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Effect of Toasting and Incubation of Soybean Meal Supplemented with Phytase in Practical Diets on the Growth and Mineral Deposition in Nile Tilapia

Nwanna, L. C.
Department of Fisheries and Wildlife, Federal University of Technology, PMB 704 Akure, Nigeria

Abstract: Feeding trials were conducted in glass tanks to assess the effect of toasting and incubation of soybean meal supplemented with phytase (Ronozyme P (5000) CT, Batch HB 955001) in practical diets on the growth, digestibility and mineral deposition in Nile tilapia, *Oreochromis niloticus*. The diets contained six levels of the enzyme at 0, 2000 units, 4000 units, 6000 units, 8000 units and 10,000 units/kg diet making diet /treatments (1-6). The zero level served as the control. The diets were fed to fingerlings of Nile tilapia (6.23 ± 0.1) at 5% of their body weight, twice daily, for 63 days. At the end of the experiment, fish carcass (whole body) and the faecal matters were analyzed for mineral composition. Results showed no significant differences in the growth and nutrient utilization of the fish fed all the diets. All the fish fed diets that contained phytase had better ($P>0.05$) growth and nutrient utilization indices than the fish fed diet without phytase. Similarly, the apparent digestibility coefficient (ADC) for protein and gross energy (GE) of fish fed diets with phytase were higher ($P> 0.05$) than the ADC for protein and ADC for GE of the fish fed diet without phytase. The fish fed diet with 8000 units of phytase/ kg diet had the best mean weight gain, specific growth rate, food conversion ratio, ADC for protein, lipid and GE compared with the fish fed every other diet. The mineral composition (Ca, Mg, P, Fe, Zn, Mn) in all the fish fed the different diets were similar ($P>0.05$),.though some of the group of fish fed diets that contained phytase had marginally higher mineral concentration than in the fish fed diet without phytase. Faecal P decreased significantly in the fish fed diets with 8000 and 10000 units of phytase/kg diet.

Key words: Toasted and incubated soybean, Phytase, Nile tilapia

Introduction

Fish meal accounts for 30-50% by weight of feeds for carnivorous fish Cheng and Hardy (2002) and about 65% of the opeating costs of aquaculture in the developing countries where it is not produced (Nwanna *et al.,* 2004). Cheng and Hardy (2002) reported that fish meal production has been more or less static over the past 15 years, while its demand in feeds has tripled over the same period. Fish meal also contains excess of phosphorus (P) that are often leached into the culture systems leading to environmental pollution and consequently, economic loss. Therefore the future of commercial aquaculture will depend largely on the effective use of altrenative plant proteins to replace fish meal in practical diets for fishes. Plant proteins (soybeans, oil seeds) are more available, relatively cheaper and contains less P than fish meal. Nevertheless, plant proteins contain antinutritional factors such as phytic acid, protease and digestive enzyme inhibitors which limit nutrient bioavailabilty and digestibility by fishes. Phytic acid serves as the main storage form of P in mature seeds and grains (Chung, 2002). Cheng and Hardy (2002) reported that about 2/3 of P in plants feed stuffs are in the form of phytate, which is not digestible by fishes. Phytate also reduces the bioavailability of other dietary components including multivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}. To liberate the phytate P, and to reduce the costs of feeds through reducton in dietary minerals supplementation, fish scientists (Lanari *et al.*. 1998 and Storebaken *et al.* 1998) have suplemented phytase enzyme in practical diets. Phytase has the ability to improve phytate P utilization by fishes as well as liberating the bound minerals in plant feedstuffs, thus sparing a certain portion of dietary P and minerals. Lanari *et al.*., (1998) reported that the effect of phytase on growth, protein and dry matter digestibility in fish varies with different protein ingredients and among fish species.

There is a dearth of information on the effects of phytase addition in practical diets on the growth of Nile tilapia, Oreochromis niloticus. In a previous study, the author tested the effects of phytase addition into untreated soybean meal based diets on the growth and mineral deposition in Nile tilapia. The present study is designed to test the effect of phytase addition into toasted and incubated soybean meal based diets on the growth and mineral deposition in Nile tilapia Oreochromis niloticus.

