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Dietary Phytase Improves Growth and Water Quality Parameters for Juvenile *Clarias gariepinus* Fed Soyabean Meal-based Diets

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Abstract The effect of phytase on growth, nutrient utilization of juvenile Clarias gariepinus fed soya bean using a 4x5 experimental design was investigated. 15.13% (S1), 34.16% (S2), 58.85% (S3) and 92.15% (S4) soyabean were formulated to substitute fish meal at 25 %, 50 %, 75 % and 100 %, respectively as basal controls with no phytase (P0). Another four diets were formulated with the same composition as the basal diet, but were supplemented with 250 FTU/g (P1), 500 FTU/g (P2), 750 FTU/g (P3) and 1000 FTU/g (P4), respectively. A fish meal diet, S0 (100%) and commercial diet (comm. diet), which served as controls, were included in the experiment to compare growth performance with experiment diet. A total of 1638 fish of average weight 11.55+ 0.20g were randomly allocated to experimental diets and fed at 3% body weight to replicate group of fish stocked at 26 fish per tank for 84 days. Growth performance was significant with phytase addition to diet (ANOVA, P<0.05). Mean weight gain declined with increasing substitution of fish meal by soya bean (Duncan, P<0.05), and irrespective of phytase levels (Tukey, P<0.05). However, significant improvement in weight gain of fish was observed with phytase addition to diets compared to diets without phytase (Tukey, P<0.05). Mean weight gain and feed conversion ratio (FCR) for fish fed S3P1 and S0P0 did not differ significantly (Duncan, P>0.05). Significant interaction for survival of fish showed a decline with increasing phytase, regardless of soya bean levels (Tukey, P<0.05). However, survival of fish fed fish meal diet was less than soya bean substitution up to 75%, regardless of phytase level (Turkey, P>0.05). Improvement in growth by phytase resulted in reduction of phytate (r= -0.231, P>0.05) and oxalate (r= -0.328, P<0.05) and improvement in water quality (Tukey, P<0.05) for oxygen (r= 0.262, P>0.05) and ammonia (r= -0.105, P>0.05). In conclusion, the study has demonstrated that phytase at low level of supplementation in soyabean diet of Clarias gariepinus can effectively utilize phosphorus from phytate, cut and manage pollution in aquaculture environments, and improve overall growth of fish compared to commercial diet.

Keywords Soya bean; Phytase; Growth; Water quality; Clarias gariepinus

1 Introduction

Statistics have confirmed that world aquaculture production has been rising, with a reported increase of aquaculture food production from 66 million tonnes in 2012 to 70.5 million tonnes in 2013 (FAO, 2014). Consequently, the sustainability of feed based production systems may be threatened by shortages and price rises of fish meal and fish oil, and thus step must be taken to reduce their inclusion levels in aqua-feeds by use of alternative protein source (Lunger et al., 2007; Pham et al., 2008; Lim et al., 2011). There has been a decline in the use of pelagic fish meal used in aquaculture feed production due to the limited amount available, which has resulted in

massive research to identify alternative protein sources (Olsen and Hasan, 2012). Among the many alternatives that have been examined, plant meals appear to have the most potential with extensive research conducted to evaluate the feasibility of plants (El-Saidy and Gaber, 2003; Abdelgamy, 2004; Catacutan and Pagador, 2004). The major protein concentrates used animal feed formulation are the oil seeds meals such as soya bean meal (Adeniji, 2009). Soybeans are the most plentiful of oilseed crops, and one of the cheapest plant sources of protein (Hussain et al., 2011), with a worldwide production of 253.1 million metric tonnes in 2012 (FAO, 2013). It is widely grown in Nigeria, with a yield of soybean of





1,700 kg per hectare on research plots, which compares favourably with the United States (US) yields of 2000 kg/ha and Brazil yields of 1,800 kg/ha (PrOpCom, 2007). It is the most efficient plant and has a higher metabolizable energy, protein and balanced amino acid profile, except methionine, compared to other plant protein sources (Nahashon and Kilonzo-Nthenge, 2011). However, the major constraints that limit its use in animal feed are the presence of fiber, starch, non-soluble carbohydrates, and anti-nutrients that affect digestibility and fish growth (Haghbayan and Mehrgan, 2015), one of which is phytate (Kumar et al., 2011), which binds minerals, including phosphorus, thereby limiting its utilization for growth of animals (Olukosi, 2012). It is highly heat-stable compared to other anitinutrients that can be destroyed by heat treatment (Nahashon and Kilonzo-Nthenge, 2011). Fish cannot digest phytate phosphorus, which represents about 70-80% phosphorus (Kumar et al., 2011) in plant-based diets because they lack intrinsic gastrointestinal phytase; therefore, in intensive fish production, large amounts of phosphorus are discharged into the environment where they pose serious pollution problems in aquatic environment (Nwanna et al., 2008). Nitrogen (N) and phosphorous (P) in metabolic waste produced by fish are the origin of most dissolved N and P waste resulting from intensive aquaculture operations (Hardy and Gatlin, 2002). The excess of these two elements in the effluents of aquaculture systems leads to eutrophication and a consequent change in the aquatic ecosystem (Nwanna and Olusola, 2014). Reducing the outputs of these dissolved wastes is considered to be a key element for the long-term sustainability of aquaculture. Nigeria is the leading country in Africa with the most number of people involved in fisheries and aquaculture sector (FAO, 2014), and the African catfish (Clarias gariepinus), an omnivorous species, is the leading aquatic crop in Nigeria, cultured for its fast growth rate and stress tolerance (Megbowon et al., 2014). The commercial production of this species may contribute to the pollution of aquatic environment. The concentration of ammonia, a nitrogenous waste from protein metabolism, is often the limiting water quality parameter in intensive aquaculture production systems, which affect fish growth ((Lazzari and Baldisserotto, 2008). Phytase, a hexaphosphosphate hydrolase enzyme,

which degrades phytate and improve phosphorus availability (Olukosi, 2012), has been extensively used in animal and fish nutrition with the benefit of enhancing growth and nutrient utilization (Nwanna and Schwarz, 2007; Nwanna et al., 2008) as well as phosphorus availability (Riche and Garling, 2004; Yoo et al., 2005), mineral availability (Debnath et al., 2005a; Liebert and Portz, 2005), and growth (Nwanna et al. 2005; Vielma et al., 2000; Baruah et al., 2007a). However, the extent to which phytase generate positive growth effect in most studies have not taken into consideration its concormitant impact on water quality parameters, particularly ammonia input in aquatic environment (Lazzari and Baldisserotto, 2008). High temperature and high pH have been reported to negatively affect fish physiological stress response to ammonia, depending on several factors, including dissolved oxygen concentration (Wagner et al., 1997; Chen et al., 2012). There is need to investigate the effect of phytase and phytase efficacy on growth of fish and its effect on water quality. The African catfish has a low stomach pH of 4, which can enhance phytase function and efficacy (Van weerd et al., 1999), which may benefit the aquaculture environment in terms of reduction of waste output from ammonia. The research, therefore, is aimed at investigating the effect of phytase on growth and some water quality parameters in juvenile Clarias gariepinus.

2 Materials and Methods

2.1 Experimental diets

The research investigated the effects of phytase supplementation in soya bean, which were formulated to contain four replacement levels of fish meal at 25 %, 50 %, 75 % and 100 % soya bean meal labelled S0, S1, S2, S3 and S4 as basal controls with no phytase (P0). Another four S1, S2, S3 and S4 diets were formulated with the same composition as the basal diets, but were supplemented with 250 FTU/g (P1), 500 FTU/g (P2), 750 FTU/g (P3), and 1000 FTU/g (P4), respectively. One FTU (fytase unit, Danish word) is defined as the amount of phytase that liberates 1 µmol of inorganic phosphorus from 0.0051 mol/L of sodium phytate per minute at pH 5.5 and 37°C (Engelen et al., 1994). The fish meal diet, S0 (100%), which served as control, was included in the experiment to compare growth performance with phytase diets. About 10 kg of raw soya bean was





purchased from Bodija main market in Ibadan, which is located in the western part of Nigeria. Raw whole seeds were subjected to heat treatment (Nwanna, 2005) by roasting on a metal plate at 120°C for 30-45 minutes, after which they were grinded using a manually operated grinding machine, sundried, packed into plastic bags, and stored at ambient temperature prior to inclusion in the formulation of with other feeding stuff for the fish. All diets were without formulated added wheat, inorganic phosphorus and amino acid supplements to optimize phytate hydrolysis in the diets. 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 2mm to produce a noddle-like strand of feed. Pelleted feeds were sun-dried and packed air-tight polythene bags before use. A stock solution of liquid Natuphos® phytase (Natuphos 5000L, BASF Corporation.,

Germany) was prepared by using a dilution factor of 1:10 and bulk density of 1.2g/cm3 (1.15-1.25g/cm3) (BASF, 2014) from which 0.4917ml, 0.9833ml, 1.475ml, and 1.967ml/kg diets for 250, 500, 750, and 1000 FTU/g phytase, respectively, were taken and supplemented in the diets according to the design. Phytase was supplied by BASF Corporation, Ludwigshafen, Germany, while phytase activity of all experimental groups were analysed by BASF SE, Lampertheim, Germany. Both phytase and non phytase-supplemented groups were analysed for total phosphorus and calcium (AOAC, 1990), phytate phosphorus (Oberleas, 1973), and phytase activity (Oberleas, 1973). The control diets, fish meal diets, S0 (100% fish meal) and commercial were not supplemented with phytase. Proximate, mineral and antinutrients composition of raw and processed soyabean (roasted) are presented in Tables 1-4, while the gross and chemical composition of the basal diet is shown in Table 5.

