Journal of Animal and Veterinary Advances 4 (12): 980-987, 2005

Effects of Different Treatments of Dietary Soybean Meal And Phytase on The Growth And Mineral Deposition In African Catfish *Clarias gariepinus*

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

Abstract: Aquafeed is a major source of phosphorus (P) that results in eutrophication and pollution of water-ways. This study was conducted to investigate the effectiveness of treatments of dietary soybean mean (SBM) and phytase in improving the growth of African catfish (*Clarias gariepinus*) and mineral concentration; and in reducing environmental P loadings. SBM was prepared as raw (untreated), oven-dried, cooked, toasted or soaked in water. Same 8000 Units of phytase (Ronozyme P (5000) CT) kg^{-1} diet was added to each of the differently prepared SBM before mixing with other ingredients to prepare 40% protein diets. Diet 1 contained untreated SBM without phytase; diet 2 contained untreated SBM with phytase; diet 3 had oven-dried SBM plus phytase; diet 4 contained cooked SBM plus phytase; diet 5 contained toasted SBM with phytase, diet 6 had soaked SBM with phytase. The diets were administered to African catfish (7.0±1.09 g) at 5% of their body weight daily in two equal instalments. After 77 days of feeding, results indicate that the mean weight gain (MWG) of fish fed diets 4 and 5 was higher ($p<0.05$) than that of fish fed other diets. Fish administered diet 3 had higher ($p<0.05$) MWG than those fed diets 1, 2 and 6. The specific growth rate (SGR) followed similar trend. Food conversion ratio (FCR) was best ($p<0.05$) in fish fed diet 4. The FCR of the fish fed diets 5 and 3 was similar but better ($p<0.05$) than that of the fish fed diet 6, while the fish fed diet 1 had the worst FCR. Ca deposited in the fish fed diets 2, 3, 6 was higher ($p<0.05$) than the deposition in other fish. P concentration in the fish fed diets 4 and 5 was higher ($p<0.05$) than the concentration in fish fed diets 2, 3; whereas fish fed diets 1 and 6 had the poorest P concentration. P discharged by all the fish fed diets with phytase was less ($p<0.05$) than the discharges from the fish fed diet without phytase. However, all fish administered diets of treated SBM and phytase discharged less ($p<0.05$) P than the fish fed diet of untreated SBM and phytase.

Key words: Treated soybean meal, phytase, African catfish, growth, mineral deposition

INTRODUCTION

For some time now, there has been growing concern about the impact of phosphorus (P) released from aquaculture systems on the environment, principally because P plays a vital role in eutrophication of fresh waters. Consequently, variable studies are being conducted on how to reduce environmental P loadings through the development of environmentally friendly fish feeds. Fish nutritionists are faced with the possibilities of either reducing the fish meal components of the diets to a level that would not compromise physiological functions or to feed fish with all plant diets because they contain less and more digestible P than fish meal. However, these options have their limitations. Lall[1] explained that fish meal contains excess undigestible P which are usually discharged into the water ways with problems of eutrophication and environmental pollution. Besides, all plant diets may not be nutritionally balanced in terms of amino acids supply as it is known that plant feedstuffs are deficient in certain essential amino acids. Considering high costs of supplemental amino acids in all plant diets for commercial fish farming, many scientists favour the use of combination of different plant ingredients to achieve amino acids balance and requirements and to enhance fish production. Akiyama *et al*.[2] pointed that the combination of malt protein flour and soybean meal at a ratio of 30:30 can maximize the rearing performance of fingerlings of rainbow trout in a similar level with a control diet with high percent of fish meal. Nevertheless, such innovation is being hampered by the presence of phytate P among other anti-nutritional factors in plant feedstuffs. Phytate chelates divalent cations, binds to amino acids and proteins and inhibits digestive enzyme activities, making most of the nutrients unavailable to fishe[3]. Cain and Garling[4], Rodehutscord *et al*[5], Li and Robinson[6] and Sajjadi and Carter[7] have applied phytase into diets to liberate the

Corresponding Author: L.C. Nwanna, Dept. of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo, Japan

phytate P and make more utilizable P available for fish growth in different species. 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, Cheng and Hardy[8] and at the same time reducing the environmental phosphate pollution.

