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Improved Apparent Digestibility Coefficient of Protein and Phosphorus by Supplementation of Microbial Phytase in Diets Containing Cottonseed and Soybean Meal for Juvenile Olive Flounder (*Paralichthys olivaceus*)

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ABSTRACT : This study was conducted to investigate the effects of phytase in diets containing cottonseed and soybean meal (CS) on growth performance, feed utilization and digestibility of protein and phosphorus in juvenile olive flounder (initial body weight 2.5 g), *Paralichthys olivaceus*. Four experimental diets replacing 0%, 30%, 30% and 40% fish meal protein with CS in equal proportion were formulated to be isonitrogenous and isocaloric (designated as CS0, CS30, CS30+P, CS40+P, respectively). Phytase of 1,000 FTU/kg was supplemented in diets CS30+P and CS40+P. Three groups of fish (25 fish per group) were fed one of the experimental diets for 10 weeks. No significant differences were observed in growth performance of fish groups except for the CS40+P diet. Apparent digestibility coefficients of protein and phosphorus in fish fed phytase-containing diets were significantly higher than those of fish fed the CS0 diet. Serum cholesterol was significantly reduced in fish fed the CS-containing diets. Antioxidant activities in the diets and liver of fish were significantly increased with the increment of dietary CS. Gossypol was only detected and found in liver of the fish fed the CS-containing diets. The findings suggest that supplementation of microbial phytase could improve the apparent digestibility of protein and phosphorus in juvenile olive flounder fed the CS-containing diets. (**Key Words** : Microbial Phytase, Olive Flounder, Cottonseed Meal, Soybean Meal)

INTRODUCTION

Replacement of fish meal by plant protein sources is of great interest (Fontainhas-Fernandes et al., 1999; Mbahinzireki et al., 2001) and has become increasingly important for the development of low-cost fish feeds (Baruah et al., 2004) because of high cost and limited availability of fish meal in many countries (Naylor et al., 2000). Suitable alternative feed ingredients, such as soybean meal and cottonseed meal are promising sources of protein for aqua-feeds in the future. The major obstacle in using these plant protein sources is the presence of anti-nutritional factors, such as phytic acid, gossypol, saponins and trypsin inhibitors (NRC, 1993; Masumoto et al., 2001). Phytic acid (myo-inositol hexakisphosphate) is the major phosphorus storage compound in plant seeds comprising 80% of total phosphorus that cannot be digested and absorbed by monogastric animals including fish (Baruah et al., 2004). The discharge of unutilized phytate phosphorus into water can stimulate the growth of algae and phytoplankton, thus reducing dissolved oxygen and causing water pollution around fish farms (Sugiura et al., 1999; Debnath et al., 2005a). Furthermore, phytic acid can chelate other divalent and trivalent cations, such as iron, zinc, magnesium, copper and calcium, resulting in decreased bioavailability of these minerals (Wise, 1983). It also can react directly with charged groups of protein mediated by mineral cations, and thus adversely influence protein digestion and bioavailability (Barbara et al., 1999; Urbano et al., 2000; Chen and Li, 2003).

Phosphorus is an essential mineral required by fish for important physiological functions (NRC, 1993). Recently, many processes have been reported to liberate free phosphorus from phytic acid (Urbano et al., 2000; Hotz and Gibson, 2001), but better results were obtained by the use of enzymatic hydrolysis (Silva et al., 2005). Phytase has been reported not only to increase phosphate utilization efficiency from phytate in feeds but also to decrease phosphorus pollution (Broz et al., 1994; Yanke et al., 1998;

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Table 1. Formulation and proximate composition of the experimental diets (% dry matter)

