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Evaluation of Rice Distillers Dried Grain as a Partial Replacement for Fish Meal in the Practical Diet of the Juvenile Olive Flounder *Paralichthys olivaceus*

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Abstract

We evaluated the effects of rice distillers dried grain (DDG) as a partial replacement for fish meal in the practical diet on growth performance, feed utilization, and body composition of juvenile olive flounder *Paralichthys olivaceus*. Six isonitrogenous and isocaloric diets were formulated to contain 0%, 7%, 14%, 21%, 28%, and 35% DDG (designated DDG0, DDG7, DDG14, DDG21, DDG28, and DDG35, respectively). Three replicate groups of juvenile olive flounder averaging 9.6 ± 0.2 g were fed one of the experimental diets to visual satiety twice daily for 8 weeks. Neither survival nor daily feed intake was affected by the dietary DDG levels. Weight gain of the flounder fed the DDG28 and DDG35 diets was lower than that of flounder fed the DDG7 diet. The feed efficiency of flounder fed the DDG28 diet was lower than that of flounder fed the DDG0, DDG7, and DDG14 diets. The protein efficiency ratio of flounder fed the DDG28 diet was lower than that of flounder fed the DDG7 diet. The proximate composition of muscle was not affected by the dietary DDG levels. The plasma contents of total protein, glucose, cholesterol, glutamate oxalo-acetate transaminase, phospholipid, and triglyceride were not affected by the dietary DDG levels. The results of this experiment suggest that DDG has the potential to replace fish meal and could be used up to 21% DDG without any negative effects on the growth and feed utilization of juvenile flounder.

Key words: *Paralichthys olivaceus*, Olive flounder, Distillers dried grain, Growth

Introduction

Protein is the most expensive dietary nutrient component in aquaculture feeds and significantly affects the growth performance of aquatic species. Fish meal has been used as a major protein source in fish diets because of its good quality and palatability. However, when global fish meal production declines relative to demand and fish meal costs increase, fish nutritionists begin evaluating cost-effective alternative protein sources originating from plants. Many protein sources have been investigated as potential partial replacements for fish meal in feeds for fish (Allen et al., 2000; Lee, 2002; Albrektsen et al., 2006; Santigosa et al., 2008; Dapra et al., 2009). The efficiency of alternative protein sources as partial replacements for fish meal has been evaluated for many carnivorous cultured fish (Regost et al., 1999; Robaina et al., 1999; Elangovan and Shim, 2000).

Among the protein sources available for animal feeds, cereal by-product is a good candidate for partial replacement of fish meal protein in diets (Chevanan et al., 2010). One alternative protein source is distillers dried grain (DDG), a cereal by-product of the distillation process. Corn-based

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DDG has been widely used as a protein source for ruminant feed (Firkins et al., 1985; Larson et al., 1993; Ham et al., 1994; Lodge et al., 1997; Ganesan et al., 2008). DDG is also considered to be a good feed ingredient for fish because it has a relatively high protein content (Zhou et al., 2010; Li et al., 2011). Research studies have been conducted on the use of corn-based DDG as a protein source in feeds for freshwater fishes such as rainbow trout *Oncorhynchus mykiss* (Randall and Drew, 2010; Barnes et al., 2012), channel catfish *Ictalurus punctatus* (Tidwell et al., 1990; Lim et al., 2009), tilapia *Oreochromis niloticus* (Lim et al. 2007; Schaeffer et al., 2009), and sunshine bass *Morone chrysops* × *M. saxatilis* (Thompson et al., 2008). Schaeffer et al. (2009) reported that supplementation of corn-based DDG as a protein source may provide aquaculture operations with a cost-effective substitute for fish meal. One study reported that the supplementation of rice-based DDG in feed could improve the growth performance of the sea cucumber *Apostichopus japonicus* (Seo et al., 2011). Our earlier study suggested that rice-based DDG might be an economical ingredient for low-cost feed and allow a reasonable amount of wheat flour substitution in juvenile black seabream *Acanthopagrus schlegelii* and olive flounder diets (Rahman et al., 2013, 2015). However, no information is available on the potential use of rice DDG as a replacement for fish meal in the practical diet of flounder.