After the work of Van Weered *et al*[9] there is still paucity of information on the utilization of phytase diets by African catfish (*Clarias gariepinus*). Also studies by Cain and Garling[10], Hughes and Soares[11] and Vielma *et al*[12], showed that phytase-treatment of raw soybean meal did not produce significant growth relative to fishes fed soybean meal without phytase. In this regard, there is need for further studies on the effect of different treatments of soybean meal plus phytase on the growth of fishes. Yan *et al*[12] also reported that influence of phytase on protein digestibility and growth of fishes increased with the levels of phytase addition. While Sugiura *et al*[13] described that with low-ash diets, the apparent absorption of phosphorus by rainbow trout increased in accordance with the level of phytase added to the diets. However, Nwanna[14] obtained the optimal phytase requirements for significant differential growth increase in the diets of Nile tilapia as 8000 Units ((Ronozyme P (5000) CT) per kg^{-1} diet .

This study was therefore designed to assess the effects of soaking and heat treatment of soybean meal and addition of 8000 Units of phytase ((Ronozyme P (5000) CT) per kg^{-1} diet on the growth and mineral concentration in African catfish.

MATERIALS AND METHODS

Treatment of soybean meal: About 20kg soybean seed (SB) was purchased from a Central Local Market in Akure, Nigeria. The SB were prepared differently to form the soybean meals.

Raw SBM: About 2.5 kg of the SB was milled into fine powder, and that represented the raw SBM.

Oven-dried SBM: About 2.5 kg of the SB was milled into fine powder (SBM). Thereafter, mixed with 1kg of clean fresh water, and dried in an oven at 120°C for 4h. After drying, the SBM was ground into fine powder again.

Cooked SBM: About 2.5 kg SB was cooked in boiling water (100°C) for 30 min. Thereafter, the SB was air-dried to a constant moisture content of <10%, before grinding into fine powder.

Toasted SBM: About 2.5 kg SB was spread in metal plates and put in an oven at 120°C for 4 h. Thereafter, the beans were ground into fine powder.

Soaked SBM: About 2.5 kg SB was soaked in clean fresh water at room temperature for 24 h. After which the beans were air-dried to a constant moisture content of <10%, before grinding into fine powder.

Analysis of phytate, tannin and lignin: For the quantification of phytate, 8g of finely ground sample (of the 6 samples of SBM as above) was soaked in 200 mL of 2% HCl for 3 h and then filtered using Whatman No 1 filter paper. 50 mL of the filtrate was pipetted into 400 mL beaker and 10ml of 0.3% ammonium thiocyanate solution was added as an indicator. 107 mL of distilled water was added to give pH 4.5. The solution was then titrated with standard ferric chloride solution containing 0.00195 g Fe/ml until a brownish yellow colour persisted for 5 min. The Fe equivalent was multiplied by 1.19 to get phytate-phosphorus. This was converted to phytate by multiplying the value of phytate–phosphorus by 3.55[15].

For the determination of tannin, 200 mg of finely ground sample (of the 6 samples of SBM as above) in 10 mL of 70% acetone was extracted for 2 h at 30°C in water-bath using Gallenkamp orbital shaker at 120rev/min. The sample was cooled to 4°C and centrifuged for 20 min at about 3,000xg. Total polyphenols (as tannic acid equivalent) was determined in 0.05 mL1 aliquol in test tubes by the addition of distilled water to make it to 1.0ml, followed by the addition of 0.5 mL of the Folin Ciocalteau reagent (freshly prepared) and 2.5 mL of sodium carbonate solution. The tubes were vortexed and the absorbance recorded at 725nm after 40 min. The amount of total polyphenols (as tannic acid) was calculated from the standard curve. All the determinations were carried out in triplicates.

For the determination of lignin, 2 mL of 72% w/w H_2S0_4 was added to 200 mg of ground sample (of the 6 samples of SBM as above) in a 100-ml centrifuge tube, and the content mixed thoroughly with a glass rod. The tube and contents were then incubated in a water bath at 30°C for 1 h, after which 50ml of distilled water was added. This was autoclaved at 121°C, 15psi for 1h. The lignin was then filtered off with a glass fibre filter. The content was rinsed carefully into a crucible and then dried at 105°C in an oven overnight. The lignin was calculated gravimetrically as expressed in percentage.