Ingredients	Diets			
	CS0	CS30	CS30+P	CS40+P
White fish meal	54.0	37.8	37.8	32.4
Soybean meal	0.0	11.8	11.8	15.74
Cottonseed meal[1]	0.0	12.7	12.7	16.94
Corn gluten meal	6.6	7.2	7.2	7.4
Wheat flour	24.0	13.3	13.3	9.72
Mineral mix[2]	0.5	0.5	0.5	0.5
Vitamin mix[3]	0.5	0.5	0.5	0.5
Squid liver oil	12.0	13.0	13.0	13.3
CMC	1.0	1.0	1.0	1.0
Lysine	0.0	0.6	0.6	0.8
Methionine	0.0	0.3	0.3	0.4
Ferrous Sulfate-$7H_2O$	0.0	0.3	0.3	0.3
Phytase[4]	0.0	0.0	0.01	0.01
Cellulose	0.9	0.5	0.49	0.49
Chromic oxide	0.5	0.5	0.5	0.5
Proximate composition and phytase activity				
Dry matter (DM, %)	98.46	98.04	96.09	95.59
Protein (% DM)	48.06	48.32	48.58	48.12
Lipid (% DM)	17.12	16.98	16.33	16.45
Ash (% DM)	8.80	8.15	8.23	8.12
Total phosphorus (mg/g DM)	3.93	4.07	3.13	3.56
Gross energy (MJ/kg DM)	17.87	17.87	17.87	17.85
Phytase activity (FTU/kg DM)[5]	0.0	0.0	1,065.0	1,097.4

[1] Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, Tennessee 38108, USA.

[2] Mineral mixture (g/kg of mixture): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; $CuCl_2$, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

[3] Vitamin mixture (g/kg of mixture): L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

[4] Phytase (10,000 FTU/g) was purchased from Easy Bio System, Inc., Seoul, Korea.

[5] Phytase activity was analyzed according to the method described by Han et al. (1999) and Kim and Lei (2005).

Um et al., 2000). Hughes and Soares (1998) showed that phytase supplementation to a diet containing a high level of phytate improved the absorption and utilization of phosphorus in striped bass. Dietary phytase also improved the nutritive value of canola protein concentrate and decreased phosphorus output in rainbow trout (Forster et al., 1999). Baruah et al. (2004) also reported that microbial phytase supplementation in diets increased bioavailability of nitrogen and phosphorus and reduced feed costs.

Olive flounder (*P. olivaceus*) is one of the most important culture species and accounts for higher than 98% of the flatfish production in Korea. Recently, many studies were conducted to investigate the use of plant protein sources for fish meal replacement in diets for olive flounder (Kikuchi et al., 1994; Kikuchi, 1999; Masumoto et al., 2001; Saitoh et al., 2003; Pham et al., 2005; Pham et al., 2007; Kim et al., 2008). The deficiency of phosphorus has been reported to limit the inclusion of plant protein in fish feeds. Masumoto et al. (2001) observed that the dietary supplementation of phytase improved the bioavailability of phosphorus in soybean meal or soybean protein concentrate based diets for olive flounder. Therefore, this study was conducted to investigate the effects of supplementation of phytase on growth performance, feed utilization, protein digestibility and phosphorus availability in juvenile olive flounder fed diets containing cottonseed and soybean meal.

MATERIALS AND METHODS

Experimental diets

Four experimental diets (CS0, CS30, CS30+P, and CS40+P) were formulated to be isonitrogenous (48% crude protein) and isocaloric (18 MJ/kg). The energy value of each diet was estimated on the basis of mammalian physiological fuel value, i.e., 16.7 KJ/g protein or carbohydrate and 37.7 KJ/g lipid (Lee and Putman, 1973). The diet formulation and proximate composition are presented in Table 1. Diet CS0 was a fish meal based diet (control diet). In diet CS30, 30% fish meal protein in the control diet was replaced by cottonseed and soybean meal in equal proportion. In diets CS30+P and CS40+P, 30% and 40% fish meal was replaced by cottonseed and soybean meal with phytase supplementation of 1,000 FTU/kg diet. Microbial phytase was used in the experimental diets as

described by Cheng and Hardy (2003) and Yoo et al. (2005). Based on the finding of our previous study (Pham et al., 2005; 2007), the diet containing 40% cottonseed and soybean meal without phytase was excluded in the present study. The phytase activities measured in diets CS30+P and CS40+P were 1065 and 1097 FTU/kg diet, respectively (Table 1). All dry ingredients were thoroughly mixed with distilled water and fish oil. Pellets were extruded through a meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size, dried by fan at room temperature, crushed into desirable particle sizes (0.4-2.0 mm), and stored at -20°C until use.

