INTERACTION BETWEEN DIETARY MINERAL AND PHYTASE ON BIOLOGICAL PERFORMANCES OF JAPANESE FLOUNDER, *Paralichthys olivaceus.* PART I. GROWTH, FEED INTAKE, AND WHOLE BODY MINERAL CONTENT

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(Received February 19th, 2013; Accepted May 28th, 2013)

ABSTRACT

In order to determine the effect of dietary calcium (Ca), inorganic phosphorus (IP), and phytase (P) supplementation in marine fish, a 2 x 2 x 2 factorial design was arranged to observe the interrelationship between dietary Ca and IP with the presence of dietary phytase. Two levels of dietary Ca at 0% and 0.2% combined with either 0% or 0.25% of dietary IP and either with 0 and 2,000 FTU phytase/kg diet, respectively to formulate eight experimental diets. SPI-based diet was used as basal diet and the sources of Ca, IP, and phytase were similar to those used in the previous experiment. Juvenile Japanese flounder with initial body weight around 0.6 g was fed the test diets. After 30 days of feeding trial, the results showed that both dietary IP and phytase, but not dietary Ca significantly enhanced the growth and feed intake. The highest growth was achieved in fish fed a diet containing the Ca, IP, and phytase supplement among groups. Fish fed diet without the three dietary supplements had the lowest SGR and did not significantly improve by supplementing dietary Ca. Feed intake (FI) and was significantly influenced by dietary IP and phytase, not dietary Ca while feed conversion ratio (FCR) was significantly affected by all dietary treatments. Interaction effect was detected between dietary Ca and IP, between dietary IP and P on FCR. Total phosphorus content in whole body was significantly increased by supplementing all dietary treatments which was highest in fish fed 0.25 IP/0.2 Ca/P. Significant interaction was observed between dietary IP and P on this parameter. Whole body Ca content was significantly improved by either dietary IP or Ca and not dietary P. As conclusion even without inorganic Ca supplement, dietary IP at level of 0.25% or 2,000 FTU phytase/kg diet could enhance growth and FI of fish as well as whole body phosphorus content of juvenile Japanese flounder when diet basal contained organic Ca around 1.2%.

KEYWORDS: mineral, phytase, whole body mineral, Japanese flounder

INTRODUCTION

Ca and P are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes (NRC, 1993). In our previous study, it has been discussed the importance of dietary P and the benefit of phytase supplementation in SPI based diet for red sea bream. Maximum utilization of dietary P originated from phytic acid (IP6) with addition of exogenous phytase could help in reducing the amount of dietary IP supplementation needed which in case of red sea bream reduced from 0.5% to 0.25% when phytase was supplemented at 2,000 FTU phytase/kg diet (Laining *et al.*, 2012).

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Eventhough 0.2% Ca was supplemented in basal diet, P deficiency sign of scoliosis observed in group fed diet without both dietary IP and phytase supplement indicated that P requirement particularly for skeletogenesis in red sea bream was not influenced by the presence of Ca in the diet. Other study found that post-most Atlantic salmon fed low P diets showed significant reduction in Ca, P, and Mg levels of whole body, bone, skin, and scale, and gradually developed abnormally soft and malformed bones (Baeverfjord *et al.*, 1998).

In constrast to P requirement, Ca requirement of most fish is affected by dietary P level, species different (Lall, 2002) and water chemistry (Shiau & Tseng, 2007). Even though, many studies reported that fish met the Ca requirement in large part by absorb this ions directly from aquatic environment, the results are still contradictory. In the study dealing with tiger puffer, inclusion of 5g IP kg^{-1} diet was sufficient to maintain the normal fish growth, even when Ca was not supplemented in diet (Laining *et al.*, 2011). However, high inclusion level of Ca at 12 g/kg diet caused the poorest growth performances and mineral utilization supporting other studies which reported that dietary Ca may interact with other essential minerals such as P, Mg, and Zn (Nakamura, 1982; Gatlin & Phillips, 1989; Vielma & Lall, 1998).

In addition, effect of phytase in increasing the vertebral P content was obvious when diet was not supplemented with Ca. Based on previous two experiments determining the interactive effect between dietary P or Ca and phytase, it seemed that supplementation of phytase was more effective when diet contained low dietary levels of Ca and P as well as Ca:P ratio (Laining *et al.*, 2011; 2012).

