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Use of Distillers Dried Grain as Partial Replacement of Wheat Flour and Corn Gluten Meal in the Diet of Juvenile Black Seabream (*Acanthopagrus schlegeli*)

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Abstract

This research was carried out to investigate the effects of dietary inclusion of distillers dried grain (DDG) level on growth performance, feed utilization, body composition and antioxidant enzyme activity of juvenile black seabream (*Acanthopagrus schlegeli*). Five isonitrogenous and isocaloric diets were formulated that contain 0 (control), 6%, 12%, 18% and 24% DDG designated as DDG0, DDG6, DDG12, DDG18 and DDG24, respectively. Three replicate groups of fish averaging 1.2 ± 0.01 g were fed with one of the experimental diets for visual satiety three times a day for 8 weeks. Weight gain was not affected by dietary DDG level ($P>0.05$). Feed efficiency and protein efficiency ratio of fish fed with DDG24 diet were lower than those of fish fed DDG0 diet ($P<0.05$). Proximate and amino acid composition of whole body were not affected by dietary DDG level. The activities of superoxide dismutase and glutathione peroxidase in the liver were not affected by dietary DDG level ($P>0.05$). The experiment suggested that DDG is a good ingredient to replace plant origin such as wheat flour and gluten meal and could be used up to 24% for the optimum growth performance of juvenile black seabream.

Keywords: Dietary ingredient, distillers dried grain, Black seabream.

Introduction

Aquaculture is currently the fastest growing animal manufacturing field in the world. The rapid expansion of aquaculture market is pronounced a lot in Asia, which attributes concerning 90% of the overall worldwide aquaculture production (Ng, 2003). This increase of aquaculture production must be supported by a corresponding increase in the production of designed diets for the cultured aquatic animals. It is usually acknowledged which the highest continual costs in aquaculture arises from feeds. Consequently, feed accounts for about 60-80% of operational expenditure in demanding aquaculture (Rola and Hasan, 2007). In recent years, the cost of imported feed ingredients used in commercial aqua feeds in many developing countries of Asia has continued to rise due to increased global demand and fluctuation in foreign currency exchange. The rising cost of imported ingredient such as wheat flour decreases into the profit margin of local fish farmers to such an extent that many local aquaculture enterprises are no longer profitable (Ng, 2003). Recently, attempts by fish nutrition experts to diminish feed costs have resulted in increased use of plant ingredients in dietary formulation for fish. Least-cost feed might provide economic positive aspects in the preparation of a nutritionally balanced diet by allowing change in diet formulations when ingredient charges change. There has been interest to replace wheat flour using less expensive carbohydrate source could be effective in decreasing feed cost. Carbohydrates have a number of effective features in aquaculture diets, including pellet binders, precursors to help dispensable amino acids and nucleic acids needed for growth (NRC, 1993). Recently, however because of the rising cost and uncertain availability of wheat flour as a carbohydrate source, we have thought we would investigate alternative carbohydrate source.

Distillers dried grains (DDG) is a cereal by-product of the distillation processing. Belyea *et al*. (2004) reported that DDG is often a useful feed ingredient which is a by-product of the dry-grind resulting from the yeast fermentation of cereal grains. DDG is becoming an increasingly potential ingredient for fish feed due to its low-cost and nutrient contents of protein, vitamin and mineral (Cromwell *et al*., 1993; Zhou *et al*., 2010; Li *et al*., 2011). Generally, DDG has been shown to be a new practical feasible alternative protein and/or energy source for ruminant

animals and fish (Klopfenstein *et al*., 2008; Chevanan *et al*., 2010). Incorporation of DDG into the diet can be far inferior expensive compare to other ingredients such as corn gluten meal or wheat flour. As very little research has been reported pertaining to the use of DDG from rice and limited nutritional information is abailable. Rahman *et al*. (2013) reported that rice-based DDG might offer an inexpensive carbohydrate complement to produce lower-cost feed, particularly for carnivorous species like juvenile olive flounder. Prior scientific studies have demonstrated that corn-based DDG constitutes a promising feed ingredient for a few freshwater fish such as rainbow trout (Cheng and Hardy, 2004a), channel catfish (Tidwell *et al*., 1990; Webster *et al*., 1993; Lim *et al*., 2009), tilapia (Coyle *et al*., 2004; Shelby *et al*., 2008; Lim *et al*., 2007; Schaeffer *et al*., 2009) and sunshine bass (Thompson *et al*., 2008).