Table 1 Proxima	ate composition	n of feed ingredien	ts					
Ingredient	Crude protein (%)	n Fat (%)	Ash (%)	Ash (%) Fibre (%) Mo		6) CHO (%)	Energy (kcal/100g)	
Fish meal	66.46 ± 0.01	5.32±0.01	4.33±0.03	0.69±0.01	9.28±0.04	13.79±0.62	2 380±0.10	
SBM (Full-fat)	42.93±0.07	18.41 ± 0.01	5.34±0.01	7.86±0.01	8.47±0.01	17.00±0.00	6 420±0.01	
SBM (Raw)	$35.5{\pm}0.05$	19.48±0.01	5.06±0.01	5.59±0.01	12.13±0.03	22.25±0.04	4 420±0.00	
Maize	10.24±0.01	3.32±0.01	1.28±0.01	1.15±0.01	10.35±0.01	73.67±0.03	3 370±0.00	
Table 2 Mineral	composition of	of feed ingredients						
Ingredient		Phosphorus (%)	Calcium (%)	Magnesiu	ım (%)	Potassium (%)	Sodium (%)	
Fish meal 2.2		2.25±0.01	4.60±0.01 1.06±		l	79.20±0.02	16.13±0.03	
SBM (roasted, 1	SBM (roasted, full fat) 0.75±0.01		$0.64{\pm}0.01$	0.27±0.02	2	1.61±0.05	26.20±3.61	
SBM (raw)	SBM (raw) 0.53±0.0		0.86 ± 0.01	0.26 ± 0.01	l	2.11±0.01	20.60±0.01	
Maize		0.28±0.01	0.30±0.01	0.13±0.01	1	0.39±0.01	0.01±0.01	
Table 3 Mineral	l composition o	of feed ingredients						
Ingredient		Manganese (%)	Iron (ppm)		Copper (p	opm)	Zinc (ppm)	
Fish meal		16.13±0.03	96.6	1±0.05	2.75±0.05	5	48.45±0.05	
SBM (roasted, i	full fat)	50.75±6.03	89.7	7±2.25	7.21±1.48	3	27.65±0.24	
SBM (raw)		80.01±0.01	101.	23±0.02	4.09±0.01		9.30±0.01	
Maize		5.00±0.02	37.00±0.01		2.01±0.01		21.02±0.02	
Table 4 Anti-nu	trients in feed	ingredients						
Feed ingredient	S	Tannin (mg/g)		Phytate (mg/g)		Oxalate (mg/g)		
SBM (Raw)		0.43±0.02		0.61±0.01		7.48±0.12		
SBM (Roasted)		0.05 ± 0.06		0.50 ± 0.02		3.58±0.20		
Fish meal		0.00 ± 0.00		0.13±0.03		0.49 ± 0.09		
Maize		0.00 ± 0.00		0.27±0.03		0.795±0.05	5	





2.2 Experimental fish

Before the commencement of the feeding trial, a total of 1092 *Clarias gariepinus* fish of average weight (4.5±0.2g), were procured from a reliable fish farm with healthy fish stocks, and acclimated under laboratory conditions for 3 weeks during which time they were fed commercial diet in 10 fish holding tanks supplied with clean water with temperature, pH and oxygen maintained at optimum range between 25°C-32°C, 7.40-7.45, 4.80- 5.0mg/l. Water was sourced from a borehole through an overhead tank with a pipe, which supplied clean water to the Aquaculture and Fisheries Management Laboratory, University of Ibadan, Oyo State, Nigeria. All fish were left unfed for 2-3 days prior to start of experiment until they attained the juvenile status of average weight of 11.55+ 0.2g and length of 11.79 + 1.03 cm before experimental feeding with phytase-treated diets. All fish were randomly allocated to all 21 treatment combination groups of diets, including control diet in a 4x5 design, and fed to duplicate groups of fish stocked at 26 fish per tank. Each of the feed rations were shared so that experimental fish were fed twice daily (morning at 08:00 hrs) and evening (evening at 16:00 hrs) to obtain optimum growth with faeces removed at each feeding period. Experimental fish were weighed biweekly throughout the experiments using an electronic compact balance S. Mettler scale with accuracy of 0.01g (Model: K-BH). Water was changed once every two (2) days using static water renewal method and water quality monitored and measured for all treatment tanks at the beginning end of the experiment.

Table 5	Gross and	chemical co	mposition	of roasted	full-fat so	vabean-basal	diets for	iuvenile	Clarias	garieninus
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Ingredient (dry matter)	S ₀ (0%)	S ₁ (25%)	S ₂ (50%)	S ₃ (75%)	S4 (100%)
Fish meal	54.29	45.38	34.16	19.62	-
Soyabean meal (full fat)	-	15.13	34.16	58.85	92.15
Maize	42.21	35.99	28.18	18.03	4.35
Vit Min Mix [#]	0.50	0.50	0.50	0.50	0.50
Fish oil	1.00	1.00	1.00	1.00	1.00
CaCO ₃	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Cellulose*	0.20	0.20	0.20	0.20	0.20
Starch	0.80	0.80	0.80	0.80	0.80
Chemical composition (%)					
Phosphorus	1.31	1.15	1.01	0.75	0.43
Available phosphorus	0.91	0.63	0.44	0.33	0.03
Calcium	1.85	1.64	1.28	0.99	0.68
Magnesium	0.25	0.24	0.28	0.27	0.27
Potassium	0.87	1.10	1.35	1.60	1.90
Sodium	54.78	51.18	43.39	31.41	20.84
Manganese	63.12	77.37	113.66	101.03	97.40
Iron (ppm)	45.02	40.77	47.59	51.14	65.53
Copper (ppm)	3.53	5.04	8.33	11.15	14.71
Zinc (ppm)	15.82	14.22	12.59	12.12	12.40
Intrinsic phytase (FTU/g)	<100	<100	<100	<100	<100

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

2.3 Proximate and Mineral composition

Proximate composition of raw and processed soyabean was determined by method of AOAC (2006). For moisture, sample was mixed the sample thoroughly and water content determined by weighing out 2.5g 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 hours at 60°C-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 ten (10) minutes 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 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°C- 600°C for 12hrs 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).

Triplicate sample of feeds were also analyzed using flame atomic absorption spectrophotometer model Buck 205, Buck Scientific, USA., while phosphorus (P) was estimated spectrophotometrically using molybdovanadate method (AOAC 1990).

Table 6 Effect of soya bean (full fat) and phytase o	n growth performance of juvenile	Clarias gariepinus
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Sources of	Mean weight	Daily weight	Daily feed intake	FCR	Specific	Survival rate
variation, P value	gain (g/fish)	gain	(g/fish)		growth rate (%)	
		(g/fish/day)			(%)	
Phytase	0.007	0.00	0.771	0.00	0.079	0.000
Soya bean	0.000	0.000	0.000	0.000	0.00	0.000
Phytase* Soya bean	0.001	0.095	0.183	0.00	0.094	0.000
Pooled SE	0.483	0.008	0.072	0.058	0.031	0.604
Tukey HSD(means,	-1.05, 0.952	-0.07*, 0.037	-0.173, 0.934	2.23*, 0.000	-0.16, 0.448	5.96*, 0.031
P values) Phytase						
P0 vs P1						
P0 vs P2	4.74*, 0.032	0.05, 0.224	0.127, 0.978	2.20*, 0.000	0.06, 0.961	5.96*, 0.031
P0 vs P3	1.25, 0.914	0.0015, 1.000	-0.08, 0.997	2.07*, 0.000	0.03, 0.997	7.41*, 0.05
P0 vs P4	3.80, 0.114	0.029, 0.735	-0.09, 0.994	1.97*, 0.000	-0.05, 0.987	12.84*, 0.000
P1 vs P2	5.80*, 0.010	0.125*, 0.000	-1.86*, 0.000	-0.43, 0.186	0.23, 0.198	0.00, 1.000
P1 vs P3	2.31, 0.590	0.075*, 0.046	0.300, 0.705	-0.57*, 0.048	0.20, 0.331	1.44, 0.945
P1 vs P4	4.86, 0.038	0.103*, 0.004	0.08, 0.996	-0.66*, 0.016	0.12, 0.777	6.87*, 0.016
P2 vs P3	-3.49, 0.207	-0.05, 0.295	-0.20, 0.905	-0.13, 0.958	-0.03, 0.997	1.44, 0.945
P2 vs P4	-0.943, 0.973	-0.023, 0.892	-0.22*, 0.885	-0.23, 0.737	-0.11, 0.803	6.87*, 0.016
P3 vs P4	2.55, 0.496	0.028, 0.801	-0.01, 1.000	-0.10, 0.984	-0.08, 0.933	5.43, 0.076
Soya bean S0 vs S1	6.93, 0.063	0.12*, 0.035	-0.06, 1.000	-0.14, 0.988	0.17, 0.797	-5.00, 0.485
S0 vs S2	10.32*, 0.003	0.11, 0.064	0.05, 1.000	-0.06, 1.000	0.17, 0.807	-1.54, 0.986
S0 vs S3	16.55*, 0.000	0.23*, 0.000	-0.40, 0.799	-0.60, 0.275	0.38, 0.139	-3.84, 0.713
S0 vs S4	39.67*, 0.000	0.495*, 0.00	-1.13*, 0.038	-5.49*, 0.000	1.26*, 0.000	15.12*, 0.001
S1 vs S2	3.38, 0.151	-0.01, 0.987	0.11, 0.983	0.08, 0.987	-0.003, 1.000	3.46, 0.310
S1 vs S3	9.61*, 0.000	0.11*, 0.001	-0.34, 0.502	-0.46, 0.085	0.21, 0.175	1.15, 0.963
S1 vs S4	32.73*, 0.000	0.37*, 0.000	-1.07*, 0.000	-5.35*, 0.000	1.08*, 0.000	20.11, 0.000
S2 vs S3	6.23*, 0.002	0.1*2, 0.000	-0.45, 0.236	-0.54*, 0.031	0.21, 0.165	-2.31, 0.683
S2 vs S4	29.35*, 0.000	0.39*, 0.000	-1.18, 0.000	-5.43*, 0.000	1.09, 0.000	16.65, 0.000
S3 vs S4	23.12*, 0.000	0.27*, 0.000	-0.73*, 0.018	-4.89, 0.000	0.87, 0.000	18.96, 0.000

