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Toasted Soybean Meal Based Diets Supplemented with Phytase Enhanced Growth and Reduced Faecal Mineral Deposition by Nile Tilapia

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Abstract: The effect of phytase addition in toasted soybean meal based diets on growth performance of Nile tilapia Oreochromis niloticus fingerlings (6.22±0.04) were investigated for 63 days in indoor glass tanks. Phytase was incorporated at 0, 2000, 40000, 6000,8000 and 10000 units/kg diet making up diets 1-6. The diets were fed to the fish at 5% of their body weight, twice daily. The growth indices such as mean weight gain, specific growth rate and food conversion ratio were used to assess the growth performance of the fishes. Results indicated no significant differences ($p>0.05$) in the mean weight gain , specific growth rate, and food conversion ratio of all fish fed the various diets. The apparent digestibility coefficient (ADC) for protein of all fish fed diets with phytase were higher than that in the fish fed diet without phytase. Fish fed diets 3, 4, 5 and 6 had significantly higher ADC for protein than the ADC for protein of fish fed diets 1 and 2. Generally, the mineral compositions in the faeces of fish fed diets with phytase were generally lower than that of group of fish fed diet without phytase. However, there was no clear effect of phytase on the mineral deposition in the body of the fish. The results of the study revealed that addition of phytase to feeds marginally improved the growth performance of the Nile tilapia and reduced the mineral compositions in the faeces of the fish.

Key words: Toasted soybean meal, Phytase, Nile tilapia

Introduction

Soybean is a novel plant protein feed ingredient that is widely used in animal and livestock feeds. Nevertheless, it contains phytate, an antinutritional factor that is not digested by fish (Rodehutscord and Pfeffer 1995; Oliva-Teles et al 1998), that has negative effect on protein digestibility (Spinelli et al 1983) and availability of other minerals (Gattlin and Philips 1989; Sugiura et al 2001). Fish scientists have been developing different methods on how to dephytinize phytate and make the phosphorus more bioavailable and other nutrients more digestible by fishes. Jackson et al (1996) used phytase to catalyse the breakdown of phytic acid (Myonitisol hexaphosphate) to sequentially produce myonitsol penta, tetra, tri; di and monophosphate which neutralized the negative effect of phytic acid on protein and other nutrients in the diets. Hauler and Carter (1997) described that phytase supplementation in fish diets stimulated appetite and therefore increased growth directly through increased feed intake.

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 soybean meal based diets on the growth and mineral deposition in Nile tilapia Oreochromis niloticus.

Materials and Methods

Diet Preparation: The feed ingredients used were; soybean, fish meal, wheat, maize, vitamin, minerals premix, starch and vegetables oil. The soybean (Glycine max) was toasted at 70°C for 10 h before grinded into fine powder to form a meal.. The soybean meal was mixed together with other ingredients 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 Experiment: The experiment was carried out in indoor glass tanks of (60 x 30 x 30 cm) each, which were supplied with Tap-water. Water level in each tank was maintained at 40 cm depth throughout the experimental period. Hatchery bred fingerlings of Tilapia (*Oreochromis niloticus*) (6.21g±0.04g) were acclimated under laboratory conditions for 14 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 twice daily for 63 days. Water in each tank was replaced every day throughout the period of the experiment 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 and stored at −20°C before analysis.

Digestibility Analysis: Determination of acid in soluble ash (AIA) and apparent digestibility coefficient were carried out according to methods of Halver et al (1993). Acid Insoluble Ash was obtained by adding 25ml of 10% HCl to a known weight of the ash content of fish feed and feaces. The solution was then covered with a wash glass and boiled gently over low flame for five minutes, after which it was filtered through ashless 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 was re weighed.

$$\%AIA = \frac{\text{weight of ash- weight of AIA} \times 100}{\text{weight of ash}}$$

$$ADC = 10^2\text{-}100\frac{(\%AIA \text{ in feed} \times \% \text{ nutrient in feaces})}{\%AIA \text{ in feaces} \times \% \text{ nutrient in feed.}}$$

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

$$\text{Weight gain} = \text{Final body weight} - \text{initial body weight}$$

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

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

Proximate Analysis: The diets and the experimental fish were analysed for proximate compositions according to the methods of AOAC (1990). Five 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 K, Zn, 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 at wave length of 430nm, after the reaction mixture was thoroughly mixed with a machine and allowed to stand at room temperature for 10 minutes

