INFLUENCE OF DIETARY PHYTIC ACID AND PHYTASE ON GROWTH, DIGESTIBILITY, AND VERTEBRAL PHOSPHORUS OF JUVENILE JAPANESE FLOUNDER, *Paralichthys olivaceus*

Asda Laining*

Research and Development Institute for Coastal Aquaculture

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ABSTRACT

Triplicate groups of juvenile Japanese flounder (0.56 g) were fed with six experimental diets to examine the effects of phytic acid, with or without phytase on growth performances, nutrient digestibility, and vertebral phosphorus (P) content. Diet without both phytic acid (PA) and phytase supplementation was used as control. One diet was added with 10 g PA/kg without phytase supplement. Four diets were formulated to contain two levels of phytase (1,000 FTU or 2,000 FTU phytase/kg diet) combined with 2 levels of PA (10 and 20 g/kg diet). All diets were added with 10 g/kg in-organic P to meet flounder requirement. After 50 days culture, fish fed 10PA/2,000P grew significantly ($P<0.05$) higher than other groups, but did not differ from fish fed 10PA/1,000P. In contrast, fish fed 10PA/0P had the lowest growth but was not different from control diet (0PA/0P). Addition of either PA or combined with phytase had no significant ($P>0.05$) effects on feed intake and FCR. However, fish fed 10 g PA/kg combined with phytase had significant ($P<0.05$) higher whole body lipid, ash, and P than other groups. Moreover, P digestibility and vertebral P content were significantly increased by dietary phytase. This finding suggested that dietary phytase had potential to enhance the growth and nutrient utilization in juvenile Japanese flounder fed diet containing phytic acid. Specifically, inclusion of 2,000 FTU phytase/kg diet gave better performances when diet containing PA at level of 10 g/kg diet.

KEYWORDS: phytic acid, phytase, vertebral phosphorus, Japanese flounder

INTRODUCTION

Plant feedstuffs are considered as the most suitable and economical candidates for replacing fishmeal in commercial aqua-feeds. However, presence of PA is limited their use because fish lacks of phytase (NRC, 1993). Most cereals, grains, and oil seeds contain high level of PA with various different levels including commonly used and potentially usable plant-derived fish feed ingredients such as soybean meal, rapeseed meal, canola meal, rice bran. Canola concentrates generally contain 53-75 g/kg PA, whereas the level of this compound in its meal usually ranges from 31-37 g/kg (Higgs *et al.*, 1995). Soybean contains PA around 10-15 g/kg whereas its meal has 14-16 g/kg. These ranges relatively lower comparing to soybean protein isolate (SPI) which contains 20 g/kg.

Growth of commonly cultured fish species such as carp, tilapia, trout, and salmon is negatively affected by inclusion of PA

* Corresponding author. Research and Development Institute for Coastal Aquaculture, Jl. Makmur Dg. Sitakka No. 129, Maros 90512, South Sulawesi, Indonesia. Phone: +62 411 371 544
E-mail: asdalaining@yahoo.com

containing ingredients in diets. The involvement of PA in inducing the negative effects has been corroborated by feeding studies where synthetic PA was added to fish diets. Inclusion of PA in diet at rate of 2.1 g/kg decreased significantly the digestibility of Zn and Mg in Atlantic salmon, but no profound dose response relationships were observed between dietary PA content and digestibility of main nutrients such as nitrogen, lipid, and starch (Denstadli *et al.*, 2006). Similar study investigating the dose response of synthetic PA showed the adverse effect of phytic acid on growth performances and nutrient utilization of juvenile Japanese flounder (Laining *et al.*, 2010a).

Concentration of PA in ingredients is not markedly decreased by most processes applied to produce soybean meal or other plant meals. But it can be reduced to some extent by enzymatically hydrolyzing by phytase. Several phytases are known to remove phosphate groups from specific positions of the PA molecule. Microbial phytases are commercially available and have been used by several ways such as pre-treatment of feedstuff (Denstadli *et al.*, 2007); mix with other ingredients before pelletizing (Sajjadi & Carter, 2004; Liebert & Portz, 2005) and spraying onto the surface of pellet (Vielma *et al.*, 2004). Application of phytase on fish diets have been reported to have some benefits particularly on common freshwater fish like tilapia (Liebert & Portz, 2007); Atlantic salmon (Sajjadi & Carter, 2004); rainbow trout (Cheng & Hardy, 2003); common carp (Sardar *et al.*, 2007); striped bass (Papatryphon *et al.*, 1999); and *Pangasius pangasius* (Debnath *et al.*, 2005). In contrast, only few studies have been reported on marine fish including humpback grouper (Rachmansyah *et al.*, 2005); Japanese sea bass (Ai *et al.*, 2007) and tiger puffer (Laining *et al.*, 2010b).