Black seabream (*Acanthopagrus schlegeli*) is a commercially important aquaculture marine fish species in Asia. This species offer a number of positive aspects such as fast growth, established seedling production and culture techniques. A part of the effort to reduce the production costs for black seabream, we investigated the extent to which wheat flour can be replaced by carbohydrate sources. Reduction of feed costs could increase the potential for economic gain and make commercial culture of black seabream more feasible. Based on our findings, we conclude that use of DDG in the diet might reduce the feed cost with reasonable growth of fish. Therefore, the aim of this study was to evaluate the possibility of replacing part of dietary DDG with wheat flour and corn gluten meal and to determine possible effects on growth performance, body composition and antioxidant enzyme activity of juvenile black seabream.

Materials and Methods

Experimental Diets

Proximate and essential amino acid compositions used in the experimental diets are presented in Table 1. Ingredients and chemical composition of the experimental diets are presented in Table 2. Five isonitrogenous and isocaloric diets were formulated to contain 0 (control), 6%, 12%, 18% and 24% DDG designated as DDG0, DDG6, DDG12, DDG18 and DDG24, respectively. Pollack fish meal was used as the primary protein source. Cod liver oil was used as lipid source. DDG used in this study was produced by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts in manufacturing process of Makgeolli which is a traditional alcoholic beverage native to Korea. DDG produced from Gangneung Makgeolli factory (Gangneung, Korea) was dried at 60℃ for 24 h and finely ground prior to incorporating in the experimental diets. All ingredients were thoroughly mixed with 30% 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℃ until utilized.

Experimental Fish and Feeding Conditions

Juvenile black seabream were transported from a private hatchery (Namhae, Korea) to the Marine Biology Center for Research and Education at Gangneung-Wonju National University. The fish were acclimated to laboratory conditions by feeding commercial pellets for 2 weeks before starting the feeding trial. After this conditioning period, juvenile black seabream (mean body weight, 1.2±0.01 g) were randomly distributed in fifteen 400-L rectangular plastic tanks at a density of 40 fish per tank, respectively. Each experimental diet was fed to three replicated groups of fish to visual satiation three times per day (09.00, 13.00 and 17.00) for 8 weeks. Each tank was operated as flow-through receiving 4 L min^{-1} filtered seawater and continuously aerated. Water temperature was 16.8±0.39℃.

Fish Sampling and Growth Parameter Evaluation

At the end of the feeding trials, fish in each tank were collectively weighed and counted after being fasted and anaesthetized with tricaine methanesulfonate (MS-222, Sigma, St.Louis, MO, USA) solution 100 ppm to calculate growth performance, feed utilization, and morphological parameters according to the following equations.

Weight gain = (final body weight – initial body weight) × 100 / initial body weight.

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].

Feed efficiency = wet weight gain × 100 / feed intake.

Protein efficiency ratio = wet weight gain / protein intake.

Condition factor = (fish weight / fish length (cm) ^{3}) × 100.

Hepatosomatic index = (liver weight / body weight) × 100.

Visceralsomatic index = (viscera weight / body weight) × 100.

Digestibility Measurements

At the end of feeding trials, remained experimental fish were fed with their respective experimental diets containing 0.5% chromic oxide as

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

Ingredients	Polack fish meal	Wheat flour	Distillers dried grain[1]
Proximate composition (% DM)			
Dry matter	91.7	89.3	97.0
Crude protein	67.3	19.3	21.5
Crude lipid	5.3	3.9	4.5
Ash	20.2	2.2	0.9
Essential amino acid composition (% protein)			
Arg	6.7	5.7	5.9
His	2.3	2.9	2.4
Ile	4.5	2.3	4.0
Leu	8.3	6.0	8.2
Lys	8.8	3.7	3.2
Met + Cys	5.1	2.8	4.3
Phe + Tyr	8.1	6.8	9.2
Thr	4.8	3.5	4.4
Val	4.5	3.2	4.9

