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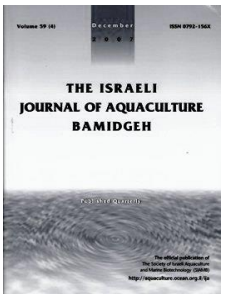

Use of Distillers Dried Grain as a Cost Effective Ingredient in the Diet of Juvenile Olive Flounder (*Paralichthys olivaceus)*

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Key words: distiller dried grain, olive flounder, growth, antioxidant activity

Abstract

This study assessed the effects of the dietary inclusion of distillers dried grain (DDG) on growth performance, feed utilization, body composition, and antioxidant activity in juvenile olive flounder (*Paralichthys olivaceus).* Five diets (DDG0, DDG1, DDG2, DDG3, and CF) were formulated to contain 0% (DDG0), 20% DDG (DDG1) replacing wheat flour only, and 20% DDG (DDG2) and 28% DDG (DDG3) partially replacing both fishmeal and wheat flour. Experimental diets were also compared with a commercial feed (CF) diet. Three replicate groups of fish average weight 16.0±0.04 g were fed each of the diets to apparent satiation twice a day for 7 weeks. Weight gain of fish fed the DDG1 and DDG2 diets was the same as fish fed DDG0 diet, but there was less weight gain in fish fed the DDG3 diet than those fed the DDG0 and CF diets. Feed efficiency, daily feed intake, daily protein intake, and protein efficiency ratio were not affected by dietary DDG and CF diets ($P>0.05$). Whole body content (WBC) of crude lipid in fish fed the DDG1 and CF diets was the same as fish fed DDG0 diet. WBC was higher in fish fed the DDG2 and DDG3 diets than in fish fed the DDG0 diet. Amino acid composition of whole body was not affected by dietary DDG and CF diets ($P>0.05$). Plasma content of total protein, cholesterol, and triglyceride, tended to decrease with dietary DDG level, and was lower in fish fed the DDG3 diet than fish fed the DDG0 and CF diets. DPPH, hydroxyl, alkyl and superoxide radical scavenging activities in the plasma of fish were not affected by dietary DDG and CF diet ($P>0.05$). The results suggest that up to 20% DDG in the diet could be used to replace plant components such as wheat flour and corn gluten meal, without negatively affecting growth performance of juvenile olive flounder.

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Introduction

Aquaculture is rapidly developing and is recognized as the fastest growing production sector in the food industry. The increase in aquaculture production has resulted in increased dependency on the use of formulated diets for fish. Feed cost typically amounts to around 50% of total farm production costs for olive flounder (Cho et al., 2013). Growing costs and unreliable supply of wheat flour have spurred the search for alternative ingredients for target fish (Kaushik et al., 1995). Many studies have researched alternative protein sources for fish meal, but there has been limited study of alternative plant sources. Finding less expensive feed ingredients in the preparation of a nutritionally balanced diet will enable preparation of lower cost, alternative, feed formulation. Therefore, alternatives to wheat flour as an energy source in diets should be investigated.

Distillers dried grain (DDG) is a cereal by-product of the ethanol and beverage industries. It has been successfully used as a feed ingredient for ruminants (Świątkiewicz and Koreleski, 2008). DDG is a possible alternative ingredient in fish feed due to its availability, low cost, as well as its protein, and vitamin content (Barnes et al., 2012; Chevanan et al., 2010; Kannadhason et al., 2009). Use of DDG in diets may be less expensive than other ingredients such as fish meal or wheat flour. Our previous study showed that rice-based DDG may be a cost effective ingredient in feed for black seabream (Rahman et al., 2013). Corn-based DDG has been found to be a beneficial feed ingredient for various fresh water fish species such as rainbow trout (Barnes et al., 2012), channel catfish (Li et al., 2010) and tilapia (Coyle et al., 2004).

Olive flounder (*Paralichthys olivaceus*) is an important aquaculture marine fish species. Its rapid growth rate, consumer popularity, and ease of production make it suitable for aquaculture (Cho et al., 2013). Since the late 1980s when a farming technique for this species was developed and implemented, its production has gradually increased in Asia (Kim et al., 2002). We hypothesized that DDG might be a suitable dietary ingredient for good fish growth at a lower cost. This study was carried out to evaluate the effects of dietary DDG on growth, body composition, and antioxidant activity of juvenile olive flounder.