Fish and feeding trial

Olive flounder juveniles were transported from a private hatchery in Jeju Island to the Marine and Environmental Research Institute, Cheju National University, Jeju, Korea. The fish were fed with a commercial diet for 2 weeks to adapt to the experimental facilities. Three hundred fish (mean body weight 2.5 g) were randomly distributed into twelve 35 L tanks (25 fish per tank) in a flow through system supplied with sand filtered seawater at a flow rate of 3 L/min. One of the experimental diets was fed to 3 groups of fish to apparent satiation (twice per day at 8:00 and 18:00 h) for 10 weeks. Aeration was also provided to maintain enough dissolved oxygen. The growth of fish was measured every 2 weeks.

Feces collection and apparent digestibility test

The indirect method described by Cho and Kaushik (1990) was used to calculate the apparent digestibility coefficient of protein and phosphorus with chromic oxide (0.5% in diets) as the inert indicator. Feces were collected with a modified fecal collection system for olive flounder (Yamamoto et al., 1998). After ten weeks of the feeding trial, fish of each treatment (three groups) were transferred to four 150 L fecal collection tanks. To collect the feces, all the fish were fed their respective diets containing 0.5% chromic oxide to satiation in the evening at 19:00 h and the feces were collected on the next morning and afternoon at 7:00 and 14:00 h, respectively. The collected feces were immediately frozen at -20°C until analysis.

Dietary and fecal protein were analysed using a Kjeltec 2003 Analyzer Unit (Foss Tecator AB, Sweden). Chromic oxide in feces and diets was determined according to the method described by Furukawa and Tsukahara (1966). Total phosphorus in diets and feces was measured using an inductively coupled plasma (ICP) emission spectrophotometer as described by Leske and Coon (1999).

Whole body composition

At the end of the feeding trial, all fish were weighed and counted for the calculation of feed intake, feed conversion ratio, protein efficiency ratio and specific growth rate. Three fish from each tank (9 fish per diet) were sampled and stored at -20°C for whole body proximate analysis. Analyses of crude protein, moisture and ash were performed by the standard procedures (AOAC, 1995). Lipids were determined according to the method described by Folch et al. (1957).

Serum cholesterol

At the end of the feeding trial, 3 fish per tank (9 fish per diet) were randomly selected and anaesthetized in tricaine methane sulfonate (MS-222) solution (100 mg/L). Blood was taken from the caudal vein with a non-heparinised syringe, kept at room temperature for 2 h and centrifuged at 5,000 rpm for 10 min at 4°C using a microcentrifuge (Micro-TR17, Hanil Science Industrial Co., Ltd, Korea). The serum cholesterol was determined using an automatic Photometer CH100 Plus (Calenzano, Firenze, Italy).

Antioxidant capacity assay

Antioxidant capacity of experimental diets and fish livers was determined using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay as described by Brand-William et al. (1995) with some modifications. Two g of diet (2 replicates per diet) was homogenized in 20 ml aqueous methanol (80%) and kept at room temperature for 10 min. The homogenate was centrifuged (5,000 rpm) at 4°C for 10 min and filtered through a 0.45 μm syringe filter (Whatman Inc., Clifton, NJ) prior to the assay. Whole livers of 3 bled fish per tank (9 fish per diet) were homogenized in aqueous methanol (80%) at a ratio of 1:4 (whole livers: aqueous methanol) for 60 sec using an homogenizer (X-120, Germany). The homogenate was centrifuged (5,000 rpm) at 4°C for 10 min. The supernatant was filtered through a 0.45 μm syringe filter. One hundred μl of filtered extract was pipetted into a 1.5 ml cuvette, then 900 μl of DPPH methanolic solution (100 μM) was added to obtain a final volume of 1 ml. The absorbance of the mixture was measured at 517 nm at 1 min intervals for 10 min by a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The antioxidant capacity of the extract against the DPPH radicals was calculated as percent inhibition. Percent inhibition = $((A_0-A_s)/A_0)\times100$, where A_0, A_s are the absorbance of sample at 0 and s min, respectively.