The olive flounder is one of the most important marine cultured fish species in Asia because of its advantages such as fast growth, the establishment of seedling production, and developed culture techniques. Based on our findings, we hypothesized that rice DDG in the olive flounder diet might improve growth and reduce feed costs. Therefore, the objectives of present feeding trial were to evaluate the effects of dietary rice DDG as a partial replacement for fish meal in the practical diet on growth performance, feed utilization, and body composition of juvenile olive flounder.

Materials and Methods

Experimental diets

The essential amino acid and proximate composition of protein sources used in the experimental diets are presented in Table 1. The ingredients and chemical composition of the experimental diets are presented in Table 2. Six isonitrogenous (50% crude protein) and isocaloric experimental diets were formulated to contain 0%, 7%, 14%, 21%, 28%, and 35% DDG, designated DDG0, DDG7, DDG14, DDG21, DDG28, and DDG35, respectively. Lysine was added to the diets containing DDG to normalize its concentration across the diets. DDG used in this study was produced by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts during the manufacturing process of Makgeolli, a traditional alcoholic beverage native to Korea. The DDG was produced by the Gangneung Makgeolli Factory (Gangneung, Korea). The ingredient was dried at 60°C for 24 h and finely ground prior to incorporation into the experimental diets. All ingredients were thoroughly mixed with 300 g/kg of distilled water, and pellets were prepared using a laboratory moist pelleting machine. The pellets were dried at room temperature for 48 h and ground into desirable particle sizes. All diets were stored at –30°C until used.

Fish and feeding trial

Juvenile olive flounder were transported from a private

Table 1. Composition of proximate and essential amino acid of the major ingredients of experimental diets

	Ingredients			
	Fish meal	Soybean meal	Wheat flour	Distillers dried grain powder*
Proximate composition (% DM)				
Crude protein	72.6	51.2	19.3	30.4
Crude lipid	6.5	1.2	3.9	3.3
Ash	16.8	6.8	2.2	0.8
Essential amino acid composition (% of protein)				
Arg	4.2	5.2	5.7	4.1
His	2.0	1.8	2.9	1.3
Ile	3.8	3.8	2.3	3.5
Leu	7.2	6.8	6.0	7.5
Lys	6.1	4.9	3.7	2.1
Met + Cys	2.5	1.6	2.8	4.3
Phe + Tyr	5.1	5.7	6.8	6.4
Thr	4.7	4.1	3.5	4.1
Val	5.6	4.2	3.2	5.2

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

hatchery (Chungnam, Korea) to the Marine Biology Center for Research and Education at Gangneung-Wonju National University and acclimated to laboratory conditions by feeding commercial pellets for 2 weeks before starting the feeding trial. After the conditioning period, juvenile olive flounder (mean body weight, 9.6 ± 0.2 g) were randomly distributed in eighteen 300-L fiberglass reinforced tanks (250 L seawater each) at densities of 30 fish per tank. Each experimental diet was fed to three replicate groups of fish to visual satiation twice daily (9:00 and 17:00 h) for 8 weeks. Filtered seawater was supplied at a flow rate of 5 L/min in each tank; the mean water temperature, salinity, dissolved oxygen concentration, and pH were 16.7 ± 1.8°C, 34.0 ± 0.1 ppt, 7.5 ± 0.8 ppm, and 7.7 ± 0.2, respectively. The photoperiod was left under natural conditions during the feeding trial. Records were kept of daily feed consumption, mortalities, and feeding behavior.

Fish sampling and chemical analysis

At the end of the feeding trial, fish in each tank were collectively weighed after being fasted and anesthetized with tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO, USA) solution at a concentration of 100 mg/L to calculate growth performance and feed utilization. The total length and whole body, liver, and visceral weights of five fish per tank were measured to calculate the condition factor and the hepatosomatic and visceralsomatic indexes. Five fish per tank were sampled and stored at –25°C for whole-body proximate analy-