Diet preparation: Same 8000 Units of phytase (Ronozyme P (5000) CT) kg^{-1} diet was added to each of the differently prepared SBM before mixing with other ingredients (Table 2) to formulate the experimental diets. Six isoproteic (40% protein) diets were formulated. Diet 1 contained untreated SBM without phytase; diet 2 contained untreated SBM with phytase; diet 3 had oven-dried SBM plus phytase; diet 4 contained cooked SBM plus phytase; diet 5 contained toasted SBM with phytase while diet 6 had

soaked SBM with phytase. The whole ingredients (Table 2) were thoroughly mixed in Hobart A-200 (Troy Ohio USA) pelleting machine until homogenous masses were obtained. The homogenised masses were extruded through 0.8mm die and pelleted into noddle-like strands which were mechanically broken into sizes, oven dried at 45°C for 48 h, packed in cellophine bags and stored at –20°C prior to use.

Feeding trials: A total of 300 fingerlings (7.0 ±1.09g) of African catfish, *Clarias gariepinus* were obtained from the Federal University of Technology Akure Teaching and Research Fish Farm. The fish were acclimated for two weeks in glass tanks in the laboratory. After acclimation, the healthy and strong fish were randomly stocked into glass tanks (70x45x40 cm) with 70 litres of water at 10 fish per tank. The fish were fed the six diets at 5% body weight twice daily in two equal instalments six days a week. Each treatment was replicated thrice. The tanks were cleaned by siphoning out faecal matters and replacing 30% of the water in the tanks. All fish in each tank were weighed bi-weekly and the new feed rate adjusted. The feeding lasted for 77 days. The water quality parameters, Temperature, Dissolved oxygen and pH, were maintained daily at 25-27°C, 5.6-6.4 (mg L^{-1}) and 6.7-8.5 respectively. Measurements were done using combined digital YSI DO meter (YSI model 57); and an electronic pH meter (Metler Toledo 320 model).

Proximate analysis: The six diets and fish samples (whole body) before and after the experiments were prepared in triplicates and analysed for their proximate compositions as described by AOAC, (1990) Methods. While the gross energy of the diets were determined by combustion in an adiabatic bomb calorimeter (GALLENKAMP).

Mineral analysis: Three replicates of the fish carcass (whole body) and faeces were analysed for minerals according to the methods of AOAC[16]. About 2.0 g of the samples were ashed for 48 h at 480°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 2.5mL 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 deionized water. Then the minerals (Mg, Ca, Zn, Mn) were measured in Atomic Absorption Spectrophotometer (AAS). Phosphorus contents of the six prepared SBM, fish and faeces were 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 vanadatemolybdate reagent was added and phosphorus concentration was measured spectrophotometrically at 430 nm, after the reaction mixture was thoroughly mixed and allowed to stand at room temperature for 10 min.

Statistical analysis: Data (mean weight gain, SGR, FCR, 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[17].

RESULTS

The anti-nutrients and total P of raw and treated SBM are presented in Table 1, which showed no significant differences in the phytate, tannin, lignin and total P contents of both untreated and treated SBM. However, the phytate P of the untreated SBM was highest while the sample treated by cooking had the least value. Similarly, the sample treated by toasting had numerically lower phytate level than the samples treated by either oven-drying or soaking. The SBM samples treated by cooking had the highest numerical total P followed by the sample treated by toasting.

Table 2 presents the gross and proximate composition of the experimental diets, which described the different treatments of the SBM used in the diet formulation. These treatments include untreated SBM, oven dried SBM, cooked SBM, toasted SBM and soaked SBM. The values of the proximate composition of the crude protein (CP), either extract, ash, crude fibre, nitrogen free extract (NFE); and the gross energy were closely related, indicating no clear effect as a result of the different treatments of the SBM.

The growth parameters of the fish fed the various diets are presented in Table 3. Fish fed diets 4 and 5 had similar (P>0.05) MWG, which was significantly highest compared with the MWG of other groups of fish. Fish fed diet 3 had higher (p<0.05) MWG than the fish fed diets 1, 2 and 6, which also had similar MWG. The SGR of the fish administered the various diets followed the same trend as in the MWG. FCR was significantly best in the fish fed diet 4. The FCR of the fish given diets 5 and 3 were similar but different (p<0.05) from the FCR of fish fed diets 2 and 6, while the fish fed diet 1 had the worst FCR. The trend of the growth parameters explains that cooked and toasted SBM supplemented with the same unit of phytase per kg. diet produced almost the same growth rates, which were significantly higher than the growth rate of fish fed other diets. Similarly, fish treated with oven dried SBM supplemented with phytase had better (p<0.05) growth rate than the fish fed untreated , or soaked SBM treated with phytase.