Statistical Analysis: Data on mean weight gain, SGR, FCR, ADC for protein, carcass minerals and mineral composition in the faeces of the fish 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 results of the proximate composition of the six experimental diets (Table 2) showed that the dietary compositions are similar.. The protein level in the diets ranged between 31.1 and 34.1 %, ether extract varied between 11.8 and 12.%, ash content ranged between 8.6 and 7.0.%, nitrogen free extract ranged from 43.3 to 49.3 while

the crude fibre content ranged between 1.37 and 1.43%.
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 25 and 28 °C, dissolved oxygen 5.6 and 6.8 mg/L and pH between 7.86 and 8.07.
The growth parameters and apparent digestibility coefficient (ADC) for protein are presented in Table 4, and in Fig. 1. There were no significant differences ($P > 0.05$) among the mean weight gain and SGR of the fish fed all the diets. All the fish fed diets with phytase had higher mean weight gain and SGR than the fish fed diet without phytase. Similarly, there were no significant differences ($P > 0.05$) among the food conversion ratio (FCR) of the fish fed all the diets. And all the fish fed diets with phytase had better FCR than the fish fed diet without phytase. There were significant differences ($P < 0.05$) among the ADC for protein of the fish fed the various diets. The ADC for protein of all the fish fed diets with phytase were higher than that of the fish fed diet without phytase; and increased with increase in the level of phytase.The ADC for protein of the fish fed diets 1 and 2 were the same but lower ($P<0.05$). than the ADC for protein of the fish fed other diets.
The result of the proximate composition of the fish before and after the experiment is shown in Table 5. There were increases in the crude protein levels and ash contents of the fish as a result of dietary treatments. The crude fiber and nitrogen free extracts of the fish decreased after the experiment.
The mineral composition of all the fish fed experimental diets (Table 6) were similar ($P>0.05$) , and indicated no remarkable effect as a result of phytase inclusion in the diets..However, inclusion of phytase at 8000 units/kg diet marginally improved Ca, Mg, K and Zn composition.
The mineral composition of fish feaces after the feeding trials is presented in Table.7. Generally the minerals from the faeces of all the fish fed diets with phytase were lower than those from the faeces of fish fed diet without phytase. This means that more minerals were actually deposited in the fish fed diets with phytase, but this could not be seen from the mineral composition in the body of the fish. Also the lower minerals especially P means that phytase can be used to reduce pollution in fish culture systems.

Discussion

The effect of toasting of soybean meal and supplementation with phytase in practical diets on growth and mineral deposition in Nile tilapia, *Oreochromis niloticus* was evaluated. The water quality parameters of the fish culture tanks, dissolve oxygen, temperature, and pH, were good enough to support good fish yield. There was no fish mortality as a result of poor water quality. The mean values of the parameters recorded during the experimental period were within the recommended values (Boyd 1981) for warm water fish culture.
The growth performance of the fish was enhanced by the addition of phytase in the diets. This is explained by the fact that fish fed diets that contained phytase had higher mean weight gain, specific growth rate (SGR), better-food conversion ratio (FCR) and apparent digestibility coefficient (ADC) for protein than the group of fish fed diet without phytase. Fig. 1 also explained that all the fish fed diets with phytase had higher mean weight gain line-curve than the fish fed diets without phytase. This improvements could be ascribed to better mineralisation of the dietary nutrients by phytase addition, which made more nutrients bioavailable for better digestibility and improved growth in the fish. The observation is in line with the work of Rodehutscord and Pfeffer (1995) who reported that addition of phytase to the diets of rainbow trout increased the phosphorus availability, which resulted in, increased feed intake and higher growth performance in the fish. Similarly, Hughes and Soares (1998) reported that addition of phytase to diets improved food conversion ratio in striped bass *Morone saratitis*. The higher ADC for protein obtained from the fish fed diets with phytase supports the findings of Van Weerd et al (1999) who asserted that phytase addition in the diets enhanced ADC for protein of African catfish *Clarias gariepinus*. Studies by Sugiura et al 2001 and yan et al 2002 showed that addition of phytase in feeds improved carcass protein and ash. This finding is supported by the results of the present study which revealed that addition of phytase in the diets enhanced carcass protein and ash composition. Supplementations of phytase in fish diets have been shown to improve mineral deposition due to phytate dephosphorylaton by the phytase. Storebakkeen et al 1998 reported that phytase addition to Atlantic salmon diets enhanced Ca, Mg, P and Zn retentions in the body carcass. This finding is in line with the results of the present study, which indicated that inclusion of 8000 units of phytase/kg diet marginally improved Ca, Mg, K and Zn deposition in the fish.
Also, the results of the present study indicated good evidence that phytase addition to diets could reduce environmental pollution in the culture systems. All the fish fed diets with phytase had lower mineral deposition in their faeces than in the group of fish fed diet without phytase. The less mineral composition in the faeces of the fish fed diets with phytase means less environmental effluent discharges and less pollution; since high mineral loadings specially, phosphorus has been linked with eutrophication and environmental pollution problems. This finding on low mineral excretion in the faeces supports the work of Sugiura et al (2001) that addition of phytase

