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Effect of Defatted and Dephytinized Soy Proteins Fortified with Phytase on the Growth, Nutrient Digestibility and Phosphorus Load of Rainbow Trout

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Abstract

Feeds with minimal phosphorus (P) and nitrogen (N) stressors on the environment are paramount for eco-friendly aquaculture, as P and N are the major sources of aquatic pollution. This study investigated the effects of defatted soybean meal (DSM) plus phytase and dephytinized soy protein (DHP) plus phytase on the growth, N and P retention, N and P excretion and total P loadings by rainbow trout (*Oncorhynchus mykiss)*. Seven isoproteic diets were formulated to contain, DSM (diet 1), DSM plus 5 g P kg^{-1} diet (diet 2), DSM plus 1500 U/kg phytase (diet 3), DSM plus 3000 U/kg phytase (diet 4), DHP (diet 5), DHP with 1500 U/kg phytase (diet 6) and DHP plus 3000 U/kg phytase (diet 7). Chromic oxide (1%) was added to the diets as inert marker for digestibility studies. The diets were tested on rainbow trout (25.6 ± 0.24 g) for 12 weeks. Results indicated no significant differences in the mean weight gain (MWG), specific growth rate (SGR) and food conversion ratio (FCR) of the fish subjected to the different dietary treatments. However, the MWG, SGR and FCR of the fish treated on diets of DSM were marginally higher than those maintained on DHP. Similarly, the fish treated on DSM retained higher N which resulted in lower excretion values of N. Fish in T 7 retained most P and generally fish treated on DHP retained slightly higher P than those treated on DSM diets. P excretion was closely related in treatments with DSM (3.64-5.41kg/Ton) and that with DHP (3.65-4.85kg/Ton), with T(s) 3 (3.64) and 7 (3.56) having the least ($P<0.05$) values, while T2 with P supplement produced the highest ($P<0.05$) P excretion of 5.41kg/Ton, least P and Zn retention and N excretion values. Effluent P discharge was least ($P<0.05$) in T 7 (5.33mg/kg). This study revealed that addition of DSM in the diets would promote better fish growth, while addition of DHP would promote better environmentally friendly aquaculture.

Keywords: Eco-friendly diets, defatted, dephytinized soybean meal, phytase

Introduction

Approximately two-thirds of phosphorus (P) in various grains and oilseeds is present as phytate or inositol hexaphosphate (Sajjadi and Carter 2004), which is not digested by fish and other monogastric animals (Kumar *et al*., 2012) because of insufficient phytase activity under normal conditions (Ravindran *et al.,* 1995, Fandrejewski *et al*., 1997). Phytate complexes with cations, protein, lipids

(Cosgrove 1966), (Denstadli *et al*., 2006), starch (Thompson and Yoon 1984) and trypsin and pepsin (Singh and krikorian 1982, Nwanna et al. 2008), thus reducing their availability and utilization. These complexes may decrease the activity of digestive enzymes with a consequent decrease in the digestibility of dietary protein and energy (Singh and Krikorian 1982). Apart from its low P availability, calcium–bound phytate increases chelation with trace elements like zinc (Zn) to form co-precipitates (Nwanna *et al*., 2007), which decreases endogenous Zn reabsorption and dietary Zn availability (Morris 1986, Nwanna *et al*., 2008). Satoh, *et al*., (1989) reported that increasing phytate levels by 1.1% in the diets of channel catfish led to decreased weight gain, feed efficiency and Zn content in the vertebrae. Similarly, Gatlin and Wilson (1984) described that with 1.1% phytate in diets, channel catfish requires close to 200 mg Zn/kg feed, which is 10 times higher than their dietary requirement for available Zn (Gatlin and Wilson 1984).

Since phytate P is not digested by fish, it is discharged directly into the water, where it adds up to environmental P loadings with impact of eutrophication and pollution. According to Yoshida (2000), the relationship between nutrients and water quality has shown that N and P released from long-period aquaculture activities can profoundly affect the trophic response in the water, changing it from oligotrophic to more eutrophic levels. Sugiura *et al.,* (2005), reported that excessive dietary P input into the water causes algal bloom, dissolved oxygen depletion and death of aquatic organisms. Therefore, for sustainable aquaculture through the production of low-costs and ecologically friendly fish feeds, phytate P must be hydrolysed.