Materials and Methods

Diets Preparation: The feed ingredients used were; soybean, fish meal, wheat, maize, vitamin, minerals premix, starch and vegetables oil. The soybean (Glycine max) was processed by heat treatment method. The soybean was toasted for 10 hours at 70^{0}C before grinded into fine powder to form a meal. The ground soybean meal was mixed in distilled water at ratios 1kg : 1kg, spread on metal plates and incubated in an oven at 70°C for 8 hours. After incubation the dried soybean meal was blended again into fine powder, packed in plastic bags and stored at ambient temperature prior to use. Ingredients were mixed together at the right proportion (Table 1) to formulate 30% crude protein diet. Then phytase enzymes were added into each mixture at 0, 2000, 4000, 6000, 8000, and 10000 Units/kg diet to make diets 1-6. Each dietary mixture was extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine to form a noodle like strands which were mechanically broken into suitable sizes for the tilapia fingerlings. The pelleted diets were sun dried to a constant moisture of less than 10%, packed in plastic bags and stored at room temperature prior to use.

Feeding Trials: The experiment was carried out in glass tanks of (60 x 30 x 30 cm) each, which were supplied with Tap-water. Water level in each tank was maintained at 40cm depth throughout the experimental period. Hatchery bred fingerlings of Tilapia (*Oreochromis niloticus*) (6.23$\pm$0.06g) were acclimated under laboratory conditions for 7 days and randomly stocked into each tank at 15 fish/tank. Each treatment was replicated thrice. The fish were fed at 5 % of their body weight for 63 days. Water in each tank was replaced every day throughout the period of the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. Each experimental tank was well aerated using air stone and aerator pumps. The water quality parameters of the culture tanks were measured at 0900h using standard methods. Water temperature and dissolved oxygen were measured daily using a combined digital YSI DO meter (YSI model 57); pH was monitored weekly using an electronic pH meter (Metler Toledo 320 model). Measurement of the fish weight changes was performed weekly and the new feeding rate adjusted accordingly. Fish faeces were collected early in the morning from each set of treatment tanks before feeding by siphoning with tubes. The faeces from each treatment sets were pooled and oven dried at 48°C for 16 h before storing for further analysis.

Digestibility Study: Acid Insoluble Ash (AIA) method (Halver *et al.*., 1993) was used for the determination of feed digestibility. A 25ml of 10% HCl was added to known weights of the ash contents of the feed and fish feaces. The solution was then covered with a wash glass and boiled gentle over low flame for five minutes, after which it was filtered through ash less filter paper and washed with hot distilled water. The residue from the filter paper was returned into the crucible and it was ignited until it was carbon free and it was re-weighted

% AIA = (Weight of Ash- Weight of AIA x 100) / Weight of Ash

Apparent Digestibility coefficient (ADC) was calculated as

$$ADC = 10^2 - 10^2 \frac{(\%\ AIA\ in\ feeds \times \%\ nutrient\ in\ feaces}{(\%\ AIA\ in\ feaces\ \%\ in\ nutrient\ in\ feed.)}$$

Fish Performance Evaluation: Growth performance and nutrient utilization of the experimental fish were measured in terms of final mean weight gain (g), specific growth rate (SGR) and food conversion ratio (FCR), according to Olivera Novoa *et al.*. (1990).

Weight gain = Final body weight – initial body weight

$$SGR\ (\%/day) = 100 \frac{(Log_e\ Final\ body\ weight - Log_e\ initial\ body\ weight)}{Time\ (day)}$$

$$FCR = \frac{Dry\ weight\ of\ feed\ fed\ (g)}{Fish\ weight\ gain}$$

Proximate Analysis: The diets and the experimental fish were analysed for proximate compositions according to the methods of AOAC (1990). Six fish and three fish each from each treatment sets were taken before and after the experiment respectively, and analysed for their proximate composition. The dried fish faeces were also analysed for proximate composition.