In order to clarify the interrelationship between Ca and P particularly with the presence of phytase in diet, this experiment was arranged to investigate the effect of dietary Ca, IP, and phytase supplementation on growth, feed intake, and whole body mineral content of juvenile Japanese flounder and interactive effects were also examined.

MATERIALS AND METHODS

Test Diet

This experiment was arranged according to factorial design 2 x 2 x 2 with triplicates. Composition of the basal diet was formulated to contain equivalent protein and lipid content as the previous study. Eight diets were formulated to contain two levels of dietary Ca at 0% and 0.2% and P at 0% and 0.25% and phytase supplementation at 0 and 2,000 FTU/kg diet. Formulation of the test diets were presented in Table 1. Diet 1 and 2 were supplemented without both Ca and P combined either without or with phytase supplementation, respectively; diet 3 and 4 were supplemented with 0.25% P without Ca combined either without or with phytase supplementation, respectively; diet 5 and 6 were added with 0.2% Ca without P combined either without and with phytase supplementation; diet 7 and 8 were included with 0.2% Ca and 0.25% P combined either without and with phytase supplementation. As previously, commercial phytase isolated from Peniophora lycii was used as phytase source. Sodium mono-phosphate (NaH_2PO_4) and calcium carbonate ($CaCO_3$) were used as IP and Ca sources, respectively. Test diets were prepared according to the previous procedures. All diets were stored at 5°C during the feeding trial. Analyzed chemical composition of the test diet and mineral content of main ingredients used in this experiment are presented in Table 2 and Table 3, respectively.

Fish and Feeding Trial Condition

Five hundred juvenile Japanese flounder were obtained from a domestic hatchery (Matsumoto Suisan Co., Miyazaki, Japan). After one week acclimatization and maintainance using commercial diet (Higashimaru, Kagoshima, Japan), fish with average initial body weight of 0.61 g were randomly distributed into 24 tanks of 100 L capacity at a density of 20 fish/tank. Each tank was supplied with filtered seawater with flow through system at a flow rate of 1.2 L/minutes. Fish were fed the diet twice a day to nearly satiation (8.00 and 16.00) for 50 days. Sampling of fish weight was carried out in every 10 days. Water temperature during feeding trial ranged from 19°C to 21°C.

Table 1. Formulation of experimental diets contained different level of dietary Ca, IP, and phytase supplementation

Ingredients (%)	Experimental diets (IP= inorganic phosphorus; Ca= Calcium; NP= non-phytase; P= Phytase)							
	0 IP/ 0 Ca/ NP	0 IP/ 0 Ca/ P	0.25 IP/ 0 Ca/ NP	0.25 IP/ 0 Ca/ P	0 IP/ 0.2 Ca/ NP	0 IP/ 0.2 Ca/ P	0.25 IP/ 0.2 Ca/ NP	0.25 IP/ 0.2 Ca/ P
Brown fishmeal	18	18	18	18	18	18	18	18
Krill meal	8	8	8	8	8	8	8	8
Soybean protein concentrated[1]	30	30	30	30	30	30	30	30
Dextrin-hydrate	10	10	10	10	10	10	10	10
α-Starch	5	5	5	5	5	5	5	5
Pollack liver oil	5	5	5	5	5	5	5	5
HUFA[2]	1	1	1	1	1	1	1	1
Activated gluten	6	6	6	6	6	6	6	6
Vitamin mix[3]	3	3	3	3	3	3	3	3
Stay-C[4]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ca-P-free mineral mix[5]	4	4	4	4	4	4	4	4
Sodium monophos-phate[6]	0	0	1.1	1.1	0	0	0	0
Calcium carbonate[7]	0	0	0	0	0.5	0.5	0.5	0.5
Phytase[8]	0	0.04	0	0.04	0	0.04	0	0.04
Attractant[9]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
α-Cellulose	9.7	9.66	8.6	8.56	9.2	9.16	8.1	8.06
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