Table 1: Gross composition of experimental diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal (65% CP)	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal (45% CP)	44.5	44.5	44.5	44.5	44.5	44.5
Wheat (18% CP)	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
Vitamin-min premix	2.00	2.00	2.00	2.00	2.00	2.00
Carboxymethylcellulose	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	2000	4000	6000	8000	10000

Table 2: Proximate composition of experimental diets (%)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Crude protein	34.1±0.20	33.6±0.23	33.6±0.33	31.1±0.36	33.0±0.46	32.8±0.12
Ether extract	12.0±1.12	13.4±0.23	12.4±0.44	11.3±0.54	12.0±0.37	11.8±0.43
Ash	8.62±0.32	8.33±0.43	7.47±0.37	7.00±1.11	8.61±1.23	7.92±0.16
Crude fibre	1.38±0.23	1.37±0.32	1.43±0.22	1.40±0.24	1.39±1.11	1.37±1.24
Nitrogen free extract	43.9±1.12	43.3±2.00	45.1±1.32	45.2±0.98	45.0±0.56	46.1±1.26

Table 3: Mean water parameters of the culture tanks

Parameters	Range
Temperature	25- 28 °C
Dissolved oxygen	5.6- 6.8 mg/l
pH	7.86- 8.07

Table 4: Growth and ADC protein of Nile tilapia fed phytase diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Initial mean weight (g)	6.23±0.03	6.19±0.01	6.22±0.04	6.19±0.01	6.22±0.04	6.22±0.02
Final mean weight (g)	9.57±1.78	9.56±0.56	11.1±1.40	11.3±0.07	10.3±1.02	10.1±2.02
Mean weight gain (g)	3.34±0.03	3.37±0.02	4.89±0.20	5.12±0.32	4.06±0.26	3.85±0.27
SGR (%/day)	0.67±0.33	0.69±0.02	0.92±0.19	0.97±0.08	0.80±0.17	0.75±0.32
FCR	1.65±0.89	1.36±0.06	1.20±0.26	1.21±0.08	1.16±0.75	1.20±0.84
ADC protein	39.8 ± 1.29^{a}	40.4 ± 0.55^{a}	46.2 ± 4.77^{b}	51.2 ± 5.21^{bc}	57.6 ± 0.06^{d}	56.7 ± 4.89^{cd}

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

Table 5: Proximate composition of the fish before and after the experimental (%)

	Initial	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Crude protein	55.8±0.12	57.5±2.34	58.3±0.88	61.3±1.37	57.9±0.74	58.7±0.82	58.2±1.34
Ether extract	3.50±0.04	2.17±1.36	3.40±0.30	4.04±0.30	3.10±1.10	4.07±0.52	2.83±0.78
Ash	18.5±0.05	21.0±1.99	24.8±1.99	23.6±1.28	24.9±1.13	23.2±0.95	24.8±0.52
Crude fibre	1.90±1.11	1.73±0.20	1.70±0.56	0.96±0.80	1.00±1.12	1.03±0.46	1.07±0.67
Nitrogen free extract	20.3±0.02	17.6±0.12	11.8±0.74	10.1±0.46	13.1±0.32	13.0±0.62	13.1±0.74

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

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Calcium	32.3±1.16	30.6±5.01	31.6±2.42	31.6±1.35	36.8±4.34	32.7±3.23
Phosphorus	23.4±2.94	23.7±1.19	22.5±1.02	24.6±2.08	22.6±4.22	23.8±0.18
Magnesium	31.8±3.99	30.4±2.11	32.3±3.94	32.6±2.85	34.0±7.47	33.1±3.03
Potassium	24.4±3.81	24.0±2.02	25.6±3.28	24.8±3.80	24.0±3.17	25.3±2.37
Zinc (µg/l)	39.5±0.81	41.6±1.06	41.2±5.26	42.1±0.23	43.6±2.75	42.2±6.24
Manganese	1.61±3.28	2.11±1.36	2.20±1.21	1.26±0.08	1.36±0.34	1.49±2.11