Determination of Minerals: Three replicates of the fish carcass (whole body) and faeces were analysed for minerals according to the methods of AOAC (1990). About 2.0g of the samples were ashed for 6h at 550°C. After the ash had cooled to room temperature, 6 mL of 6 N HCl was added and the mixture was brought to boiling point. After cooling to room temperature, another 2.5 mL of 6 N HCl was added and the mixture was warmed to dissolve all the solutes. The solution was then cooled and diluted to 25 mL with distilled water. Then the minerals (Ca, Mg Zn, Fe, Mn) were measured in Atomic Absorption Spectrophotometer (AAS). Phosphorus composition was analysed using the vanadomolybophosphoric acid colorimetric method 4500-P with slight modifications. To 3 mL of the diluted solution of the sample, 3 ml of vanadate-molybdate reagent was added and phosphorus concentration was measured, spectrophotometrically (BECKMAN, UV-DU20 No. 4635009) at 430nm, after the reaction mixture was thoroughly mixed with a machine (Heidolph REAX 2000, No. 54119, Germany) and allowed to stand at room temperature for 10 minutes.

Statistical Analysis: Data (mean weight gain, SGR, FCR, ADC protein, ADC gross energy, ADC lipid, carcass minerals and mineral composition in the faeces) resulting from the experiment were subjected to one way analysis of variance (ANOVA) test using the SPSS (Statistical Package for Social Science 1998 version). Individual differences ($p=0.05$) among treatment means were separated using Duncan`s multiple range test (Duncan, 1955).

Results

The proximate composition of the experimental diets (Table 2) shows that the crude protein of the diets was similar and ranged between 30.8 and 31.5%. The values of the ether extract, ash content, crude fibre and nitrogen free extract were similar and ranged between 16.31 and 16.96%, 8.1 and 8.3%, 10.9 and 12.05 and 31.69 and 33.79% respectively.

The mean water quality parameters (Temperature, dissolved oxygen and pH) measured during the experiment is presented in Table 3. The table shows that Temperature ranged between 27 and 30 oC, dissolved oxygen 5.6 and 7.5 mg/L and pH between 7.89 and 8.01. These values are good for warm water fish culture

The growth response of the fish fed on diet containing varying levels of phytase is shown in Table 4. and in Fig. 1. Though there were no significant differences in the mean weight gain of the fish among the treatments; the highest mean weight gain was obtained from fish fed diet 5, followed by the fish fed diet 6 while the lowest value was obtained from fish fed diet without phytase (control diet).

The specific growth rate (SGR) of fish fed all the diets was similar ($P>0.05$). However, the SGR of the fish fed diet 5 was higher than the SGR of fish fed other diets. The value of the feed conversion ratio followed the same trend. Apparent digestibility coefficient (ADC) for protein, lipid and gross energy showed no significant differences as a result of dietary treatments..Nonetheless, the ADC for protein was highest in the fish fed diet 5 and closely followed by fish fed diet 6 and the lowest value was obtained in fish fed diet 1. Apparent digestibility coefficient for lipid was highest in the fish fed diet 5, followed by the fish fed diet 6 and the lowest value was recorded in the fish fed diet 4. The ADC for gross energy followed the same trend as for the apparent digestibility coefficient for protein.

The result of the proximate composition of the initial and final carcass composition of the Nile tilapia fingerlings fed on the experimental diets is presented in Table 5. The fish fed diet 1 had the highest numerical value of crude protein while the least value was obtained from fish fed diet 2. The highest ash content was obtained from fish fed diet 6 while the lowest value was obtained from fish fed diet 4 and 5.The fat content was highest in fish fed diet 6 while the least composition was in fish fed diet 3. The highest crude fibre (%) was obtained from fish fed diet 6 while the lowest value was obtained from fish fed diet 2. Fish fed diet 6 had the lowest value of nitrogen free extract (NFE) while those fed diet 2 had the highest. There was no defined pattern of inclination in the carcass values as a result of the dietary treatments.

The mineral composition of the fish after the experiment is presented in Table 6. There were no significant differences in the carcass Ca, Mg, P, Fe, Zn and Mn. However, addition of phytase in the diets improved minerals deposition in the body of the fish. Further calculation of the percentage differences in the values in the Table revealed that Ca, Mg, P, Zn, Fe, and Mn were improved by between 1.8- 8 %, 0.87-10.8 %, 1.5- 11.1 %, 0.36- 16.6 %, 7.58- 27.5 % and 2.63- 20.2 % respectively.