Table 2. Ingredients and chemical composition of the experimental diets

	Diets					
	DDG0	DDG7	DDG14	DDG21	DDG28	DDG35
Ingredients (%)						
Fish meal	54.0	52.0	50.0	48.0	46.0	44.0
Distillers dried grain power*	0.0	7.0	14.0	21.0	28.0	35.0
Wheat flour	26.0	20.8	15.7	10.5	5.4	0.2
Soybean meal	5.0	5.0	5.0	5.0	5.0	5.0
α-starch	5.0	5.0	5.0	5.0	5.0	5.0
Wheat gluten	4.0	4.0	4.0	4.0	4.0	4.0
Squid liver oil	2.9	3.0	3.0	3.1	3.1	3.2
Vitamin premix†	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix‡	1.5	1.5	1.5	1.5	1.5	1.5
Choline	0.1	0.1	0.1	0.1	0.1	0.1
L-Lysine HCl§	0.0	0.1	0.2	0.3	0.4	0.5
Nutrient content (% DM)						
Crude protein	50.5	50.1	49.6	49.5	48.2	48.5
Crude lipid	7.4	7.7	8.2	8.8	9.2	8.5
Ash	11.3	10.8	10.2	9.7	9.1	8.7
Carbohydrate¶	30.8	31.4	32.0	32.0	33.5	34.3
Gross energy(kcal/g diet)#	4.8	4.8	4.9	4.9	5.0	4.9
Essential amino acid composition (% of protein)						
Arg	3.7	4.0	4.0	4.1	4.1	3.8
His	2.7	2.9	2.6	2.4	2.3	2.1
Ile	3.4	3.7	3.7	3.7	3.8	3.5
Leu	6.1	6.6	6.7	6.8	6.8	6.3
Lys	5.1	5.3	5.4	5.3	5.5	5.0
Met + Cys	1.7	2.0	2.0	2.1	2.1	2.0
Phe + Tyr	4.4	4.9	5.0	5.1	5.2	4.9
Thr	3.8	4.1	4.1	4.1	4.2	3.8
Val	4.9	5.2	5.3	5.4	5.5	5.1

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

†Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 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.

‡Mineral premix contained the following ingredients (g/kg mix): $MgSO_4·7H_2O$, 80.0; $NaH_2PO_4·2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4·7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3·6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4·H_2O$, 2.0; $CoCl_2·6H_2O$, 1.0.

§L-lysine mono-hydrochloride, Sigma, USA.

¶Carbohydrate = 1,000 - (crude protein + crude lipid + ash).

#Calculated based on 5.64 kcal/g protein, 9.44 kcal/g lipid and 4.11 kcal/g carbohydrate.

sis. Blood samples were taken from the caudal veins of five fish per tank using heparinized syringes. Plasma was collected after centrifugation at 7,500 *g* for 10 min, and the plasma was separated and stored at –70 ºC for analysis.

The proximate composition was analyzed according to standard methods (AOAC, 1995). Crude protein was determined by the Kjeldahl method using an Auto Kjeldahl System (Büchi Labortechnik AG, Flawil, Switzerland). Crude lipid was analyzed with ether extraction in a Soxhlet extractor (VELP Scientifica, Milano, Italy), and moisture was determined using a dry oven at 105ºC for 12 h. The ash content was determined after combustion at 550ºC for 4 h in a muffle furnace. The amino acid composition in the experimental diets and dorsal muscle of juvenile flounder was analyzed using an automatic amino acid analyzer (Hitachi, Tokyo, Japan). The plasma total protein, glucose, cholesterol, glutamate oxaloacetate transaminase, phospholipid, and triglyceride concentrations were determined using a clinical investigation commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

Statistical analysis

The data were subjected to one-way analysis of variance, and if statistically significant (P < 0.05) differences were found, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. The data are presented as the mean ± standard error of three replicate groups. All statistical analyses were carried out using SPSS Version 19 (SPSS, Michigan Avenue, Chicago, Illinois, USA).

Results

The growth performance, feed utilization, and morphological parameters of juvenile olive flounder fed the experimental diets containing different levels of DDG are presented in Table 3. The survival, daily feed intake, and daily protein intake of flounder were not affected by the dietary DDG levels (P > 0.05). The weight gain of flounder fed diets containing DDG was not different from those of flounder fed DDG0, but the weight gain of flounder fed the DDG28 and DDG35 diets was lower than that of flounder fed the DDG7 diet (P < 0.05). The feed efficiency of flounder fed the DDG28 diet was lower than that of flounder fed the DDG0, DDG7, and DDG14 diets (P < 0.05). The protein efficiency ratio of flounder fed the DDG28 diet was lower than that of flounder fed the DDG7 diet (P < 0.05). The condition factor, hepatosomatic index, and viscerалsomatic index were not affected by the dietary DDG levels (P > 0.05).