Materials and Methods

Experimental diets. Proximate and essential amino acid compositions of ingredients used in the experimental diets are presented in Table 1.

Table 1. Nutrient composition of the ingredients of experimental diets.

	Ingredients		
	Fish meal	*Wheat flour*	*Distillers dried grain powder*[1]
Proximate composition (% DM)			
Dry matter	95.8	89.3	98.2
Crude protein	75.3	19.3	19.1
Crude lipid	8.8	3.9	7.8
Ash	14.6	2.2	0.5
Essential amino acid composition (%) of protein			
Arg	6.7	5.7	6.9
His	2.3	2.9	2.0
Ile	4.5	2.3	3.6
Leu	8.3	6.0	8.0
Lys	8.8	3.7	3.1
Met + Cys	5.1	2.8	3.4
Phe + Tyr	8.1	6.8	10.8
Thr	4.8	3.5	4.7
Val	4.5	3.2	5.8

[1]Residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts produced from Incheon Makgeolli factory (Incheon, Korea).

Ingredients and chemical composition of the experimental diets are presented in Table 2.

Table 2. Ingredient and chemical composition of experimental diets.

	Diets				
	DDG0	*DDG1*	*DDG2*	*DDG3*	*CF*[5]
Ingredients (%)					
Fish meal	62	62	57	57	
Wheat flour	18				
Corn gluten meal	4	2	7		
Distillers dried grain powder[1]	0	20	20	28	
α-Potato-starch	7	7	7	7	
Brewer yeast	1	1	1		
Fish oil	5	5	5	5	
Vitamin premix[2]	1	1	1	1	
Mineral premix[3]	1	1	1	1	
Stay-C (50%)	0.3	0.3	0.3	0.3	
Choline salt (50%)	0.2	0.2	0.2	0.2	
Taurine	0.3	0.3	0.3	0.3	
Nutrient content (%)					
Crude protein	54.7	54.7	54.5	51.2	54.7
Crude lipid	10.8	11.1	11.4	11.9	10.5
Ash	10.6	10.5	9.8	9.7	12.4
N-free extract[4]	24.1	24.0	24.7	27.4	22.4
Essential amino acid composition (%) of protein					
Arg	6.0	6.3	6.1	6.5	
His	4.6	4.6	4.3	4.5	
Ile	4.0	4.1	4.0	4.2	
Leu	8.3	8.3	8.9	8.2	
Lys	7.5	7.2	6.7	7.0	
Met+Cys	4.0	3.9	4.0	4.0	
Phe+Tyr	7.3	7.4	7.6	7.4	
Thr	4.7	4.7	4.7	4.8	
Val	4.8	5.0	4.9	5.1	

[1]Residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts produced from Incheon Makgeolli factory (Incheon, Korea).
[2]Vitamin premix contained the following diluted in cellulose (g/kg mix): 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 (98%), 0.68; p-aminobenzoic acid, 18.2; menadione,1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003
[3]Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferriccitrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0
[4]Nitrogen-free extract (NFE) =100 - (crude protein + crude lipid + ash)
[5]Commercial feed

Five isonitrogenous and isocaloric diets were formulated to comprise 0% (DDG0), 20% DDG (DDG1) replacing wheat flour, and 20% DDG (DDG2) and 28% DDG (DDG3) partially replacing both fishmeal and wheat flour. Commercial feed (CF) for flounder was also compared with experimental diets. Fish meal was the primary protein source and fish oil was the lipid source. DDG, which is a by-product of Makgeolli, a traditional alcoholic beverage native to Korea, prepared by filtration of the aqueous mixture of fermented rice with *Aspergillus oryzae a* filamentous fungus and yeast, was obtained from Incheon Makgeolli factory (Incheon, Korea). The ingredient was dried at 60^0C for 24 h and finely ground prior to its incorporation in the experimental diets. All ingredients were thoroughly mixed with 30% distilled water and pellets were prepared with a moist pelleting machine. The pellets were dried at room temperature for 48 h and stored at -30^0C until used.