The objective of this study was to determine the influence of microbial phytase on growth performances, digestibility and vertebral P content of juvenile Japanese flounder. *Paralichthys olivaceus* fed different level of PA.

MATERIALS AND METHODS

Feed Ingredients and Diets

Formulation and chemical composition of experimental diets are shown in Table 1 and Table 2, respectively. Six diets were formulated to contain iso-nitrogen and iso-caloric diets by using SPI, fishmeal and krill meal as protein sources, pollack liver oil as lipid source, and dextrin, α-starch as the carbohydrate sources. Diet without PA and phytase supplement was used as control (0PA/0P). One diet was supplemented with 10 g PA/kg without phytase supplement (10PA/0P). Four diets were prepared to contain two levels of PA (10 and 20 g IP6/kg) combined with two levels of phytase (1,000 or 2,000 FTU phytase/kg diet). SPI as the main protein source was detected to contain 17.5 g native PA/kg. In order to meet the tested level of PA, synthetic Na-PA (Sigma Chemical Co) was supplemented at different levels to all diets excluding 0PA/P.

Microbial phytase used in this experiment (Ronozyme P, CT, DSM Nutritional Product Ltd, Basel, Switzerland) has activity of 5,000 FTU/g. One unit FTU is the amount of enzyme that liberates 1 µmol inorganic orthophosphate/min. at pH 5.5 and 37°C at a substrate concentration, sodium phytate ($C_6H_6Na_{12}O_{24}P_6.10H_2O$) at 5.1 mmol/L (Engelen *et al.*, 1994). According to the product specification, this phytase was produced by a genetically modified strain of the yeast, Hansenula polymorpha; has an elevated pH optimum; higher stability against proteolytic activity and heat treatments. Supplementation of PA and phytase was done with expense of α-cellulose. In order to assess only the effect of PA and or phytase, each diet was also supplemented with 10 g/kg of inorganic P to meet the requirement level of juvenile Japanese flounder.

Diets are prepared by mixing all dry ingredients excluding essential amino acid (EAA) mixture for about 30 minutes and put the oil source. EAA mixture was added after coating with CMC in order to minimize leaching during feeding time, then mix again until all ingredients homogenized. Distilled water was used to moist and pelleted into about 1 mm granules. After pelleting, the feeds were dried in oven at 40°C until the moisture around 10%-11%.

Growth Experiment

The experiment was designed to a completely randomized design with triplicates. Five hundred juvenile Japanese flounder were obtained from Matsumoto Suisan Co., Miyazaki, Japan. After one week adaptation, fish was selected and allocated into 18, 100 L circular tank in a seawater flow-through system (1.5 L/min.) with density of 15 fish/tank. Each tank

Table 1. Formulation of experimental diet (g/100 g)

Ingredients	Phytic acid (PA) level / Phytase (P) level					
	0 PA/ 0P	10 PA/ 0P	10 PA/ 1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P
Brown fishmeal	12	12	12	12	12	12
Krill meal	8	8	8	8	8	8
Soybean protein isolate[1]	35	35	35	35	35	35
EAA mix[2]	2.5	2.5	2.5	2.5	2.5	2.5
Dextrin-hydrate	4	4	4	4	4	4
α-Starch	4	4	4	4	4	4
Pollack liver oil	10	10	10	10	10	10
HUFA[3]	1	1	1	1	1	1
Activated gluten	5	5	5	5	5	5
Vitamin mix[4]	3	3	3	3	3	3
AMP[5]	0.1	0.1	0.1	0.1	0.1	0.1
P-free mineral mix[6]	3	3	3	3	3	3
Sodium monophosphate[7]	4	4	4	4	4	4
Phytic acid[8]	0	1	1	1	2	2
Phytase[9]	0	0	0.02	0.04	0.02	0.04
Attractant[10]	0.5	0.5	0.5	0.5	0.5	0.5
α-Cellulose	2.9	1.9	1.88	1.86	0.88	0.86
Total	**100**	**100**	**100**	**100**	**100**	**100**