Note: *Mean differences are significant at P<0.05

2.4 Analysis of antinutrients

Phytate, tannin, and oxalate composition of raw, processed and experimental diets were determined in duplicate samples as follows.

2.4.1 Phytate

Phytate in feed samples was measured by alkaline picrate method of Oberleas (1973). Sample was

extracted with 0.2N HCl to give $(3-30\mu g \text{ ml-1 phytate} \text{ solution})$. 0.5ml of extract was pipetted into a test tube fitted with a ground glass stopper. 1ml 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 minutes. Care was taken to ensure that for the first 5 minutes, the





tube remained well stoppered. After cooling in ice water for 15 minutes, the tube was allowed to adjust to room temperature. Once the tube reached room temperature, the content of the tube was mixed and centrifuged for 30 minutes at 3000g. 1 ml of the supernatant was transferred to another test tube and 1.5ml of 2, 2'- Bipyridine solution added. The absorbance was measured at 519nm against distilled water.

2.4.2 Tannin

Tannin in raw, processed and experimental diets was determined using methods described by AOAC (2006). About 2g of feed was defatted for 2 hours using Soxhlet extraction apparatus. The residue was dried in oven for 12 hours at 100°C, boiled with 300ml of distilled water, diluted to 500ml in standard volumetric flask and filtered through non -absorbent cotton wool. A volume of 25ml of the infusion was measured into 2 liter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution turned green; then few drops of 0.1N potassium permanganate was added. The difference between the two titrations was multiplied by 0.006235 to obtain the amount of tannin in the sample using equation (1).

0.1 N oxalic acid=0.006235g tannin.....(1)

2.4.3 Total oxalate

The total oxalate of the powdered samples was determined by the modified method of Abeza et al. (1968). About 2g aliquot of the sample was weighted into a 250 ml flash; 190 ml distilled water and 10ml of 6M hydrochloric acid were added. The mixture was digested for 1 hour on boiling water bath, cooled and transferred into a 250 ml volumetric flash, diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was then heated to 100°C, allowed to cool and filtered to remove precipitate containing ferric ions. The filtrate was boiled, 10 ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through whatman No. 40 filter paper. The precipitate was washed several times with distilled water. The precipitate was transferred quantitatively to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The

resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate used, the oxalate content of the unknown sample was calculated using equation.

1ml of KMn04=2.2mg oxalate......(2)

2.5 Determination of phytase activity

Phytase activity in feed samples were determined modified by the Relative Method of BASF (1997) with slight modification to methods by Engelen et al. (1994). About 100 g feed was milled to a particle size less than 0.5 mm. Two 5.0 g portions of each sample of feed was weighed with an accuracy of 10 mg into an Erlenmeyer flask. 50.00 ml acetate buffer was metered by a dispenser into each sample, and the mixture was then stirred on a magnetic stirrer for 60 min. The stirring was followed by decantation into 10 ml centrifuge tubes and centrifugation at 4000 rpm (equivalent to about 2500g) for 20 minutes. The centrifugate was then diluted with buffer using the dilutor to a content of about 0.02 FTU/ml. 2.00 ml of each of the two solutions was pipetted as sample and sample blank into a 10 ml centrifuge tube. For the blank, 2.00 ml portions of acetate buffer were pipetted into two 10 ml centrifuge tubes. One centrifuge tube was incubated, and the other centrifuge tube was treated in analogy to the blanks as enzyme standard. The centrifuge tubes with the enzyme, sample blank and control solutions were each placed at a defined time interval (e.g. every 10 sec) in a water bath at 37.0 +/- 0.1°C and equilibrated for exactly 5 min. Then, at the same time intervals (every 10 sec), 4.00 ml sodium phytate solution (equilibrated at 37.0 +/- 0.1°C) was added by a dispenser and mixed. After incubation for exactly 60 min, the reaction was stopped, again at the same time intervals (every 10 sec), with 4.00 ml stop reagent and mixed to produce a colored complex with the phosphate formed. After waiting for at least 10 min, the solutions were centrifuged at 4000 rpm (equivalent to about 2500 g) for 20 minutes and then the absorbance at a wavelength of 415 nm was measured in a spectrophotometer against air. The enzyme phytase liberates inorganic phosphate from the substrate sodium phytate during incubation and the of of intensity the vellow color the



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vanadomolybdophosphorus complex is a measure of

the amount of phosphate liberated.

Table 7 Growth parameters and Nutrient utilization of *Clarias gariepinus* fed experimental diet (full fat)

Trt	Mean initial weight (g/fish	Mean final weight (g/fish)	Mean weight gain (g/fish)	FCR	SGR (%)	Daily feed intake (g/fish/day)	Survival rate (%)	Analysed Phytase Activity
S0P0	12.28±0.13	60.48±2.80g	48.20±2.67f	1.29±0.01ª	1.90±0.01 ^{ef}	2.04±0.02a	92.31±	(FTU/g) <100
S1P0	12.04±0.01	51.98±0.21fg	39.94±0.21ef	1.38±0.08ª	1.74±0.01 ^{cdef}	2.07±0.11a	0.00 ^{cd} 100.00±	<100
S1P1	12.71±0.24	54.36±2.78fg	41.65±3.02ef	1.39±0.06ª	1.72±0.03 ^{cdef}	1.97±0.11a	0.00 ^d 98.08± 2.72 ^d	580
S1P2	11.88±0.44	48.16±0.27def	36.29±0.71de	1.57±0.22 ^{ab}	1.43±0.11 ^{cde}	2.13±0.02a	$100.00\pm$	720
S1P3	14.27±0.48	55.15±3.01fg	40.88±2.53ef	1.47±0.11ª	1.67 ± 0.09^{cdef}	2.06±0.06a	96.16±	890
S1P4	11.34±1.42	51.44±0.10efg	37.97±0.01ef	1.37±0.13ª	1.73±0.12 ^{cdef}	2.06±0.02a	98.08±	1510
S2P0	11.43±0.38	49.62±0.83def	37.89±0.45de	1.34±0.08 ^a	$1.66{\pm}0.04^{cdef}$	2.01±0.05a	2.72 86.54± 2.72°	<100
S2P1	10.41±0.28	42.16±2.24cd	31.75±1.96cd	1.40±0.01ª	$1.66{\pm}0.03^{cdef}$	2.01±0.04a	2.72 94.23±	270
S2P2	10.96±0.68	43.60±3.00cde	32.64±2.32cd	1.38±0.01 ^a	$1.69{\pm}0.04^{cdef}$	2.08±2.06a	86.54±	580
S2P3	12.11±0.98	54.93±6.64fg	42.82±5.66ef	1.29±0.08ª	$1.66{\pm}0.06^{cdef}$	2.25±0.25a	$100.00\pm$	1260
S2P4	11.31±0.54	49.14±0.65def	37.83±1.19de	1.34±0.18 ^a	$1.69{\pm}0.06^{cdef}$	1.96±0.01a	94.23±	1260
S3P0	12.02±0.37	40.01±3.89c	27.99±3.52c	1.67±0.12 ^{ab}	1.39±0.22 ^{cd}	2.14±0.01a	2.72 98.08± 2.72 ^d	<100
S3P1	9.68±1.88	52.56±2.44fg	42.88±0.56ef	1.30±0.06 ^a	$2.04{\pm}0.00^{g}$	2.15±0.03a	73.08± 10.88 ^b	330
S3P2	13.18±0.70	42.07±3.00cd	28.89±3.70c	1.74±0.22 ^{ab}	1.67±0.13 ^{cdef}	2.12±0.04a	96.15± 0.00 ^d	660
S3P3	11.99±0.15	38.43±2.51c	26.44±2.36c	$2.40{\pm}0.18^{b}$	$1.80{\pm}0.06^{cdef}$	2.00±0.09a	94.23± 2.72 ^{cd}	1050
S3P4	12.61±0.95	38.16±0.41c	25.56±0.54c	2.36±0.23 ^b	$1.38{\pm}0.10^{cd}$	2.98±0.07b	94.23±2. 72 ^{cd}	1340
S4P0	11.65±0.83	14.38±1.19a	2.44±0.31a	16.71±1.43 ^e	0.22±0.1ª	3.66±0.04b	75.00± 2.72 ^b	<100
S4P1	12.20±1.12	24.44±1.47ab	12.24±0.35b	3.30±0.37°	$0.83{\pm}0.04^{b}$	3.98±1.46b	$92.31\pm$ 0.00 ^{cd}	330
S4P2	11.27±0.38	18.79±0.62ab	7.52±0.24ab	$4.43{\pm}0.06^{d}$	$0.61 {\pm} 0.00^{b}$	2.64±0.02b	96.16± 5.44 ^d	640
S4P3	11.89±0.33	20.26±1.66ab	8.38±1.34ab	$4.49{\pm}0.54^{d}$	$0.63{\pm}0.07^{b}$	2.75±0.04b	94.23±	830
S4P4	12.03±22.65	17.63±0.04ab	5.60±1.35ab	$4.97{\pm}0.48^d$	$0.90{\pm}0.78^{b}$	2.74±0.27b	55.19±	930

