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Effects of the Dietary Microbial Phytase Supplementation on Bioavailability of Phosphorus in Juvenile Olive Flounder *Paralichthys olivaceus* Fed Soybean Meal based Diets

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Abstract

An 8 weeks feeding trial was carried out to evaluate the effects of dietary microbial phytase (P) supplementation on nutrient digestibility, and body composition in juvenile olive flounder *Paralichthys olivaceus* fed soybean meal-based diets. Seven experimental diets were formulated to be isonitrogenous and isocaloric to contain 50.0% crude protein (CP) and 16.7 kJ of available energy/g with or without dietary phytase supplementation. White fish meal (FM) provided 92.4% of the total protein in the basal diet (S_0), in the other 6 diets, 30% or 40% FM protein was replaced by soybean meal: 70% FM + 30% soybean meal (S_{30}); 70% FM + 30% SM + 1000 U phytase/kg diet ($S_{30}P_{1000}$); 70% FM + 30% SM + 2000 U phytase/kg diet ($S_{30}P_{2000}$); 60% FM + 40% SM (S_{40}); 60% FM + 40% SM + 1000 U phytase/kg diet ($S_{40}P_{1000}$); and 60% FM + 40% SM + 2000 U phytase/kg diet ($S_{40}P_{2000}$). After two weeks of the conditioning period, triplicate groups of 25 fish initially averaging 6.15 ± 0.04 g (mean ± S.D.) were randomly distributed into the aquarium and were fed one of the experimental diets for 8 weeks. After feeding trial, supplementation of phytase significantly improved the apparent digestibility coefficients of phosphorus in flounder diets ($P<0.05$) containing 30% and 40% soybean meal regardless the levels. However, phytase had no significant influence on growth performance and whole body composition of fish. Based on the experimental results, we conclude that dietary supplementation of phytase could improve the apparent digestibility coefficient of phosphorus in olive flounder.

Key words: *Paralichthys olivaceus*, Phytase, Posphorus, Digestibility, Olive flounder

Introduction

Production of finfish culture increased gradually these years in Korea. Aquaculture of olive flounder *Paralichthys olivaceus* in Korea started in the 1970s and commercial production became extensive in the 1980s with development of seed production and rearing techniques. Olive flounder is currently the most important marine aquaculture species in Korea and its aquaculture production has been stable during last 10 years ranging from 40,000-50,000 metric tons (Ministry of Oceans and Fisheries, 2013). However, feed costs constitute a large part of the total expenses of an aquaculture facility, because of the use of the expensive fish meal with a large dietary proportion. Many studies have been conducted to seek less expensive protein source alternatives to fish meal, due to the expensive cost and availability issue of this ingredient (Lim et al., 2004; Jang et al., 2005; Barrows et al., 2007). Soybean meal is one of the plant sources that can be used as an alternative protein source. It has a high level of available protein with well-balanced amino acid profile, low price and wide availability

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(Choi et al 2004; Lim et al., 2011). One of the major problems limiting the use of soybean meal in fish feed is the presence of phytate. Phytate is the primary form of phosphorus (P) in soybean, account for approximately two-thirds of the total P bound as phytate P (Cheng and Hardy, 2002; Cao et al., 2008). Phytate is an indigestible P form, which is practically not available for fish because of lack of an intestinal phytase for efficient hydrolysis of phytate during digestion (NRC, 1993). Consequently, the available P in plant diets may not meet the requirements of fish, and inorganic P is usually supplemented to the diets. On the other hand, undigested phytate P excreted via faeces and contributes to environmental pollution. Excessive excretion of phosphorus in water can stimulate the growth of phytoplankton, thus reducing dissolved oxygen and causing water pollution. Many studies have demonstrated that addition of phytase to fish feeds can improve the utilization of phytate P and decrease the total P load to the environment for several species (Chong et al., 2003; Tantikitti et al., 2005; Kikuch and Furuta, 2009).

The present study investigated the effects of dietary microbial phytase supplementation on the nutrient digestibility, growth performance and body composition in the juvenile olive flounder fed soybean meal-based diets.

Materials and Methods

Experimental diets

Composition of the experimental diets is shown in Table 1. Seven experimental diets were formulated to be isonitrogenous and isocaloric to contain 50% crude protein (CP) and 16.7 kJ of available energy/g with or without dietary phytase supplementation (Natuphos-5000™, BASF, NJ, USA). Estimated available energy of the experimental diets was calculated as 16.7, 16.7 and 37.7 kJ/g for protein, carbohydrate and lipid, respectively (NRC, 1993). White fish meal (FM) provided 89.1% of the total protein in the basal diet (S_0), in the other 6 diets 30% or 40% FM protein was replaced by soybean meal as: 70% FM+30% soybean meal (S_{30}); 70% FM+30% SM+1000 U phytase/kg diet ($S_{30}P_{1000}$); 70% FM+30% SM+2000 U phytase/kg diet ($S_{30}P_{2000}$); 60% FM+40% SM (S_{40}); 60% FM+40% SM+1000 U phytase/kg diet ($S_{40}P_{1000}$); and 60% FM+40% SM + 2000 U phytase/kg diet ($S_{40}P_{2000}$). All the ingredients were mixed completely and then pelleted by using a 2 mm diameter module. After processing, all the diets were packed into small bags and kept at -80℃ until use.