Notes:

1. Soybean protein isolated obtained from Fuji Pro Company, Tokyo, Japan
2. Amino acid mixture (g/kg): Arginine 10; Lysine 10; Methionine 5
3. Poweash A, Oriental Yeast Co, Ltd, Tokyo, Japan
4. Vitamin mixture (g/kg diet): ρ-aminobenzoic acid 0.67; biotin 0.01; inositol 6.68; nicotinic acid 1.30; Ca-pantothenate 0.47; pyridoxine-HCl 0.08; riboflavin 0.33; thiamin-HCl 0.10; menadione 0.08; vitamin A-palmitate 0.32; ρ-tocopherol 0.67; cyanocobalamin 0.46; calciferol 0.02; ascorbyl-2-phosphate-Mg 0.12; folic acid 0.03 and choline chloride 13.65
5. Ascorbic acid monophosphate-Mg, Wako Pure Chemical Industries, Ltd, Japan
6. Phosphorus free mineral mixture (g/kg diet): KCl 1.392; MgSO4.5H2O 3.8; Fe Citrate 0.82; Ca lactate 9.07; Al(OH)3 0.0052; ZnSO4.7H2O 0.099; CuSO4 0.0028; MnSO4.5H2O 0.022; K(IO3)2 0.0045; CoSO4.7H2O 0.028; Cellulose 14.75
7. Wako Pure Chemical Industries, Ltd
8. Sodium phytate, P8810, Sigma-Aldrich, St. Louis, MO, USA (72.5% purity)
9. Ronozyme P, CT, DSM Nutritional Product Ltd, Basel, Switzerland
10. Attractant (g/kg diet): betaine 2; taurine 3

was supplied with aeration. Fish were fed twice a day to apparent satiation for 50 days at 8.00 am and 16.00 pm. Average initial weight of the tested fish was 0.57 g. Fish were counted and weighed individually every 10 days. The performance data was evaluated based on weight gain, FCR, nutrient deposition in percent of nutrient intake including protein and phosphorus, nutrient digestibility coefficient, and phosphorus content in vertebrae.

Table 2. Chemical composition of experimental diets (g/100 g diet)

Ingredients	Phytic acid (PA) level / Phytase (P) level					
	0 PA/0 P	10 PA/0 P	10 PA /1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P
Moisture	8.07	8.42	8.6	7.49	8.04	8.06
Crude protein	52.15	52.03	52.12	52.2	52.31	52.26
Total lipid	11.64	11.68	11.41	11.72	11.53	11.61
Ash	8.05	8.73	8.72	8.76	9.4	9.42
Energy (kcal/g)*	4.85	4.81	4.8	4.85	4.8	4.8
Phytic acid/IP6	0.59	1.35	1.3	1.38	2.06	2.04
Phytase (FTU/kg)	226.3	248.7	1,105	2,100	1,185	2,460
Phosphorus	1.57	1.82	1.87	1.87	1.99	1.91

Note:

* Energy was calculated by multiplying the conversion value for protein, lipid, and carbohydrate of 5.65, 9.45, and 4 kcal/g, respectively (Halver & Hardy, 2002)

Sample Collection

Twenty fish representing the mean body weight at the beginning of the experiment and five fish from each tank at the end of experiment were taken for chemical analysis. Fish were freeze-dried, then kept in freezer at -20°C until analyzed. Analysis of whole body included proximates analysis, total lipid, and in-organic phosphorus.

Vertebrae were prepared by cooking with microwave for 3 minutes, vertebrae was dissected, cleaned of connective tissues, and finally washed with distilled water. All vertebrae were also pooled by tanks. The vertebral samples were defatted by chloroform-methanol (2:1) extraction according to Folch *et al.* (1957). Defatted vertebrae were dried and pulverized with mortar and pestle, and stored at -20°C for total phosphorus analysis.