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

Table 2. Ingredient and chemical composition of experimental diets

	Diets				
Ingredients	DDG0	DDG6	DDG12	DDG18	DDG24
Pollack fish meal	59.0	59.0	59.0	59.0	59.0
Distillers dried grain powder[1]		6.0	12.0	18.0	24.0
Wheat flour	24.0	18.5	13.0	7.5	2.0
Corn gluten meal	2.0	1.5	1.0	0.5	
α-Potato-starch	5.0	5.0	5.0	5.0	5.0
Cod liver oil	7.0	7.0	7.0	7.0	7.0
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0
Stay-C (50%)	0.5	0.5	0.5	0.5	0.5
Choline salt (50%)	0.3	0.3	0.3	0.3	0.3
Taurine	0.2	0.2	0.2	0.2	0.2
Chromic oxide[4]	-	-	-	-	-
Nutrient content (% DM)					
Crude protein	47.9	48.2	47.3	47.7	48.3
Crude lipid	12.0	11.6	12.0	11.9	12.1
Ash	12.7	12.6	12.7	12.6	12.3
Carbohydrate[5]	27.4	27.6	28.0	27.8	27.3
Essential amino acid composition (% protein)					
Arg	6.5	6.5	6.7	6.7	6.9
His	2.3	2.3	2.3	2.2	2.3
Ile	3.8	3.9	3.8	3.7	3.4
Leu	8.2	8.4	8.2	8.2	8.1
Lys	8.1	8.1	8.2	8.1	8.2
Met + Cys	3.1	3.3	3.2	3.2	2.6
Phe + Tyr	6.9	7.2	7.2	7.2	7.2
Thr	4.7	4.7	4.8	4.8	4.8
Val	4.4	4.5	4.5	4.4	4.1

[1]Residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts produced from Gangneung Makgeolli factory (Gangneung, Korea).
[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): 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-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 premix): $MgSO_4 \cdot 7H_2O$, 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, 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]Replaced 0.5% wheat flour when nutrient digestibility was determined.
[5]Carbohydrate =100 - (crude protein + crude lipid + ash).

an indicator to visual satiation at two times per day. Two hours after feeding, the rearing tanks were brushed to remove uneaten and fecal residues. And faeces in each tank were collected next every morning for two consecutive weeks. Fecal samples were prepared for analysis as described in previous study (Lee, 2002).

Chemical Analysis

Proximate composition of fish was analyzed according to standard methods (AOAC 1995). Ten fish per tank at the end of the feeding trials were sampled and stored at -25°C for proximate composition analysis. Crude protein was determined by Kjeldahl method using 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 using an oven dry at 105°C for 6h and also ash content was determined after combustion at 550°C for 4h in a muffle furnace. Amino acid composition in the experimental diets and whole body of fish was performed with acid hydrolysis with 6 N HCL (reflux for 23h at 110°C) followed by using an automatic amino acids analyzer (Hitachi, Tokyo, Japan).

Antioxidant Enzymes Activity

For the determination of hepatic superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity, 0.1g liver of fish were homogenized in 9 volumes of 5 mM Tris and 35 mM glycine (pH 7.6). The homogenate was centrifuged at 10000×g for 30 min to remove debris. The resultant supernatants were used for SOD and GPx assay. Protein content in the supernatants was measured using the Bradford (1976) method.

SOD activity of liver was assayed by using kit (Sigma Aldrich Inc. Saint-Louis, Switzerland). The rate of the reduction with O_2 was linearly related to the xanthine oxidase (XO) activity and was inhibited by SOD. Therefore, the IC50 (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method. In this method briefly, 20 µl samples were added to 220 µl substrate solution and then inhibition activity was determined by a kinetic method up to 20 min at 410 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader.

GPx was assayed with kit (Biovision, Inc. Milpitas, CA, USA). In this assay, cumene hydroperoxide is used as the peroxide substrate (ROOH), glutathione reductase (GSSG-R) and NADPH (b-Nicotinamide Adenine Denucleotide Phosphate, Reduced) were included in the reaction mixture. Therefore, the change in A_{340} due to NADPH oxidation was monitored and was indicative of GPx activity. Briefly, 50 µl samples were added to 40 µl reaction mixture and incubated for 15 min and then 10 µl cumene hydroperoxide was added and OD_1 read at 340 nm. After 5 min incubation OD_2 read in 340 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. Activity of GPx was calculated as a U mg^{-1} protein.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant ($P<0.05$) differences were found, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. All statistical analyses were carried out using the SPSS version 19 (SPSS, Michigan Avenue, Chicago, Illinois, USA).