Phytase (myo-inositol, hexaphosphate and phosphohydrolase), is an enzyme that releases P from phytate (Gibson and Ullah 1990). The enzyme activity is expressed as FTU units, where 1 FTU is the amount of enzyme that liberates 1 µmol inorganic orthophosphate/min from 0.0051mol sodium phytate/l at pH 5.5 and a temperature of 35°C. Phytases have the capacity to dephosphorylate phytate in a step-wise manner to a series of lower inositol phosphate esters (myo-inositol pentaphosphate to myo-inositol monophosphate) and ultimately, to inositol and inorganic P. Phytase may also improve the utilization of protein, amino acid and apparent metabolizable energy (Ravindran *et al*., 1997). Mineral-phytate complexes could limit lipid utilization; and by preventing the formation of mineral phytate complexes, phytase would reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids (Ravindran *et al*., 2001). The amount of phytate-P released is also influenced by the level and source of added phytase and substrate coupled with dietary levels of non-phytate-P, Ca, cholecalciferol and Ca: P (Ravindran *et al*., 1995). Therefore this study was conducted to investigate the effect of defatted and dephytinized soy proteins fortified with phytase (Ronozyme PL HBN 01101) on the growth, nutrient digestibility and phosphorus load of rainbow trout, *Oncorhynchus mykiss.* And to circumvent the thermo-instability concerns about phytase, the enzyme was top-sprayed on the diets after pelleting.

Materials and Methods

Diet Preparation

Seven different diets were formulated to contain 40% protein. Diets 1-4 contained defatted soybean meal (DSM) while diets 5-7 contained dephytinized soya protein (DHP). (The dephytinized soya protein HP340 (0.12% phytic bound P is a type of feed grade non-GMO soya product produced at HAMLET PROTEIN). Other ingredients used in the diets (Table 1) include fish meal (FM), corn gluten meal, poultry feather meal, wheat flour, pre-gelatinized starch, fish oil, soybean oil, p-free mineral, vitamin pre-mixtures, choline chloride, vitamin E, cellulose and chromic oxide. Diets 3 and 4 were fortified with 1500 and 3000 U kg^{-1} phytase. Similarly, diets 6 and 7 were supplemented with 1500 and 3000 U kg^{-1} phytase respectively. The phytase Ronozyme PL HBN01101 used in the study was supplied by DSM Nutritional Product Tokyo, Japan. All the ingredients were mixed in machine (ACM-50 LAT, Aikohsha, Mfg, Tokyo, Japan) and pelleted through 3mm die (Meat chopper, Hiraga, Kobe, Japan). The strands were broken into smaller units before freeze drying. After pelleting, the phytase (enzyme) was mixed with 100g of soy bean oil (deducted from total soy bean oil in the diet) and top spread on diets 3, 4, 6 and 7 according to dosage specifications and dried in a vacuum freeze-dryer (REL-206, Kyowa Vacuum

Tech. Co. Ltd., Tokyo, Japan). The freeze dried diets were stored in a cold room (5°C) before use.

Feeding Trial

Four hundred and fifty rainbow trout fingerlings (25.6±0.24 g) were acclimatized in 60L glass tanks for 2 weeks. Then the healthy and strong ones were stocked in seven tanks at 26 fish per tank and each treatment was duplicated. Fish were fed the experimental diets to apparent satiation twice daily (09.00-1000am and 4.00 -5.00pm). Fish sampling was carried out bi-weekly, and the weight measured by using top loading electronic balance (Libror EB 32000, Shimadzu Corp., Kyoto, Japan). All fish were starved for 24h and anesthetized with ethylene glycol monophenyl ether (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 300 ppm before each weighing. After 12 weeks of feeding, 5 fish were randomly selected from each tank for whole body mineral and proximate analyses. The fish selected for whole body analysis were totally minced using an ultra centrifugal mill (ZM 100, Retsch GmbH & Co., Haan, Germany) fitted with 0.5 mm screen and the homogenate was kept -20°C before analysis.