Table 7 presents the mineral composition of the fish faeces after the experiment. The results indicated that the mineral composition in the faeces of the fish fed diet without phytase was generally higher than the compositions in the faeces of fish fed diets with phytase. The table also showed a downward trend in the value of the minerals with increase in the level of phytase in the diets. The Table highlighted that addition of phytase in the diets reduced the concentrations of Ca, P, Zn, Fe, Mn in the faeces by between 4.1- 32.6 %, 9.7- 21.2 %, 6.39- 21.6 %,12.3- 41.5 %, and 16.9- 51.3.% respectively.

Discussion

The effects of toasting and incubation of soybean meal supplemented with phytase on the growth, nutrient digestibility and minerals deposition in Nile tilapia, *Oreochromis niloticus* were investigated in glass tanks..The water quality parameters; temperature, dissolved oxygen and pH measured during the experiment were good, and there was no mortality as a result of poor water quality. The value obtained for the parameters were within the ranges recommended for warm water fishes (Boyd, 1981) The results of the study showed that fish fed diets with phytase had higher mean weight gain, specific growth rate and nutrient digestibility than the group of fish fed diet without

Table 1: Gross composition of experimental diets

	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6
Fish meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean	44.50	44.50	44.50	44.50	44.50	44.50
Wheat	19.28	19.28	19.28	19.28	19.28	19.28
Maize	18.22	18.22	18.22	18.22	18.22	18.22
Vit-mineral premix	2.00	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00	1.00
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Phytase(U/kg diet)	0.00	2000	4000	6000	8000	10000

Table 2: Proximate composition of the experimental diets

	Diets					
	1	2	3	4	5	6
Crude protein	31.0± 0.02	31.3 ±0.04	30.8 ±0.08	31.5± 0.10	31.0± 0.02	31.1±0.10
Ash	8.10±0.05	8.50±0.06	8.00±0.07	8.30±0.08	8.30±0.08	8.30±0.01
Ether extract	16.7±1.02	16.3±0.07	17.0±0.75	16.3±0.08	17.0±0.86	16.3±0.67
Crude fibre	10.9±0.92	11.1±0.75	11.5±0.08	11.9±0.02	12.1±0.10	12.1±0.95
NFE	33.3±0.01	34.8±0.10	32.7±0.10	32.0±0.98	31.7±0.89	32.2±0.10

Table 3: Mean water parameters of the culture tanks

Parameters	Range
Temperature	27- 30 °C
Dissolved oxygen	5.6- 7.4 mg/l
pH	7.89- 8.01

Table 4: Growth performance and nutrient utilization of Nile tilapia fed phytase

	Diets					
	1	2	3	4	5	6
Initial mean weight (g)	6.19±0.02	6.23±0.04	6.18±0.01	6.25±0.04	6.27±0.01	6.23±0.04
Final mean weight (g)	9.11±0.21	9.22±0.12	9.53±0.45	9.26±0.02	11.6±0.67	9.87±0.20
Mean weight gain	2.92±0.19	2.99±0.08	3.35±0.46	3.01±0.01	5.32±0.67	3.64±0.24
SGR	0.27±0.01	0.27±0.0	0.30±0.03	0.27±0.03	0.42±0.04	0.32±0.01
FCR	1.60±0.09	1.56±0.03	1.40±0.19	1.56±0.01	1.18±0.01	1.28±0.09
ADC protein	45.2±0.09	48.1±0.01	54.0±0.20	49.0±0.02	60.5±0.04	59.0±0.06
ADC lipid	53.4±0.10	50.7±0.07	46.0±0.01	45.0±0.01	63.7±0.05	60.0±0.05
ADC gross energy	45.9±0.04	53.0±0.03	48.0±0.02	49.0±0.02	64.3±0.02	61.4±0.06

Means of three replicates along the same row are not significantly different (P>0.05)