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





Table 8 Effect of soya bean (full fat) and phytase on antinutrients (phytate and soyabean) in the diet of juvenile *Clarias gariepinus*

Sources of	Phytate (%)	Oxalate (%)
variation, P		
value		
Phytase	0.000	0.000
Soya bean	0.000	0.000
Phytase* Soya	0.000	0.000
bean		
Pooled SE	0.002	0.015
Tukey HSD		
(means,		
P values)		
Phytase		
P0 vs P1	0.18*, 0.000	0.55*, 0.000
P0 vs P2	0.02, 0.107	0.88*, 0.000
P0 vs P3	-0.01, 0.469	0.89*, 0.000
P0 vs P4	0.20*, 0.000	0.89, 0.000
P1 vs P2	-0.16*, 0.000	0.32*, 0.000
P1 vs P3	-0.19*, 0.000	0.33*, 0.000
P1 vs P4	0.02*, 0.288	0.34*, 0.000
P2 vs P3	-0.03*, 0.005	0.01*, 1.000
P2 vs P4	0.18*, 0.000	0.02*, 0.997
P3 vs P4	0.21*, 0.000	0.01*, 1.000
Soya bean		
S0 vs S1	0.004, 0.997	-0.98*, 0.000
S0 vs S2	-0.002,1.000	-1.72*, 0.000
S0 vs S3	-0.06*, 0.002	-2.18*, 0.000
S0 vs S4	0.08*, 0.000	-1.48*, 0.000
S1 vs S2	-0.01, 0.916	-0.74*, 0.000
S1 vs S3	-0.06*, 0.000	-1.20*, 0.000
S1 vs S4	0.08*, 0.000	-0.50*, 0.000
S2 vs S3	-0.05*, 0.000	-0.46*, 0.000
S2 vs S4	0.08*, 0.000	0.24*, 0.000
S3 vs S4	0.14*, 0.000	0.70*, 0.000

Note: *Mean differences are significant at P<0.05

2.6 Growth and nutrient utilization

Growth performance was monitored biweekly with the following parameters measured:

Mean final weight= Mean total weight after 84 days Mean weight gain= Mean final weight -mean initial

weight

Daily weight gain= mean weight gain/ experimental

period (84 days)

Daily Feed Intake, DFI (%) = Feed intake (dry matter) x 100/[initial fish weight + final fish weight) x days fed/2]

Feed Conversion Ratio (FCR): Feed Intake (g)/Fish weight gain (g)

Specific Growth Rate, SGR (%): In (W2-W1)/(t2-t1), where W2 and W1 are weights on day t2 and t1, respectively.

Where, W2= final weight, W1= initial weight, t2= time at the end of experiment, t1= time at end of experiment

Protein efficiency ratio (PER)= Weight gain/ protein fed

Survival rate (%) = Initial number of fish –mortality/ initial number of fish X 100

2.7 Water quality

Water quality was regularly monitored throughout the experiment. Parameters were analyzed in two replicates per treatment at the beginning and end of the experiment. Dissolved oxygen was measured using digital dissolved oxygen meter (Model Lab tech DO meter); pH and temperature (pen type) correct to ± 0.1 and 0.01° C, respectively, were measured using a hand held portable dual function pH meter (pen type); ammonia and nitrate were measured using the automated phenate method and UV spectrophotometer screening method, respectively (Technical Instrument Corporation, 1973; Searle, 1984; Greenberg et al., 1992).

2.8 Economic analysis

Economic evaluation of producing catfish by phytase supplementation in all diets used in the experiments were carried out by determining profit index and incidence of cost.

2.8.1 Profit index (PI)

Profit index was determined from the relationship between value of fish produced and cost of feed consumed by fish as described by Miller (1976).

Profit Incidence = Value of fish produced (N/kg)/Cost of feed used in production (N/kg)

2.8.2 Incidence of cost (IC)

Incidence of cost was also determined as described by Vincke (1969) according to the formula:

Incidence of Cost = cost of feed used in production (N/kg)/ total weight of fish produced (kg)





2.8.3 Cost savings

 $Cost \ savings= (IC_{fish} \ {}_{meal}\text{-}IC_{diet}) \ x \ Cost \ of \ feed \\ consumed_{fishmeal}$

Where, $IC_{fish meal}$ = incidence of cost for fish meal diet (S0P0)

IC_{diet}= Incidence of cost for experimental diet

Cost of feed consumed_{fishmeal}= cost of feed consumed for fish meal diet

Assumptions:

 $\cdot \text{Cost}$ of feeds formulated was based on the prevailing market prices.

·Value of fish was based on the selling price of N500/kg.

•Total weight of fish produced was got from the total weight of fish recovered at the end of the experiment.

Table 9 Anti-nutrients in experimental diet based on soya bean (fullfat)

Treatment	Tannin	Phytate	Oxalate
	(mg/g)	(mg/g)	(mg/g)
S0P0	0.00±0.00	0.40±0.02e	0.40±0.02ª
S1P0	0.00 ± 0.00	$0.52{\pm}0.01^{hi}$	$1.92{\pm}0.02^{\rm f}$
S1P1	0.00 ± 0.00	$0.14{\pm}0.01^{a}$	$1.94{\pm}0.12^{\rm f}$
S1P2	0.00 ± 0.00	$0.61{\pm}0.01^k$	1.39±0.04e
S1P3	0.00 ± 0.00	$0.51{\pm}0.01^{hi}$	0.91 ± 0.01^{bc}
S1P4	0.00 ± 0.00	$0.18{\pm}0.01^{b}$	$0.74{\pm}0.01^{b}$
S2P0	0.00 ± 0.00	$0.57{\pm}0.01^{j}$	2.26±0.11 ^{gh}
S2P1	0.00 ± 0.00	$0.44{\pm}0.01^{fg}$	$2.26{\pm}0.08^{gh}$
S2P2	0.00 ± 0.00	$0.41{\pm}0.01^{ef}$	$1.95{\pm}0.09^{\rm f}$
S2P3	0.00 ± 0.00	0.39±0.01 ^e	$2.02{\pm}0.13^{\rm f}$
S2P4	0.00 ± 0.00	$0.18{\pm}0.01^{b}$	$2.09{\pm}0.12^{fg}$
S3P0	0.00 ± 0.00	$0.43{\pm}0.02^{efg}$	$3.09{\pm}0.11^{j}$
S3P1	0.00 ± 0.00	$0.34{\pm}0.04^{d}$	$2.28{\pm}0.03^{gh}$
S3P2	0.00 ± 0.00	$0.51{\pm}0.02^{h}$	$2.42{\pm}0.04^{\rm hi}$
S3P3	0.00 ± 0.00	$0.55{\pm}0.01^{ij}$	$2.47{\pm}0.23^{hi}$
S3P4	0.00 ± 0.00	$0.44{\pm}0.01^{\rm fg}$	$2.61{\pm}0.11^{i}$
S4P0	0.00 ± 0.00	0.40±0.01°	$4.95{\pm}0.21^{k}$
S4P1	0.00 ± 0.00	$0.21{\pm}0.01^{b}$	1.40±0.01e
S4P2	0.00 ± 0.00	0.25±0.01°	$0.83{\pm}0.05^{b}$
S4P3	0.00 ± 0.00	$0.45{\pm}0.01^{g}$	1.14±0.12 ^d
S4P4	0.00 ± 0.00	0.26±0.01°	1.08±0.05 ^{cd}
Sig.		< 0.05	< 0.05

Note: Means with the same superscript in the same column are not significant (P<0.05). n=2

2.8.4 Statistical analysis

At the end of the all experiments, all subjected to one way analysis of variance (ANOVA) using statistical software (SPSS 20) to detect significant differences in all parameters. Duncan new multiple range tests (Duncan, 1955) was used to detect individual differences between treatment means of growth, antinutrients, water quality and economic analysis. Main effects and interaction of soyabean and phytase was assessed by factorial analysis. Tukey HSD test of comparison was used to detect significant difference between mean pair differences for antinutrints, water quality and growth parameters. Correlation was employed to define the relationship between growth, water quality, and dietary phytase supplementation in Clarias gariepinus fed graded levels of soya bean meal-based diets. All data were presented as means + S.D and a rejection level of P>0.05 was used for all statistical analysis.