Results

Growth performance, feed utilization and morphological parameters of juvenile black seabream fed the experimental diets containing different levels of DDG are presented in Table 3. Weight gain was not affected by dietary DDG level ($P>0.05$). Feed efficiency and protein efficiency ratio of fish fed the DDG24 diet were lower than those of DDG0 diet ($P<0.05$). Daily feed intake and daily protein intake of fish fed the DDG18 and DDG24 diets were higher than those of DDG0 diet ($P<0.05$). Condition factor, hepatosomatic index and visceralsomatic index of fish were not affected by dietary DDG level ($P>0.05$). The results of body composition and essential amino acid composition of juvenile black seabream fed the experimental diets are presented in Table 4. Proximate and amino acid composition of whole body in juvenile black seabream were not affected by dietary DDG level ($P>0.05$). The results of antioxidant enzyme activities are presented in Table 5. The activities of superoxide dismutase and glutathione peroxidase in the liver were not affected by dietary DDG level ($P>0.05$).

Discussion

The present results showed that the dietary supplementation of DDG up to 24% in the formulated diets did not affect the growth performance, morphological parameters, body composition and amino acid profile of juvenile black seabream. The findings of this study suggested that rice-based DDG is considered to be useful candidate as a feed ingredient for juvenile black seabream. Many scientific studies suggested positive performance with the potential for including corn-based DDG to replace fish meal used in tilapia feeds (Coyle *et al*., 2004; Schaeffer *et al*., 2009). It has been demonstrated that corn-based DDG can be included in channel catfish diets without having negative effects on growth performance (Webster *et al*., 1993). Li *et al*. (2010) observed that the use of 30% corn-based DDG in the diet improved weight gain and feed efficiency ratio of channel catfish compared to control diet. Schaeffer *et al*. (2011) indicated that corn-based DDG with soybean meal might be utilized in yellow perch diet without compromising growth performance. Zhou *et al*. (2010) reported that the use of 30% corn-based DDG appeared to be suitable for replacing soybean meal and corn meal in hybrid catfish diets. It had been proven that corn-based DDG could utilize in rainbow trout diets (Barnes *et al*., 2012; Randall and Drew,

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

Diets	DDG0	DDG6	DDG12	DDG18	DDG24
IBW (g)[1]	1.2 ± 0.01[ns]	1.2 ± 0.01	1.2 ± 0.03	1.2 ± 0.02	1.2 ± 0.03
WG[2]	403 ± 40.3[ns]	324 ± 22.4	364 ± 38.3	387 ± 48.5	354 ± 29.6
FE[3]	112 ± 2.3[b]	96 ± 8.1[ab]	102 ± 5.0[ab]	96 ± 5.0[ab]	88 ± 4.1[a]
PER[4]	2.3 ± 0.03[b]	2.0 ± 0.15[ab]	2.2 ± 0.13[ab]	2.0 ± 0.10[ab]	1.8 ± 0.07[a]
DFI[5]	1.8 ± 0.03[a]	1.9 ± 0.09[ab]	2.0 ± 0.03[ab]	2.1 ± 0.06[bc]	2.2 ± 0.03[c]
DPI[6]	0.9 ± 0.00[a]	0.9 ± 0.03[a]	0.9 ± 0.00[a]	1.0 ± 0.00[b]	1.1 ± 0.03[c]
CF[7]	2.0 ± 0.1[ns]	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
HSI[8]	2.2 ± 0.1[ns]	2.0 ± 0.3	2.3 ± 0.3	2.5 ± 0.2	2.2 ± 0.1
VSI[9]	5.9 ± 0.5[ns]	5.9 ± 0.6	6.7 ± 0.5	6.4 ± 0.7	7.2 ± 0.7

Values (mean ± SE of replication) in the same row having different superscript letters are significantly different (P<0.05).
ns = Not significant ($P > 0.05$).
[1]IBW (g): Initial body weight
[2]Weight gain = (final fish wt. - initial fish wt.) × 100 / initial fish wt.
[3]Feed efficiency = wet weight gain × 100 / feed intake.
[4]Protein efficiency ratio = (wet weight gain / protein intake).
[5]Daily feed intake = feed intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].
[6]Daily protein intake = protein intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].
[7]Condition factor = [fish weight (g) / fish length (cm) ^{3}] × 100.
[8]Hepatosomatic index = (liver weight / body weight) × 100.
[9]Visceralsomatic index = (viscera weight / body weight) × 100.