The whole-body and muscle proximate compositions of juvenile olive flounder fed the experimental diets are presented in Table 4. No significant differences were found in the whole-body moisture, crude protein, or crude lipid contents of flounder fed different dietary DDG levels, but flounder fed the DDG35 diet had higher body ash contents than flounder fed the DDG0 diet (P < 0.05). The muscle proximate composition of flounder was not affected by the dietary DDG levels (P > 0.05). The amino acid compositions of the dorsal muscle of juvenile olive flounder fed the experimental diets are presented in Table 5. DDG did not alter the muscle amino acid

Table 3. Growth performance, feed utilization and morphological parameters of juvenile olive flounder fed the experimental diets for 8 weeks

	Diets					
	DDG0	DDG7	DDG14	DDG21	DDG28	DDG35
Initial body weight (g fish^{-1})	9.7 ± 0.01^{ns}	9.6 ± 0.15	9.6 ± 0.06	9.5 ± 0.06	9.5 ± 0.03	9.8 ± 0.12
Survival (%)	99 ± 1.00^{ns}	99 ± 1.0	99 ± 1.0	93 ± 5.2	91 ± 3.0	95 ± 3.9
Weight gain (%)*	292 ± 1.2^{abc}	307 ± 27.3^{c}	300 ± 21.2^{bc}	274 ± 3.0^{abc}	245 ± 16.0^{a}	248 ± 8.3^{ab}
Feed efficiency (%)†	107 ± 2.9^{b}	109 ± 3.6^{b}	105 ± 2.3^{b}	99 ± 1.2^{ab}	93 ± 4.3^{a}	100 ± 2.2^{ab}
Daily feed intake‡	1.81 ± 0.05^{ns}	1.81 ± 0.01	1.87 ± 0.02	1.86 ± 0.06	1.85 ± 0.04	1.78 ± 0.05
Daily protein intake§	0.91 ± 0.03^{ns}	0.91 ± 0.01	0.93 ± 0.01	0.92 ± 0.03	0.89 ± 0.02	0.86 ± 0.03
Protein efficiency ratio¶	2.12 ± 0.06^{ab}	2.16 ± 0.07^{b}	2.11 ± 0.05^{ab}	2.00 ± 0.02^{ab}	1.92 ± 0.09^{a}	2.07 ± 0.05^{ab}
Condition factor#	1.15 ± 0.03^{ns}	1.18 ± 0.02	0.58 ± 0.30	0.80 ± 0.28	1.10 ± 0.02	1.09 ± 0.05
Hepatosomatic index**	1.82 ± 0.08^{ns}	2.00 ± 0.33	1.92 ± 0.20	2.20 ± 0.38	2.20 ± 0.20	1.90 ± 0.25
Visceralsomatic index††	2.78 ± 0.06^{ns}	3.06 ± 0.21	3.33 ± 0.14	3.22 ± 0.36	3.23 ± 0.21	3.37 ± 0.39

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).
*Weight gain = (final fish wt. - initial fish wt.) × 100 / initial fish wt.
†Feed efficiency = wet weight gain × 100 / feed intake.
‡Daily feed intake = feed intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.)/2 × days reared].
§Daily protein intake = protein intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.)/2 × days reared].
¶Protein efficiency ratio = (wet weight gain / protein intake).
#Condition factor = fish weight (g) × 100 / fish length (cm)^3.
**Hepatosomatic index = liver weight × 100 / body weight.
††Visceralsomatic index = viscera weight × 100 / body weight.

profile of juvenile olive flounder, except for histidine. Histidine in the muscle of flounder fed the DDG21, DDG28, and DDG35 diets was lower than that in flounder fed the DDG0, DDG7, and DDG14 diets ($P < 0.05$). Hematological parameters of the plasma in juvenile olive flounder fed the experimental diets are presented in Table 6. The plasma contents of total protein, glucose, cholesterol, glutamate oxaloacetate transaminase, phospholipid, and triglyceride were not affected by the dietary DDG levels ($P > 0.05$).