3 Result

3.1 Growth and nutrient utilization

Significant interaction of soya bean and phytase was observed for mean weight gain, feed conversion ratio and surivival rate (Table 6). Mean weight gain also declined (Table 6) with increasing substitution of fish meal by soyabean (Tukey, P<0.05), irrespective of phytase levels (Figure 1). However, supplementation of 250 FTU/g phytase improved mean weight gain compared to 500 FTU/g and 1000 FTU/g (Tukey, P<0.05). Compared to diet without phytase (Table 6, fig 2), mean weight gain of fish fed 250 FTU/g diet was higher, but did not differ significantly (Tukey, P<0.05). Phytase increased significantly mean weight gain of fish fed 25% soya bean (S1) from 39.94±0.21g (S1P0) to 40.88±2.53g (S1P3). No significant effect of phytase was observed for mean weight gain of fish fed 50% soyabean (Duncan, P>0.05). Phytase inclusion in 75 % soya bean (S3) resulted in highest weight gain for S3P1 fish (Table 7), which was statistically different from S3P2, S3P3, and S3P4 (Duncan, P<0.05). No statistical difference in mean weight gain and FCR for fish fed S3P1 and S0P0 (Duncan, P>0.05) was recorded. Mean weight gain increased significantly with phytase addition to diet based on 100% (S4) soya bean meal (P<0.05). Fish fed S4P4 had the lowest mean weight gain compared to S4P1, S4P2, and S4P3 (Duncan, P>0.05). Irrespective of





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Figure 1 Mean Effect of soya bean on weight gain of juvenile Clarias gariepinus fed soyabean bean diet



Figure 2 Effect of phytase on mean weight gain of juvenile Clarias gariepinus



Figure 3 Effect of phytase daily feed intake of juvenile Clarias gariepinus



Figure 4 Effect of soya bean on daily feed intake of juvenile Clarias gariepinus



Figure 5 Effect of phytase on feed conversion ratio of juvenile Clarias gariepinus



Figure 6 Effect of soya bean on feed conversion ratio of juvenile Clarias gariepinus



Week 0 Week 2 Week 4 Week 6 Week 8 Week 10 Week 12

Figure 7 Weight gain performance (biweekly) of fish fed experimental diet based on soyabean meal (full fat) for 84 days





soyabean level, phytase at 250 FTU/g showed a significantly higher mean weight gain (Tukey, P<0.05) and daily feed intake (Tukey, P>0.05, fig 3) compared to 0 FTU/g, 500 FTU/g, 750 FTU/g and 1000 FTU/g (Figure 4). FCR of 250 FTU/g was also lower (Turkey, P < 0.05) compared to other phytase diet (Figure 5). Survival rate (Table 6) of fish showed a significant decline in value with increasing levels of soya bean (Duncan, P<0.05). A significant decrease in survival rate (Table 7) was observed when phytase was supplemented in diet based on 25% and 75% soya bean with phytase (Duncan, P<0.05). Fish fed S3P1 showed the lowest survival of fish (73.08±10.88%) compared to S3P0 (98.08±2.72%), S3P2 (96.15±0.00%), S3P3 (94.23±2.72%) and S3P4 (94.23±2.72%). Fish fed 100% soya bean supplemented with 250, 500 and 750 FTU/g phytase had significantly higher values (P<0.05) of survival compared to control diet (S4P0). Regardless of phytase level, survival rate improved with inclusion of soyabean (Tukey, P>0.05) up to 75% compared to fish meal diets (Table 6). Fig1-6 shows effect of phytase and soyabean on growth performance of the fish fed phytase diets. While figure 7 shows weight gain performance (biweekly) of fish fed phytase supplemented diets.

Significant interaction of the diets and phytase was observed for phytate and oxalate concentration (factorial, P<0.05). A significant phytate reduction was achieved with phytase supplementation of 250 FTU/g and 1000 FTU/g compared to control with no phytase (Tukey, P<0.05). Oxalate levels were lower with phytase supplementation at all levels compared to control (Tukey, P<0.05). Although, phytate content varied significantly in experimental diets based on soyabean without phytase from 0.52±0.01mg/g in diet S1P0 to 0.40±0.01mg/g in S4P0 (Table 9), significant reduction in phytate content was recorded in diets based on 25% (25 FTU/g and 500 FTU/g), 50% (250FTU/g-1000 FTU/g), and 100% soyabean meal compared to basal controls (Duncan, P<0.05). Diet S3P1 showed significant reduction in phytate $(0.34\pm0.04$ mg/g), compared with S3P0 (0.43 ± 0.02) , S3P2 (0.51±0.02mg/g), S3P3 (0.55±0.01mg/g), and S3P4 (0.44±0.01mg/g) (P<0.05). There is no significant difference in phytate content of diets S2P3, S2P2, S0P0, and S4P0 (P>0.05). The lowest phytate in experimental diets was measured in S1P1 $(0.14\pm0.01 \text{mg/g})$, while the highest was observed in diet S1P2 (0.61±0.01mg/g). Phytate is negatively correlated with phytase (r= -0.231, P>0.05). Oxalate was also reduced significantly by phytase (r = -0.328, P<0.05) (Table 10).

3.2 Effects of phytase on phytate hydrolysis

Table 10 Correlation between treatment, phytase level, and antinutrients in experimental diet nased on full fat soya bean meal supplemented with phytase

Pearson Correlation	Treatments	Phytase level	Phytate	Oxalate
Treatments	1			
Phytase level	.327*	1		
	0.035			
Phytate	-0.19	-0.231	1	
	0.228	0.142		
Oxalate	0.223	328*	0.206	1
	0.155	0.034	0.191	

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

3.3 Water quality

Multivariate analysis of water quality showed significant interaction between phytase and soyabean for all parameters, except pH (Table 12) was observed for fish. A significant effect of phytase in diet of the fish was observed (Table 12) for dissolved oxygen (ANOVA, P < 0.05). Disolved oxygen improved

significantly with dietary phytase supplemention diets compared to diets without phytase (Tukey, P<0.05). There was little or no negative effect of phytase on water quality (Table 11 and 12) as shown by improved growth parameters (Table 13). Dissolved oxygen concentration decreased significantly with soya bean inclusion without phytase from 3.85 ± 0.07 mg/l in fish





tank that received S1P0 to 2.65 ± 0.07 mg/l in tank of fish fed S4P0 (Duncan, P<0.05). Phytase addition increased significantly the level of dissolved oxygen in tank fed 75%, and 100% soya bean compared to diets without phytase (Duncan, P<0.05). Fish reared in tanks allocated 25% soya bean showed increase in dissolved oxygen from 3.85 ± 0.07 mg/l in S1P0 fish tank to 3.95 ± 0.07 mg/l for tank with fish fed S2P4 (Duncan, P>0.05). The lowest level of dissolved oxygen were obtained in tanks of fish fed S0P0 and S3P0, both of which recorded similar value of 2.15 \pm 0.07mg/l; tanks with fish fed diets S3P2 and S3P3, which both had the highest dissolved oxygen level of 3.95 \pm 0.07mg/l. Irrespective of phytase levels, all soyabean diets showed a significantly higher level of dissolved oxygen compared to fish meal diet (Tukey, P<0.05). Dissolved oxygen showed a positive with phytase (r= 0.262, P>0.05) (Table 12). It also showed a positive correlation with final weight, weight gain, feed intake, SGR, PER, and survival rate (Table 14).