1. Soybean protein isolated obtained from Fuji Pro Company, Tokyo, Japan
2. Poweash A, Oriental Yeast Co, Ltd, Tokyo, Japan
3. Vitamin mixture (g/kg diet): β-carotene 0.192; vitamin D3 0.019; menadione 0.0917; α-tocopherol acetate 0.77; thiamin nitrate 0.115; riboflavin 0.385; pyridoxine-HCl 0.092; cyanocobalamin 0.00018; d-biotin 0.0115; inositol 7.698; nicotinic acid 1.539; Ca-pantothenate 0.5391; folic acid 0.0288; choline chloride 15.738; ρ- aminobenzoic acid 0.7665; cellulose 2.849
4. Ascorbic acid monophosphate-Mg
5. Calcium-phosphorus free mineral mixture (g/kg diet): KCl 1.856; MgSO4.5H2O 5.067; Fe Citrate 1.098; Al(OH)3 0.0069; ZnSO4.7H2O 0.132; CuSO4 0.0037; MnSO4.5H2O 0.029; K(IO3)2 0.006; CoSO4.7H2O 0.037; Cellulose 31.75
6. Wako Pure Chemical Industries, Ltd, Japan
7. Wako Pure Chemical Industries, Ltd, Japan
8. Ronozyme P5000, DSM Nutritional Product Ltd, Basel, Switzerland (0.04% = 2,000 FTU/kg diet).
9. Attractant (g/kg diet): betaine 2.0

Table 2. Chemical composition of experimental diets contained different level of dietary Ca, IP, and phytase supplementation

Nutrients (%)	Experimental diets (IP = inorganic phosphorus; Ca= Calcium; NP= non-phytase; P= Phytase)							
	0 IP/ 0 Ca/ NP	0 IP/ 0 Ca/ P	0.25 IP/ 0 Ca/ NP	0.25 IP/ 0 Ca/ P	0 IP/ 0.2 Ca/ NP	0 IP/ 0.2 Ca/P	0.25 IP/ 0.2 Ca/ NP	0.25 IP/ 0.2 Ca/ P
Crude protein:								
Total lipid	11.5	11.7	11.5	11.2	11.4	11.1	11	11.3
Ash	6.9	6.9	7.6	7.3	7.4	7.2	8.1	7.9
Phytic acid/ IP6	0.55	0.52	0.53		0.53	0.58	0.58	0.59
Phytase (FTU/kg)	248.4	1,961.7	197.5	2,093.1	214.7	1,918.5	362.1	2,031.9
Minerals content:								
Total P	0.86	0.84	1.08	1.13	0.88	0.89	1.13	1.15
Ca	1.19	1.19	1.21	1.2	1.37	1.39	1.37	1.39
Mg	0.15	0.18	0.18	0.17	0.17	0.16	0.16	0.17
Zn (mg/g)	0.08	0.09	0.09	0.08	0.08	0.08	0.08	0.07
Ca:P ratio	1.38	1.42	1.12	1.06	1.56	1.56	1.21	1.20

Table 3. Analysed mineral content of fishmeal, SPI, and krill meal used in the experimental diet contained different levels of dietary Ca, IP, and phytase supplements

Nutrients (%)	Ingredients		
	Fishmeal	SPI	Krill meal
Total P	2.64	0.54	Not analized
Ca	5.55	0.07	1.56
Mg	0.34	0.06	0.49
Zn (mg/g)	0.1	0.04	0.06

Whole Body Sampling

At the beginning of the feeding trial, 15 fish were randomly taken, freeze-dried, and ground using blender for whole body sample. Samples of five fish from each tank representing the mean body weight were taken and prepared for analysis. All samples were stored at -20°C until analysis.

Chemical Analysis

Proximate analysis including moisture, crude protein, lipid, and ash for experimental diet and whole body were carried out based on AOAC (1995) procedures. Total lipid was analyzed based on Bligh & Dyer (1959). Measurement of pH in stomach and intestine of fish was determined by making slurry of the gut content with distilled water and using a pH electrode. Analysis of total P for diet, whole body, feces, and vertebrae were carried out according to Lowry & Lopez (1946). Phytic acid content of test diet was detrmined spectro-photometrocally based on Haugh & Lantzch (1959). In-feed analysis of phytase activity was carried out due to the procedure of Engelen *et al.* (1994). Mineral content of ingredients, diets, and whole body was analyzed using AAS (Hitachi Z-2300, Tokyo, Japan) after acid digestion. LaCl3 (Wako Pure Chemical Industries, Ltd) solution was at the rate of 10% to the sample and standard solution before injection to prevent any chelating effect of other elements.