Table 4. Proximate compositions (% DM) of the whole body and muscle of juvenile olive flounder fed the experimental diets for 8 weeks

Diets	Moisture	Crude protein	Crude lipid	Ash
Whole body				
DDG0	74.7 ± 0.9^{ns}	18.2 ± 0.9^{ns}	2.9 ± 0.2^{ns}	3.1 ± 0.2^{a}
DDG7	74.3 ± 0.9	17.1 ± 0.5	2.9 ± 0.2	2.7 ± 0.2^{a}
DDG14	75.8 ± 0. 2	18.1 ± 0.8	2.9 ± 0.4	3.2 ± 0.2^{ab}
DDG21	75.3 ± 0.3	16.8 ± 0.4	2.5 ± 0.2	3.1 ± 0.1^{a}
DDG28	76.4 ± 1.5	16.6 ± 0.4	2.9 ± 0.4	3.2 ± 0.1^{a}
DDG35	74.6 ± 0.7	17.3 ± 0.5	2.6 ± 0.3	3.8 ± 0.2^{b}
Dorsal muscle				
DDG0	75.9 ± 0.5^{ns}	20.0 ± 0.1^{ns}	0.5 ± 0.1^{ns}	1.3 ± 0.04^{ns}
DDG7	77.2 ± 0.4	19.2 ± 0.3	0.5 ± 0.2	1.5 ± 0.05
DDG14	76.8 ± 0.7	19.7 ± 0.5	0.6 ± 0.05	1.4 ± 0.04
DDG21	77.5 ± 0.1	19.1 ± 0.1	0.4 ± 0.03	1.4 ± 0.04
DDG28	77.4 ± 0.6	19.0 ± 0.1	0.4 ± 0.02	1.4 ± 0.03
DDG35	77.1 ± 0.1	19.2 ± 0.2	0.4 ± 0.2	1.5 ± 0.10

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

Table 5. Amino acid composition (% of protein) of the dorsal muscle of juvenile olive flounder fed the experimental diets for 8 weeks

Diets	DDG0	DDG7	DDG14	DDG21	DDG28	DDG35
Arg	5.1 ± 0.09^{ns}	5.1 ± 0.07	5.2 ± 0.09	5.0 ± 0.09	5.1 ± 0.03	4.9 ± 0.17
His	1.9 ± 0.03^{b}	2.0 ± 0.03^{b}	2.0 ± 0.03^{b}	1.8 ± 0.03^{a}	1.8 ± 0.03^{a}	1.8 ± 0.02^{a}
Ile	5.0 ± 0.03^{ns}	4.9 ± 0.07	5.0 ± 0.09	4.9 ± 0.15	5.0 ± 0.01	4.8 ± 0.15
Lys	7.5 ± 0.07^{ns}	7.7 ± 0.09	7.6 ± 0.30	7.6 ± 0.23	7.4 ± 0.06	7.5 ± 0.23
Met + Cys	2.7 ± 0.03^{ns}	2.8 ± 0.07	2.8 ± 0.06	2.7 ± 0.06	2.6 ± 0.07	2.6 ± 0.12
Phe + Tyr	6.1 ± 0.15^{ns}	6.1 ± 0.03	6.2 ± 0.12	6.1 ± 0.12	6.1 ± 0.06	5.9 ± 0.21
Thr	5.3 ± 0.03^{ns}	6.2 ± 0.95	6.1 ± 0.88	5.3 ± 0.07	5.2 ± 0.33	5.1 ± 0.19
Val	5.7 ± 0.03^{ns}	5.6 ± 0.07	5.7 ± 0.03	5.6 ± 0.09	5.6 ± 0.03	5.4 ± 0.23

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

Table 6. Hematological parameters of the plasma in juvenile olive flounder fed the experimental diets for 8 weeks

	Diets					
	DDG0	DDG7	DDG14	DDG21	DDG28	DDG35
Total protein (g/dL)	3.6 ± 0.25^{ns}	4.3 ± 0.43	3.5 ± 0.13	3.5 ± 0.15	3.5 ± 0.2	3.4 ± 0.02
Glucose (mg/ dL)	26.0 ± 3.06^{ns}	31.7 ± 6.17	23.3 ± 2.33	22.7 ± 1.20	32.7 ± 8.84	30.3 ± 5.24
Cholesterol (mg/dL)	234 ± 7.2^{ns}	255 ± 18.9	236 ± 15.0	237 ± 5.2	230 ± 18.3	230 ± 18.0
GOT (IU/L)	7.3 ± 2.85^{ns}	2.3 ± 0.33	3.3 ± 0.88	3.7 ± 0.33	5.7 ± 0.33	9.3 ± 3.48
Phospholipid (mg/dL)	622 ± 31.3^{ns}	700 ± 49.3	674 ± 39.1	677 ± 16.4	662 ± 51.3	624 ± 41.1
Triglyceride (mg/dL)	115 ± 20.3^{ns}	265 ± 36.0	166 ± 15.7	193 ± 44.2	209 ± 30.9	166 ± 36.4