Analytical methods

Proximate and mineral analyses of the fish samples (whole body, faeces) were conducted in triplicates. The samples were digested with nitric acid using the MLS-120 Mega Microwave Labstation system (Milestone, Sorisole, Italy). Vessels with digested samples were allowed to cool and deionized water was used to make up to required volume. The deionized samples were diluted with standard solutions and allowed in warm water (40°C) for 30 mins before measurement. Phosphorus concentration was measured using an atomic spectrophotometer (UV-256FW, Shimadzu Corp., Kyoto, Japan) at a wave length of 750nm. Other minerals were determined with a polarized Zeeman Atomic Absorption Spectrophotometer (Z-5010, Hitachi Ltd., Japan). Proximate composition of the diets, initial fish sample, final fish sample, and fish faeces were analysed according to AOAC (1990) methods. Crude protein was analysed using the 2020 Digestor and Kjeltic Auto Sampler System 1035 (Tecator AB, Sweden). Chromic oxide contents of the diets and faeces were measured after the nitric acid and perchloric acid digestion method using visible light at a wave length of 350 nm.

$$\%\,Cr_2O_3 = \frac{[(\text{Absorbance of digested sample solution} - 0.0032) \times 100]}{(0.2089 \times \text{sample weight} \times 1000)}$$

Digestibility experiment

Faecal samples were collected from each of the tanks from 4 weeks before the end of the feeding trials. Diets were fed to apparent satiation twice daily. After the second feeding, the remaining uneaten feed or faeces in the tanks were siphoned out using plastic tubes. Then detachable faeces collectors (glass tubes) were installed an hour later. Faeces were collected for six consecutive days and pooled samples from each tank were freeze-dried, blended into powder and store at -5°C before analysis. Apparent digestibility coefficient (ADC) for nutrients was calculated using the methods of Kleiber (1961) as follows:

$$ADC = 100 - \frac{(g\,kg^{-1}\ Cr\ feed \times g\,kg^{-1}\ nutrient\ in\ feces)}{(g\,kg^{-1}\ Cr\ feces \times g\,kg^{-1}\ nutrient\ in\ feed)} \times 100$$

ADC (dry matter %) = 100-100(Cr_2O_3 in faeces/Cr_2O_3 in diets) (Cho et al. 1982)

Statistical analyses

Data were subjected to one way analysis of variance (ANOVA) using Statistical 6 Software (1998). Duncan multiple range test was used to separate means among treatments (Duncan, 1955).

Results

The chemical and mineral compositions of the experimental diets are presented in Table 2 which shows that the compositions are similar. The crude protein ranged between 42.2 and 43%, lipid between 14.1 and 17.5, crude ash between 6.07 and 6.60%, gross energy between 4.70 and 5.04 kcal/g, pH between 5.43 and 5.58%. The phytase activities varied from 185 to 4498 U/kg. The activities were increased by addition of phytase, and improved with increase in phytase levels. The mineral contents of the diets were similar, with no indication of phytase effects, and without any trend of distribution.

Table 1. Gross composition of experimental diets (%)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Jack mackerel meal (66% CP)	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Defatted soybean mean (DSBM) (45% CP)	26.0	26.0	26.0	26.0	0.00	0.00	0.00
Dephytinized Soy protein (HP340) (56% CP)	0.00	0.00	0.00	0.00	20.9	20.9	20.9
Corn gluten meal (70% CP)	19.8	19.8	19.8	19.8	19.8	19.8	19.8
Poultry feather meal (80% CP)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Wheat flour (17% CP)	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Pregelatinized starch	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
P-free mineral[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premixture[2]	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin E (50%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Cellulose	2.30	2.30	2.30	2.30	7.40	7.40	7.40
Chromic oxide	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ca(H_2P04)2 (g/kg)	0.00	0.50	0.00	0.00	0.00	0.00	0.00
Phytase(U/kg)	0	0	1500	3000	0	1500	3000

1. g/100g NaCl 5,$MgSO_4.7H_2O$ 74.5,$FeC_6H_5O_7.2H_2O$ 12.5,(Zn,Mn,Cu 3),$ZnSO_4.7H_2O$ 35.3, $MnSO_4$-5H $_2$O 16.2, $CuSO_45H_2O$ 3.1, $AlCl_3.6H_2O$ 1.0, $CoCl_2.6H_2O$ 0.1, KIO_3 0.1, cellulose 44.