Table 5: Proximate composition of the experimental fish

		Diets					
	Before	1	2	3	4	5	6
Crude protein	55.8± 0.02	63.4 ±0.95	58.3 ±0.70	63.1± 0.40	61.9± 3.25	60.7± 0.10	62.4± 2.45
Ash	17.5±0.05	20.0±1.75	19.9±0.05	20.2±0.75	19.2±0.50	19.2±0.15	20.3±0.85
Ether extract	3.50±0.06	7.40±0.01	7.70±2.00	7.80±0.15	8.40±0.31	8.10±0.55	9.70±2.71
Crude fibre	1.90±0.01	2.19±0.05	1.98±0.20	2.21±0.15	2.12±0.06	2.04±0.50	2.22±0.08
NFE	20.3±0.60	7.01±0.05	12.1±0.01	9.96±0.09	8.38±0.08	9.96±0.07	5.48±0.17

Table 6: Mineral composition of Nile tilapia (whole body) fed phytase diets (mg/l)

	Diets					
	1	2	3	4	5	6
Calcium	48.0±8.36	46.0±7.08	57. ±0.74	48.9±5.28	41.9±2.68	52.5±6.44
Magnesium	56.9±11.3	46.1±2.65	62.8±2.58	57.4±3.71	49.2±2.25	63.8±3.82
Phosphorus	59.0±9.98	52.2±4.38	66.4±3.57	59.9±5.41	64.9±3.03	61.3±1.89
Zinc	27.7±1.06	27.3±1.94	33.2±1.07	33.2±0.03	27.8±1.98	31.7±1.47
Iron	5.61±0.08	3.51±0.06	6.07±0.54	7.74±0.38	5.05±1.15	6.45±1.29
Manganese	4.81±0.42	4.94± 0.66	6.03±0.60	4.38±4.91	5.80±0.88	5.79±0.10

Means of three replicates along the same row are not significantly different (P>0.05)

Table 7: Mineral composition of the faeces of Nile tilapia fed phytase diets (mg/l)

	Diets					
	1	2	3	4	5	6
Calcium	63.1±2.25 [a]	60.5±6.80 [a]	58.0±3.57 [a]	44.2±6.13 [a]	29.7±5.62 [b]	40.0±2.16 [b]
Magnesium	67.5±2.71 [a]	67.8±4.70 [a]	58.6±1.68 [a]	59.8±0.06 [a]	55.0±12.4 [a]	57.6±0.41 [a]
Phosphorus	52.4±0.75 [a]	46.1±10.4 [a]	41.1±11.8 [a]	47.3±3.49 [a]	30.7±1.78 [b]	38.3±6.23 [b]
Zinc	76.7±1.49 [a]	76.4±6.40 [a]	71.8±3.42 [a]	66.1±5.02 [a]	60.1±15.7 [a]	61.1±4.25 [a]
Iron	15.5±0.96 [a]	13.6±2.69 [a]	12.8±0.70 [a]	11.0±2.78 [a]	10.1±0.24 [b]	9.07±0.60 [b]
Manganese	23.6±0.16 [a]	19.6±1.69 [b]	18.4±0.80 [b]	14.3±0.98 [c]	13.3±0.01 [c]	11.5±2.41 [d]

Means along the same row followed by the same superscript are not significantly different (P>0.05)