Table	11	Water	Quality	Analysis
1 aute	11	water	Quanty	Allalysis

Treatments	Dissolved Oxygen	pН	Temperature (⁰ C)	Ammonia (mg/l)	Nitrate (mg/l)
	(mg/l)				
Initial	4.80±0.02	7.40±0.03	23.8±0.04	0.00±0.00	0.00±0.00
S0P0	2.15±0.07 ^a	$7.44{\pm}0.01$ abcdef	$23.55{\pm}0.07^{ab}$	9.39±0.03 ^r	33.11 ± 0.18^{j}
S1P0	$3.85{\pm}0.07^{jk}$	$7.45{\pm}0.00^{bcdef}$	23.50±0.28ª	4.31 ± 0.01^{m}	1.70±0.01 ^b
S1P1	$3.75{\pm}0.07^{ij}$	$7.43{\pm}0.00^{abcdef}$	$23.80{\pm}0.00^{bcd}$	$1.83{\pm}0.01^{h}$	1.23±0.01ª
S1P2	$3.45{\pm}0.07^{\rm fg}$	7.38±0.03ª	23.85±0.07 ^{cde}	1.59±0.01 ^g	$2.65{\pm}0.03^{f}$
S1P3	$3.35{\pm}0.07^{\rm ef}$	$7.48{\pm}0.07^{\rm f}$	$24.30{\pm}0.14^{gh}$	1.05±0.01 ^e	2.30±0.01°
S1P4	$3.95{\pm}0.07^{k}$	7.39±0.01 ^{ab}	$24.50{\pm}0.00^{hi}$	6.14±0.01°	40.85±0.05°
S2P0	$3.85{\pm}0.07^{jk}$	$7.44{\pm}0.01$ abcdef	23.40±0.00 ^a	$4.47{\pm}0.01^{n}$	$3.46{\pm}0.01^{gh}$
S2P1	$3.55{\pm}0.07^{gh}$	$7.43{\pm}0.04^{abcdef}$	23.65±0.35 ^{abc}	$2.74{\pm}0.01^{1}$	34.18 ± 0.38^{1}
S2P2	$3.65{\pm}0.07^{hi}$	$7.42{\pm}0.01$ abcdef	$23.95{\pm}0.07^{def}$	9.70±0.02s	35.83±0.02 ^m
S2P3	3.05±0.07°	$7.40{\pm}0.03^{abcd}$	$24.50{\pm}0.00^{hi}$	6.14±0.01°	$2.25{\pm}0.01^{de}$
S2P4	$3.15{\pm}0.07^{cd}$	$7.41{\pm}0.04^{abcde}$	$24.30{\pm}0.00^{gh}$	0.34±0.01ª	37.56±0.03 ⁿ
S3P0	2.15±0.07ª	$7.40{\pm}0.00^{\rm abcd}$	23.45±0.07 ^a	7.39±0.01 ^p	$33.75 {\pm} 0.01^k$
S3P1	$3.25{\pm}0.07^{de}$	$7.42{\pm}0.02^{\rm\ abcde}$	$23.90{\pm}0.00^{cdef}$	0.82±0.01°	$3.72{\pm}0.01^{i}$
S3P2	$3.95{\pm}0.07^{k}$	7.39±0.03 ^{abc}	$24.10{\pm}0.00^{efg}$	$1.45{\pm}0.01^{\rm f}$	$3.28{\pm}0.04^{g}$
S3P3	$3.95{\pm}0.07^k$	$7.40{\pm}0.03$ abcd	$24.55{\pm}0.07^{hi}$	$2.05{\pm}0.01^{i}$	$3.60{\pm}0.02^{hi}$
S3P4	$3.25{\pm}0.07^{de}$	7.42±0.01 abcde	$24.15{\pm}0.07^{fg}$	$2.44{\pm}0.01^{k}$	1.78±0.01 ^b
S4P0	2.65 ± 0.07^{b}	$7.45{\pm}0.00^{\rm \ cdef}$	$23.80{\pm}0.00^{bcd}$	$2.20{\pm}0.01^{j}$	$1.84{\pm}0.01^{b}$
S4P1	$3.65{\pm}0.07^{hi}$	$7.45{\pm}0.00^{\rm \ cdef}$	$23.80{\pm}0.00^{bcd}$	0.78 ± 0.01^{b}	$2.09{\pm}0.01^{cd}$
S4P2	$3.25{\pm}0.07^{de}$	$7.46{\pm}0.01^{def}$	$24.10{\pm}0.00^{efg}$	$0.88{\pm}0.02^{d}$	1.70±0.01 ^b
S4P3	$3.55{\pm}0.07^{gh}$	$7.47{\pm}0.04^{\rm ef}$	$24.60{\pm}0.00^{i}$	0.76 ± 0.01^{b}	2.05±0.01°
S4P4	3.15 ± 0.07^{cd}	$7.44{\pm}0.01$ abcdef	$24.05{\pm}0.07^{defg}$	8.55±0.01 ^q	1.25±0.01ª
Sig	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Note: Means with the same superscript in the same column are not significant (P<0.05). n=2

Ammonia concentration in fish tanks varied significantly with phytase addition to experimental diets (P<0.05). The highest level of ammonia was observed in tank of fish fed S2P2 (9.70 ± 0.02 mg/l), while the lowest observed value was recorded for fish tank fed S2P4 (0.34 ± 0.01 mg/l). Ammonia levels

increased significantly with diets based on 25%, 50%, and 75% without phytase (P<0.05). Phytase addition to soya bean decreased ammonia levels significantly (Duncan, P<0.05), which also showed a significant reduction (Tukey, P<0.05) compared to diets without phytase, regardless of soyabean levels (Table 11).





Levels of ammonia decreased with phytase from 4.31 ± 0.01 mg/l in fish tank fed S1P0 to 1.05 ± 0.01 mg/l in tank fed S1P3 (P<0.05). There were also significant decrease in ammonia levels from 7.39 ± 0.01 mg/l for fish fed S3P0 to 2.44 ± 0.01 mg/l in fish fed S3P4 (P<0.05). Fish fed diet S3P1 had the lowest (Duncan, P<0.05) ammonia level (0.82 ± 0.01 mg/l) compared to ammonia levels in tanks of fish fed S3P2

(1.45 \pm 0.01mg/l), S3P3 (2.05 \pm 0.01mg/l), and S3P4 (2.44 \pm 0.01mg/l). Concentration of ammonia decreased significantly (Duncan, P<0.04) from 2.20 \pm 0.01g/l for diet S4P0 to 0.76 \pm 0.01mg/l (S4P3). Ammonia correlated negatively with treatment (r= -0.249, P>0.05) and phytase (r= -0.105, P>0.05) (Table 13). Levels of ammonia were significantly lower (Tukey, P<0.05) in all phytase diets, irrespective of soyabean

Tuote 12 Elleet of boya obtain (fait fat) and phytabe of mater quality of fatenine of a top inter	Table	12 Effect	of soya	bean ((full fat) and	phytas	se on	water	quality	y of	juvenile	Clarias	gariepin	ius
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Sources of	Dissolved	pH	Temperature (⁰ C)	Ammonia (mg/l)	Nitrate (mg/l)
variation, P value	Oxygen (mg/l)				
Phytase	0.000	0.162	0.000	0.000	0.000
Soya bean	0.000	0.007	0.270	0.000	0.000
Phytase	0.000	0.159	0.002	0.000	0.000
* Soya bean					
Pooled SE	0.011	0.004	0.017	0.002	0.014
Tukey HSD	-0.62*, 0.000	0.002, 0.999	-0.25*, 0.001	4.01*, 0.000	4.46*, 0.000
(means,					
P values)					
Phytase					
P0 vs P1					
P0 vs P2	-0.65*, 0.000	0.023, 0.362	-0.46*, 0.000	2.15*, 0.000	3.91*, 0.000
P0 vs P3	-0.55*, 0.000	-0.002, 1.000	-0.95*, 0.000	3.05*, 0.000	12.22*, 0.00
P0 vs P4	-0.45*, 0.000	0.023, 0.362	-0.71*, 0.000	1.19*, 0.000	-5.59*, 0.000
P1 vs P2	-0.03, 0.953	0.02, 0.537	-0.21*, 0.008	-1.86*, 0.000	-0.56*, 0.000
P1 vs P3	0.08, 0.248	-0.01, 0.995	-0.70*, 0.000	-0.96*, 0.000	7.76*, 0.000
P1 vs P4	0.18*, 0.001	0.02, 0.537	-0.46*, 0.000	-2.82*, 0.000	-10.05*, 0.000
P2 vs P3	0.10, 0.068	-0.03, 0.323	-0.49*, 0.000	0.90*, 0.000	8.31*, 0.0000
P2 vs P4	0.20*, 0.000	0.00, 1.000	-0.25*, 0.002	-0.97*, 0.000	-9.50*, 0.000
P3 vs P4	0.10, 0.068	0.03, 0.323	0.24*, 0.003	-1.87*, 0.000	-17.81*, 0.000
Soya bean	-1.52*, 0.000	0.016, 0.923	-0.44*, 0.000	6.41*, 0.000	23.36*, 0.000
S0 vs S1					
S0 vs S2	-1.30*, 0.000	0.022, 0.801	-0.41*, 0.001	4.71*, 0.000	10.45*, 0.000
S0 vs S3	-1.16*, 0.000	0.036, 0.39	-0.48*, 0.000	6.56*, 0.000	23.88*, 0.000
S0 vs S4	-1.10*, 0.000	-0.012, 0.973	-0.52*, 0.00	6.76*, 0.000	31.32*, 0.000
S1 vs S2	0.22*, 0.000	0.006, 0.984	0.03, 0.973	-1.70*, 0.000	-12.91*, 0.000
S1 vs S3	0.36*, 0.000	0.02, 0.430	-0.04, 0.927	0.15*, 0.000	0.52*, 0.000
S1 vs S4	0.42*, 0.000	-0.03, 0.143	-0.08, 0.509	0.35*, 0.000	7.96*, 0.000
S2 vs S3	0.14*, 0.002	0.01, 0.739	-0.07, 0.630	1.85*, 0.000	13.43*, 0.000
S2 vs S4	0.20, 0.000	-0.03, 0.051	-0.11, 0.214	2.05, 0.000	20.87, 0.000
S3 vs S4	0.06, 0.349	-0.05*, 0.003	-0.04, 0.927	0.20, 0.000	7.44, 0.000

Note: *The mean differences are significant at P<0.05





levels compared to diets without phytase (Table 12). It was also negatively correllated to FCR, PER and SGR (Table 14). Nitrate levels were significantly affected by soya bean and phytase inclusion in experimental diets (ANOVA, P<0.05). Concentration of nitrate increased significantly in tanks fed diets based on 25%, 50%, and 75% soya bean meal without phytase (Duncan, P>0.05), but only declined with phytase addition to diets based on 75% and 100% soya bean meal compared to diets without phytase (Duncan, P<0.05). Phytase addition to diet based on 75% soya bean decreased nitrate level from 33.75 ± 0.01 mg/l in tank of fish fed S3P0 to 1.78 ± 0.01 mg/l in tank fed

 Table 13 Correlation between water quality, treatment and phytase

S3P4 (Duncan, P<0.05). Similarly, nitrate level decreased significantly in fish tank fed 100% soya bean from 1.84 ± 0.01 mg/l in fish tank fed S4P0 to 1.25 ± 0.01 mg/l in tank fed S4P4 (Duncan, P<0.05). Irrespective of soybean levels, phytase addition of 750 FTU/g had a significantly lower nitrate level compared to other diets (Tukey, P<0.05). Nitrate level was lowest in tank of fish that received 100% soyabean compared to other level of soya bean, irrespective of phytase level (Tukey, P<0.05). There was no significant difference between nitrate levels in fish tanks fed S1P0, S3P4, S4P0, and S4P2 (Duncan, (P>0.05).