2. (mg/100g)Vitamins (B16,B210,B6 4,B12 .01),Vitamin C 500,Niacin 40, Calcium panthotenate 10,Inositol 200, Biotin 0.6, Folic acid 1.5, P-aminobensoic acid, 5 Vitamin K3 5, Vitamin A acetate 4000IU, Vitamin D3 4000IU

Table 2. Chemical and mineral composition of experimental diets[a]

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Crude protein (%)	43.0	42.9	42.2	43.1	42.8	43.0	42.8
Lipid (%)	14.1	14.4	17.5	15.5	14.4	15.0	15.1
Crude ash (%)	6.60	6.77	6.26	6.51	6.34	6.37	6.23
Gross energy (cal/g)	4703	4984	5044	4951	5042	4955	4939
pH	5.58	5.44	5.57	5.55	5.48	5.44	5.43
Phytase activity (U/kg)	249	231	1872	3849	193	1740	4498
P (mg/g DM)	7.13	8.64	6.86	7.06	6.92	6.61	6.51
Ca (mg/g DM)	6.78	7.99	6.67	6.96	7.05	6.89	6.64
Mg (mg/g DM)	2.39	2.38	2.33	2.41	2.31	2.30	2.31
Fe (µg/g DM)	36.6	36.7	37.0	36.4	39.9	41.1	41.5
Mn (µg/g DM)	20.9	21.1	21.2	22.0	21.3	21.6	22.1
Zn (µg/g DM)	14.8	15.7	12.3	12.5	13.0	11.8	13.1
Cu (µg/g DM)	6.77	6.96	8.19	7.95	9.26	9.07	10.0

[a] Values are means of 3 replicate samples
Values in a row sharing same superscripts are not significantly (P>0.05) different

Figure 1 showed the growth trend while (Table 3) indicated no significant differences in the mean weight gain and specific growth rate (SGR) of the fish subjected to the different dietary treatments. However, the fish treated on diets of DSM had marginally higher mean weight gain and SGR than those maintained on diets of DHP. There were no significant differences in the amount of feed consumed by the fish under the different treatments. Nonetheless, the fish raised on diets of DSM

consumed more feed than those fed diets of DHP. Similarly, fish treated on phytase diets consumed more feed than others, a suggestion that addition of phytase into the diets could stimulate appetite. The feed conversion ratio (FCR) was almost the same (P>0.05) in all the treatments. Observations also indicated that treatments of DSM produced numerically better FCR than those of DHP. Fish fed diet of DSM plus 1500 U/kg phytase retained significantly higher N than those fed diets of DHP with or without phytase. Similarly treatment of DSM plus P produced fish with higher (P<0.05) N content than the fish treated on DHP diets. Generally, the fish treated on diets of DSM retained higher N resulting in lower N excretion than that of the fish fed diets of DHP.

Apparent digestibility coefficients (ADC) of nutrient and minerals are presented in Table 4 which indicated high digestibility for all the nutrients. Protein digestibility was closely related with higher digestibility values observed for diets that contained DHP. The values ranged between 94.3 and 95.6%. ADC protein of all the diets treated with phytase was significantly higher than that of the diet with inorganic P. The ADC of lipid was similar in all the treatments with values varying from 94.5 to 96.2%. All treatments with phytase had the same (P>0.05) rates of lipid digestibility which were higher than the digestibility in treatment with inorganic P. The ADC of energy also varied significantly, with treatments of DSM having higher ADC than those of DHP. The ADC of the T (s) with DSM and phytase were significantly higher than those of the T (s) with DHP and phytase. However, as in ADC(s) of protein and lipid, all the treatments with phytase had significantly higher ADC energy than that with inorganic P. ADC of dry matter were high and variable, with values ranging between 80.0 and 83.4%. The digestibility was similar (p>0.05) in T (s) 1 and 4, which also had significantly higher values than other treatments.