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

Discussion

The results of the present study show that dietary supplementation up to 21% DDG in the formulated diets did not affect the growth performance, feed utilization, or morphological parameters of juvenile olive flounder. These results indicate that the rice DDG used in this study may be considered a potential feed ingredient as a replacement for fish meal for juvenile olive flounder. Many researchers have worked on incorporation of corn-based DDG in aquaculture feeds (Chevanan et al., 2007, 2010; Kannadhason et al., 2009). Studies have found satisfactory growth performance in tilapia fed diets containing corn-based DDG as replacement for fish meal (Coyle et al., 2004; Lim et al., 2007; Shelby et al., 2008; Schaeffer et al., 2009; Abo-state et al., 2009). Webster et al. (1993) and Robinson and Li (2008) showed that corn-based DDG could be added to channel catfish diets without negative effects on growth performance. It was also shown that corn-based DDG could be incorporated into rainbow trout diets (Cheng and Hardy, 2004; Stone et al., 2005; Barnes et al., 2012). Rahman et al. (2015) reported that DDG is an effective replacement for wheat flour and could be used up to 14% for the growth of juvenile olive flounder. In a previous study of flounder (Rahman et al., 2015), experimental diets were formulated to contain different levels of DDG, replacing plant origin ingredient with same level of 60% fish meal in all diets. In this study, DDG was used as a replacement for fish meal in the control diet containing 54% fish meal. When DDG was used at 21% in the flounder diet, fish meal could be decreased from 54% to 48% in the diet without any negative effects on growth or feed efficiency. Our results suggest that DDG is a good replacement not only for plant materials such as wheat flour and corn gluten meal, but also for fish meal in the practical diet of juvenile olive flounder.

The generally poor growth performance observed in fish fed plant protein is related to palatability, amino acid imbalance, phosphorus availability, and anti-nutritional factors (Gomes et al., 1995). Ye et al. (2011) reported that replacement of fish meal protein could adversely affect the growth and metabolism of protein and lipid of olive flounder. Similarly, it was observed that growth of the turbot *Scophthalmus maximus* gradually decreased with increasing levels of soybean protein concentrate (Day and Plascencia Gonzalez, 2000). Previous studies have reported reductions in growth and feed utilization of fish fed plant protein due to imbalanced dietary amino acids, reduced mineral content, increased fiber, reduced palatability, and the presence of anti-nutrient factors (Lim and Lee, 2009). Fibers and anti-nutrients are related to reduce digestibility in fish (Francis et al., 2001). In the present study, decreased growth performance and feed utilization at high dietary inclusion levels of DDG may be associated with various factors including increased carbohydrate levels, reduced feed palatability, the presence of anti-nutrients, and an imbalanced dietary amino acid profile (Francis et al., 2001; Hemre et al., 2003; Opstvedt et al., 2003).

Li et al. (2011) reported that fish fed diets containing corn-based DDG tended to have higher body ash contents than fish fed the control diet. In the present study, flounder fed the DDG35 diet showed a significantly higher body ash content than did the control group. This result may be due to the relatively lower body weight of fish fed high-DDG diets than fish fed low-DDG diets. The amino acid profile of fish muscle generally reflects the amino acid profile of the diet. Hence, the lower level of histidine in fish muscle may be a consequence of the amino acid content of the experimental diets. Lim et al. (2007) and Li et al. (2011) reported that different levels of corn-based DDG did not significantly influence the hematological parameters of Nile tilapia. Similar findings were observed for the hematological parameters of juvenile olive flounder in the present study.

The results of this experiment suggest that DDG has the potential to replace fish meal and wheat flour and could be used up to 21% DDG without any negative effects on the growth or feed utilization of juvenile flounder.

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References

Abo-state HA, Tahoun AM and Hammouda YA. 2009. Effects of replacement of soybean meal by DDGS combined with commercial phytase on Nile tilapia (*Oreochromis niloticus*) fingerlings growth performance and feed utilization. American-Eurasian J Agricult Environ Sci 5, 473-479.

Albrektsen S, Mundheim H and Aksnes A. 2006. Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gaddus morhua*) fed two different fish meal qualities at three dietary levels of vegetable protein sources. Aquaculture 261, 626-640.