Means on the same row are not significantly different (P>0.05)

Table 7: Mineral composition of the fish faeces (mg/l)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Calcium	21.1 ± 6.89^{a}	20.1 ± 8.13^{a}	17.0 ± 4.05^{a}	17.4 ± 1.00^{a}	12.4 ± 3.22^{b}	13.4 ± 2.28^{b}
Phosphorus	18.0 ± 5.42^{a}	14.6 ± 0.26^{a}	11.9 ± 1.70^{b}	12.4 ± 0.04^{b}	12.3 ± 0.05^{b}	12.7 ± 4.18^{b}
Magnesium	20.5 ± 7.71^{a}	20.1 ± 2.11^{a}	17.4 ± 4.27^{a}	17.6 ± 2.85^{a}	18.5 ± 3.26^{a}	18.3 ± 4.03^{a}
Potassium	20.5 ± 1.25^{a}	17.6 ± 6.37^{a}	17.6 ± 6.55^{a}	16.8 ± 3.74^{a}	11.3 ± 5.68^{b}	14.2 ± 2.34^{b}
Zinc (µg/l)	30.6 ± 1.63^{a}	27.5 ± 4.26^{a}	27.4 ± 3.47^{a}	30.1 ± 6.11^{a}	28.6 ± 1.14^{a}	29.8 ± 4.03^{a}
Manganese	4.61 ± 1.91^{a}	3.88 ± 0.33^{a}	3.74 ± 0.13^{a}	2.68 ± 0.67^{a}	3.73 ± 1.27^{a}	3.89 ± 0.67^{a}

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

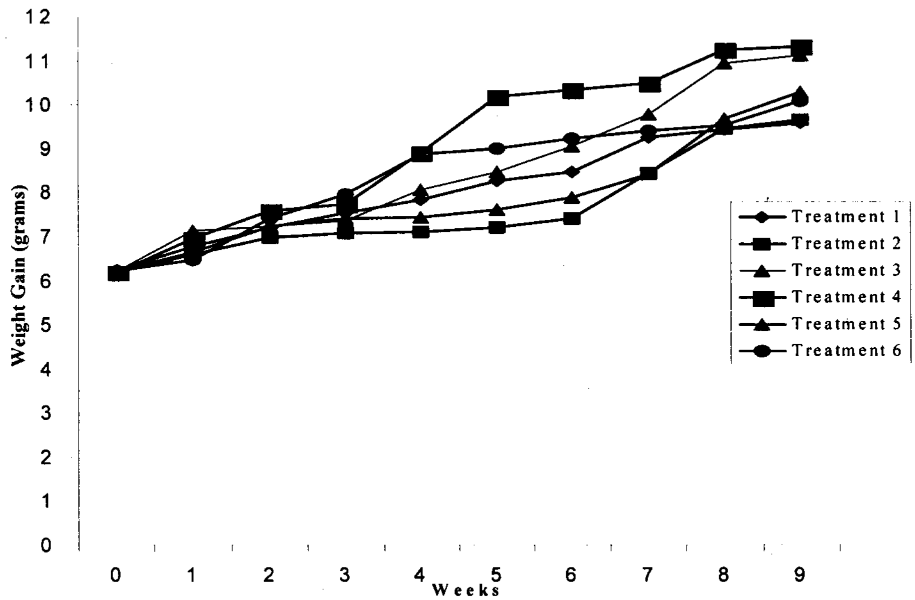

Fig. 1: Weekly mean weight gain of Nile tilapia fed experimental diet for 63 days

in rainbow trout diets reduced the faecal P excretions between 95% and 98% compared with same group of fish fed the same diets but without phytase. Vielma et al (2002) also reported reduction in P loadings into the water from fish fed dephytinized soy proteins compared with the P loadings from fish fed untreated soy proteins.

Conclusion

Supplementation of phytase in the diets enhanced fish growth performance marginally but improved the apparent digestibility coefficient (ADC) for protein of the fish significantly.

Also addition of phytase in the diets significantly reduced the minerals and P levels in the fish faeces; but did not lead to proportionate increase in the mineral composition in the body of the fish.

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