The effect of phytase reflected more on mineral digestibility. The ADC of P increased with increase in supplemental phytase. The treatment of DHP with

Table 3. Growth parameters of rainbow trout fed phytase diets[1]

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Initial meam wt. (g)	26.1	25.5	25.7	25.8	25.4	25.3	25.7
Final mean wt. (g)	118.8	118.3	131.3	128.9	112.7	104.7	122.4
Weight gain (g)	92.6^{a} ±2.83	92.9^{a} ±5.52	105.5^{a} ±12.1	103.1^{a} ±14.8	87.4^{a} ±18.2	79.4^{a} ±3.82	96.7^{a} ±4.91
SGR (%/day)[2]	1.80^{a} ±0.01	1.83^{a} ±0.04	1.94^{a} ±0.12	1.91^{a} ±0.11	1.77^{a} ±0.18	1.69^{a} ±0.04	1.86^{a} ±0.03
FCR [3]	1.29^{bc} ±0.03	1.23^{c} ±0.00	1.26^{bc} ±0.00	1.28^{bc} ±0.07	1.37^{b} ±0.03	1.34^{bc} ±0.00	1.35^{bc} ±0.03
Feed intake (g/fish)	119.0^{a} ±0.17	113.8^{a} ±6.27	131.4^{a} ±14.7	131.1^{a} ±12.5	116.3^{a} ±22.5	106.7^{a} ±5.47	128.9^{a} ±5.37
Nitrogen retention (%)[4]	32.2^{bc} ±0.85	33.5^{ab} ±0.10	35.4^{a} ±0.06	33.9^{ab} ±2.12	29.6^{cd} ±0.90	29.9^{cd} ±0.03	31.1^{bc} ±1.03

[1] Values (mean ± S.D) in a row sharing same superscripts are not significantly (P>0.05) different.
[2] Specific growth rate = 10^{2}x (Ln final body weight-Ln initial body weight)/ experimental period (days)
[3] Feed conversion ratio = (Dry feed fed/weight gain)
[4] Retention = [(Final nutrient content- initial nutrient content)/nutrient intake] x100

3000 U/kg phytase had higher (P<0.05) ADC of P than the treatment of DSM with 3000 U/kg phytase. The treatments supplemented with 1500 U/kg phytase had the same (P>0.05) ADC of P. Comparatively, ADC of P for diets with DHP were better than that of the diets with DSM. All the treatments had significantly higher ADC of P than the treatment with inorganic P supplement, an indication that the fish could not adequately digest inorganic P. The ADC of Ca was almost the same in all the treatments except that the values from T (s) 1, 3, 4, 6 and 7 were higher (P<0.05) than that from T 5, while the values from all the treatments were significantly higher than that from the treatment with P supplement (T 2). Observation from Table 4 also showed that Mg was mostly digested by the

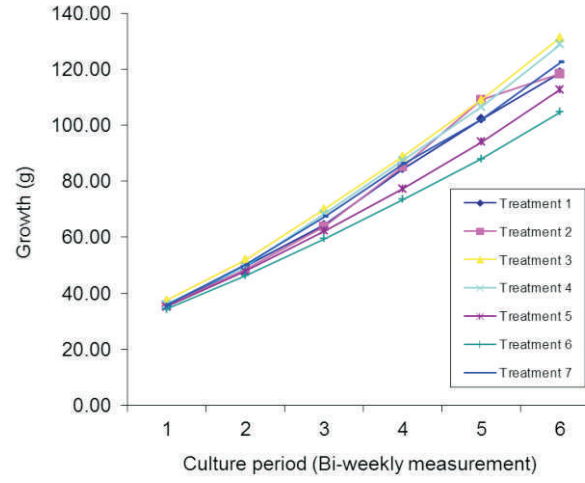

Figure 1. Growth trend of individual fish for 12 weeks

Table 4. Apparent digestibility coefficient (ADC) of crude nutrients and minerals (%)[1]