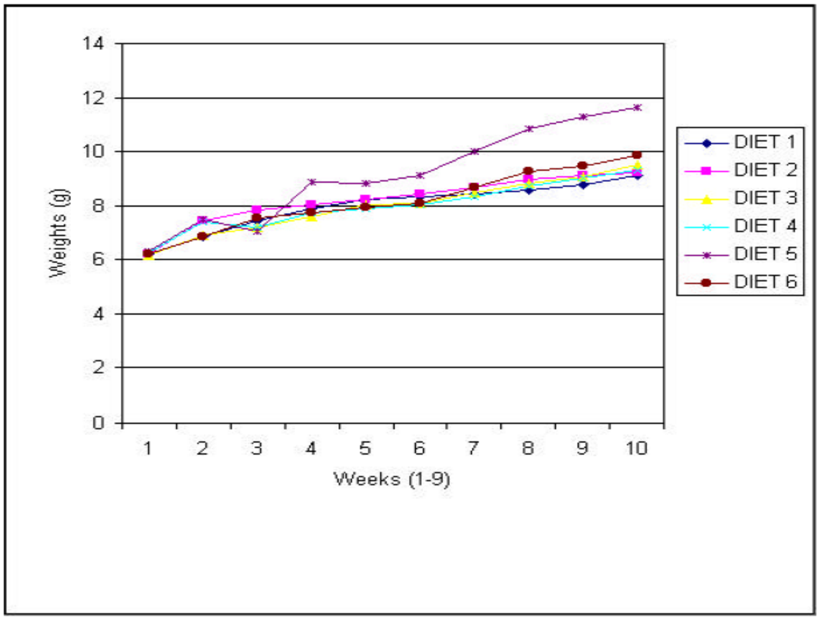

Fig. 1: Weekly mean weight of nile tilapia fed treated soybean meal with phylase

phytase.

The enhancement of the growth performance in the fish fed diet that contained phytase increased with increase in the levels of phytase addition. This improvement could be attributed to the liberation of more phytate phosphorus (P) from the diets by the phytase enzymes, which the fish utilized for the better performance. The higher performance could also be ascribed to higher dietary nutrients bioavailability and digestibility made possible by the phytase enzymes. Addition of phytase to the diets improved the ADC for protein, lipid and gross energy. This observation is in line with the work of Van Weerd *et al*. (1999) who reported that phytase addition enhanced protein and energy digestibilities in African catfish fed phytase-treated soybean meal based diets. The improvement in the ADC for protein, lipid, and energy of the fish fed phytase-treated diets would have contributed to the improvement in the growth parameters. This is in consonant with the ascertion by Nwanna (2004) that supplementation of phytase to untreated soybean meal based diets improved the ADC for protein, lipid and energy which led to significant improvement in the growth rate of Nile tilapia fingerlings. The findings from the present study on the improvement of the growth performance of fish fed phytase treated diets support the report of Li and Robinson (1997) that phytase supplementation may stimulate appetite and therefore increase growth directly through increased feed intake. Similarly, Rodehutscord and Pfeffer (1995) concluded that addition of phytase to soybean meal based diets for rainbow trout (*Oncorhynchus mykiss Walbaum*) increased feed intake and weight gain. Redehutscord (1996) also attributed higher weight gain in rainbow trout fed phytase fortified diets to increased phosphorus (P) availability in the diets caused by the phytase enzyme.

The results of the minerals composition of the fish after the experiment, indicated that addition of phytase in the diets improved the carcass minerals deposition. This supports the work of Hauler and Carter, (1997) who reported that adding phytase to fish feeds has been shown to improve phosphorus uptake in fishes. Similarly, Jackson *et al*., (1996) reported that inclusion of phytase in the diet of fish at 1,000 units per kg or higher significantly increased the mineral content of bone-especially the concentration of calcium, magnesium, phosphorus and zinc.

The minerals deposition in the faeces of the fish after the feeding trials showed that the deposition were generally higher in the feaces of fish fed diet without phytase than in the feaces of fish fed diet with phytase. In other words, addition of phytase in the diets of *Oreochromis niloticus* successfully reduced the minerals available in the faeces. This finding compares well the report of BASF and Gist Brocades, (1999) that phytase can decrease the amount of phosphorus and other minerals in livestock manure including fish manure at 30-35% and amount of nitrogen by 5%.

Conclusion

The study revealed that addition of phytase to toasted and incubated soybean meal based diets improved the growth, nutrient digestibility and minerals deposition in Nile tilapia (*Oreochromis niloticus),* but not statistically significantly. It also established the best dosage of the enzyme used as at 8000 units/kg diet. Deposition of less minerals in the faeces of the fish fed diets with phytase is an indicaton that more minerals were made available in the body of the fish for growth. Therefore phytase can be added to the diets of fishes that desire mineral supplementation as a means of reducing costs through the liberation of the minerals from the natural plant feed ingredients. Also less phosphorus discharges in the faeces means that phytase addition in feeds can be used to achieve less pollution in aquaculture environments.

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