Experimental fish and feeding conditions. Juvenile olive flounder were transported from a private hatchery (Namhae, Korea) to the Marine Biology Center for Research and Education at Gangneung-Wonju National University (Gangneung, Korea). The fish were acclimated to laboratory conditions by feeding them commercial pellets for 2 weeks before starting the feeding trial. After this conditioning period, juvenile olive flounder (mean body weight, 16.0±0.04 g) were randomly distributed in 400 l cylindrical plastic

tanks (300 l water volume) at a density of 25 fish per tank. Each experimental diet was fed to three replicate groups of fish to visual satiation twice a day (9:00 and 17:00 h) for 7 weeks. Filtrated seawater was supplied at a flow rate of 4 l/min to each tank, and the mean water temperature and salinity were 20.4±2.0^{0}C and 34±0.1 ppt, respectively. The feeding trial was conducted under natural photoperiod conditions. Records were kept on the daily feed consumption, mortalities, and feeding behavior.

Sample collection and analytical methods. At the end of each feeding experiment, all fish in each tank were starved for 24 hours, collectively anesthetized with MS222, and weighed. Proximate composition of the whole body was analyzed according to standard methods (AOAC, 1995). At the end of the feeding trials five fish per tank were sampled and stored at -25°C until analyzed. Crude protein was determined by the Kjeldahl method with an auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was analyzed with ether extraction in a Soxhlet extractor (SER 148, VELP Scientifica, Milano, Italy). Moisture was determined by drying in an oven at 105°C for 6 h and the ash content was determined after combustion at 550°C for 4 h in a muffle furnace. Amino acid composition of the experimental diets and whole body of fish was analyzed using an automatic amino acid analyzer (Hitachi, L-8800, Tokyo, Japan).

Blood chemistry. Blood was drawn from the caudal veins of five fish per tank with 1 ml heparinized syringes, transferred to microcentrifuge tubes and centrifuged at 7,500 g for 10 min. The plasma was then separated and stored at -75°C for chemical analysis. Plasma total protein, glucose, GOT, cholesterol, and triglyceride concentrations were determined using a commercial clinical kit (Asan Pharmaceutical, Seoul, Korea).

Radical scavenging activities. At the end of the feeding trials, five fish per tank were sampled and stored at -75°C for antioxidant activity analysis. Samples of plasma were homogenized with extract buffer in 5 mM Tris-HCl and 35 mM glycine (pH 8.4) followed by centrifugation (13,000 g for 10 min at 4°C). The supernatant was then collected and analyzed for radical scavenging activity.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of plasma was evaluated with the method described by Nanjo et al. (1995). A 30 µl peptide solution (or ethanol alone, as the control) was added to 30 µl of DPPH (60 µM) in ethanol solution. After mixing vigorously for 10 sec, the solution was moved into a 100 µl quartz capillary tube, and the scavenging activity of peptide on the DPPH radical was determined with a spectrometer (Jeol, Tokyo, Japan). After 2 min, the spin adduct was determined with an Electronic Spin Resonance (ESR) spectrometer. The measurement conditions were: magnetic field, 336.5±5 mT; power, 5 mW; modulation frequency, 9.41 GHz; amplitude, 1×1000; and sweep time, 30 sec.

Hydroxyl radicals of plasma were generated by iron-catalyzed Fenton Haber-Weiss reaction and were reacted rapidly with an electron spin trap 5, 5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (Rosen and Rauckman, 1984). The DMPO-OH adducts were analyzed by ESR spectrometer. The peptide solution (20 µl) was blended with DMPO (0.3 M, 20 µl), $FeSO_4$ (10 mM, 20 µl), and hydrogen peroxide (H_2O_2, 10 mM, 20 µl) in phosphate buffer solution (pH 7.4), and was then transferred into a 100 µl quartz capillary tube. After 2.5 min, the ESR spectrum was recorded. Experimental conditions were: magnetic field, 336.5±5 mT; power, 1 mW; modulation frequency, 9.41 GHz; amplitude, 1 × 200; and sweep time, 4 min.

Alkyl radicals of plasma were determined by 2, 2-azobiz-(2-amidinopropane)-hydrochloride (AAPH). The phosphate buffered saline (pH 7.4) reaction mixtures included 10 mM AAPH, 10 mM 4-Pyridyl-1-oxide-tert-butyl nitrone, and known concentrations of samples (100 µg/ml), which were incubated at 37^{0}C in a water bath for 30 min (Hiromoto et al., 1993) and then moved to a capillary tube. The spin adduct was recorded with a spectrometer (Jeol, Tokyo, Japan). Parameters measured were: modulation frequency, 100 kHz; microwave power, 10 mW; microwave frequency, 9441 MHz; magnetic field, 336.5±5 mT; and sweep time, 30 sec.