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Protein	94.4^{d} ±0.58	93.6^{e} ±0.48	94.3^{d} ±0.29	94.8^{c} ±0.57	95.2^{b} ±0.58	95.1^{bc} ±0.22	95.0^{bc} ±0.12
Lipid	95.4^{d} ±0.53	94.5^{e} ±0.38	95.8^{bc} ±0.17	95.2^{d} ±0.24	96.2^{a} ±0.27	95.9^{abc} ±0.43	95.7^{cd} ±0.05
Energy	86.4^{b} ±0.58	84.9^{d} ±0.48	86.6^{ab} ±0.29	87.0^{a} ±0.57	85.7^{c} ±0.58	85.9^{c} ±0.22	84.6^{d} ±0.12
Dry matter	83.4^{a} ±0.61	80.0^{d} ±0.59	81.1^{b} ±0.38	83.0^{a} ±0.43	80.7^{c} ±0.69	81.6^{b} ±0.28	80.0^{d} ±0.20
P	71.0^{e} ±0.54	59.9^{g} ±2.18	79.6^{bc} ±1.51	80.5^{b} ±0.62	67.9^{f} ±0.80	78.1^{cd} ±1.54	83.6^{a} ±0.99
Ca	48.9^{ab} ±4.35	30.7^{d} ±6.60	46.8^{b} ±4.95	50.3^{ab} ±8.22	38.5^{c} ±5.90	52.5^{ab} ±7.62	55.7^{a} ±4.31
Mg	91.7^{c} ±0.46	87.0^{e} ±1.00	94.6^{a} ±0.40	94.2^{a} ±0.27	90.3^{d} ±0.96	93.7^{ab} ±0.21	92.7^{b} ±0.14
Fe	46.7^{de} ±5.50	30.5^{f} ±4.60	50.9^{cd} ±5.03	54.2^{bc} ±2.21	42.1^{e} ±5.96	57.8^{ab} ±3.17	61.5^{a} ±1.11
Mn	58.2^{a} ±1.30	35.9^{c} ±3.89	58.1^{a} ±2.17	57.5^{a} ±5.04	43.9^{b} ±2.48	59.9^{a} ±3.72	60.1^{a} ±1.82
Zn	51.0^{cd} ±5.97	38.7^{e} ±13.7	64.2^{ab} ±3.02	69.1^{a} ±3.04	47.0^{d} ±7.00	59.0^{bc} ±6.97	60.9^{ab} ±6.75

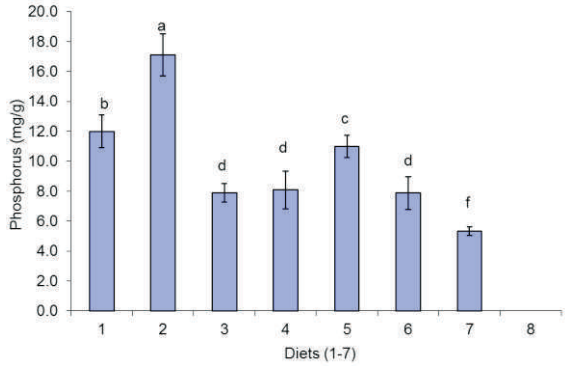

Figure 2. Phosphorus load of rainbow trout fed phytase diets

fish with ADC values ranging between 87.0 and 94.6%. The value from the treatment of DSM plus 3000 U/kg phytase was higher (P<0.05) than that from the treatment of DHP with 3000 U/kg phytase. Similarly, as in ADCs of P and Ca, the ADC of Mg from other treatments was significantly higher than that from T 2. ADC of Fe also varied significantly, with higher (P<0.05) values from treatments of DHP plus phytase than those from treatments of DSM plus phytase. The ADC of Fe from other treatments was significantly higher than that from T 2. Also the ADCs of Mn and Zn were least significant in T 2, while the ADC of Zn was similar (P>0.05) in all the treatments that contained phytase.