Table 4. Proximate and essential amino acid composition of the whole body in juvenile black sea bream fed the experimental diets for 8 weeks

Diets	DDG0	DDG6	DDG12	DDG18	DDG24
Proximate composition (%)					
Moisture	71.5 ± 0.41[ns]	70.1 ± 0.52	71.0 ± 0.83	71.7 ± 0.76	71.6 ± 0.22
Crude protein	15.7 ± 0.20[ns]	15.5 ± 0.50	15.7 ± 0.20	16.1 ± 0.34	15.3 ± 0.31
Crude lipid	6.9 ± 5.0[ns]	6.9 ± 0.34	6.7 ± 0.20	6.7 ± 0.37	6.4 ± 0.25
Ash	4.2 ± 0.12[ns]	4.2 ± 0.27	4.3 ± 0.11	4.5 ± 0.12	4.4 ± 0.24
Essential amino acids (% protein)					
Arg	6.3 ± 0.10[ns]	6.4 ± 0.08	6.4 ± 0.10	6.4 ± 0.03	6.4 ± 0.09
His	2.4 ± 0.23[ns]	2.4 ± 0.03	2.5 ± 0.33	2.5 ± 0.03	2.5 ± 0.00
Ile	4.3 ± 0.15[ns]	4.1 ± 0.34	4.2 ± 0.39	4.4 ± 0.15	4.0 ± 0.20
Leu	8.9 ± 0.05[ns]	9.0 ± 0.06	9.0 ± 0.12	9.0 ± 0.06	8.9 ± 0.03
Lys	9.7 ± 0.20[ns]	9.5 ± 0.05	9.5 ± 0.09	9.5 ± 0.15	9.6 ± 0.07
Met + Cys	4.3 ± 0.00[ns]	4.4 ± 0.09	4.4 ± 0.07	4.4 ± 0.03	4.4 ± 0.07
Phe + tyr	7.8 ± 0.05[ns]	7.9 ± 0.06	8.0 ± 0.09	7.9 ± 0.03	7.8 ± 0.03
Thr	4.8 ± 0.10[ns]	4.8 ± 0.03	4.9 ± 0.00	4.9 ± 0.17	4.8 ± 0.12
Val	4.2 ± 0.15[ns]	4.0 ± 0.29	4.1 ± 0.34	4.2 ± 0.12	3.9 ± 0.18

Values are presented as mean ± SE of replication. ns = Not significant (P>0.05).

Table 5. Activities of superoxide dismutase and glutathione peroxidase in the liver of juvenile black seabream fed the experimental diets for 8 weeks

Diets	DDG0	DDG6	DDG12	DDG18	DDG24
Inhibition activity of superoxide dismutase (%)	80.8 ± 3.8[ns]	83.5 ± 4.0	86.3 ± 2.2	84.2 ± 3.4	87.5 ± 2.8
Glutathione peroxidase (U mg^{-1} protein)	26.1 ± 5.4[ns]	36.2 ± 7.8	27.8 ± 12.6	15.4 ± 3.0	18.3 ± 5.6

Values are presented as mean ± SE of replication. ns = Not significant (P>0.05).

2010). Thompson *et al*. (2008) observed that corn-based DDG might be improved palatability in sunshine bass diet. Lim *et al*. (2009) showed that up to 40% corn-based DDG can be included in the diet of channel catfish as a replacement of soybean meal and corn meal without affecting weight gain. It has been reported that up to 28% rice-based DDG did not affect growth performance of juvenile olive flounder

(Rahman *et al*., 2013).