		<u>,</u>	1 5			
Pearson	TRT	Phytase	DO	pН	Temperature	Ammonia
TRT	1					
Phytase	.327*	1				
	0.035					
DO	-0.047	0.262	1			
	0.767	0.093				
pН	0.138	-0.183	-0.102	1		
	0.382	0.247	0.519			
Temperature	.359*	.831**	0.282	-0.112	1	
	0.019	0	0.07	0.478		
Ammonia	-0.249	-0.105	352*	-0.123	-0.254	1
	0.112	0.507	0.022	0.438	0.105	

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

4 Discussion

4.1 Growth and nutrient utilization

Phytase has been extensively used in animal nutrition to enhance growth and feed and nutrient utilization (Nwanna and Schwarz, 2007; Nwanna et al., 2008). Phytase enhanced apparent digestibility of minerals and their deposition in the fish (Nwanna, 2005). It increased mineral utilization through the breakdown of phytate-mineral complex (Singh, 2008), which resulted in increased growth and feed efficiency of the fish (Cao et al., 2007; Nwanna et al., 2008) through improvement in phosphorus bioavailability (Kumar et al., 2011). Improved growth performance and nutrient utilization were observed in this research investigating the possibility of substituting entirely or almost entirely level of fish meal in fish diet (Cao et al., 2007). Reduction in mean weight gain by increasing soyabean levels in the diet (Figure 1) may be due to high fibre (Table 1) and low phosphorus (Kumar et al., 2011) and other antinutrient (Haghbayan and Mehrgan, 2015) in soya bean. Mean weight gain with phytase at 250 FTU/g in 75% soya bean (full fat) was better than at lower (25% and 50%) and higher (100%) fish meal substitution by soya bean (full fat) with phytase addition at any level (Table 6). This may be explained by fact that phytase is effective in hydrolyzing phytate at low dietary phosphorus (Table 5) and low available phosphorus level (Cao et al., 2007; Selle and Ravindran, 2007; Singh, 2008). The total and available phosphorus (Table 5) in 75% soya bean diet (full fat) without phytase are much lower $(0.75\pm0.01\%)$ and 0.33±0.01%) than at 25% (1.15±0.01% and $0.63\pm0.01\%$) and 50% (1.01±0.01% and 0.44±0.01%). Phytase addition to 25%, 50%, and 75% soya bean diet (full fat) significantly improved feed intake compared to control (100% fish meal). The improvements in daily feed intake (Figure 3) and feed conversion (Figure 5) may be attributed to phytate





hydrolysis (Hussain et al., 2011a) leading to improved overall phosphorus utilization from phytate in the diet (Table 8). In this research, improved growth in fish fed 75% soya bean meal (full fat) with 250 FTU/g is comparable to fish meal, which could possibly substitute and effectively replace a fish meal based diet of the fish. This finding was also reported by Castro et al. (2011) who reported that soya bean at 75% inclusion could effectively replace fish meal in the diet of Rain bow trout (*Oncorhynchus mykiss*) without affecting growth. The higher growth performance achieved with 250 FTU/g phytase showed the better growth performance compared to other diets (Table 6).

Table 14 Correlation bet	ween water qualit	y growth and nutrient	utilization parameters
Table 14 Correlation bet	ween water quant	y, growth and nutrien	, utilization parameters

Pearson	TRT	Phytase	DO	pН	Temp	NH ₃	Nitrate	FNLWT	WTG	FI	FCR
Nitrate	330*	0.017	-0.25	-0.268	-0.102	.473**	1				
			6								
	0.033	0.914	0.101	0.086	0.522	0.002					
Mean	850*	-0.092	0.074	-0.257	-0.128	0.172	0.303	1			
final wt	*										
	0	0.56	0.641	0.1	0.421	0.276	0.051				
Mean	849*	-0.102	0.072	-0.264	-0.136	0.178	.328*	.997**	1		
weight	*										
gain											
	0	0.52	0.652	0.092	0.389	0.259	0.034	0			
DFI	630*	0.042	0.089	-0.251	0.051	-0.007	0.023	.798**	.775**	1	
	*										
	0	0.791	0.575	0.109	0.747	0.963	0.886	0	0		
FCR	.480**	-0.167	-0.28	0.274	-0.008	-0.137	-0.274	695**	698*	449*	1
			5						*	*	
	0.001	0.29	0.067	0.079	0.958	0.385	0.079	0	0	0.003	
SGR	-0.176	-0.102	0.191	-0.287	-0.102	-0.003	0.213	0.133	0.141	0.019	353*
	0.266	0.518	0.225	0.066	0.521	0.986	0.176	0.399	0.372	0.905	0.022
PER	-0.282	-0.168	0.226	320*	-0.139	-0.092	0.262	0.239	0.246	0.114	375*
	0.07	0.289	0.15	0.039	0.382	0.561	0.094	0.128	0.116	0.472	0.014

Improvement in growth performance in Clarias gariepinus (Figure 2, 3 and 5) showed its capacity to utilize plant-based diet when supplemented with phytase compared with that of other fish like Nile tilapia, which only showed better growth at 50% soya bean with phytase (Goda et al., 2002). Additionally, the increase in growth support the report of an increased utilization of phosphorus from phytate (Kumar et al., 2011), which is highly heat stable (Nahashon and Kilonzo-Nthenge, 2011). The high phytate in soya bean (Table 4) may also explain improved growth in the fish (Selle and Ravindra, 2007). Castro et al. (2011) reported phytase at (4,000 FTU/g, Ronozyme) replaced 75% for Rainbow trout, which showed better growth performance than diet without phytase. In the studies of Li and Robinson (1997) and Robinson et al. (2002), channel catfish fed the diets containing 250 FTU/g phytase or above

consumed more feed, gained more weight, and had a lower feed conversion ratio in comparison to fish fed the basal diet containing no microbial phytase. Nwanna et al. (2006) also reported an improvement in growth of juvenile Clarias gariepinus fed phytase supplemented diet based on oven dried soya bean meal with the highest growth obtained with 8000 FTU/g Ronozyme phytase (Figure 6). Differences in optimum phytase for growth, which has been reported in several studies (Kumar et al., 2011; Hussain et al., 2011a), may be due to differences in phytase sources and diet formulation (Cao et al., 2007; Hussain et al., 2011b). Survival rate in fish 75% soya bean supplemented with 250 FTU/g showed a significant decline in value compared to other phytase supplemented diet, which may be due to the high fat in soya bean compared with fish meal (Table 1) and the inhibition of phosphorus by fat (Rezq et al., 2010),





rather than the effect of water quality (Lemarie et al., 2004). An evidence of this assertion is the fact that high dissolved oxygen and lower ammonia was observed in S3P1 compared to S3P0, S3P2, S3P3, and S3P4 (Table 12). Fish fed 100% soya bean also showed the lowest feed intake, specific growth rate, protein efficiency, and the highest feed conversion. The low growth in soya bean fish may be due to unbalanced amino acid profile of soya bean compared to fish meal (Eyo, 2003; Castro et al., 2011), high fibre (Haghbayan and Mehrgan, 2015), the presence of antinutrients (Lei and Porres, 2011) an, including phytate and oxalate (Table 4), high fat content (Table

1) and low phosphorus (Table 2) and zinc (Table 3) compared to fish meal ,which limits its total substitution of fish meal limiting its total substitution. Zinc is essential for the growth and development in animals (Akpoilih et al., 2016). Survival rate, however, was improved in fish fed 100% soya bean with phytase at 250 FTU/g (S4P1), 500 FTU/g (S4P2) and 750 FTU/g (S4P3) showed improvement compared to value for fish fed diet without phytase, which may be due by low phosphorus level at 100% substitution (Table 5). Low dietary phosphorus level in fish (Cao et al., 2007) and animal (Lim et al., 2000) has been reported to stimulate positive response to phytase.