Total polyphenol compounds

Total polyphenol compounds in the experimental diets were measured by a colorimetric method described by Skerget et al. (2005). Briefly, 1 g of diet was extracted with 250 ml methanol for 2 h at 40°C. The solution was cooled and filtered through a 0.45 μm syringe filter (Whatman Inc., Clifton, NJ). To 0.5 ml filtered extract, 2.5 ml of Folin-

Table 2. Growth performance and feed utilization of juvenile olive flounder fed the experimental diets for 10 weeks*

Diets	CS0	CS30	CS30+P	CS40+P
Initial body weight (g)	2.47±0.15	2.47±0.04	2.50±0.11	2.49±0.02
Final body weight (g)	15.71±0.59^{a}	14.96±1.07^{ab}	15.30±0.31^{a}	14.08±0.57^{b}
Specific growth rate (SGR)[1]	1.09±0.02^{a}	1.06±0.05^{ab}	1.06±0.03^{ab}	1.02±0.03^{b}
Protein efficiency ratio (PER)[2]	1.67±0.01^{a}	1.56±0.04^{ab}	1.59±0.04^{ab}	1.47±0.17^{b}
Feed conversion ratio (FCR)[3]	1.24±0.01	1.30±0.07	1.26±0.01	1.32±0.08
Feed intake (g/g BW)[4]	1.94±0.13	2.36±0.13	1.95±0.05	2.25±0.35
Survival (%)	100±0.0	91.7±7.8	98.3±1.3	93.3±8.89

* Values are presented as mean±SD. Values in the same row having different letters are significantly different ($p<0.05$).

[1] SGR (%) = ((ln final body weight-ln initial body weight)/days)×100.

[2] PER = Wet weight gain/total protein given. [3] FCR = Dry feed fed/wet weight gain.

[4] FI (g/g body weight) = Dry feed consumed (g)/body weight (g).

Table 3. Whole body composition of juvenile olive flounder fed the experimental diets for 10 weeks*

Diets	Initial	CS0	CS30	CS30+P	CS40+P
Moisture content (%)	79.0±0.1	74.6±0.5	75.1±0.9	74.6±0.5	74.6±0.3
Protein (% DM)	75.1±1.4	65.2±4.0	64.1±3.2	64.3±3.0	66.1±2.2
Lipid (% DM)	8.3±0.2	23.5±2.2	20.9±1.4	23.2±0.9	21.9±1.7
Ash (% DM)	18.2±0.6	9.8±0.7^{c}	10.9±1.3^{bc}	11.8±0.9^{ab}	12.7±0.6^{a}

* Values are presented as mean±SD. Values in the same row having different letters are significantly different ($p<0.05$).

Ciocalteu reagent (0.2 N, Sigma) was added and kept for 5 min at room temperature, then 2 ml of Na_2CO_3 solution (75 g/L) was added. The mixture was incubated for 5 min at 50 °C and cooled. The absorbance was measured at 760 nm using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The results were expressed in gram of gallic acid per kilogram of dry diet.