Table 1. Composition and proximate analysis of experimental diets for juvenile olive flounder *Paralichthys olivaceus*

Ingredients	Diets[1]						
	S_0	S_{30}	$S_{30}P_{1000}$	$S_{30}P_{2000}$	S_{40}	$S_{40}P_{1000}$	$S_{40}P_{2000}$
White fish meal[2]	64	44.8	44.8	44.8	38.4	38.4	38.4
Corn gluten meal[2]	4	3.76	3.76	3.76	3.69	3.69	3.69
Soybean meal[3]	-	27.5	27.5	27.5	36.67	36.67	36.67
Dextrin[4]	13.5	5.27	5.27	5.27	2.51	2.51	2.51
Methionine	-	0.06	0.06	0.06	0.08	0.08	0.08
L-Lysine·HCl[5]	-	0.11	0.11	0.11	0.15	0.15	0.15
Glutamin	0.2	0.2	0.1	-	0.2	0.1	-
Phytase[6]	-	-	0.1	0.2	-	0.1	0.2
Other ingredients[7]	18.3	18.3	18.3	18.3	18.3	18.3	18.3
Proximate analysis (dry matter basis)							
Crude protein	50.1	50.8	50.3	49.8	49.5	49.1	49.9
Crude lipid	12.7	11.9	11.8	11.9	11.4	11.5	11.6
Crude ash	15.0	13.9	13.8	13.7	13.1	13.3	13.2
Total phosphorus (g/kg diet)	23.3	18.4	18.8	18.5	16.8	16.7	16.5
Analyzed phytase activity (U/kg diet)	0	0	987	2013	0	1036	2018

[1]White fish meal (FM) provided 92.4% of the protein in the basal diet (S_0), in the other six diets 30% or 40% FM protein was replaced by soybean meal (SM) as: 70% FM + 30% soybean meal (S_{30}); 70% FM + 30% SM + 1000U phytase/kg diet ($S_{30}P_{1000}$); 70% FM + 30% SM + 2000U phytase/kg diet ($S_{30}P_{2000}$); 60% FM + 40% SM (S_{40}); 60% FM + 40% SM + 1000U phytase/kg diet ($S_{40}P_{1000}$); 60% FM + 40% SM + 2000U phytase/kg diet ($S_{40}P_{2000}$).
[2]Fisheries Co-operative of Korea Feeds Co., Uiryeong County, Uiryeong Town, Korea.
[3]American Soybean Association/Korea.
[4]United States Biochemical, Cleveland, Ohio 44122.
[5]0.5% L-lysine·HCl (0.4% lysine).
[6]Natuphos 5000™, BASF, NJ, USA.
[7]Other ingredients include: 4.80% wheat meal (Young Nam Flourmills Co., Busan, Korea), 8.00% squid liver oil, 1.00% vitamin premix (Kim et al., 2002), 3.00% mineral premix (Kim et al., 2001), 1.00% attractant (composed of Keratine hydrolysis, betaine, glycine and inosinate), and 0.50% Cr_2O_3.

Fish and feeding trial

Juvenile olive flounder were transferred from Pukyong Aquaculture Company, Tongyoung, Korea to the Department of Fisheries Biology, Pukyong National University, Korea. Before the start of the experiment, all the fish were reared in a 3000-L round tank and were fed a basal diet (S_0) for 2 weeks to acclimated with the experimental diet and conditions. The feeding trial was conducted by using a flow-through system with 60-L aquaria receiving filtered sea water at a rate of 1 L/min. Supplemental aeration was provided to maintain dissolved oxygen near saturation. Water temperature was maintained at 15 ± 1℃. Fish averaging 6.15 ± 0.04 g (mean ± SD) were distributed randomly to each aquarium as a group of 25 fish and were fed one of seven experimental diets in triplicate groups at a rate of 3% of wet body weight for 8 weeks. Total fish weight in each aquarium was measured every 2 weeks, and the amount of diet fed to fish was adjusted accordingly.