Digestibility Experiment

After termination of the feeding trial, following trial on digestibility was done by applying similar diet to those feeding trial. Chromium oxide was supplemented as inert marker at level of 0.5%. Experimental condition was also similar to that of feeding trial, however, three replications of each treatment was pooled and divided randomly into duplicates of 10 fish/tanks. Fish were adapted to the tested diet for 5 days before starting collecting the feces. Fish was fed twice a day at satiation in the morning at 8.00 am and afternoon at 16.00 pm. Uneaten diet was siphoned out from tank 30-minutes after feeding. Feces were collected 3 times a day in the morning and afternoon just before feeding and at 10.00 pm at night. Feces was collected by siphoning and filtered with plankton net mesh 300 µm and rinsed with distilled water. Feces was freeze-dried and kept in vial then put in freezer at -20°C until analyzed. Feces were analyzed for protein, lipid, phosphorus, and chromium oxide.

Apparent digestibility coefficient (ADC) is calculated according to: ADC (%) = 100 - {100 (% Cr in diet/% Cr in feces) X (% N in feces/% N in diet)} Where: Cr is chromium oxide (Cr_2O_3) and N is nutrient

Chemical Analysis

Proximate analysis of diet, whole body, and feces were analyzed by using oven at 110°C for 12-h. or until constant weight (AOAC, 1995). Total lipid was determined according to Bligh & Dyer (1959) method. Concentration of phosphorus within diet, whole body, bone, and feces was determined spectrophotometrically (Lowry & Lopez, 1946). Chromium was analyzed by spectrophotometer after acid digestion based on Furukawa & Tsukahara (1966). Determination of phytic acid content of soy protein isolated, diet and feces was carried out spectrophotometrically based on Haug & Lantzsch (1983). Phytase activity in diet was analysed based on method of Engelen *et al.* (1994) combined with method of Eeckhout & De Paepe (1994).

Statistical Analysis

Data were analyzed as one-way ANOVA ($P<0.05$) by using Supernova. Duncan multiple range was used to identify significant different means.

RESULTS AND DISCUSSION

Growth Performances

Data on weight gain, specific growth rate, and survival rate are presented in Table 3. Growth was linear for all groups over the period of trial as shown in Figure 1. Phytase supplementation of diets enhanced flounder growth during the trial as shown by both final weight and weight gain. Within 50 days culture, fish fed 10PA/2,000P had six times increment from their initial weight, while rest of the groups had a weight more than 5 times. Fish fed diet containing 10PA/2,000P had the fastest growth (3.91%/d) among group, however it was not significantly different with fish fed 10PA/1,000P. Fish fed control diet grew a bit faster (3.72%/d) than fish fed diet with 10PA/0P (3.69%/d) but there was no significant difference between these two groups.

There were no significant differences in feed intake, FCR and SR. The total feed intake of fish was between 3.62-3.82 g/individual and FCR was between 0.94-1.02 (Tabel 4). There were only 2 mortalities occurred over 50 days in control and group without phytase supplement, respectively.

Whole Body Composition and Nutrient Retention

Generally fish fed IP6 supplemented diet without phytase had significant lower dry

Table 3. Weight gain, specific growth rate, and survival rate of Japanese flounder, *P. olivaceaus* fed experimental diets

Parameter	Phytic acid (PA) level / Phytase (P) level						Pooled S.E.M
	0 PA/0 P	10 PA/0 P	10 PA/ 1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P	
Initial weight (g)	0.57	0.58	0.57	0.56	0.56	0.55	
Final weight (g)	3.75	3.76	3.96	3.97	3.6	3.71	
Weight gain (%)	541.6^{a}	532.8^{a}	588.3^{bc}	607.3^{c}	542.1^{a}	573.1^{b}	8.78
SGR (%/d)	3.72^{a}	3.69^{a}	3.86b^{c}	3.91^{c}	3.72^{a}	3.81^{b}	0.03
Survival rate (%)	98^{a}	98^{a}	100^{a}	100^{a}	100^{a}	100^{a}	0.74

