Effect of Phytase on Growth and Phosphorus Utilization in Japanese Flounder (*Paralichthys olivaceus*)

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ABSTRACT

An experiment was conducted to investigate the effect of phytase supplementation on weight gain, phosphorus and protein digestibility and retention in Japanese flounder (*Paralichthys olivaceus)* fed a soybean meal (SBM)-containing diet. Six levels of phytase-supplemented diets containing 0, 150, 300, 450, 900 and 1500 FTU (phytase unit)/100g diet were assigned to triplicate tanks and fed to Japanese flounder (20 fish/tank, initial average weight 151.4 g) for 40 days. The increase of soluble phosphorus and decrease of phytic acid remained relatively constant for all levels receiving the 300 FTU diet and greater. Significantly (P<0.05) greater weight gain and higher feed conversion ratios (FCR) were observed in fish fed diets supplemented at 300 FTU or greater compared to the control (0 FTU) diet. Significantly (P<0.05) improved apparent protein and phosphorus digestibility, as well as serum calcium concentration were found in fish fed the 300 FTU diet. All

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diets supplemented at 300 FTU or greater also showed a significantly ($P<0.05$) improved concentration of bone calcium and zinc. The 150 FTU inclusion level showed only better protein and phosphorus retention, bone phosphorus and magnesium than the control (0 FTU) diet. Therefore, this study indicated that supplementation of phytase is effective and that the 300 FTU/100g SBM-containing diet resulted in the maximum release of soluble phosphorus, and as a consequence improved weight gain, FCR, bone minerals, phosphorus and protein digestibility and retention in Japanese flounder.

INTRODUCTION

Soybean meal (SBM) is an important dietary protein source for cultured Japanese flounder *(Paralichthys olivaceus*). After oil extraction, SBM is used as a protein source in animal feeds due to its well-balanced amino acid profile and relatively high crude protein level (Cheng and Hardy 2003). Soybean meal is widely used in fish nutrition research, as well as for commercial fish feed purposes (Robinson *et al*. 1985, Hughes 1988, Riche and Brown 1996). However, it is also reported that SBM is rich in phytic acid, which reduces the availability of nutrients such as protein and minerals. Phytic acid has been shown to have a strong anti-nutritive effect due to its tendency to form insoluble complexes with di- and trivalent minerals, rendering these minerals unavailable to fish (Kerovuo 2000). Approximately two-thirds of the total phosphorus (P) in various plant ingredients is present as phytic acid (Ketola 1994). It is known that fish cannot utilize phosphorus bound in the phytic acid complex molecule because fish lack the phytase enzyme needed to hydrolyze bound phytic acid phosphorus to an available form of phosphorus. On the contrary, commercial phytase can hydrolyze the phytate complex bond producing a simpler form of P, thereby increasing the bioavailability of mineral elements (Persson *et al*. 1998). This holds promise for reduced phosphorus in effluents and reduced phosphorus pollution. Use of commercial phytase has been reported to improve phytate P bioavailability in poultry, swine, and fish, which should decrease the amount of phosphorus excreted (Nelson *et al*. 1971, Simons *et al*. 1990, Crowell *et al*. 1993, Rodehutscord *et al*. 1995, Schafer *et al*. 1995, Jackson *et al*. 1996, Lanari *et al*. 1998, Veilma *et al*. 1998, Veilma *et al*. 2000). The increased nutrient utilization due to the addition of phytase in the diet has yet to be widely studied in fish. Therefore, the present study was conducted to examine the effects

of phytase supplementation on weight gain, phosphorus and protein digestibility and retention in Japanese flounder fed a diet containing soybean meal.

MATERIALS AND METHODS

Experimental diets

Six diets with common base ingredients were prepared in this study (Table 1). The six diets were: (1) Control diet with no added phytase (0 FTU/100g diet) and (2-6) five phytase-treated diets incorporating a dry enzyme powder (Natuphos ™, BASF Corporation, Parsippany, NJ, USA) at incremental concentrations of 150, 300, 450, 900 and 1500 FTU/100g. The phytase premix was found to contain 10,000 U of phytase activity/g, with 1 FTU (phytase unit) of phytase activity defined as the amount of enzyme that liberates 1 μmol of inorganic phosphorus/min from 5.1 mmol/L of sodium phytate at a pH of 5.5 and a water bath temperature of 37°C (Hughes and Soares 1998). The diet materials were mixed in a Hobart mixer, moistened and then formed into granules 7.5 mm in diameter using a laboratory pellet mill. A portion of each of the six experimental diets was removed for chemical analysis which included protein, lipid, ash, total P, soluble P, and phytic acid content analysis. Three storage times were maintained (0 h, 40 h, and 60 h) at room temperature (26.4°C) to determine the optimal time for releasing the soluble P. Finally, the pellets were stored at –20°C until use.