Calculation and Statistical Analysis

Biological data were evaluated based on the following parameters:

1. Weight gain (%) = (final body weight - initial body weight)/Initial body weight x 100
2. SGR (Spesific growth rate,%/day) = (LnWf - LnWi)/t x 100
3. Feed conversion ratio (FCR g/g) = (feed intake/weight gain)
4. Data were subjected to three-way ANOVA ($P<0.05$) to identify significant differences among treatments.

RESULTS AND DISCUSSION

Results Growth, Feed Utilization, and Survival Rate

Growth performances of juvenile Japanese flounder are showed in Table 4. Dietary IP and phytase levels, but not dietary Ca level significantly enhanced FBW, WG, SGR, FI, and SR, whereas FCR was significantly improved by the three dietary treatments. Even though supplementation of dietary Ca increased from 0% to 0.2%, fish growth was not significantly enhanced when dietary IP and phytase were not included in diet (SGR was 3.18% and 3.36%/day, respectively). In contrast, increasing of dietary IP from 0% to 0.25% significantly improved SGR even if dietary Ca and phytase were not supplemented (3.18% vs 3.62%/day). However, supplementation of 2,000 FTU phytase/kg diet at two levels of IP significantly enhanced the SGR, regardless of dietary Ca (3.88% vs 3.97%/day). The highest SGR among groups was achieved when dietary Ca, IP, and phytase were supplemented together.

Three way ANOVA demonstrated that neither interaction between two dietary treatments nor second-order interaction between three dietary treatments (or interaction between Ca, IP, and phytase) were observed on growth, feed utilization, and survival rate (Table 5 and Figure 1)

Trend of FI in all groups strongly related to growth that dietary Ca did not significantly enhance FI, but dietary IP, and phytase did. However, significant effect of the three dietary treatments was detected on FCR. Moreover, significant interaction was observed between Ca and IP supplement as well as interaction between dietary IP and phytase, but no second-order interaction was detected (Table 5 and Figure 2). FCR of fish fed diet contained dietary Ca, IP, and phytase was the lowest compared to other groups.

Table 4. Growth, survival rate, and feed conversion ratio of juvenile Japanese flounder fed diet contained different level of Ca, IP, and dietary phytase supplements

	Experimental diets (IP= inorganic phosphorus; Ca= Calcium; NP= non-phytase; P= Phtase)							
	0 IP/ 0 Ca/ NP	0 IP/ 0 Ca/ P	0.25 IP/ 0 Ca/ NP	0.25 IP/ 0 Ca/ P	0 IP/ 0.2 Ca/ NP	0 IP/ 0.2 Ca/ P	0.25 IP/ 0.2 Ca/ NP	0.25 IP/ 0.2 Ca/ P
IBW (g/fish)	0.61	0.62	0.61	0.62	0.61	0.61	0.61	0.62
FBW (g/fish)	1.6	1.96	1.82	2.04	1.68	1.9	2.01	2.25
Weight gain (%)2	160.3	220	198.2	230.2	174.4	209.1	230.1	264.3
SGR (% day-1)3	3.19	3.88	3.62	3.97	3.36	3.77	3.97	4.31
Survival rate (%)	67.5	88.3	82.5	91.7	75	91.7	86.7	90
Feed intake (g fish-1)	1.36	1.6	1.58	1.64	1.52	1.56	1.53	1.64
Feed conversion ratio (g g-1)	1.32	1.19	1.16	1.16	1.34	1.21	1.09	1.01

Table 5. *Three-way ANOVA* for growth performances and feed utilization of juvenile Japanese flounder fed diet contained different level of Ca, P, and dietary phytase supplementation (P< 0.05)

Independent factor	IP	Ca	Phytase	CaxIP	IPxPhytase	CaxPhytase	IPxCa
Final weight (g)	S	NS	S	NS	NS	NS	NS
Weight gain (%)	S	NS	S	NS	NS	NS	NS
SGR (%/day)	S	NS	S	NS	NS	NS	NS
FI (g/fish)	S	NS	S	NS	NS	NS	NS
FCR (g/g)	S	S	S	S	S	NS	NS
Survival rate	S	NS	S	NS	NS	NS	NS