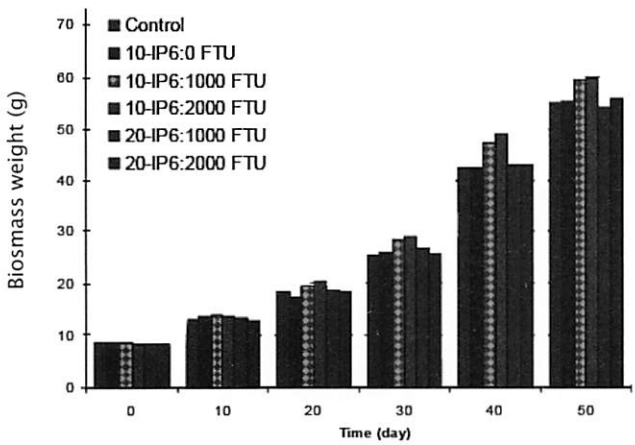

Figure 1. The pattern of growth of juvenile Japanese flounder, *P. olivaceus* within 50 days fed experimental diets

Table 4. Feed intake, FCR, nitrogen, and phosphorus retention in Japanese flounder fed tested diets

Parameter	Phytic acid (PA) level / Phytase (P) level						Pooled S.E.M
	0 PA/ 0 P	10 PA/ 0 P	10 PA/ 1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P	
Feed intake (g/ind.)	3.07[a]	3.11[a]	3.17[a]	3.21[a]	3.10[a]	3.10[a]	0.04
FCR (g/g)	0.97[a]	0.98[a]	0.94[a]	0.94[a]	1.02[a]	0.98[a]	0.02
Nitrogen retention (%)	42.21[bc]	38.82[a]	42.22[bc]	44.93[c]	38.87[a]	40.39[ab]	0.65
Phosphorus retention (%)	31.89[b]	27.76[a]	35.01[c]	38.69[d]	32.23[b]	34.25[bc]	0.66

matter, crude protein, lipid, and ash content in whole body compare to others, but the ranges among groups were small (Table 5). Whole body P was significantly higher in fish fed phytase supplemented diet whereas control and group consumed diet with 20 g PA/ kg combined with phytase had lower body nutrients compare to groups with 10 g PA combined with phytase. There was an increase body protein in fish fed phytase, but the differences among treatments were small. Fish fed 10 PA/2,000P had the highest ash and P as well as lipid body.

There were significant differences on P retention among treatments (Table 4). Fish obtained lower PA combined with phytase had higher P retention compare to other groups. However, P retention of control groups was lower than groups fed higher PA with phytase. Retention of nitrogen in fish fed 10 g PA/kg with phytase supplement was significantly different with fish fed supplemented PA without phytase, but it did not differ from control. Nitrogen retention of fish obtained higher PA with phytase were lower than fish fed lower PA with phytase, even lower than control.

Nutrient Digestibility and Vertebral P Content

Supplementation of phytase enhanced the digestibility of protein, lipid, and particularly P as shown in Table 6. Digestibility of dry matter was similar among groups. Digestibility of protein was slightly higher in fish fed lower PA with phytase than other treatments. Lipid digestibility was similar among groups when fed dietary phytase, while fish fed with supplemented 10PA/0P had lower lipid digestibility compared to other groups. Digestibility of P significantly increased by dietary phytase, even though fish fed higher PA (20 g/kg) with lower phytase level (1,000 FTU/kg) had lower P digestibility compared to control diet.

Concentration of P in vertebrae increased significantly by dietary phytase (Figure 2).

Table 5. Chemical composition of whole body of Japanese flounder, *P. olivaceus* fed experimental diets (% dry matter)

Nutrients	Initial	Phytic acid (PA) level / Phytase (P) level						Pooled S.E.M
		0 PA/ 0 P	10 PA/ 0 P	10 PA/ 1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P	
Dry matter	28.36	28.52[b]	27.89[ab]	27.12[a]	28.09[ab]	27.24[ab]	28.03[ab]	0.36
Crude protein	61.63	65.09[b]	63.08[a]	69.42[c]	69.19[c]	67.13[b]	65.42[b]	0.53
Total lipid	8.98	14.05[b]	13.41[a]	14.99[c]	15.87[d]	13.07[a]	13.65[ab]	0.23
Ash	Nd*	15.83[ab]	15.53[a]	16.19[b]	16.28[b]	16.07[ab]	16.16[b]	0.12
Phosphorus	2.18	1.59[a]	1.66[a]	2.12[bc]	2.17[c]	2.07[b]	2.07[b]	0.03

Notes:

* Nd = not determined due to insufficient sample for ash analysis

Table 6. Apparent nutrient digestibility coefficients of juvenile Japanese flounder fed experimental diets

Nutrient digestibility (%)	Phytic acid (PA) level / Phytase (P) level						Pooled S.E.M
	0 PA/ 0 P	10 PA/ 0 P	10 PA/ 1,000P	10 PA/ 2,000P	20 PA/ 1,000P	20 PA/ 2,000P	
Dry matter	78.97^{a}	78.42^{a}	80.04^{a}	79.91^{a}	78.70^{a}	79.09^{a}	0.88
Crude protein	90.89^{a}	90.45^{a}	93.44^{b}	93.74^{b}	90.76^{a}	91.42^{a}	0.48
Total lipid	84.86^{ab}	84.30^{a}	85.66^{b}	85.63^{b}	84.75^{ab}	84.90^{ab}	0.23
Total P	46.3^{a}	44.6^{a}	63.87^{c}	63.56^{c}	48.78^{a}	54.48^{b}	1.08

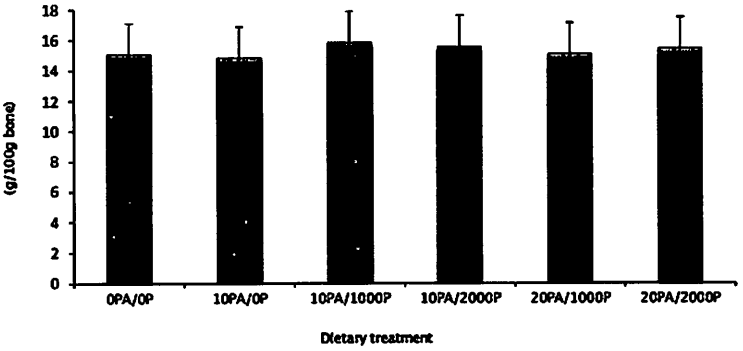

Figure 2. Vertebral P content of Japanese flounder fed experimental diets for 50 days

Fish fed with 10PA/P had the lowest content compared to control and other dietary phytase groups. Fish fed 20PA/2,000P had similar vertebral P content with groups fed 10PA/1,000P. On the other hand, vertebral P in group consumed 20PA/1,000P was lower than former groups but similar to 0PA/0P.

Incorporation of phytase into fish diet has been widely practiced due to its benefits on improvement of P availability and overall performances in particular growth (Storebakken *et al.*, 1998; Papatryphon *et al.*, 1999; Yan & Reigh, 2004; Liebert & Portz, 2005). In this study, the advantageous effects of phytase supplementation was also confirmed with significant enhancement of growth, P digestibility, P retention, body composition, and vertebral P content. SPI which was used 350 g/kg contributed at least 60% of crude protein in diet had no negative effect on survival rate and FCR was in normal ranges between 0.94–1.02. This indicated that juvenile Japanese flounder can accept higher level of plant protein. Fish fed four diets containing PA combined with phytase grew significantly faster than two groups fed un-supplemented phytase. This meant that phytase effectively hydrolyzed the PA contained in diet. Similar response on growth was also reported in rainbow trout (Forster *et al.*, 1999); Atlantic salmon (Sajjadi & Carter, 2004); *Pangasius pangasius* (Debnath *et al.*, 2005); rockfish (Yoo *et al.*, 2005). Moreover, supplementation of phytase at 1,000 and 2,000 FTU/kg in diet containing same rate of PA had similar response on growth which were higher than control. Similar study found that the optimum level of phytase supplement for Japanese flounder was 3,000 FTU/kg (Sarker *et al.*, 2006). The difference was probably due to different phytase sources used in these two experiments. Liebert & Porzt (2007) found that the efficacy of phytase in breaking down the P from inositol ring of PA molecule depended on the source of the phytase. Another study using plant based diets found that supplementation of 750-1,000 FTU phytase/kg significantly enhanced the growth in tilapia (Liebert & Porzt, 2005).

Lower growth in fish fed same levels of phytase (1,000 and 2,000 FTU/kg) combined with higher level of PA (20 g/kg) indicated that efficacy of phytase depended on PA concentration in diet. Study on rainbow trout fed graded levels of phytase showed that digestibility of dietary PA directly related to the dosage of dietary PA (Forster *et al.*, 1999). They observed that PA in canola protein concentrate diet without phytase supplementation was almost totally indigestible, whereas around 45% of PA in phytase supplemented 4,500 FTU/kg was degraded to several derivates.