The mineral retention, P and N excretion values are presented in Table 5 which showed that the group of fish in T 7 retained most (P<0.05) P in their body. The group also had the highest (P<0.05) P digestibility value and least (P<0.05) P discharge into the environment (Fig. 2). Similarly, the group excreted significantly less P than those in other T (s), except in T 3. Fish in all the treatments with DHP generally discharged less (P<0.05) P than those fed diets of DSM. All the fish retained almost the same amount of Ca and Mg, except those in T 1 which contained least (P<0.05) Ca. Fish in most of the T (s) had closely related Zn contents. However, Z concentration was significantly least in the fish that received diet with supplemental P. N excreted by the fish treated on DSM was generally less than that excreted by those that received diets of DHP. This trend of N excretion correlates with higher N retention values observed in the same groups of fish.

Table 5. Mineral retention, P and nitrogen (N) excretion[1] after 12 weeks of feeding

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
P retention (%)	59.0^{ef} ±1.55	54.5^{f} ±0.06	69.2^{bc} ±0.28	62.8^{de} ±3.43	64.5^{cd} ±0.78	67.7^{cd} ±0.34	79.6^{a} ±1.71
Ca retention (%)	67.5^{c} ±1.78	75.0^{b} ±0.01	87.4^{a} ±0.64	86.7^{a} ±4.03	85.2^{a} ±0.18	75.5^{b} ±0.47	90.2^{a} ±1.72
Mg retention (%)	16.4^{c} ±0.43	19.5^{ab} ±0.00	19.7^{a} ±0.18	19.1^{ab} ±0.85	18.8^{ab} ±0.02	18.4^{b} ±0.14	19.1^{ab} ±0.35
Zn retention (%)	34.2^{e} ±0.90	28.4^{f} ±0.11	41.9^{bc} ±0.08	43.0^{b} ±2.60	39.4^{cd} ±0.91	50.1^{a} ±0.21	35.9^{e} ±1.17
P excretion (kg/Ton)	4.77^{b} ±1.55	5.41^{a} ±0.06	3.64^{d} ±0.28	4.40^{bc} ±3.43	4.85^{b} ±0.78	3.65^{d} ±0.34	3.56^{d} ±1.71
N excretion (kg/Ton)	60.7^{cd} ±0.01	53.4^{e} ±0.04	53.3^{e} ±0.12	58.1^{d} ±0.11	68.5^{b} ±0.18	90.3^{a} ±0.04	65.8^{b} ±0.03

Values are means ± SD (n= pooled samples of 5 fish per tank)

[1] Excretion (kg^{-1} t) = $\frac{\text{(FCR x nutrient in diet (kg)-(nutrient retained in fish (kg)}}{\text{Fish production (kg)}}$ x 1000

Values in a column sharing the superscripts are not significantly different (P>0.05)

Discussion

The use of defatted and dephytinized soybean meal in fish nutrition is a model to reduce environmental P and N loadings in aquaculture systems, and consequently modulating environmental pollution. Similarly, phytase is used to dephosphorylate the phytate P, to make P and bound minerals in plant feedstuffs more available (Kumar et al. 2012) for fish utilization resulting in the discharge of less minerals and pollution of the environment (Nwanna et al. 2008). Therefore, the present study assessed the effects of defatted or dephytinized soybean meal fortified with phytase (liquid Ronozyme PL, HBN01101) on the growth, mineral digestibility and P loadings by rainbow trout. The growth indices after 12 weeks of feeding indicated that the mean weight gain, specific growth rate and feed intake of the fish administered diets of defatted, dephytinized, with or without phytase were statistically the same. This observation supports the works of Cain and Garling (1995), Vielma *et al*.,(2002) and Feng *et al.,* (2009) who reported no significant differences in the weight gain of rainbow trout (*Oncorhynchus mykiss*) treated on phytase diets. The fish that received DSM diets had marginal growth increment over those treated on DHP diets. This observation is contrary to that of Vielma *et al*., (2002) who reported that dephytinization of soy protein produced significantly higher mean weight gain and feed efficiency than in treatments with different soy proteins. The findings from their study could have been affected by the use of microbial phytase, Natuphos. However, the results of the present study are in line with that of Forster *et al* .,(1999) who reported no significant differences on the growth and feed efficiency of rainbow trout fed P fortified soy protein-based diets top sprayed with 0, 500, 15000, or 4500 U phytase kg $^{-1}$ diet. In the present study also, inclusion of phytase into DHP diets slightly improved growth over that of the fish fed diets of DHP without phytase; and similar trend was observed in the case of treatment with DSM. Results also showed that the fish which received similar diet of DSM with phytase had higher weight gain, SGR and feed intake than the fish treated on diet with 5 g P kg^{-1}. This observation correlates with the report of Vielma *et al.*, (2002) that phytase pre-treatment of soy proteins enhanced the growth of rainbow trout higher than the treatment with 3 g supplemental P kg^{-1} diet. The observation is also in agreement with the reports of Nwanna (2005) and Nwanna *et al.,* (2007).), who compared the effects of supplemental phytase and phosphorus on the production of Nile tilapia and common carp respectively.