Table 15 Economic analysis c	of experimental diet	with phytase based on	full fat soyabean meal for	Clarias gariepinus
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Treatments	Cost of feed consumed (N)*#	Value of fish (N)	Incidence of cost	Profit index	Cost savings (N)
S0P0	721.86 ± 59.31^{i}	$626.56{\pm}49.02^{\rm f}$	$0.46{\pm}0.01^{bcd}$	$0.87{\pm}0.00^{fgh}$	0.00
S1P0	596.93 ± 32.61^{fgh}	521.89±0.14 ^e	$0.45{\pm}0.02^{bc}$	$0.88{\pm}0.05{}^{\rm fgh}$	7.21
S1P1	622.18±40.12 ^{gh}	541.36 ± 55.42^{ef}	$0.44{\pm}0.01^{abc}$	$0.87{\pm}0.03^{\text{ fgh}}$	14.44
S1P2	$609.39{\pm}68.81^{fgh}$	471.70±13.03 ^{de}	$0.49{\pm}0.06^{cde}$	$0.78{\pm}0.11^{ef}$	-21.66
S1P3	$655.70{\pm}26.52^{hi}$	541.39 ± 60.64 ef	$0.46{\pm}0.02^{bcd}$	$0.81{\pm}0.04^{\rm fg}$	0.00
S1P4	586.50 ± 30.41^{efgh}	521.31±24.17e	$0.44{\pm}0.02^{abc}$	$0.89{\pm}0.08^{\rm ~fgh}$	14.44
S2P0	499.54±40.96 ^{cde}	492.64 ± 8.22^{de}	$0.39{\pm}0.02^{ab}$	$0.99{\pm}0.06^{\rm h}$	50.53
S2P1	435.75±41.16 ^{bc}	412.75±36.01 ^{cd}	$0.40{\pm}0.01^{ab}$	$0.95{\pm}0.01^{gh}$	43.31
S2P2	441.95±39.99 ^{bc}	424.29±42.66 ^{cd}	$0.39{\pm}0.00^{ab}$	$0.96{\pm}0.01^{gh}$	50.53
S2P3	$539.84{\pm}62.94^{defg}$	556.65 ± 104.06^{ef}	$0.38{\pm}0.01^{ab}$	$1.03{\pm}0.07^{hi}$	57.75
S2P4	497.09±47.64 ^{cde}	491.80±21.81 de	$0.39{\pm}0.04^{ab}$	$1.00{\pm}0.13^{h}$	50.53
S3P0	400.45 ± 41.72^{b}	363.87±64.61°	$0.39{\pm}0.01^{ab}$	$0.91{\pm}0.06^{\rm ~fgh}$	50.53
S3P1	481.45±12.09 bcd	$557.44{\pm}10.41^{\text{ ef}}$	0.36±0.04ª	$1.16{\pm}0.05^{i}$	72.19
S3P2	429.05±23.41 bc	375.55±68.09 °	$0.40{\pm}0.02^{ab}$	$0.87{\pm}0.11^{\text{ fgh}}$	43.31
S3P3	547.90 ± 22.95^{defg}	343.80±43.39 °	0.55±0.04 ^e	$0.63{\pm}0.05^{de}$	-64.97
S3P4	521.00 ± 34.51^{cdef}	332.21±9.72 °	$0.53{\pm}0.02^{de}$	$0.64{\pm}0.06^{de}$	-50.53
S4P0	284.31±27.39 ^a	31.71±5.73ª	$0.76{\pm}0.01^{\rm f}$	$0.11{\pm}0.01^{a}$	-216.56
S4P1	284.33±43.57 ª	158.35±5.47 ^b	0.45 ± 0.03^{bc}	$0.56{\pm}0.07^{cd}$	7.22
S4P2	234.27±13.25 ª	97.50±4.62 ^{ab}	$0.48{\pm}0.00^{cde}$	$0.42{\pm}0.01^{bc}$	-14.44
S4P3	260.32±26.90 ª	108.89±24.49 ab	$0.50{\pm}0.01^{cde}$	$0.42{\pm}0.05^{bc}$	-28.87
S4P4	248.89±32.24 ª	72.79±24.66 ^{ab}	0.55±0.11e	$0.30{\pm}0.14^{b}$	-64.97
Sig	P<0.05	P<0.05	P<0.05	P<0.05	

4.2 Water quality

Improvement in growth of fish was consistent with improved water quality (Table 12, Table 13, Table 14). Water quality measured in the study indicates not only a positive effect of promotion pollution management, but also on sustainable aquaculture production. There was observed decrease in oxygen consumption with concomitant increase in dissolved oxygen levels in experimental treatment (Table 11), which may indicate better oxidation of nutrient and less excretion of nitrogen (Castro et al., 2011) as recorded in Table 12. The highest dissolved oxygen recorded for 250 FTU/g phytase compared to 500, 750, and 1000 FTU/g with acorresponding growth improvement (Figure 2, Figure 5) may indicate better phosphorus utilization (Olukosi, 2012) from phytate (Table 8), resulting in the reduction of pollution (Nwanna et al., 2008) in the form of ammonia (Table 12). There was observed increase in dissolved oxygen (decreased consumption) in treatment 25%, 75%, and 100% soyabean diet with phytase addition compared to fish meal, which showed the lowest dissolved oxygen (increased





consumption). In Castro et al. (2011), fish 100% fish meal diet consumed the most oxygen than other phytase supplemented diets, indicating the lowest dissolved oxygen for the diet, which also observed in this study. Dissolved oxygen measured in this study are in the range of requirement for *Clarias gariepinus* between 0.50-3.0mg/l as reported by Manuel et al. (2014). However, it has also been shown that an oxygen level of 3.2 mg/l (60 mmHg) or higher is desired for African catfish held at 25 "C (Manuel et al., 2014). Measurement of ammonia concentration showed significant reduction in all diet, except for fish meal diet and diet without phytase where levels were significantly elevated due to increased excretion as observed by Castro et al. (2011), compared to phytase soyabean diet. According to Castro et al. (2011), nitrogen excretion and oxygen consumption may be used to estimate the oxidation of nutrients from the diet and, usually, low values indicate a better use of the protein source (less excretion) and less energy spent in its oxidation (less consumption). In this study, fish fed 100% soya bean with phytase showed the lowest ammonia level, while control fish fed 100% fish meal (S0P0) showed the highest ammonia level as observed by Castro et al. (2011). The high ammonia level in tank of fish fed diet SOP0 may possibly explain the reduction in survival of the fish (Manual et al., 2004) compared to phytase-supplemented diets (Table 7). Farmer et al. (2011) reported that elevated ammonia levels is stressful to fish, which could lower fish growth and survival (Lemarie et al., 2004). The lack of significant change in pH may suggest an improved water quality, which was not impaired by phytase supplementation to diet. Temperature was slightly raised, but are within the range for the fish (Manual et al., 2013). The improvement in dissolved oxygen with phytase supplementation in the diet without significant change in pH may possibly obviate

the negative effect of temperature (Wagner et al., 1997). Temperatures ranging between 18°C and 28°C occur in the natural habitat of this species (Manual et al., 2013). The significant reduction in nitrate, resulting from a reduction of ammonia by oxidation (WHO, 2011) as observed in Table 12, may indicate improved oxygen carrying capacity of blood and reduced gill permeability, making it less toxic to fish compared to nitrite (Schram et al., 2012). Levels of nitrate observed in this experiment are still tolerable by the fish and are far below the maximum of 140 mg/l nitrate-nitrogen, which may explain improved daily feed intake (Figure 3) with phytase supplementation (Schram et al., 2012). The correlation between treatment, phytase and water quality shows phytase is a non-polluting feed supplement. Improvement in growth by phytase without impairing water quality (Table 14) shows it is an environmentally sustainable method of sustaining aquaculture production. Economic analysis (Table 15) showed that soyabean effectively replaced greater amount of fish meal with low incidence of cost, but higher profit index (Table 16) compared to fishmeal at low phytase of 250FTU/g. Cost savings is equally derived with phytase supplementation in soya bean based diet of the fish. The highly reduced cost of phytase (N143.12/kg) mean it is not only a cheaper and environmental alternative to inorganic phosphorus (Lei et al., 2013), but a better economy for enhancing aquaculture production with minimal cost implications. In conclusion, the study has demonstrated that although phosphorus level in water was not measured, which may limit its scope, phytase supplementation at low level in soyabean diet of Clarias gariepinus could effectively utilize phosphorus from phytate, cut and manage pollution in aquaculture environments, and improve overall growth and economy of fish when compared to the use of fish meal-based diets and diet with no phytase supplementation.

Table 16 Economic analysis of phytase supplementation in soyabean based diet of Clarias gariepinus

Phytase (FTU/g)	Cost of feed consumed (N)	Value of fish (N)	Incidence of cost	Profit index	Cost savings (N)
0	500.62±162.91	407.33±218.47	0.49±0.15	0.75±0.34	-21.66
250	455.93±131.82	417.47±172.68	0.41 ± 0.04	0.88±0.23	34.29
500	428.66±145.56	342.26±158.40	0.44±0.05	0.76±0.23	14.44
750	500.94±159.04	387.68±200.23	0.47 ± 0.07	0.72±0.25	-9.02
1000	463.37±139.73	354.53±190.80	0.47 ± 0.08	0.71 ± 0.30	-12.63
Sig	P>0.05	P>0.05	P>0.05	P>0.05	





Conflict of interest

The authors have declared that there is no conflict of interest arising from the submission and publication of this manuscript.

Authors' contribution

ABU carried out the research work with the supervision of OBO. AEK provided technical depth, guidance and support for its completion. ABU performed statistical analysis and typesetted the manuscript, while OBO and AEK read and approved the manuscript for submission. Revision of manuscript was read by OBO and AEK, correction was made by ABU. All authors approved its content.

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