Experimental design, fish rearing system, and feeding

Japanese flounder with a mean weight 151.4 ± 0.1g that had been maintained and acclimated at Akaoka Fish Center, Kochi University for 1 year, were used in this study. Three hundred sixty fish were randomly distributed in 18 flow-through fiber reinforced plastic (FRP) tanks (800 L holding capacity of 3 tanks/diet and 20 fish/tank). Filtered water (32 ppt salinity) was supplied to each tank at a flow rate of 10 L/min in a flow-through system located at this facility. An adequate level of oxygen in each system was maintained through artificial aeration throughout the experimental period. Even though this experiment was conducted in flow-through systems, the overall study is also relevant to recirculating aquaculture systems. Before the commencement of the feeding study, fish were gradually acclimated over three weeks to experimental conditions

Table 1. Composition of experimental phytase-supplemented diets for Japanese flounder, Paralichthys olivaceus.

Phytase (FTU/100g diet)	**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**1**	**2**	**3**	**4**	**5**	**6**
Brownfish meal	40	40	40	40	40	40
Soybean meal [1]	29	29	29	29	29	29
Krill meal	10	10	10	10	10	10
Blood meal	5	5	5	5	5	5
L-Lysine	0.2	0.2	0.2	0.2	0.2	0.2
L-Methionine	0.4	0.4	0.4	0.4	0.4	0.4
Fish oil	2	2	2	2	2	2
Vitamin mixture[2]	2	2	2	2	2	2
Mineral mixture[3]	2	2	2	2	2	2
Phytase	0	0.015	0.03	0.045	0.09	0.15
α Cellulose	2.4	2.385	2.37	2.355	2.31	2.25
α Starch	7	7	7	7	7	7
Total	100	100	100	100	100	100
Proximate composition (%)						
Dry matter	63.9	64.7	64.6	64.7	64.4	64.1
Crude protein	52.5	51.8	51.6	51.5	52.0	52.4
Crude lipid	6.9	6.2	6.8	6.7	6.8	6.8
Crude sugar	11.7	12.3	12.2	11.7	11.3	11.5
Crude ash	9.3	8.7	8.9	9.3	9.4	9.3
Energy (kcal/100g)	345	338	342	339	340	343

[1] Syowa Sangyo. Co. Ltd (Tokyo, Japan).

[2]Contains (mg/100g): Thiamine HCl 2.2; Riboflavin 2.2; Pyrodoxin HCl 2.3; Nicotinic acid 9.6; Ca-pantothenate 7.2; Inositol 60.0; Biotin 0.14; Folic acid 2.4; Cholin chloride 300.0; Cyanocobalamin 0.04; Ascorbic acid 21.6; Vitamin A palmitate 1.1; α- Tocopherol 20; α- Cellulose 1571.22

[2] Contains (mg/100g): KH_2PO_4 412; Ca $(H_2PO_4).2H_2O$ 618; Ca-lactate 282; Fe-fumaric acid 160; Trace minerals 50 α-Cellulose 478

and fed with a commercial diet (Marubeni Inc., Tokyo, Japan). Fish were held on a 12-h dark/12-h light photoperiod. Dissolved oxygen levels and water temperature were monitored daily with ranges of 5.20 - 6.89 mg/l and 20.5 - 24.3°C, respectively (TOA Electronic Ltd., Kobe, Japan). Fish were hand-fed to apparent satiation twice a day for a period of 40 days, and feed consumption was recorded at each feeding.