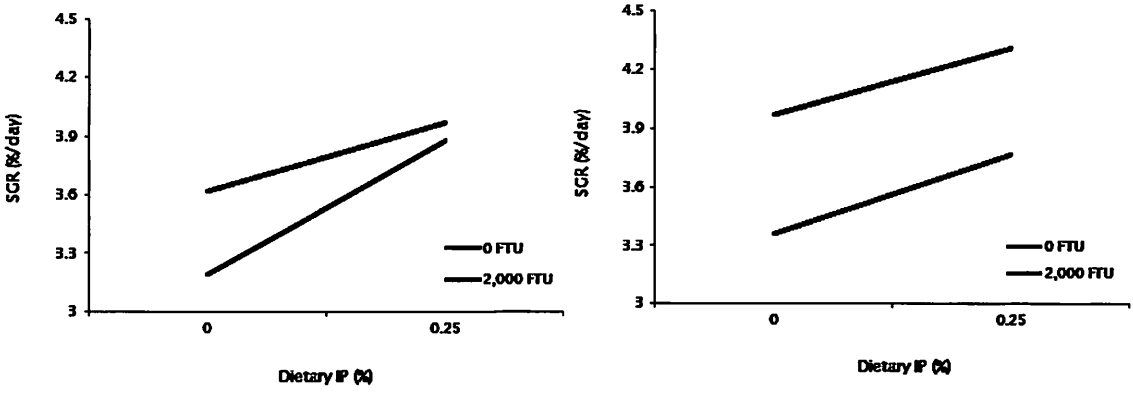

Figure 1. Mean plot of non second-order interaction (interaction between dietary IPxCax-phytase) on SGR of juvenile Japanese flounder at two levels of phytase. Each graph illustrated the non interaction effect between dietary IP and Ca on SGR (data from Tabel 5)

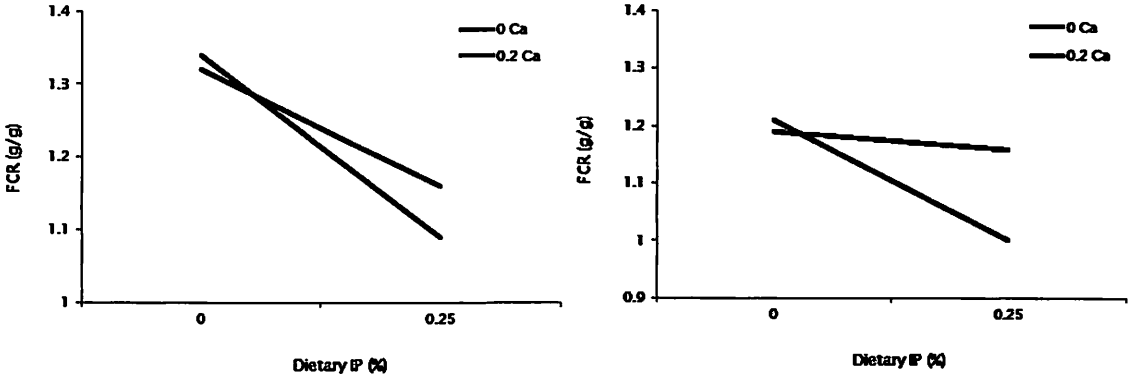

Figure 2. Mean plot of non second-order interaction (interaction between dietary IPxCax-phytase) on FCR of juvenile Japanese flounder at two levels of phytase. Each graph illustrated the significant interaction effect between dietary IP and Ca on FCR (data from Tabel 5)

pH of Digestive Tract

pH of stomach and intestines of fish measured at the end of digestibility is presented in Table 6. Stomach pH was in a range of 4.0 to 4.4 whereas pH in intestine ranged from 6.8 to 7.1.

Whole Body Mineral Contents

Total phosphorus content in whole body were significantly influenced by the three dietary treatments which the highest was found in fish fed dietary IP, Ca, and P at level of 0.25%, 0.2%, and 2,000 FTU/kg diet, respectively. Interaction effect was only detected between dietary IP and phytase and no second-order interaction was analyzed as shown in Tabel 7 and Figure 3. Whole body calcium content was not affected by dietary phytase, but dietary IP and Ca. Whole body Ca content increased when diet contained either dietary IP or Ca. No interaction effect was detected among the three dietary treatments meaning that each factor has independent effect on this parameter. In addition, Ca:P ratio was only significantly influenced by dietary IP.