Gossypol analysis

Gossypol content in the experimental diets and fish liver was determined by High Performance Liquid Chromatography (HPLC) according to the method described by Kim and Calhoun (1995) with some modifications by Lee and Dabrowski (2002). Briefly, the samples were weighed and 5-10 volumes of complexing reagent was added to obtain 2-amino-1-propanol derivatives of gossypol. The complexing reagent was composed of 2 ml 2-amino-1-propanol (Sigma Chemical, St. Louis, MO), 10 ml glacial acetic acid (Sigma Chemical) and 88 ml N, N-dimethylformamide (Sigma Chemical). The samples were homogenized in complexing reagent for 30 sec, heated at 95°C for 30 min, cooled on ice and then centrifuged at 1,500×g for 5 min. After centrifugation, an aliquot of the supernatant was diluted with mobile phase to obtain a desirable concentration, centrifuged again at 1,500×g for 5 min and filtered through a syringe filter (0.45 μm, Whatman Inc., Clifton, NJ) before injection into HPLC.

Statistical analysis

Data were subjected to one-way ANOVA in SPSS version 11.0. The significant differences between group means were compared using Duncan's multiple test. Data were presented as means±standard deviation (SD). The percentage data were arcsine transformed before the ANOVA analysis. Differences were considered significant at $p<0.05$.

RESULTS

Final body weight, specific growth rate and protein efficiency ratio of fish fed diets CS30 and CS30+P were not significantly different from the values of fish fed the control diet (Table 2). Feed conversion ratio and feed intake did not differ among fish groups fed all the experimental diets. Survival of all fish groups was over 90% and was not significantly different.

For whole body composition (Table 3), there were no significant differences among fish groups fed the experimental diets, except for ash content. Ash in the whole body of juvenile olive flounder was gradually increased with increasing dietary cottonseed and soybean meal inclusion.

Serum cholesterol of fish fed diets CS30 and CS40+P was significantly lower than that of fish fed the control diet. Serum cholesterol of fish fed diet CS30+P was comparable to that of fish fed the control diet (Figure 1).

The apparent digestibility coefficient (ADC) of protein in diet CS40+P was significantly higher than that of other experimental diets (Figure 2). No differences in ADC of protein were found between diets CS0, CS30, and CS30+P, even though diets CS30 and CS30+P resulted in numerically increased values. Phosphorus availability was gradually increased ($p<0.05$) with the increment of dietary CS inclusion and/or phytase supplementation (Figure 2).

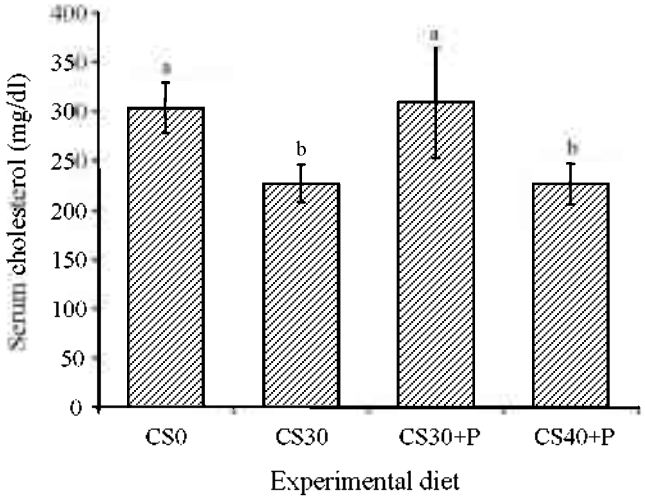

Figure 1. Serum cholesterol of juvenile olive flounder fed the experimental diets for 10 weeks. Values are the mean of three replicates per treatment. Bars with different letters are significantly different ($p<0.05$).