Biological sampling and tissue collection

Prior to the feeding trial, 10 fish were taken from the common tank, blood was collected for analyzing plasma mineral concentrations, and the fish humanely euthanized with MS-22 (Sigma Chemical Co., St. Louis, MO, USA). Of the 10 fish, five were finely ground for proximate analysis, while the remaining five fish were individually dissected to collect vertebrae for mineral concentration estimation. At the beginning of the experiment and at 10-day intervals during the trial, fish were counted and bulk-weighed after a 12-h fast. At the end of the feeding trial, after counting and weighing the fish, five fish were randomly selected from two tanks of fish fed each of the experimental diets for vertebrae collection and another five fish were collected for carcass analysis as previously described. The collected samples were immediately transferred to a freezer (-40°C) until analysis. After collecting vertebrae, soft tissue was carefully removed from the vertebral axis and the bones oven-dried at 110°C for 24 h, finely ground, and analyzed for mineral content.

Analytical methods

Blood was withdrawn through the caudal tail vessel using 2.5 ml heparinzed syringes, then centrifuged for 5 min at 13750 x g. The resultant plasma was used to measure mineral contents of each fish. For proximate analysis and determination of whole-body P concentrations, fish were pooled and homogenized in a mincing machine. The phosphorus retention in flounder after 8 weeks of feeding was calculated as described previously (Papatryphon *et al*. 1999). The proximate analyses were determined according to the AOAC method (AOAC 1995). To determine phosphate (ascorbic acid method), ammonium molybdate and potassium antimonyl tartrate were made to react in an acid medium with orthophosphate to form a heteropoly acid- phosphomolybdic acid which was then reduced to intensely colored molybdenum blue by ascorbic acid. The plasma was analyzed for P (ammonium molybdate method)

and Zn (2-(5-bromo-2-pyridylazo)-5-(*N*-propyl—3-sulfopropylamino)-phenol method) using a commercial diagnostic kit (Wako Pure Chemical Industries, Osaka, Japan). Chromic oxide was determined following the method of Furukawa and Tsukahara (1966). Diets were analyzed for phytic acid (myo-inositol 1,2,3,5/6- hexakis dihydrogen phosphate) by the method of Graf and Dintzis (1982).

Digestibility study

The effect of phytase on the digestibility of P and protein in SBM-containing diets was measured by an indirect method with chromic oxide (Cr_2O_3) as an inert reference substance. The use of an inert indicator, which passed unaffected by digestion through the alimentary tract, provided a convenient method of measuring digestibility. This method has been successfully applied to fish using Cr_2O_3 as the indicator (Furukawa and Tsukahara 1966). The marker (0.5%) was added to the feed for the digestibility study. After 40 days of the feeding trial, the remaining experimental fish were fed each diet to satiation at 8:00 PM and fecal samples were obtained by gentle manual stripping of the lower abdomen at 8:00 AM the next morning as recommended by Austreng (1978). Fecal samples were collected once a day for 2 weeks and were pooled for subsequent analyses and stored at –30°C. The diets and fecal samples were freeze-dried and were subjected to analyses for total P, crude protein and Cr_2O_3 content.

Statistics

Results were subjected to a one-way ANOVA (Statistica Release 6.0 StatSoft, Inc., Tulsa, OK, USA). Differences among treatments were compared by Tukey's HSD (Honestly Significant Difference). Means were considered significant at $P<0.05$.

RESULTS

Soluble P release pattern

Comparatively little soluble P was released after 0-h storage time (Figure 1). The release of soluble P increased after 20-h storage time at room temperature (26.4°C), however, no additional release was observed after 40-h storage at room temperature. Moreover, the diets containing 300, 450, 900, and 1500 FTU released similar amounts of soluble P after