The carcass composition (Table 4) showed close

Table 1: Anti-nutrients and total P of the raw and pretreated SBM

	Treatments					
Parameters	1	2	3	4	5	6
Phytate (mg g^{-1})	2.02±0.2	1.93±0.1	1.92±2.0	1.65±1.5	1.82±1.2	1.92±2.2
Tannin (mg/100g)	1.50±1.2	1.20±0.5	1.30±1.0	1.75±1.1	1.75±1.0	1.05±0.6
Lignin (%)	0.20±1.1	0.25±2.1	0.05±0.3	0.05±0.1	0.35±0.5	0.05±0.2
Total P (mg g^{-1})	4.87±12.7	4.73±6.6	4.70±11.3	4.95±5.4	4.91±10.1	4.89±10.2

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

Table 2: Gross and proximate composition of experimental diets (g/100g DM)

	Treatments					
	1	2	3	4	5	6
Fish meal (65% CP)	36.46	36.46	36.46	36.46	36.46	36.46
Raw SBM (45% CP)	31.33	31.33	0.00	0.00	0.00	0.00
Oven dried SBM (45% CP)	0.00	0.00	31.33	0.00	0.00	0.00
Cooked SBM (45% CP)	0.00	0.00	0.00	31.33	0.00	0.00
Toasted SBM (45% CP)	0.00	0.00	0.00	0.00	31.33	0.00
Soaked SBM (45% CP)	0.00	0.00	0.00	0.00	0.00	31.33
Maize	25.13	25.13	25.13	25.13	25.13	25.13
Vitamin-min premix	1.75	1.75	1.75	1.75	1.75	1.75
Carboxylmethylcellulose	1.05	1.05	1.05	1.05	1.05	1.05
Vegetable oil	4.28	4.28	4.28	4.28	4.28	4.28
Phytase (U/kg diet)	0.00	8000	8000	8000	8000	8000
Proximate composition (%)						
Crude protein	40.2	40.3	40.4	40.5	40.4	40.4
Ether extract	14.3	15.6	14.0	14.5	14.3	14.0
Ash	14.9	14.1	14.7	13.5	13.3	14.1
Crude fibre	6.56	6.29	5.33	6.33	5.18	5.94
NFE	24.0	23.7	25.6	25.2	26.8	25.6
Gross energy (Kcal $100g^{-1}$)	435.5	436	435.5	435.7	435.6	435.1

Table 3: Growth and protein digestibility of *C. gariepinus* fed SBM based diets supplemented with phytase

	Treatments					
Parameters	1	2	3	4	5	6
Initial weight (g)	7.15	7.25	7.23	7.30	7.41	7.22
Final weight (g)	18.5	19.8	23.1	28.5	28.2	17.3
Mean wt. gain (g)	11.4 ± 1.0^{ad}	12.6 ± 1.2^{a}	15.9 ± 0.2^{c}	21.2 ± 1.2^{b}	20.8 ± 0.1^{b}	10.1 ± 0.2^{d}
SGR	1.23 ± 0.2^{a}	1.30 ± 0.0^{a}	1.51 ± 0.6^{c}	1.77 ± 0.0^{b}	1.74 ± 0.0^{b}	1.13 ± 0.1^{ac}
FCR	2.97 ± 0.5^{a}	2.21 ± 0.3^{bc}	1.90 ± 0.3^{cd}	1.40 ± 0.2^{e}	1.50 ± 0.2^{de}	2.40 ± 0.2^{b}

Means along the same row followed by same superscripts are not significantly different (P>0.05) Mean weight gain = final mean weight – initial mean weight
Specific growth rate = 10^2 (Log$_e$ final weight -Log$_e$ initial weight)/culture period (days)
Food conversion ratio = Dry weight of feed fed (g)/ fish weight gain
The above methods are after (Olvera-Novoa *et al* 1990)

relationship between the values of CP, ether extract, ash, crude fibre and NFE of the fish under the different treatments. This is also an indication that treatment of the SBM and or addition of phytase had no clear effect on the values of the carcass yield. However, the CP of the fish showed slight increase over the CP of the fish before the experiment, while the crude fibre of the fish before the experiment was slightly higher than that after the experiment.