DISCUSSION

Presence of either dietary supplemental IP or phytase significantly enhanced the fish growth. This supported the previous results found in tiger puffer that Japanese flounder seemed to have the capacity to uptake Ca

Table 6. pH of stomach and intestines of juvenile Japanese flounder 1-h post feeding with contained different level of Ca, IP, and dietary phytase supplementation

Treatments	pH of digestive tracts 1-h after feeding	
	Stomach	Intestines
0IP/0Ca/NP	4.06	7.08
0IP/0Ca/P	4.07	7.04
0.25IP/0Ca/NP	4.34	6.98
0.25IP/0Ca/P	4.25	6.95
0IP/0.2Ca/NP	4.35	7.06
0IP/0.2Ca/P	4.22	7.11
0.25IP/0.2Ca/NP	4.08	7.08
0.25IP/0.2Ca/P	4.16	6.8

Table 7. Mineral content in whole body of juvenile Japanese flounder (%)

	Experimental diets (IP = inorganic phosphorus; Ca= Calcium; NP= non-phytase; P= Phytase)							
	0 IP/ 0 Ca/ NP	0 IP/ 0 Ca/ P	0.25 IP/ 0 Ca/ NP	0.25 IP/ 0 Ca/ P	0 IP/ 0.2 Ca/ NP	0 IP/ 0.2 Ca/ P	0.25 IP/ 0.2 Ca/ NP	0.25 IP/ 0.2 Ca/ P
Whole body								
Total P	2.84	3.1	3.19	3.24	2.92	3.19	3.35	3.43
Ca	5.25	5.46	5.2	5.72	5.63	5.82	6.18	6.1
Ca:P ratio	1.9	1.76	1.72	1.77	1.92	1.83	1.85	1.73
Three-ways ANOVA	IP	Ca	Phytase	CaxIP	IPx-Phytase	Cax-Phytase	IPxCax-Phytase	
Whole body								
Total P	S	S	S	NS	S	NS	NS	
Ca	S	S	NS	NS	NS	NS	NS	
Ca:P ratio	S	NS	NS	NS	NS	NS	NS	

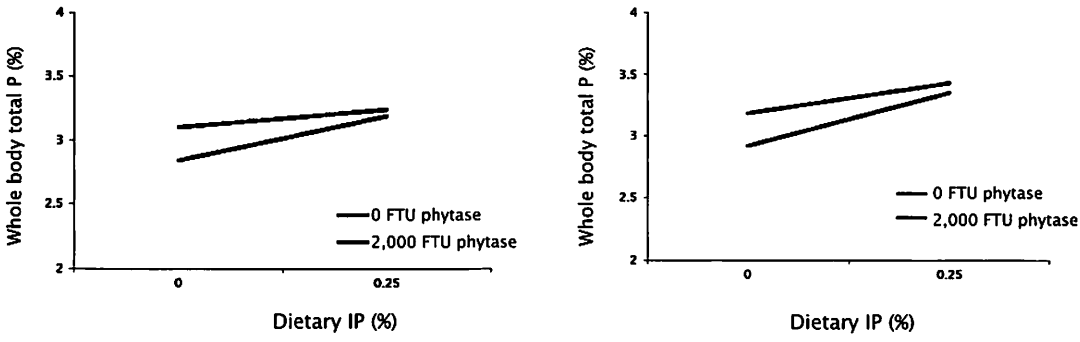

Figure 3. Mean plot of non second-order interaction (interaction between dietary IPxCax-phytase) on whole body total P content at two levels of dietary Ca. Each graph illustrated the independent effect between dietary IP and phytase on whole body total P content (data from Tabel 7)