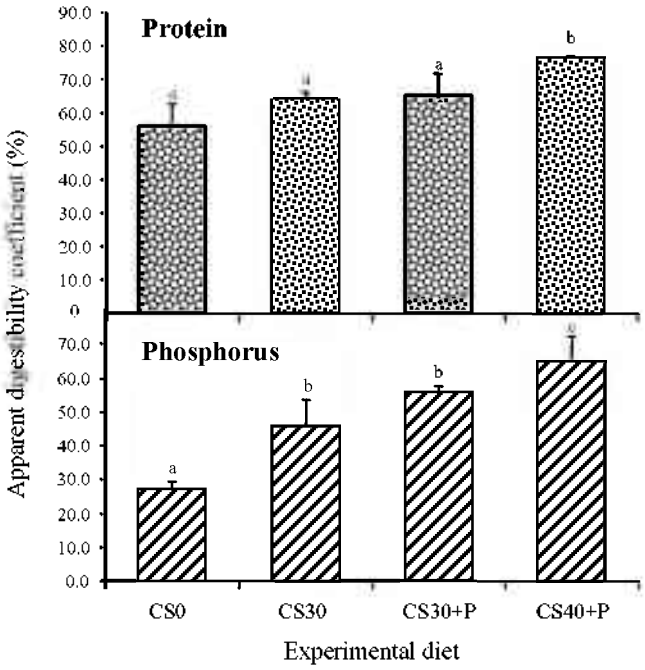

Figure 2. Apparent digestibility coefficient of protein and phosphorus in juvenile olive flounder fed diets containing cottonseed and soybean meal for 10 weeks. Values are the mean of three replicates per treatment. Bars with different letters are significantly different ($p<0.05$).

Antioxidant activity in the experimental diets was significantly increased with the increment of dietary CS (Figure 3). The DPPH radical scavenging capacity of liver tended to increase with the increment of dietary CS (Figure 3). Total polyphenol content was significantly increased in the CS40+P diet compared to the other diets (Figure 4).

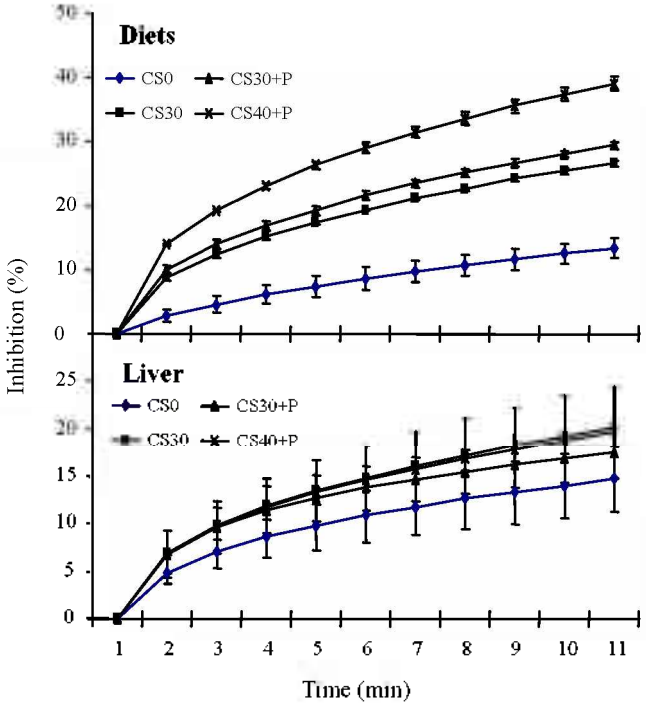

Figure 3. DPPH radical scavenging activity (%) in the experimental diets and liver of juvenile olive flounder fed the experimental diets for 10 weeks. Absorbance was measured at 517 nm for 10 min at intervals of 1 min.

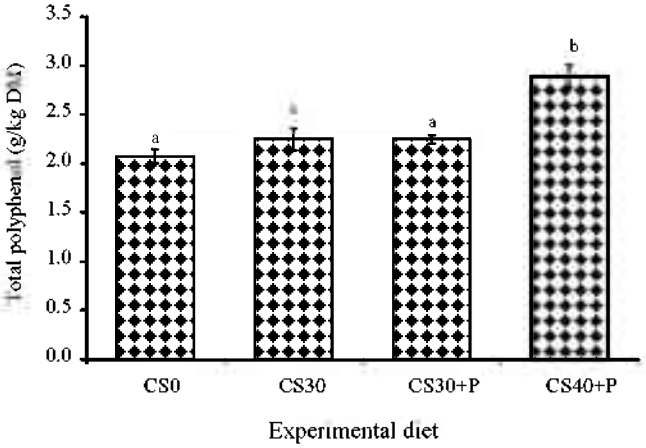

Figure 4. Total polyphenol compounds in the experimental diets. Values are the mean of two replicates per diet. Bars with different letters are significantly different ($p<0.05$).

