 31.10 % respectively. This is an indication of the effective utilization and absorption of these minerals as a result of phytase supplementation, with an attendant reduction in the phosphorus load in culture effluent. Nwanna and Schwarz (2007) reported a 9 – 22 % reduction in faecal phosphorus from treatments containing 1000 and 4000 FTU/kg^{-1}. Schaefer *et al.* (1995) recorded a significant difference in phosphorus excretion in common carp fed phytase diets over the fish fed diets without phytase. Similar result was reported for salmonid (Cain and Garling, 1995), channel catfish (Jackson *et al.*, 1996) and *P. pangasius* (Debnath *et al.,* 2005).

Incidence of cost were significantly lower ($p<0.05$) in fish fed phytase supplemented diets than those fed diet without phytase. Profitability index were also higher in fish fed phytase supplemented diets. In the present study, phytase supplement in the diet of *C. gariepinus* improved nutrient digestibility, utilization and retention, with economic benefits.

ACKNOWLEDGEMENTS

The authors appreciate the BASF (Germany) for the provision of phytase (Natuphos$^{@}$ 5000L) used for this study and the analysis of samples for phytase activity.

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