Sample collection and analysis

At the end of the feeding trial, all fish were weighed and counted to calculate percent weight gain (WG), feed efficiency ratio (FER), specific growth rate (SGR), protein efficiency ratio (PER), hepatosomatic index (HSI), condition factor (CF), and survival. Blood samples were obtained from the caudal vein by using a heparinized syringe. Hematocrit (PCV) was determined from five individual fish per aquarium by the microhematocrit method (Brown, 1980) and hemoglobin (Hb) was measured in the same five fish by the cyanmethemoglobin procedure using Drabkin's reagent. A hemoglobin standard prepared from human blood (Sigma Chemical, St. Louis, MO) was used. Five fish per aquarium were used for proximate analysis. Crude protein, moisture and ash of whole-body samples were analyzed by AOAC methods (1995). Crude lipid was determined by using an ether extraction procedure (Soxtec System 1046, Foss, Sweden) after freeze-drying samples for 12 h. Fecal collections and digestibility determination were conducted according to the method described by Lim et al. (2004).

Statistical analysis

All data were subjected to one way ANOVA using SPSS for Windows (release 14.0). When a significant treatment effect was observed, an Honestly Significant Difference (HSD) test was used to compare means. Treatment effects were considered significant at $P<0.05$.

Results and Discussion

Apparent digestibility coefficients (ADCs) of phosphorus, dry matter and crude protein of diets containing different levels of soybean meal and phytase fed to juvenile olive flounder for 8 weeks are summarized in Table 2. ADCs of phosphorus in diets $S_{30}P_{1000}$, $S_{30}P_{2000}$, $S_{40}P_{1000}$ and $S_{40}P_{2000}$ were significantly higher than those in diets S_0, S_{30} and S_{40} ($P<0.05$). The supplementation of phytase significantly improved the ADC of phosphorus in experimental diets ($P<0.05$) containing 30% or 40% soybean meal regardless of the level in olive flounder. Fish can only utilize approximately 1/3 of the phosphorus contained in soybean meal. Because about 60% phosphorus in soybean meal is in the form of phytate, fish cannot digest (Sajjadi and Carter, 2004). Based on the present experiment, supplementation of phytase can improve the ADCs of phosphorus in soybean meal based diets, and then it is possible to improve the phosphorus retention of diets and reduce the phosphorus discharge into water that was considered as one of the main pollution elements in water environment. Many studies found that addition of phytase had positive effects on ADC of phosphorus in red sea bream (Biswas et al., 2007), rockfish (Yoo et al., 2005) and rainbow trout (Sugiura et al., 2001; Wang et al., 2008) fed soybean meal based diets. There was no significant effect of phytase supplementation on ADCs of dry matter and protein in the present study.

Growth performance, hepatosomatic index (HSI) and survival rate of juvenile olive flounder fed seven experimental diets for 8 weeks were shown in Table 3. There was no significant difference in weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FE) and protein efficiency ratio (PER) of fish fed S_0, S_{30}, $S_{30}P_{1000}$ and $S_{30}P_{2000}$. However, WG, SGR, FE and PER of 40% soybean meal groups were significantly lower than those of fish fed S_0 and 30% soybean meal. The reduced growth of 40% soybean meal groups could be because of the effect of difference in amino acid profiles and amount of antinutritional factors in the diets. Soybean contains numerous antinutritional factors like proteinase inhibitors and

Table 2. Apparent digestibility coefficients of phosphorus (ADPh), dry matter (ADM) and crude protein (ADPr) of the diets containing different levels of soybean meal and phytase in juvenile olive flounder *Paralichthys olivaceus*[1]

Diets[2]	ADPh (%)	ADM (%)	ADPr (%)
S_0	62.8[b]	68.8[a]	88.1[a]
S_{30}	58.2[b]	64.1[a]	84.9[ab]
$S_{30}P_{1000}$	91.0[a]	66.9[a]	89.9[a]
$S_{30}P_{2000}$	90.7[a]	66.5[a]	89.2[a]
S_{40}	53.6[b]	56.8[b]	81.4[b]
$S_{40}P_{1000}$	86.7[a]	58.8[b]	85.4[ab]
$S_{40}P_{2000}$	85.3[a]	58.0[b]	84.2[ab]
Pooled S.E.M.[3]	7.26	1.07	1.06

[1]Values are means of triplicate groups, values in the same column not sharing a common superscript are significantly different ($P < 0.05$).
[2]Refer to the Table 1, footnote #1.
[3]Pooled standard error of mean: S.D./$\sqrt{n}$.