Superoxide radical scavenging activity of plasma was generated by UV irradiated riboflavin / EDTA system (Guo et al., 1999). The reaction mixture containing 0.3 mM riboflavin (20 l), 1.6 mM EDTA (20 l), 800 mM DMPO (20 l) and indicated concentrations of FMP solution (20 l) was UV-irradiated for 1 min at 365 nm. The reaction mixture was then transferred to 100 l quartz capillary tube of the ESR spectrometer for measurement. Experimental conditions were: magnetic field, 336.5±5 mT; power, 10 mW; modulation frequency, 9.41 GHz; amplitude, 1×1000; and sweep time, 1 min.

DPPH, hydroxyl, alkyl, and superoxide radical scavenging activities (RSA) were computed according to the following equation, in which H and H_0 were the relative peak heights of radical signals with and without sample, respectively.

$$\text{RSA (\%)} = \frac{(1-H)}{H_0} \times 100$$

Statistical analysis. The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 18.0 (SPSS, Chicago, IL, USA). Significant differences ($P<0.05$) among the means were determined using Duncan`s multiple range test (Duncan, 1955). The data are presented as mean±S.E. of three replicate groups.

Results

Growth performance and feed utilization of juvenile olive flounder fed the experimental diets are presented in Table 3.

Table 3. Growth performance and feed utilization of juvenile olive flounder fed the experimental diets for 7 weeks.

	Diets				
	DDG0	*DDG1*	*DDG2*	*DDG3*	*CF*
Initial body weight (g/fish)	16.0 ±	15.9 ±	16.0 ± 0.03	16.0 ±	15.9 ± 0.03
Survival	94 ± 2.3^{ns}	75 ± 9.3	69 ± 3.5	65 ± 10.9	79 ± 1.3
Weight gain[1]	153 ± 1.0^{b}	138 ±	134 ± 10.9^{ab}	110 ±	157 ± 10.7^{b}
Feed efficiency[2]	114 ± 2.2^{ns}	102 ± 8.8	104 ± 6.5	101 ± 16.8	121 ± 16.9
Daily feed intake[3]	0.99 ±	0.95 ±	0.99 ± 0.01	0.91 ±	0.91 ± 0.06
Daily protein intake[4]	0.54 ±	0.52 ±	0.54 ± 0.01	0.46 ±	0.50 ± 0.03
Protein efficiency ratio[5]	2.09 ±	1.88 ±	1.94 ± 0.12	1.98 ±	2.22 ± 0.31

Values (mean ± SE of three replications) in the same row not sharing a common superscript are significantly different ($P<0.05$)

^{ns} Not significant ($P>0.05$)

[1]Weight gain = (final fish wt. - initial fish wt.) × 100 / initial fish wt.

[2]Feed efficiency = wet weight gain × 100 / feed intake

[3]Daily feed intake = feed intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.)/2 × days reared]

[4]Daily protein intake = protein intake × 100/ [(initial fish wt. + final fish wt. + dead fish wt.)/2 × days reared]

[5]Protein efficiency ratio = (wet weight gain / protein intake)

Survival was not affected by dietary DDG and CF diet ($P>0.05$). Weight gain of fish fed the DDG1, DDG2, and CF diets was not different than for fish fed DDG0 diet, but was lower in fish fed the DDG3 diet than fish fed the DDG0 and CF diets ($P<0.05$). No significant differences were identified in feed efficiency, daily feed intake, and protein efficiency ratio among the groups ($P>0.05$). Proximate and essential amino acid composition of whole body in juvenile olive flounder fed the experimental diets are presented in Table 4.

Table 4. Proximate and essential amino acid composition of whole body in juvenile olive flounder fed the experimental diets for 7 weeks.