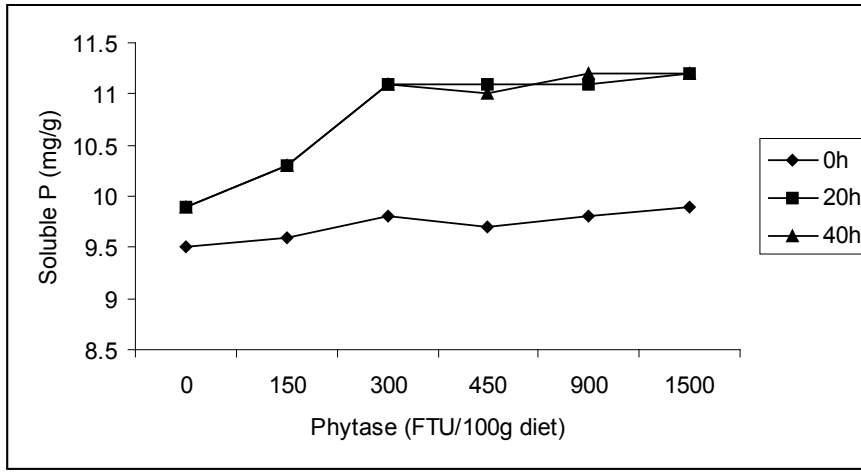

Figure 1. Soluble P release patterns in control (0 FTU/100g) and phytase-supplemented diets (150, 300, 450, 900 and 1500 FTU/100g) held for various times (0 h, 20 h and 40 h) post-preparation at 26.4°C.

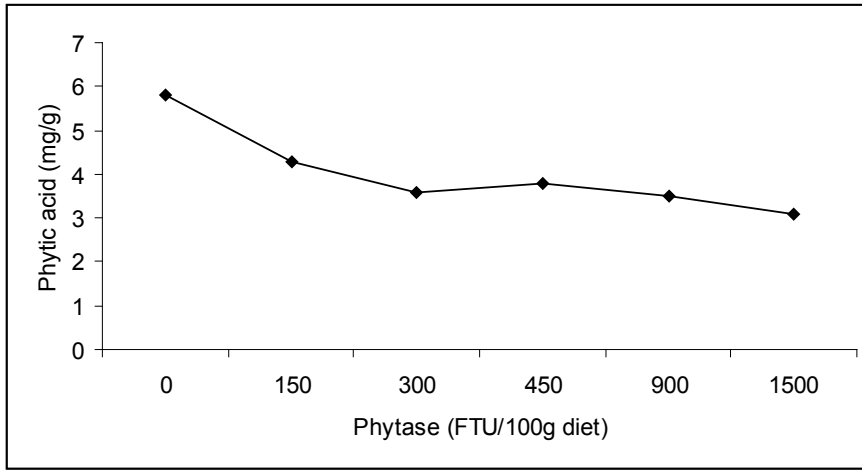

Figure 2. Phytic acid content in control (0 FTU/100g) and phytase-supplemented diets (150, 300, 450, 900 and 1500 FTU/100g).

storage at room temperature. However, the diets containing 0 and 150 FTU released significantly lower amounts of soluble P than the diets containing 300 FTUs or more.

The phytic acid content of the experimental diets varied between 3.1 and 5.8 mg/g, with the diet containing 0 FTU having the highest amount (5.8 mg/g) (Figure 2). The phytic acid content was reduced in the diets of 300, 450, 900 and 1500 FTU, where the values were 3.6, 3.8, 3.5 and 3.1 mg/g, respectively.

Fish growth and feed performance

Weight gain in fish fed the 150 FTU diet was not significantly different from the control diet (0 FTU). However, significantly greater weight gain was observed in the 300 FTU group and higher levels of phytase supplemented diets (Table 2). A significantly higher FCR (poorer conversion) was observed in the control dietary group (0 FTU) than in all other dietary treatments. Fish receiving a diet containing 300 FTU or greater exhibited a significantly better FCR than either the control (0 FTU) diet or the 150 FTU treatment. The 150 FTU treatment had a significantly better FCR than the control (0 FTU) diet, but was significantly lower than the 300 FTU or greater treatments.

Proximate composition and phosphorus content in fish

Moisture content in all the experimental groups was lower than in the initial fish, resulting in higher crude protein and crude lipid content (Table 3). However, at the end of the study, differences in proximate composition of the fish were not significant among the dietary treatments. Although the dietary treatments did not have a significant influence on the total P content, all groups receiving phytase in the diets showed higher total phosphorus concentrations than either the initial group or the control (0 FTU) group.

Vertebrae mineral

Although only the 900 FTU diet resulted in a significantly higher concentration of vertebrae P, all phytase-treated diets showed a higher vertebrae P concentration compared to the 0 FTU diet (Table 4). The vertebrae calcium (Ca) and zinc (Zn) were significantly higher for all diets containing phytase compared to the 0 FTU diet. The vertebrae Ca and Zn concentration were not significantly higher for the groups receiving 300 to 1500 FTU compared to the 150 FTU and 0 FTU groups, and there were no differences shown between the 300 and 1500 FTU groups. Vertebrae magnesium (Mg) concentrations were significantly higher for all phytase containing diets compared to the 0 FTU dietary group.