Feed intake and FCR were found similar among groups, in agreement with Sajjadi & Carter (2004) and Debnath *et al.* (2005). However, several studies reported that dietary phytase stimulates feed intake in fish fed diets with suboptimal or requirement level of P. Phytase in plant protein based diet with a slightly deficient level of available P (5 g/kg) increased feed intake in Atlantic salmon (Hauler & Carter, 1997). Rainbow trout fed a diet containing phytase at a suboptimal P level had higher feed intake (Rodehutscord & Pfeffer, 1995). Increase of P availability due to the phytase was concluded as the reason for increased feed intake and growth. In this experiment, all groups were supplemented with the same rate of in-organic P to meet requirement level of juvenile Japanese flounder. Therefore, there was no difference in feed intake because of phytase.

It is not surprising that digestibility of P significantly increased by dietary phytase. Increasing levels of phytase from 1,000 to 2,000 FTU in diet supplemented with 10 g PA/kg did not show further increase on P digestibility. Similarly, P digestibility in nile tilapia increased when fed phytase up to 1,000 FTU/kg diet, and no further improvement by higher supplementation (Portz & Liebert, 2003). Increased in P absorption was positively correlated to P concentration in whole body and further, increased retention of P in body. The low availability of P bound to PA was also observed in salmonids (Teskeredzic *et al.*, 1995) including Atlantic salmon (Storebakken *et al.*, 1998). In nile tilapia, P digestibility improved significantly when fish fed phytase up to 1,000 FTU/kg. Digestibility of protein slightly increased in phytase supplemented group which related to protein level in whole body. Contrast response was obtained by Sarker *et al.* (2006) who found that protein retention in Japanese flounder improved by dietary phytase at level of 3,000 FTU. This experiment did not determine whether the discrepancy between these two studies was due to the phytase sources or the lower level of phytase activity applied in this study was not enough to enhance the protein availability. Factor such as differences in protein sources may also contributed, even though Papatryphon & Soares (2001) showed that protein digestibility of some plant ingredients in stripped bass was not affected by phytase. More contrast result was observed by Teskeredzic *et al.* (1995) who found reduced protein retention by treating canola protein concentrate with phytase.

Digestibility of dry matter was found similarly among groups which were similar to that of reported in Nile tilapia (Portz & Liebert, 2003), but different with what obtained in *Pangasius pangasius* (Debnath *et al.*, 2005). They found that dry matter digestibility increased by at least 500 FTU phytase/kg diet.

Lower lipid content in body of fish fed containing PA without phytase including control (contained 5.9 g/kg native PA) was in accordance with Usmani & Jafri (2002). They found lower lipid content in fish fed more than 0.5% PA. Similar trend was also reported on Chinook salmon (Richardson *et al.*, 1985) and Atlantic salmon (Sajjadi & Carter, 2004). Furthermore, a study found that dietary phytase influenced the lipid profile but not the growth in tiger shrimp, *Penaeus monodon* (Biswas *et al.*, 2007). However, Forster *et al.* (1999) stated that dietary phytase even at high level of 4,500 FTU/kg did not affect the composition of body nutrients in rainbow trout.

Availability of P was also indicated by increasing level of P in vertebrae observed in this study. High concentration of vertebral P indicated that dietary P in all diets was met the requirement for bone mineralization. Even though the range of vertebral P content was high (around 15%), groups fed 10 g PA with supplemented phytase had higher vertebral P compare to other groups. This indicated that phytase is able to enhance P concentration in vertebrae as reported by several other studies (Vielma *et al.*, 2002; Debnath *et al.*, 2005). Similar study in juvenile Japanese flounder found that supplementation of phytase at 3,000 FTU/kg along with 2 g inorganic-P significantly increased vertebral P, Ca, and Zn content (Sarker *et al.*, 2006).

In conclusion, supplementation of phytase enhances growth and availability of nutrient in juvenile Japanese flounder. Supplementation of 2,000 FTU phytase/kg diet gave better growth performances and nutrient utilization of juvenile Japanese flounder when fed diet containing 10 g PA/kg diet.

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