Allan GL, Parkinson S, Booth MA, Stone DAJ, Rowland SJ, Frances J and Warner-Smith R. 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus:* I. Digestibility of alternative ingredients. Aquaculture 186, 293-310.

AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis of the association of Official Analytical Chemists (16^{th} edn). Association of Official Analytical Chemists, Arlington, VA, USA.

Barnes ME, Brown ML and Rosentrater KA. 2012. Initial observations on the inclusion of high protein distillers dried grain into rainbow trout diets. Open Fish Sci J. 5, 21-29.

Cheng ZJ and Hardy RW. 2004. Nutritional value of diets containing distiller's dried grain with solubles for rainbow trout, *Oncorhyn-*

chus mykiss. J Appl Aquac 15, 101-113.

Chevanan N, Rosentrater KA and Muthukumarappan K. 2007. Twin-screw extrusion processing of feeds blends containing distillers dried grains with solubles (DDGS). Cereal Chem 84, 428-436.

Chevanan N, Rosentrater KA and Muthukumarappan K. 2010. Effects of processing conditions on single screw extrusion of feed ingredients containing DDGS. Food Bioprocess Technol 3, 111-120.

Coyle SD, Mengel GJ, Tidwell JH and Webster CD. 2004. Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* ×*Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquac Res 35, 365-370.

Dapra F, Gai F, Costanzo MT, Maricchiolo G, Micale V, Sicuro B, Caruso G, Genovese L and Palmegiano GB. 2009: Rice protein-concentrate meal as a potential dietary ingredient in practical diets for blackspot seabream *Pagellus bogaraveo*: a histological and enzymatic investigation. J Fish Biol 74, 773-789.

Day OJ and Plascencia Gonzalez HG. 2000. Soybean protein concentrate as a protein source for turbot (*Scophthalmus maximus* L). Aquac Nutr 6, 221-228.

Duncan DB. 1955. Multiple-range and multiple F tests. Biometrics 11, 1-42.

Elangovan A and Shim KF. 2000. The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). Aquaculture 189, 133-144.

Firkins JL, Berger LL and Fahey Jr GC. 1985. Evaluation of wet and dry distillers grains and wet and dry corn gluten feeds for ruminants. J Anim Sci 60, 847-860.

Francis G, Makkar HPS and Becker K. 2001. Antinutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227.

Ganasan V, Rosentrater KA and Muthukumarappan K. 2008. Flowability and handling characteristics of bulk solids and powders- a review with implication for DDGS. Biosystems Engineering 101, 425-435.

Gomes EF, Rema P and Kaushik SJ. 1995. Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture 130, 177-186.

Ham GA, Stock RA, Klopfenstein TJ, Larson EM, Shain DH and Huffman RP. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J Anim Sci 72, 3246-3257.

Hemre GI, Karlsen O, Mangor-Jensen A and Rosenlund G. 2003. Digestibility of dry matter, protein, starch and lipid by cod, *Gadus morhua*: comparison of sampling methods. Aquaculture 225, 225-232.

Kannadhason S, Muthukumarappan K and Rosentrater KA. 2009. Effects of ingredients and extrusion on aquafeeds containing DDGS and tapioca starch. J Aquac Feed Sci Nutr 1, 6-21.

Larson EM, Stock RA, Klopfenstein TJ, Sindt MH and Huffman RP. 1993. Feeding value of wet distillers byproducts from finishing ruminants. J Anim Sci 71, 2228-2236.

Lee SM. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture 207, 79-95.

Li E, Lim C, Cai C and Klesius PH. 2011. Growth response and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*, fed diets containing different levels of wheat distillers's dried grains with solubles with or without lysine supplementation. Anim Feed Sci Technol 170, 246-255.

Lim SJ and Lee KJ. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture 290, 283-289.

Lim C, Garcia JC, Yildirim-Aksoy M, Klesius PH, Shoemaker CA and Evans JJ. 2007. Growth response and resistance to streptococcus iniae of Nile tilapia, *Oreochromis niloticus*, fed diets containing distiller's dried grains with solubles. J. World Aquac Soc 38, 231-237.

Lim C, Yildirim-Aksoy M and Klesius PH. 2009. Growth response and resistance to *Edwardsiella ictaluri* Channel Catfish, *Ictalurus punctatus,* fed diets containing distiller's dried grains with solubles. J World Aquac Soc 40, 182-193.