The carcass (whole body) mineral concentration of the fish is presented in Table 5. The groups of fish fed diets 2, 3 and 6 had higher (p<0.05) Ca deposition than the composition in fish fed other diets. Fish treated with diet 5 also had higher (p<0.05) Ca than the fish fed diets 1 and 4 , which had similar but lowest Ca concentration. The pattern of Ca distribution indicated that fish fed soaked, oven-dried or untreated SBM supplemented with phytase produced higher (p<0.05) Ca in the body of the fish. Mg content of the fish fed diets 1, 2, 3, 4 and 5 was the same (P>0.05) while the Mg contents of the fish fed diets 1, 2, 4 and 5 were higher (p<0.05) than the Mg content of the fish fed diet 6. P deposition was similar (P>0.05) in the fish fed diets 4 and 5, but higher (p<0.05) than the concentration in the fish fed diets 2 and 3. Fish treated on diets 1 and 6 had similar (P>0.05), but poorest P composition. Zn concentration in the fish fed all the diets was statistically the same. Mn composition in the fish fed diets 2, 3, 4 and 6 was the same (P>0.05), while the composition in fish fed diets 2, 3 and 6 was higher (p<0.05) than the composition in the fish fed diets 1 and 5. The trend in the mineral distribution indicated that generally, and, except in Mg, fish fed diets with phytase

Table 4: Proximate composition of the experimental fish

	Treatments						
Parameters	Before	1	2	3	4	5	6
Crude protein	66.1±0.0	66.9±0.1	67.6±0.7	68.1±0.4	69.1±1.3	69.7±0.1	67.3±0.5
Ash	14.1±0.1	13.5±0.8	12.8±0.1	12.4±0.8	12.0±0.5	12.0±0.2	12.1±0.9
Ether extract	3.45±0.1	4.05±0.0	4.01±1.0	3.25±0.2	3.22±0.3	3.51±0.6	4.12±0.7
Crude fibre	5.46±0.0	4.14±0.1	4.03±0.1	4.94±0.2	4.48±0.1	4.39±0.5	5.09±0.1
NFE	11.9±0.6	11.4±0.0	11.6±0.0	11.3±0.1	11.2±0.1	10.4±0.0	11.4±0.2

Means along the same row are not significantly different (p>0.05)

Table 5: Mineral composition of *C. gariepinus* fed SBM based diets supplemented with phytasez

	Treatments					
Parameters	1	2	3	4	5	6
Calcium (mg g^{-1})	19.1±10.6^{a}	23.0±6.7^{be}	22.9±4.0^{cbe}	19.4±2.0^{a}	20.5±0.9^{d}	22.5±0.9^{e}
Magnesium (mg g^{-1})	23.0±10.6^{a}	21.9±9.2^{a}	21.2±3.7^{ac}	23.0±10.0^{a}	23.3±11.0^{ab}	19.9±8.5^{c}
Phosphorus (mg g^{-1})	22.5±6.4^{a}	25.0±7.0^{b}	25.2±7.8^{b}	26.7±6.8^{c}	26.4±8.7^{c}	23.3±3.7^{a}
Zn (µg g^{-1})	25.8±2.1^{a}	30.1±1.3^{a}	30.1±0.3^{a}	26.5±0.3^{a}	29.3±3.3^{a}	31.2±11.2^{a}
Manganese (µg g^{-1})	19.6±1.2^{a}	25.0±0.0^{b}	24.2±0.2^{bc}	21.5±1.3^{ac}	20.5±1.5^{a}	23.4±3.1^{bc}

Means along the same row followed by same superscripts are not significantly different (p>0.05)

Table 6: Mineral composition of the faeces of *C. gariepinus* fed SBM based diets supplemented with phytase

	Treatments					
Parameters	1	2	3	4	5	6
Calcium (mg g^{-1})	55.0±17.5^{a}	57.0±13.5^{b}	50.5±15.5^{c}	47.4±11.5^{d}	43.9±13.6^{e}	0.2±14.5^{f}
Magnesium (mg g^{-1})	54.0±11.6^{a}	49.1±13.0^{b}	53.3±13.4^{a}	51.8±15.1^{ac}	52.9±15.3^{a}	50.4±17.1^{cb}
Phosphorus ((mg g^{-1})	78.8±9.4^{a}	53.6±13.8^{b}	45.4±10.4^{c}	40.5±7.3^{d}	32.3±17.7^{e}	46.5±6.3^{f}
Zn (µg g^{-1})	104.6±4.5^{a}	59.2±10.0^{bc}	86.0±3.0^{cd}	83.0±2.0^{d}	96.0±3.1^{ac}	66.5±2.0^{e}
Manganese (µg g^{-1})	69.3±4.1^{a}	44.3±2.9^{bef}	49.8±1.1^{c}	35.6±0.0^{d}	41.5±0.0^{ef}	44.6±0.0^{f}