*Table 2. Performance of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)	**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**1**	**2**	**3**	**4**	**5**	**6**
Initial mean body wt. (g)	151.4 ± 0.1^{a}	151.5 ± 0.2^{a}	150.7 ± 0.7^{a}	150.8 ± 0.2^{a}	151.2 ± 0.4^{a}	151.5 ± 0.3^{a}
Final mean body wt. (g)	209.56 ± 9.0^{a}	220.0 ± 0.8^{ab}	235.5 ± 1.7^{b}	233.3 ± 2.3^{b}	232.3 ± 5.0^{b}	237.0 ± 3.2^{b}
Mean body wt. gain (%)	38.9 ± 5.8^{a}	45.2 ± 10.0^{ab}	56.2 ± 6.1^{b}	54.7 ± 3.2^{b}	53.6 ± 12.5^{b}	56.4 ± 20.4^{b}
FCR	2.4 ± 0.3^{c}	1.8 ± 0.3^{b}	1.5 ± 0.5^{a}	1.5 ± 0.2^{a}	1.6 ± 0.8^{a}	1.5 ± 0.4^{a}
Daily feeding rate (%)	1.72 ± 0.09^{a}	1.73 ± 0.03^{a}	1.7 ± 0.11^{a}	1.69 ± 0.01^{a}	1.74 ± 0.07^{a}	1.71 ± 0.02^{a}
Feed efficiency (%)	147.1 ± 49.4^{a}	182.6 ± 41.9^{a}	198.8 ± 62.7^{b}	196.9 ± 20.5^{b}	197.7 ± 59.3^{b}	213.4 ± 74.4^{b}
Survival rate (%)	100.0^{a}	100.0^{a}	100.0^{a}	97.5^{a}	100.0^{a}	97.5^{a}

* Values are means of triplicate tanks. Different superscripts within the same row are significantly different, $P<0.05$

*Table 3. Whole body proximate composition and phosphorus content in Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)		**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**Initial**	**1**	**2**	**3**	**4**	**5**	**6**
Moisture (%)	75.2	74.2 ± 0.3^{a}	70.3 ± 1.4^{a}	74.5 ± 0.5^{a}	74.8 ± 0.8^{a}	73.9 ± 0.0^{a}	73.6 ± 2.0^{a}
Crude protein (%)	16.7	17.8 ± 0.6^{a}	19.5 ± 0.4^{a}	18.6 ± 0.6^{a}	18.3 ± 0.2^{a}	19.5 ± 0.4^{a}	19.4 ± 2.2^{a}
Crude lipid (%)	2.5	2.7 ± 1.0^{a}	3.0 ± 1.3^{a}	2.7 ± 0.7^{a}	2.5 ± 0.0^{a}	3.3 ± 0.2^{a}	3.0 ± 0.5^{a}
Crude ash (%)	4.3	3.6 ± 0.4^{a}	5.6 ± 0.1^{a}	3.4 ± 0.6^{a}	3.6 ± 0.3^{a}	3.7 ± 0.2^{a}	5.3 ± 2.5^{a}
Total phosphorus (mg/g)	3.9	6.4 ± 2.1^{a}	14.1 ± 4.7^{a}	14.3 ± 7.2^{a}	13.6 ± 0.1^{a}	11.0 ± 4.0^{a}	11.2 ± 4.3^{a}

* Mean values with standard deviation from triplicate tanks (except for initial sample) and pooled five fish for each. Different superscripts within the same row are significantly different, $P<0.05$

Serum mineral

The 300 FTU group showed a significantly improved serum Ca concentration over the control (0 FTU) dietary group (Table 5). There were no significant differences in serum P or Zn among the various dietary groups.

Apparent P and protein digestibility

The apparent P and protein digestibility of fish fed the different diets are shown in Table 6. All phytase-treated diets had significantly higher P digestibility values compared to the control (0 FTU) diet. Only the 300 FTU treatment showed significantly higher apparent protein digestibility than the 0 FTU treatment.