Lodge SL, Stock RA, Klopfenstein TJ, Shain DH and Herold DW. 1997. Evaluation of corn and sorghum distillers byproducts. J Anim Sci 75, 37-43.

Opstvedt J, Aksnes A, Hope B and Pike IH. 2003. Efficiency of feed utilization in Atlantic salmon (*Salmo salar* L.) fed diets with increasing substitution of fishmeal with vegetable proteins. Aquaculture 221, 365-379.

Rahman MM, Choi J and Lee SM. 2013. Use of distillers dried grain as partial replacement of wheat flour and corn gluten meal in the diet of juvenile black seabream (*Acanthopagrus schlegeli*). Turkish J Fish Aquat Sci 13, 699-706.

Rahman MM, Choi J and Lee SM. 2015. Influences of dietary distillers dried grain level on growth performance, body composition and biochemical parameters of juvenile olive flounder (*Paralichthys olivaceus*). Aquac Res 46, 39-48.

Randall KM and Drew MD. 2010. Fractionation of wheat distiller's dried grains and solubles using sieving increases digestible nutrient content in rainbow trout. Anim Feed Sci Technol 159, 138-142.

Regost C, Arzel J and Kaushik SJ. 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). Aquaculture 180, 99-117.

Robaina L, Corraze G, Aguirre P, Blanc D, Melcion JP and Kaushik SJ. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. Aquaculture 179, 45-56.

Robinson EH and Li MH. 2008. Replacement of soybean meal in channel catfish, *Ictalurus punctatus*, diets with cottonseed meal and distillers dried grains with solubles. J World Aquac Soc 39, 521-527.

Santigosa E, Medale SF, Kaushik S, Perez-sanchez J and Gallardo MA. 2008. Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response of dietary fish meal replacement by plant protein sources. Aquaculture 282, 68-74.

Schaeffer TW, Brown ML and Rosentrater KA. 2009. Performance characteristics of Nile tilapia (*Oreochromis niloticus*) fed diets containing graded levels of fuel-based distillers dried grains with solubles. J Aquac Feed Sci Nutr 1, 78-83.

Seo JY, Shin IS and Lee SM. 2011. Effect of dietary inclusion of various plant ingredients as an alternative for *Sargassum thunbergii* on growth and body composition of juvenile sea cucumber *Apostochopus japonicas*. Aquac Nutr 17, 549-556.

Shelby RA, Lim C, Yildrim-Aksoy M and Klesius PH. 2008. Effect of distillers dried grains with solubles-incorporated diets on growth, immune function and disease resistance in Nile tilapia (*Oreochromis niloticus* L.). Aquac Res 39, 1351-1353.

Stone DAJ, Hardy RW, Barrows FT and Cheng ZJ. 2005. Effects of extrusion on nutritional value of diets containing corn gluten meal and corn distiller's dried grain for rainbow trout, *Oncorhynchus mykiss*. J Appl Aquac 17, 1-20.

Tidwell JH, Webster CD and Yancey DH. 1990. Evaluation of distiller's grains with solubles in prepared channel catfish diets. Tran Kentucky Acad Sci 52, 135-138.

Thompson KR, Rawles SD, Metts LS, Smith RG, Wimsatt A, Gannam AL, Twibell RG, Johnson RB, Brady YJ and Webster CD. 2008. Digestibility of dry matter, protein, lipid, and organic matter of two fish meals, two poultry by-product meals, soybean meal, and distiller's dried grains with solubles in practical diets for sunshine bass, *Morone chrysops* × *M. saxatilis*. J World Aquac Soc 39, 352-363.

Webster CD, Tidwell JH, Goodgame LS and Johnsen PB. 1993. Growth, body composition, and organoleptic evaluation of channel catfish fed diets containing different percentages of distillers' grains with solubles. Prog Fish-Cult 55, 95-100.

Ye J, Liu X, Wang Z and Wang K. 2011. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder (*Paralichthys olivaceus*). Aquac Int 19, 143-153.

Zhou P, Zhang W, Davis DA and Lim C. 2010. Growth response and feed utilization of juvenile hybrid catfish fed diets containing distillers's dried grains with solubles to replace a combination of soybean meal and corn meal. N Am J Aquac 72, 298-303.