Means along the same row followed by same superscripts are not significantly different (p>0.05)

had higher mineral concentration than the fish fed diets without phytase, while fish fed diet with untreated SBM plus phytase contained similar Ca with fish fed diets 3 and 6; similar Mg with fish fed diets 2, 3, 4 and 5; similar P with the fish fed diet 3 and the same Mn with fish fed diets 3 and 6. These results showed that treated or untreated SBM plus phytase can produce similar mineral deposition, unlike in growth parameters where treatment of SBM significantly improved the nutrient utilization and growth rates of the fish.

The faecal minerals (Table 6) describes that except in Ca discharges, other minerals were generally lower than the mineral discharged by the fish fed diet without phytase. Also faecal Ca and P discharged by the fish fed untreated SBM plus phytase were generally higher than the discharges from the fish fed treated SBM plus phytase. Faecal Ca discharges was significantly lowest in fish fed diet 6, followed by discharges from the fish fed diets 5, 4, 3, 2, 1 in that order. Fish fed diets 1, 3, 4 and 5 discharged the same (P>0.05) level of Mg while the Mg discharged by the fish fed diets 2 and 6 was lower (p<0.05). The faecal P discharged by the fish fed diet 1 was significantly highest, followed by the discharges from the fish fed diets 2, 6, 3, 4 and 5 in that order. Zn discharges was highest (p<0.05) from the fish fed diet 1 compared to the discharges from the fish fed diets 2, 3, 4 and 6. Zn discharged by the fish fed diet 2 was lowest in comparison with discharges from other fish.

However, Zn discharged by fish fed diets 2 and 6 were the same (P>0.05), but lower (P>0.05) than the discharges from fish fed diets 3, 4 and 5. Fish fed diet 1 discharged the highest concentration of Mn into the culture system. The discharges from the fish fed diet 3 was higher (p<0.05) than the discharges from the fish fed diets 2, 4, 5 and 6. The discharges from the fish fed diets 2, 5 and 6 were similar but significantly higher than the discharges from the fish fed diet 4. It can be summarised that most minerals discharged by the fish fed diets with phytase was significantly less than the discharges from the fish fed diet without phytase.

DISCUSSION

The similarity in mean weight gain (MWG) of fish treated on diets 1 and 2 is an indication that addition of phytase to untreated SBM was not enough to produce a significant difference in growth compared to the fish fed untreated SBM without phytase (diet 1). Also the fish fed diet 6 had the same MWG with the ones fed diet 1, emphasising that soaking was not adequate to reduce the anti-nutrients in the SBM so as to significantly improve upon the growth more than in the fish fed untreated SBM without phytase.

Although the total P of the untreated and treated SBM was statistically the same , phytate P was numerically highest in the untreated SBM. This could

explain the poorest food conversion ratio (FCR) recorded by the fish fed diet 1 in comparison with fish fed other diets. Fish fed diet with untreated SBM plus phytase had significantly better FCR than the fish fed diet 1. This shows that presence of phytase would have liberated more utilizable P and other nutrients which made the diet better converted by the fish; and consequently increase in the specific growth rate over that of the fish fed diet with untreated SBM without phytase. Hughes and Soares[10] reported a significant difference in FCR of striped bass (*Monorone saxatilix*) fed diet containing 1300 units of phytase (Natuphos) per kg. diet, over a diet without phytase. The observation from the study is in line with the work of Forster *et al*[18] who reported a clear positive dose-response of phytase on dietary phytate digestibility, and improvement in P availability. In Cain and Garling[4], fish fed phytase treated diet had excellent weight gain and feed conversion over fish fed a commercial diet. Lanari *et al*[19], Storebaken *et al*[20] and Vielma *et al*[21] also reported enhanced phytate p availability by supplemental phytase in rainbow trout.