Dietary total gossypol content was significantly increased with increment of cottonseed meal (Table 4). Gossypol was only detected and found in liver of the fish fed the CS-containing diets.

DISCUSSION

The use of exogenous phytase can increase the availability of phosphorus in fish feeds containing a larger

Table 4. Total and (+) and (-) enantiomer contents of gossypol in the experimental diets and liver of juvenile olive flounder fed the experimental diets for 10 weeks

	Diet (g/kg DM)			Liver (μg/g wet weight)		
	(+)	(-)	Total	(+)	(-)	Total
CS0	-*	-	-	-	-	-
CS30	0.82	0.50	1.32	4.28	3.50	7.78
CS30+P	0.74	0.36	1.10	3.79	3.28	7.07
CS40+P	0.93	0.60	1.53	3.64	3.58	7.22

* Not detected.

proportion of plant proteins and consequently reduce effluent phosphorus in aquaculture (Lanari et al., 1998; Vielma et al., 2002; Cheng and Hardy, 2003; Sajjadi and Carter, 2004). In the present study, the level of supplemented phytase in the experimental diets was based on a study conducted by Yoo et al. (2005). At the end of the 10 week feeding trial, the supplementation of phytase significantly increased apparent digestibility coefficient of phosphorus and protein in olive flounder fed the CS containing diets. This is in agreement with previous studies which resulted in positive effects of phytase inclusion in many fish species, such as rainbow trout (Sugiura et al., 2001), Atlantic salmon (Sajjadi and Carter, 2004), Korean rockfish (Yoo et al., 2005), striped bass (Paratryphon and Soares Jr, 2001), African catfish (Van Weerd et al., 1999), channel catfish (Li et al., 2004), olive flounder (Masumoto et al., 2001) and Nile tilapia (Portz and Liebert, 2004). Yoo et al. (2005) concluded that supplementation of microbial phytase significantly improved the apparent digestibility coefficient of phosphorus in rockfish diets, replacing 30% and 40% fish meal with soybean meal regardless of the level and method of phytase supplementation. A higher absorption of phosphorus was observed in juvenile olive flounder fed a diet containing soybean meal with phytase compared to fish fed a fish meal-based diet (Masumoto et al., 2001). However, no significant differences in phosphorus digestibility, retention and conversion were found between fish fed diets containing soybean meal with 1,000 FTU/kg by pretreatment or simple supplementation (Van Weerd et al., 1999; Yoo et al., 2005). The efficacy of nutrient digestibility in fish feeds depends on the types of ingredients used (Cheng and Hardy, 2002; 2003), processing techniques (Nwanna et al., 2005) and chemical composition of diets, particularly high ash content (Sugiura et al., 2001). A significant improvement in protein digestibility of fish diets containing plant protein sources has been demonstrated in several studies (Portz and Liebert, 2004; Debnath et al., 2005b). The results of the present study indicate that supplementation of 1,000 FTU, or higher, phytase per kg diet containing CS is likely to improve the apparent digestibility coefficients of protein and phosphorus (Figure 2).