	Diets				
	DDG0	*DDG1*	*DDG2*	*DDG3*	*CF*
Proximate composition (%)					
Moisture	75.4 ± 0.45	73.9 ± 0.14	75.3 ± 1.33	72.8 ± 0.02	75.3 ± 1.32
Crude protein	18.4 ± 0.65	17.8 ± 0.29	16.9 ± 0.61	17.3 ± 0.08	17.6 ± 0.75
Crude lipid	2.8 ± 0.05^{a}	3.5 ± 0.06^{ab}	4.2 ± 0.32^{b}	4.1 ± 0.61^{b}	3.8 ± 0.15^{ab}
Ash	4.1 ± 0.17^{c}	3.4 ± 0.04^{ab}	3.1 ± 0.35^{a}	4.0 ± 0.14^{bc}	3.3 ± 0.18^{a}
Essential amino acid composition (%)					
Arg	6.9 ± 0.12	6.8 ± 0.03	6.8 ± 0.03	6.8 ± 0.03	6.9 ± 0.03
His	2.2 ± 0.01	2.1 ± 0.01	2.1 ± 0.01	2.1 ± 0.01	2.2 ± 0.03
Ile	3.9 ± 0.06	3.8 ± 0.06	3.8 ± 0.09	3.9 ± 0.09	3.8 ± 0.18
Leu	7.7 ± 0.10	7.5 ± 0.01	7.6 ± 0.01	7.5 ± 0.09	7.6 ± 0.17
Lys	8.7 ± 0.12	8.4 ± 0.03	8.5 ± 0.01	8.4 ± 0.06	8.4 ± 0.18
Met + Cys	3.9 ± 0.03	3.9 ± 0.07	3.9 ± 0.09	4.0 ± 0.03	3.9 ± 0.03
Phe + Tyr	7.2 ± 0.09	7.1 ± 0.03	7.2 ± 0.07	7.1 ± 0.07	7.2 ± 0.12
Thr	5.1 ± 0.37	5.0 ± 0.35	5.1 ± 0.40	5.4 ± 0.38	5.5 ± 0.43
Val	3.2 ± 1.33	4.5 ± 0.06	4.5 ± 0.09	4.5 ± 0.07	4.5 ± 0.15

Values (mean ± SE of three replications) in the same row not sharing a common superscript are significantly different ($P < 0.05$)
^{ns} Not significant ($P > 0.05$)

Dietary DDG and CF diet did not affect the moisture, crude protein, and amino acid composition of the whole body of fish (P>0.05). Whole body content of crude lipid in fish fed the DDG1 and CF diets was not different from fish fed the DDG0 diet, but was higher in fish fed the DDG2 and DDG3 diets than in fish fed the DDG0 diet. Whole body content of ash in fish fed the DDG3 diet was not different from fish fed the DDG0 diet, but was lower in fish fed the DDG1, DDG2 and CF diets compared to DDG0. The results of hematological parameters of plasma in juvenile olive flounder are shown in Table 5.

Table 5. Hematological parameters of the plasma in juvenile olive flounder fed the experimental diets for 7 weeks.

	Diets				
	DDG0	*DDG1*	*DDG2*	*DDG3*	*CF*
Total protein (g/l)	29 ± 0.5^{c}	27 ± 1.5^{bc}	28 ± 0.1^{c}	24 ± 1.4^{a}	30 ± 1.3^{c}
Glucose (m mol/l)	1.2 ± 0.1^{ns}	1.5 ± 0.3	1.3 ± 0.3	1.8 ± 0.2	1.16 ± 0.1
GOT (IU/l)	13.7 ± 1.2^{ns}	16.3 ± 1.9	27.0 ± 3.0	17.3 ± 2.6	23.0 ± 6.1
Cholesterol (m mol/l)	5.1 ± 0.3^{b}	3.9 ± 0.5^{ab}	4.4 ± 0.3^{ab}	3.6 ± 0.5^{a}	6.3 ± 0.1^{c}
Triglyceride (m mol/l)	0.93 ± 0.02^{b}	0.48± 0.01^{a}	0.62 ± 0.10^{a}	0.39 ± 0.07^{a}	0.91± 0.12^{b}

Values (mean ± SE of three replications) in the same row not sharing a common superscript are significantly different ($P < 0.05$)
^{ns} Not significant ($P > 0.05$)

There was no difference among treatments for plasma glucose content and GOT activity. Plasma contents of total protein in fish fed the DDG1, DDG2 and CF diets was not different from fish fed the DDG0 diet, but was lower in fish fed the DDG3 diet. Plasma content of cholesterol in fish fed the DDG1 and DDG2 diets were not different to fish fed the DDG0 diet, but was lower in fish fed the DDG3 diet compared to the DDG0 and CF diets. Plasma content of triglyceride in fish fed the DDG1, DDG2, and DDG3 diets was lower than that of fish fed the DDG0 and CF diets ($P<0.05$). The results of radical scavenging activities of the plasma in juvenile olive flounder are presented in Table 6.

Table 6. Radical scavenging activity of the plasma in juvenile olive flounder fed the experimental diets for 7 weeks.