from seawater to meet the requirement level for growth. Flik *et al.* (1995) reported that Ca is actively absorbed through the gill ephitellium in freshwater fish whereas in marine fish Ca is transported across the intestinal ephitelium. In contrast, Hossain & Furuichi (2000) reported that absorption of Ca from seawater was not adequate for the growth of juvenile Japanese flounder. The discrepancy between these studies, perhaps due to the difference in feed ingredients used in basal diets. In the previous study, casein based diet was used as the basal diet containing around 0.02% Ca, while in the present study, fishmeal, krilmeal, and SPI were used as the protein sources which contained around 5.6%, 1.6%, and 0.1% Ca, respectively contributing around 1.2% Ca in basal diet. Based on digestibility trial reported separately from this present growth study, Ca digestibility of basal diet was only 5.4% contributing around 0.06% Ca (Laining *et al.*, 2013) which was higher compared to 0.02% Ca in basal diet used in the previous study by Hossain & Furuichi (2000). It is likely that Ca from the organic sources was at least partially available and utilized by flounder along with Ca from waterborne. This was in accordance with the result found in the previous study that flounder fed 0.2% Ca from TCP, the main form of Ca-P in fishmeal, had significant higher growth compared to un-supplemented group. However, inclusion of high level of 2.5% TCP had negative effect on growth of flounder.

Even though Japanese flounder seemed able to meet the Ca requirement for growth by partially utilizing Ca from organic sources along with Ca uptake from water, significant lower WG in fish fed no dietary IP than fish fed 0.25% IP (160% vs 198%) revealed that flounder could not fully utilize TCP as dietary P source to fulfill the P requirement for maximum growth. Similar response was shown in black sea bream that dietary TCP did not significantly enhance the growth due to the low availability of TCP in intestine (Yone & Toshima, 1979). Moreover, the poorest growth of fish fed without dietary IP can not be improved even if dietary Ca was supplemented at 0.2% indicating that dietary IP played more important role on growth improvement than dietary Ca in Japanese flounder, since P uptake from seawater can not satisfy the requirement level for maximum growth. This was supported by trend of SR which was similar to growth response that dietary IP and P, not Ca significantly affected SR. Lowest SR found in group fed no dietary treatment indicated that dietary IP and P were important in early stage of Japanese flounder. Moreover, malformations were observed in opercula of fish fed non-IP supplement even with phospholipid supplementation (Uyan *et al.*, 2007).

Dietary phytase stimulated FI of the flounder indicated that negative effect of phytic acid on FI might be minimized by inclusion of phytase then stimulate the fish to fed more. This result supported several previous findings in other species such as Atlantic salmon (Denstadli *et al.*, 2006) and channel catfish (Jackson *et al.*, 1996). Indepth observation about relationship between dietary phytic acid and phytase effect on FI are required.

Study on tiger puffer showed that inclusion of 1.2% Ca as $CaCO_3$ had direct effect in reducing the palatability of fish due to the bitterness thus decreased the feed intake (Tordoff & Sandell, 2009; Laining *et al.*, 2010). However, in the present study, inclusion of lower Ca at 0.2% using similar source of $CaCO_3$ in juvenile Japanese flounder did not affect the feed intake and did not show any inhibitory effect on other mineral availability.

Studies on P requirement in salmonids showed that in many cases, bone or total body ash has been at least as sensitive as growth for evaluating P utilization (Watanabe *et al.*, 1980; Rodehutscord, 1996; Asgard & Shearer, 1997). In this present study, whole body ash was not analyzed due to the insufficient samples. However, increasing of whole body phosphorus and Ca content reflected to increased whole body ash content. Total phosphorus in whole body significantly improved by dietary Ca showed that there is a synergistic relationship in which one element enhances the role of another. This is difficult to explain and need more research to better define the complex nutritional and metabolic mechanism.

Besides synergetic relationship between mineral, interrelationships between minerals may also manifest themselves as competition for binding sites on transport or as storage molecules, substitution at an active site of an enzyme or as a requirement for one element for the proper metabolism of another. Antagonistic relationship between minerals has been observed in several species such as Atlantic salmon (Lall, 2002), rainbow trout (Apines *et al.*, 2003) and tiger puffer (Laining *et al.*, 2011).

In this present study, interactive effect among three dietary treatments of Ca, IP, and phytase or also called second-order interaction was not significantly detected on all parameters. According to Toutenburg (2002), second order interaction can be interpreted as indicating that interaction between Ca and IP differs depending on the level of phytase or interaction between Ca and phytase depending on the level of IP, and so on. Therefore interaction between dietary IP and P on whole body total phosphorus (for example) was not influenced by dietary phytase level since no second order interaction was found in this parameter.

In conclusion, even without inorganic Ca supplement, dietary IP at level of 0.25% or 2,000 FTU phytase could enhance growth and FI of fish as well as whole body phosphorus content of juvenile Japanese flounder when diet basal contained organic Ca around 1.2%.

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