Apparent P and protein retention

Apparent retention of P and protein in Japanese flounder fed the different experimental diets are given in Table 7. Protein retention was significantly improved in fish fed any of the phytase-supplemented diets, and P retention was significantly higher in fish fed diets supplemented at the 150, 300, and 450 FTU levels compared to the control (0 FTU) level.

DISCUSSION

Soluble P release patterns showed that by using microbial phytase at the level of 300 FTU, the SBM-containing diets with a high content of phytic acid could be optimized after 20 h of storage time at room temperature (26.4°C). Several investigators have also reported that a significant amount of dephosphorylation of phytate occured in a phytase-supplemented diet during diet preparation (Scafer *et al*. 1995, Branden and Carter 1999, Yan *et al*. 2002). The advantageous effects of phytase in SBM-containing diets were confirmed in this feeding experiment with improved growth performance, feed conversion, feed efficiency, P and protein digestibility and mineral utilization by Japanese flounder. It has been reported that dietary phytase improved growth and overall performance while reducing P excretion in rainbow trout, *Oncorhynchus mykiss* (Rodehutscord and Pfeiffer 1995, Sugiura *et al*. 2001), carp, *Cyprinus carpio* (Schafer *et al*. 1995) and striped bass, *Morone saxatilis* (Papatryphon *et al*. 1999). This study demonstrated that the addition of 150 FTU or more in SBM-containing diets improved the FCR of Japanese flounder compared to a

*Table 4. Mineral contents of vertebrae of Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)		**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**Initial**	**1**	**2**	**3**	**4**	**5**	**6**
Phosphorus (mg/g)	117.0	126.83 ± 3.0^{a}	129.0 ± 3.5^{ab}	128.3 ± 1.4^{ab}	127.9 ± 0.7^{ab}	134.2 ± 0.8^{b}	128.2 ± 0.2^{ab}
Calcium (mg/g)	175.8	188.9 ± 7.6^{a}	193.6 ± 7.7^{b}	198.5 ± 8.4^{c}	198.3 ± 6.2^{c}	200.6 ± 5.8^{c}	198.4 ± 5.4^{c}
Magnesium (mg/g)	3.8	3.81 ± 0.1^{a}	3.95 ± 0.09^{b}	3.97 ± 0.03^{b}	4.0 ± 0.02^{b}	3.96 ± 0.02^{b}	4.0 ± 0.1^{b}
Zinc (mg/g)	0.136	0.147 ± 0.009^{a}	0.166 ± 0.004^{b}	0.175 ± 0.004^{c}	0.172 ± 0.004^{c}	0.175 ± 0.006^{c}	0.176 ± 0.001^{c}

* Mean values with standard deviation from triplicate tanks (except for initial sample) and pooled five fish for each. Different superscripts within the same row are significantly different, $P<0.05$

*Table 5. Serum minerals in Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)		**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**Initial**	**1**	**2**	**3**	**4**	**5**	**6**
Calcium (mg/100ml)	19.7 ± 4.0	16.6 ± 0.5^{a}	17.8 ± 0.1^{ab}	18.2 ± 1.0^{b}	17.7 ± 0.4^{ab}	17.6 ± 0.2^{ab}	17.3 ± 0.5^{ab}
Phosphorus (mg/100ml)	18.9 ± 5.0	12.3 ± 1.5^{a}	12.3 ± 0.4^{a}	14.0 ± 1.4^{a}	13.6 ± 1.5^{a}	12.2 ± 0.9^{a}	12.5 ± 0.3^{a}
Zinc (µg/100ml)	43.07 ± 31.7	94.4 ± 50.9^{a}	102.6 ± 53.8^{a}	124.2 ± 45.0^{a}	145.1 ± 22.5^{a}	143.0 ± 19.4^{a}	126.4 ± 48.2^{a}

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, $P<0.05$

*Table 6. Apparent phosphorus and protein digestibility of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)	**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**1**	**2**	**3**	**4**	**5**	**6**
Phosphorus (%)	33.6 ± 11.7^{a}	49.6 ± 2.0^{b}	56.1 ± 5.7^{b}	55.5 ± 11.6^{b}	54.2 ± 5.4^{b}	51.3 ± 1.5^{b}
Protein (%)	78.4 ± 0.5^{a}	81.5 ± 6.4^{ab}	85.0 ± 2.0^{b}	83.9 ± 2.2^{ab}	82.0 ± 6.2^{ab}	84.4 ± 0.9^{b}