	Diets				
	DDG0	*DDG1*	*DDG2*	*DDG3*	*CF*
Radical scavenging activity (%)					
DPPH radical	66.9 ± 1.0^{ns}	62.6 ± 3.2	66.2 ± 1.1	64.7 ± 1.8	65.3 ± 0.4
Hydroxyl radical	39.0 ± 11.2^{ns}	45.3 ± 12.3	41.1 ± 4.8	44.5 ± 7.3	56.5 ± 5.1
Alkyl radical	79.6 ± 1.2^{ns}	81.7 ± 1.4	82.6 ± 1.1	78.9 ± 3.4	72.7 ± 6.9
Superoxide radical	23.5 ± 7.5^{ns}	30.9 ± 3.0	32.1 ± 6.0	35.8 ± 2.9	33.0± 9.5

Values are presented as mean ± SE of triplicate groups
^{ns} Not significant ($P > 0.05$)

DPPH, hydroxyl, alkyl and superoxide radical scavenging activities in the plasma of fish were not affected by dietary DDG and CF diet (P>0.05).

Discussion

The results demonstrate that up to 20% DDG from rice in the diet, is sufficient for juvenile olive flounder. The current findings indicate that rice-based DDG is a potential feed ingredient for juvenile olive flounder. DDG from rice could also be included in juvenile black seabream diets as a partial replacement for wheat flour and corn gluten meal without affecting growth performance (Rahman et al., 2013). A scientific study evaluating rice-based DDG as partial replacement for soybean meal and wheat flour in juvenile sea cucumber diet showed satisfactory growth performance (Choi et al., 2013). Incorporation of up to 20% rice-based DDG could be used as a feed ingredient for juvenile sea cucumber without negatively affecting growth performance (Seo et al. 2011). Rice-based DDG was also found to be successful in rockfish diets without adversely affecting fish growth performance (Choi et al., 2014a). Up to 30% rice-based DDG was found to be a suitable replacement for soybean meal and wheat flour in juvenile abalone diets (Choi et al. 2014b). The present results in the growth performance of juvenile olive flounder are entirely consistent with these findings.

High levels of DDG in the diet significantly increased fat deposition in channel catfish (Robinson and Li, 2008). In this study, high levels of dietary DDG increased whole body lipid deposition, although daily feed intake was not affected by dietary DDG level. A significant hypocholesterolemic effect on rainbow trout with dietary soybean meal was also observed (Kaushik et al. 1995). With increasing dietary corn gluten meal, a decline in plasma triglyceride concentrations in yellowtail has been observed (Shimeno et al., 1993). In the present study, DDG elicited reduction of plasma total protein, cholesterol and triglyceride concentrations in juvenile olive flounder fed diets containing high levels of DDG in diets. Decreased plasma content of fish fed diets containing a high level of DDG may be due to lipid metabolism leading to poor growth rate in juvenile olive flounder. Similar findings of reduced plasma cholesterol and triglyceride concentrations in turbot fed diets containing high dietary corn gluten meal have been reported (Regost et al., 1999).

A direct relationship between antioxidant activity and total phenolic content material in herbs and grain products was found (Chew et al., 2009; Velioglu et al., 1998). DPPH radical may be used to determine free radical scavenging effects on antioxidant substances, plant and fruit extracts, and food materials (Wong et al., 2006; Matsukawa et al., 1997). To our understanding, there are no published data available on scavenging activities in fish fed diets containing DDG as a feed ingredient. DPPH radical scavenging activity in the plasma of juvenile rockfish was increased using diets containing high levels of rice-based DDG (Choi et al. 2014a). Hydroxyl and alkyl radical scavenging activities in the plasma of juvenile rockfish were not different among diets containing rice-based DDG (Choi et al. 2014a). Superoxide anion radicals play an important role in the formation of single oxygen and hydroxyl radicals. They can magnify cellular damage by increasing other free radicals and oxidizing agents (Athukorala et al., 2006). In the present study, DPPH, hydroxyl, alkyl and superoxide radical scavenging activities were not significantly different among the treatments. This result suggests that phenolic compound of DDG in diet did not affect in radical scavenging activities of juvenile olive flounder.

The results of this study suggest that DDG can replace plant origin sources, such as wheat flour and corn gluten meal, and could be used in levels up to 20% in the diet without affecting the growth performance of juvenile olive flounder.

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