Findings from the study also showed that heat treatment of the SBM enhanced the activities of the phytase, indicating that phytase alone may not be able to degrade all the anti-nutrients to the extent of producing significant differences in the growth of the fish. Because even at a very high level of inclusion of 8000 units per kg. diet, phytase alone could not significantly improve the growth over the fish fed untreated SBM. Whereas all fish fed diets that contained oven-dried, cooked or toasted SBM supplemented with phytase had significantly higher growth than the fish fed either diet of untreated SBM or untreated SBM plus phytase.

Phytate as a major anti-nutrient in SBM and other plant feedstuffs chelates divalent cations[22], thereby making supplementation of minerals essential in feed diets. Phytase has the ability to degrade feed phytate thereby improving mineralization in animals and fishes. The Ca, P and Mn contents of the fish fed diet 2 were higher ($p<0.05$) than those of the fish fed the same diet (diet 1), but without phytase. The beneficial effects of dietary phytase supplementation or pre-treatment on P and mineral availability have been reported in a number of fish species[9,13,23,24]. Sugiura *et al*[13] similarly reported that phytase supplementation increased the apparent absorption of P, Ca, Mg, Cu, Fe and Zn in low ash diets containing soybean meal.

A clear effect of the treatment of the SBM on increasing mineralization was also demonstrated by feeding the fish with either cooked or toasted SBM. The fish fed diets of cooked or toasted SBM supplemented with phytase had significantly higher Ca, P and Mn composition than that of the fish fed untreated SBM with phytase. This showed that the two treatment methods were able to degrade the anti-nutrients better which activated the phytase to release bound Ca, P and Mn in the diets which were well utilized by the fish for increase in their body mineralization. Except for the fish fed diet of soaked SBM with phytase, the P content of all the fish fed diets with phytase were higher ($p<0.05$) than that of the fish fed diet without phytase. The value of Zn concentration in the fish was statistically the same, indicating that the fish absorbed equal amount of Zn from the diets. This is also an evidence that heat treatment of the SBM had little physiological role in the liberation and absorption of Zn from the diets.

Faecal mineral discharges showed that except for Mg and Zn, the minerals released by all the fish administered diets with phytase were lower ($p<0.05$) than that released by the fish fed diet without phytase. This is an indication that the added phytase liberated significantly more minerals which were well utilized for growth. It also means that addition of phytase into diets could reduce mineral leachate and environmental pollution associated with culture effluents. Cain and Garling[4] reported an 88% reduction in P discharge of fish fed phytase treated diets over fish fed a commercial diet. Similarly, Sugiura *et al*[13] explained that fish fed low-ash diet containing phytase treated soybean mean excreted 95-98% less P than the fish fed commercial diet without phytase. Jackson *et al*[25] also showed that the concentration of faecal P decreased linearly as phytase supplementation increased.

It is worthy to note that even the fish fed untreated SBM plus phytase released significantly lower Mg, P, Zn and Mn than the fish fed the same diet without phytase. This showed the strong effect of the phytase in making more dietary minerals bioavailable for fish growth.
Vielma *et al*[26] reported reduction in P loadings into the water from fish fed phytase dephytinized soy proteins compared to the P loadings from fish fed soy protein without phytase. All the fish fed treated SBM plus phytase released significantly lower P than the fish fed diet with untreated SBM plus phytase. This means that a combination of any of the treatment methods with phytase would greatly reduce P discharges into the environment.

CONCLUSION

Overall assessment of the trend of the results revealed that oven drying, cooking or toasting of SBM before addition of phytase will enhance fish growth and reduction in P and mineral discharges into the culture systems. The results also showed that the effect of heat

treatment of SBM, supplemented with phytase was greater on growth than on the mineral deposition.

ACKNOWLEDGEMENT

We are very grateful to Dr. Jiri Broz of L(DSM, Nutritional Products) Switzeland for providing the phytase enzyme used in the study.