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, $P<0.05$

*Table 7. Apparent phosphorus and protein retention of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.**

Phytase (FTU/100g diet)	**0**	**150**	**300**	**450**	**900**	**1500**
Dietary group	**1**	**2**	**3**	**4**	**5**	**6**
Phosphorus (%)	41.4 ± 33.3^{a}	58.7 ± 9.6^{b}	57.8 ± 18.2^{b}	54.6 ± 10.7^{b}	46.6 ± 6.8^{ab}	44.2 ± 7.8^{ab}
Protein (%)	38.3 ± 2.7^{a}	46.4 ± 6.2^{b}	47.7 ± 8.6^{b}	45.4 ± 0.9^{b}	47.0 ± 4.4^{b}	44.1 ± 2.9^{b}

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, $P<0.05$

control diet with 0 FTU phytase addition. Inclusion of 300 FTU or more resulted in an additional FCR improvement over the 150 FTU inclusion rate. In this study, a significantly better FCR value was found in fish fed the 300 FTU diet. This suggests that growth reflects the FCR, which can be improved through the addition of 300 FTU in SBM-containing diet for Japanese flounder.

It is noteworthy that all levels of phytase addition showed significantly improved P digestibility over the control (0 FTU) diet. However, the higher dosages of phytase, such as 900 and 1500 FTU groups, did not further increase of P digestibility in the SBM-containing diet for Japanese flounder. Cheng and Hardy (2003) pointed out that when phytase was supplemented in extruded full-fat soybean meal, the apparent digestibility coefficient of total P and phytate P increased. Improved digestibility of P from pelleted diets containing SBM by supplementation with phytase has been demonstrated in both rainbow trout and common carp (Rodehutscord *et al*. 1995, Scafer *et al*. 1995). Sugiura *et al*. (1998) also reported that the digestibility of dietary P in rainbow trout was lower in SBM without supplementation of phytase.

Significantly higher protein digestibility values were also noted in fish fed the 300 FTU and 1500 FTU diets than the control (0 FTU) diet. Storebakken *et al*. (1998) demonstrated that phytase pretreatment of isolated soy protein resulted in improved protein digestibility in Atlantic salmon (*Salmo salar).* Lei *et al*. (1993) also reported that supplementation of phytase in the diet may improve bioavailability of protein and trace minerals. The results of the present study indicate that phytase in the diets improved P and protein retention. It is also reported that improved P retention was found with phytase (2000 FTU/kg diet) supplemented in soybean meal and canola meal diets in striped bass (Papatryphon 1999) and in Atlantic salmon (Sajjadi *et al*. 2004). In our study, phytase enhanced protein retention, suggesting that phytase reduces formation of phytate-protein complexes in the gut and causes an improvement in utilization.

In this study, flounder fed the 300 FTU diet showed a significantly higher serum Ca concentration. The diets containing 150, 450, 900 and 1500 FTU showed no significant difference of serum Ca compared to the fish fed the control (0 FTU) diet. Hughes and Soares (1998) reported that sea bass fed a diet containing phytase had a higher serum Ca content, which

is in agreement with our findings. However, no significant variation was observed in either serum P or Zn among the different dietary groups. The suitability of using serum P concentration as an indicator of P status seems questionable due to a combination of physiological and experimental factors. Skonberg *et al*. (1997) speculated that when rainbow trout are fed excess P, blood P does not respond to the excess P level.

Mineral bioavailability was increased with phytase supplementation as demonstrated through improved bone mineralization in this experiment. These results agree with other studies conducted concerning P bioavailability with carp, catfish (*Ictalurus punctatus)* and striped bass (Scafer *et al*. 1995, Jackson *et al*. 1996, Papatryphon *et al*. 1999). The vertebral Ca and Zn were significantly higher in the fish fed the 300 FTU diet than the control (0 FTU) diet. Vertebral P and Mg were markedly improved in all phytase-supplemented diets. Similar results appear to concur with these observations in striped bass (Hughes and Soares 1998).

CONCLUSION

This study indicates that in an SBM-containing diet, supplementation of phytase was effective and that 300 FTU/ 100g in resulted in the maximum release of soluble P, and as a consequence improved the weight gain, FCR, bone mineral content, P and protein digestion and retention in Japanese flounder.

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