The dietary crude protein (48%) and energy content (18 MJ/kg) in the present study were formulated based on the requirement of juvenile olive flounder reported by Kim et al. (2002). In the CS containing diets, limiting amino acids, such as lysine and methionine, were supplemented to meet their requirements in the fish. In the present study, up to 30% replacement of fish meal protein by CS with phytase supplementation did not affect the growth of fish (Table 2). In our previous study (Pham et al., 2005), growth performance of juvenile olive flounder (initial body weight 0.74 g) was not affected by dietary CS up to 30% fish meal replacement. However, higher incorporation of dietary CS (diet CS40+P) resulted in impairment in growth performances that were also demonstrated in the previous study (Pham et al., 2005). The lower growth performance of fish fed diet CS40+P might be due to the presence of anti-nutrient factors in cottonseed and soybean meal, particularly gossypol. Gossypol, a yellow pigment found in the gland of cottonseed, has been demonstrated to be toxic for many fish species (Dorsa et al., 1982; Dabrowski et al., 2000; Lee et al., 2002; Garcia-Abiado et al., 2004; Pham et al., 2007). In the present study, the total gossypol contents in diet CS40+P (16.94% dietary cottonseed meal inclusion as DM, Table 1) might have been higher than the tolerant level of gossypol in juvenile olive flounder and thereby resulted in the depression of fish growth. Phytase had no influence on growth performance and whole body composition of fish in the present study as previously proved by Li et al. (2004) and Yoo et al. (2005).

The increment in whole body ash content of fish fed phytase diets could be related to the excess of inorganic phosphorus and/or other minerals released from the experimental diets by phytase. Sajjadi and Carter (2004) observed that there was an interaction between phytase and inorganic phosphorus on bone ash, bone phosphorus and whole body phosphorus, and concluded that supplementation of phytase or inorganic phosphorus or both resulted in higher whole body ash.

Cholesterol lowering effect of soybean and other plant proteins has been intensively investigated in vertebrates including fish (Goldberg et al., 1982; Kaushik et al., 1995; Ali et al., 2004; Chisholm et al., 2005; Dias et al., 2005). Goldberg et al. (1982) demonstrated that decreased cholesterol level in hypercholesterolemic patients was contributed by soybean protein in diets. Anderson and Wolf

(1995) suggested that cholesterolemia is affected by various non-protein components of soy, such as trypsin inhibitors, saponins, phytoestrogens, fiber, phytosterols, phytic acid and minerals. Ali et al. (2004) also observed that soy isoflavones lowered plasma cholesterol in rats. It is evident that there are various factors including nutrition, non-nutrition and endocrines involved in the modulation of cholesterol synthesis and metabolism in vertebrate animals, except for teleosts. Cholesterol metabolism is not well understood in fishes (Este'vez et al., 1996). Kaushik et al. (1995) reported that plasma cholesterol levels were reduced in rainbow trout fed soybean protein in comparison to those fed a fish meal based diet. Similar results were also observed by Dias et al. (2005) who reported that the level of plasma cholesterol was lower in European seabass fed soybean diets than those fed a fish meal based diet. In the present study, interestingly, serum cholesterol was significantly reduced in fish fed CS containing diets (Figure 1). Recent studies have shown that cottonseed products have an ability to reduce serum cholesterol in animals (Nwoha and Aire, 1995; Edwards and Radcliffe, 1995; Radcliffe et al., 2001). The mechanism for the gossypol in cottonseed products on cholesterol has not been clearly determined, but studies have revealed a significant interaction between gossypol and lipid metabolism. The current results imply that dietary inclusion of both cottonseed and soybean meal could affect cholesterol metabolism in fish. Further study is needed on this issue.

An increased antioxidant activity in CS containing diets (Figure 3) seemed to be attributed to higher levels of dietary polyphenols (Figure 4) in the present study. This is supported by the fact that DPPH radical scavenging activities are closely related with polyphenol contents (Skerget et al., 2005). The present results on liver gossypol confirmed our previous finding that the liver accumulation of total gossypol was positively associated to the dietary inclusion levels of cottonseed meal (Pham et al., 2007).

In conclusion, supplementation of phytase in diets containing cottonseed and soybean meal could improve the apparent digestibility of protein and phosphorus in juvenile olive flounder. Growth performances might not be affected by the supplementation of phytase. Also, dietary supplementation of cottonseed and soybean meal could reduce the serum cholesterol and increase the antioxidant capacity in the fish.

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