Effect of dietary phytase treatment can be most accurately measured using P utilization as the indicator of phytic acid hydrolysis (1998, Nwanna and Schwarz 2007). In the present study, addition of 3000 U phytase kg^{-1} diet significantly improved P digestibility in comparison with treatments without phytase, including treatment with P supplement. This observation is in line with that of Cheng and Hardy (2002) who reported that phytase supplementation in rainbow trout diets significantly improved P digestibility. Cain and Garling (1995) similarly reported increased P utilization in juvenile rainbow trout fed phytase diets. In fish, poor P availability and negative effects on mineral utilization have been documented as negative properties of phytate on growth and feed efficiency (National Research Council, 1993). Therefore the significant improvement in P absorption recorded from the present study is evidence of phytate hydrolysis by the dietary phytase. This supports the works of (Nwanna *et al*., 2006, Nwanna and Schwarz 2008).

In the present study, addition of phytase into the diets significantly improved ADC protein, Ca and Mg .Similar observation was reported by Cheng and Hardy (2002) and Feng *et al*., (2009) from a study involving digestibility of four plant proteins by rainbow trout. Reports also abound that addition of phytase to Rainbow trout diets did not improve protein digestibility ((Vielma et al., 2000; Cheng and Hardy 2002). However, contrary to the result of the present study, Feng *et al*., (2009) reported a negative effect of phytase on the ADC of lipid. Also, supplementation of 5 g P kg^{-1} diet significantly decreased Ca, Mg, Fe, Mn and Zn absorption. The total P contents of the diets (mean of 6.70mg/g and 8.64 mg/g in P

treatment) were closely related to the levels reported by other authors for have supported good growth and mineralization. For example, Vielma *et al*., (2002) used P contents of between 6.0 and 9.5 mg/g without any related effect on mineral reduction, rather they observed increased utilization of Ca and Mg. This discrepancy from the present study could be ascribed to inability of the fish to digest inorganic P, though it is known that P decreases the intestinal absorption of Ca and Mg from plant feedstuffs Vielma *et al*., (2002).

Improvement in Zn utilization by the fish is an indication of successful hydrolysis of phytic acid by phytase treatment, because Zn utilization is reduced by phytate (Richardson *et al*., 1985, Nwanna *et al*., 2006) and Zn in soybean meal is poorly available to fish (Sugiura *et al*., 1998). Data from the present study support this observation, and addition of phytase into the diets also produced significantly higher Zn digestibility than the treatment with P supplement. This positive effect of pyhtase is consistent with significant improvement in Zn retention which culminated in the discharge of less ($P<0.05$) Zn into the environment. In Ramseyer *et al*., (1999), and (Nwanna *et al*., 2008) phytase pre-treatment of diets similarly enhanced higher Zn utilization and absorption.

Low P discharge was observed from the present study, especially in phytase treatments. The values range between 5.33 and 8.08 mg/g in treatments with phytase and 17.0 mg/g in treatment with P supplement. Similar low values of P release had been reported by Cain and Garling (1995), Lanari *et al*.,(1998), Vielma *et al*.,(1998), Vielma *et al*.,(2002) (Nwanna *et al*., 2006) and Feng *et al*., (2009).

Conclusion

This study revealed that addition of DSM in the diets would promote better fish growth, while addition of DHP would promote better environmentally friendly aquaculture.
Environmental phosphorus loadings reduced with increasing levels of phytase in the diets, while the supplemental phosphorud has inverse relationships with Zn with carcass deposition.

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