REFERENCES

1. Lall, S.P., 1991. Digestibility, Metabolism And Excretion Of Dietary Phosphorus in Fish. In : Cowey, C. B., Cho, C. Y., (Eds). Nutritional Strategies and Aquaculture Waste. University of Guelph, Guelph, Ontario Canada, pp: 21-36
2. Akiyama, T., T. Unuma, T. Yamamoto, P. Marcouli and S. Kishi, 1995. Combinational use of malt protein flour and soybean meal as alternative protein sources of fish meal in fingerling rainbow trout diets. Fisheries Sci., 61: 828- 832.
3. Kerovuo, J. and S. Tynkkyen, 2000. Expression of Bacillus subtilis phytase in Lactobacillus plantarum 755. letters in Applied Microbiology, 30: 325-329.
4. Cain, K.D and D.L Garling, 1995. Pretreatment of soybean meal with phytase for Salmonid diets to reduce phosphorous concentrations in hatchery effluents. The Progressive Fish- Culturist, 57: 114-119
5. Rodehutscord, M. and E. Pfeffer, 1995. Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). Water Sci. Tech., 31: 143-147.
6. Li, M. H. and E.H Robinson, 1997. Micobial phytase can replace inorganic phosphorous supplements in channel catfish Ictalurus punctatus diets. J. World Aquacult. Soc., 28: 402-406
7. Sajjadi, M. and C.G. Carter, 2004. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L). Aquaculture Nutrition, 10: 135-142.
8. Cheng, Z. and R.W Hardy, 2002a. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, meaured in vivo using rainbow trout (Oncorhynchus mykiss). Aquaculture Nutrition, 8: 271-277
9. Van Weerd, J.H.K.H.A. Khalaf, F.J. Aartsen and P.A.T. Tijssen, 1999. Balance trials with African catfish *Clarias gariepinus* fed phytase-treated soybean meal based diets. Aquaculture Nutrition, 5: 135-142.
10. Hughes, P.K. and J.H Jr. Soares, 1998. Efficacy of phytase on phosphorus utilization in practical diets fed to striped bass (*Morone saxatilis*). Aquaculture Nutrition, 4: 133-140
11. Vielma, J.T. Maekinen, P. Ekholm and J. Koskela, 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture, 183: 349-362.
12. Yan, W., R.C. Reigh and Z. Xu, 2002. Effects of fungal phytase on utilization of dietary protein and minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish (*Ictalurus punctatus*) fed an all plant protein diet. Journal of the World Aquaculture Society, 33: 10-22.
13. Sugiura, S.H.J. Gabaudan, F.M. Dong and R.W. Hardy, 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout (*Oncorhynchus mykiss*) (Walbaum) fed soybean meal-based diets. Aquaculture Rresearch, 32: 583-592.
14. Nwanna, L.C., 2004. Effect of untreated soybean meal supplemented with phytase in practical diets on growth and mineral deposition in Nile tilapia *Oreochromis niloticus* (L). J. food and Environ., 2: 51-56.
15. Young, S.M. and J.E. Greaves, 1940. Influence of variety and treatment on the phytic acid content of wheat. Food Research, 5: 103-105.
16. AOAC (Association of Official Analytical Chemists)., 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC. USA. 15th Edn., pp: 1230.
17. Duncan, D.B., 1955. Multiple F-test. Biometrics, 11: 1-42.
18. Forster, I., D.A Higgs, B.S. Dosanjh, M. Rowshandeli and J. Parr , 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11°C fresh water. Aquaculture, 179: 109-125.
19. Lanari, D, E.D. Agaro, and C. Turri, 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss)*. Aquaculture, 161: 345-356.
20. Storebakken, T.K.D. Shearer and A.J. Roem, 1998. Availabilty of protein, phosphorous and other elements in fish meal, soy-protein concentrate and phytase- treated soy- protein-concentrate-based diets to Atlantic salmon, Salmo salar. Aquaculture, 161: 363-377

21. Vielma, J.S.P. Lall, J. Koskela, F.J. Schoner and P. Mattila, 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 163: 309-323.
22. Satoh, S.T. Takeuchi and T. Watanabe, 1991. Availability of manganese and magnesium contained in white fish meal to rainbow trout *Oncorhynchus mykiss*. Nippon Suisan Gakkaishi, 57: 99-104.
23. Rodehutscord, M.A. Becker and E. Pfeffer , 1995. Einfluss des Zusates einer Aspergillus niger-phytase auf die Verwertung pflanzlichen phosphorus durch die Forelle (*Oncorhynchus mykiss*) Arch. Anim. Nutr., 48: 211-219.
24. Schäfer, A.W.M Koppe, K.H. Meyer-Burgdorff and K. D Günther, 1995. Effects of a microbial phytase on the utilization of native phosphorous by carp in a diet based on soybean meal. Wat. Sci. Tech., 31: 149-155.
25. Jackson, L.S. M.H.Li and E.H. Robinson, 1996. Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorous. J. World Aquacult. Soc., 27: 309-313
26. Viema, J.K. Ruohonen and M. Peisker, 2002. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 204: 145-156.