agglutinating lectins, as reported by many researchers (Refstie et al., 2001; Sun et al., 2007). In this study, supplementation of 1000 or 2000 U phytase did not improve WG. Similar results were observed in Korean rockfish, red sea bream and parrot fish (Yoo et al., 2005; Biswas et al., 2007; Lim and Lee, 2009). However, growth improvement was also observed in salmon, rainbow trout and Nile tilapia fed the diets with phytase pre-treated ingredients (Cain and Garling 1995; Vielma et al., 2002; Cao et al., 2008). According to Cao et al., (2008), pre-treatment of plant ingredients using microbial phytase significantly increased nutrient digestibility and growth. To improve the nutritive value of plant products for fish, ingredients are being modified by chemical, mechanical and biological methods (Cao et al., 2007). While differences in dietary formulations could be one of the reasons contributing to differences in feeding response of fish to phytase, species differences is another possible factor (Venou et al., 2006). Therefore, further studies should be conducted to compare the economic benefits among using chemical, mechanical and biological methods to the plant protein sources. There was no significant difference in HSI, condition factor (CF), hematocrit (PCV), hemoglobin (Hb) and survival rate among all the dietary treatments. Sur-

Table 3. Growth performance, hepatosomatic index (HSI), and survival rate of juvenile olive flounder *Paralichthys olivaceus* fed seven experimental diets for 8 weeks[1]

	Diets[2]							Pooled S.E.M.[3]
	S_0	S_{30}	$S_{30}P_{1000}$	$S_{30}P_{2000}$	S_{40}	$S_{40}P_{1000}$	$S_{40}P_{2000}$	
WG (%)[4]	275[a]	261[a]	281[a]	275[a]	235[b]	236[b]	235[b]	3.25
SGR (%)[5]	2.41[a]	2.29[ab]	2.42[a]	2.40[a]	2.16[c]	2.16[c]	2.16[c]	0.02
FER (%)[6]	109[a]	101[ab]	106[ab]	102[ab]	91.6[b]	93.9[ab]	93.9[ab]	1.14
PER (%)[7]	2.15[a]	2.07[abc]	2.10[ab]	2.03[abc]	1.85[c]	1.88[bc]	1.88[bc]	0.02
HSI (%)[8]	2.76	2.29	2.11	2.19	2.28	2.00	2.37	0.09
CF[9]	1.04	1.17	1.77	1.09	1.09	1.02	1.04	0.02
Survival rate (%)	100	96.7	96.7	95.0	100	96.7	86.7	1.03
PCV[10]	47.8	46.6	48.2	48.7	47.4	48.6	46.5	0.39
Hb[11]	6.45	6.32	5.50	6.54	6.62	6.33	6.43	0.13

[1]Values are means of triplicate groups, values in the same row not sharing a common superscript are significantly different ($P < 0.05$).
[2]Refer to Table 1, footnote #1.
[3]Pooled standard error of mean: S.D. /$\sqrt{n}$.
[4]Weight gain: [(final wt. - initial wt.) / initial wt.] × 100.
[5]Specific growth rate: [(ln final wt. - ln initial wt.) / days] × 100.
[6]Feed efficiency ratio: (wet wt. gain / dry feed intake) × 100.
[7]Protein efficiency ratio: wet wt gain / protein intake.
[8]Hepatosomatic index: (liver wt. / body wt.) × 100.
[9]Condition factor: [wet wt. (g) / body length (cm)^3] × 100.
[10]Hematocrit (%).
[11]Hemoglobin (g/100 mL).

Table 4. Whole body proximate composition of juvenile olive flounder *Paralichthys olivaceus* fed experimental diets for 8 weeks (% of as-is basis)[1]

Diets[2]	Moisture	Crude protein	Crude lipid	Ash
S_0	76.4	17.0	3.77	5.50
S_{30}	73.7	17.7	4.64	6.43
$S_{30}P_{1000}$	74.5	17.5	4.56	5.77
$S_{30}P_{2000}$	74.0	17.2	4.43	6.02
S_{40}	74.6	18.1	4.05	6.28
$S_{40}P_{1000}$	74.6	17.9	3.88	5.56
$S_{40}P_{2000}$	75.2	17.9	3.96	5.97
Pooled S.E.M.[3]	0.19	0.14	0.13	0.16

[1]Values are means of triplicate groups, values in the same column not sharing a common superscript are significantly different ($P< 0.05$).
[2]Refer to Table 1, footnote 1.
[3]Pooled standard error of mean: S.D./$\sqrt{n}$.

vival rate of fish ranged from 94.6 to 100 with flounder fed $S_{40}P_{2000}$ showed the lowest value of survival.

Whole-body proximate compositions were shown in Table 4. In this study, whole body composition resulted to be not affected by soybean meal and phytase level. This result is in agreement with several studies on other species (Kim et al., 2009). Whole body protein slightly increased with increasing soybean meal in diets regardless the supplementation levels of phytase. There was a negative correlation to whole body lipid and ash content a slight decrease was observed when soybean meal inclusion increased. Similar results were also reported by Lin et al. (2012) and Bonaldo et al. (2008). Based on the experimental results, we conclude that supplementation of phytase could significantly increase available P content in feed, indicating that the indigestible phytate P successfully converted to available P by phytase.

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