While in production involving DDG from rice, yeasts are utilized in order to facilitate the fermentation process. DDG contains significant quantity of yeast cells (Zohu *et al*. 2010) which are rich in protein, B-complex vitamins and β-glucans. It has been reported that yeasts can enhance immune responses (Ortuno *et al*., 2002) as well as growth (Lara-Flores *et al*., 2003) of fish and thus may serve as an exceptional well-being health promoter for fish culture. Thus, these yeast cells can improve dietary quality involving DDG in diet for juvenile black seabream.

Generally when alternative plant ingredients are used in diets with the same concentration of energy and are able to meet the nutritional requirements of the animal being fed, similar performance may be expected (Cruz- Suarez *et al*., 2001). Many scientific studies have revealed that fermented plant ingredients at a proper incorporation level may be great nutrient resources pertaining to fish (Sun *et al*., 2007; Seo *et al*., 2011) and shrimp (Molina-Poveda and Morales, 2004). Growth performance of fish is related to a number of factors including feed intake (El-Saidy and Gaber, 2003), digestibility (Lanari and D'Agaro, 2005) as well as anti-nutritional factors (Borgeson *et al*., 2006). Several practical ways to improve utilization of plant proteins have been suggested including blending (Azarm and Lee, 2012), feeding stimulants (Takeda and Takii, 1992) and fermentation (Kader *et al*., 2012; Kim *et al*., 2010). Guo *et al*. (2007) reported that fermentation process might be useful to complement the nutritional formation of plant proteins. It has been reported that fermentation of plant ingredients induces substantial removal or inactivation of anti-nutritional factors such as protease inhibitor (Reddy and Pierson, 1994), enhances the nutritional quality (Kader *et al*., 2012), improves the apparent digestibility (Kiers *et al*., 2000) and extends shelf life of the refined foods (Skrede and Nes, 1988). Zhou *et al*. (2010) suggested that fermentation could enhance the digestibility of plant dietary protein sources. Also it has been suggested that nutrient value may be improved during fermentation by various microbial activities (Seo *et al*., 2011). Therefore, fermentation process could be improved the nutritional quality of DDG in diet.

Feed efficiency and protein efficiency ratio appeared to decrease with high inclusion of DDG to partial replace wheat flour and corn gluten meal. Similar results were reported in protein efficiency ratio with the diet containing 40% corn-based DDG of Nile tilapia (Lim *et al*., 2007). Whole body proximate composition of juvenile black seabream was not affected by dietary DDG level. This is an agreement with the findings of Li *et al*. (2011) who revealed that whole body proximate composition of Nile tilapia was not significantly affected by dietary levels of wheat DDG.

Recently, it was reported that vegetable products from fermentation processing induce to increase non-specific immune response, antioxidant activities and diseases resistance pertaining to fish (Ashida *et al*., 2002; Pham *et al*., 2007). Therefore, Kim *et al*. (2010) announced that fermented soybean meal was significantly improved antioxidant activity like liver superoxide activity in olive flounder. Kader *et al*. (2012) reported that antioxidant activity was significantly increased with levels of fermented soybean meal and squid by-product in diet for flounder. Azarm and Lee (2012) reported that fermented soybean meal induced higher antioxidant activity in liver of juvenile black seabream. Nevertheless, in the present study, the antioxidant enzyme activities in liver of black seabream were not influenced by DDG in diet. A similar result was obtained in antioxidant enzyme activity of juvenile olive flounder (Rahman *et al*., 2013).

Although the data of energy and nutrient digestibilities determined in this study were not shown in the table, the digestibility values (92-94% for protein, 97-99% for lipid, 64-78% for carbohydrate and 90-92% for energy) were not affected by dietary DDG level. It indicated that black seabream can easily consume and utilize the DDG in diet. Cheng and Hardy (2004b) reported that protein and lipid apparent digestibility coefficient of corn-based DDG were 90% and 82% for rainbow trout, respectively.

In the present study, substituting DDG for wheat flour and corn gluten meal may be served as an ingredient for least-cost fed formulation of black seabream. The use of rice-based DDG in black seabream diets may allow the feed producer more flexibility in formulating a nutritious diet at the lowest possible cost by adding another possible ingredient to the least-cost formulation and reducing the dependence upon wheat flour. The results of this experiment suggested that DDG is good ingredient to replace plant origin such as wheat flour and corn gluten meal and could be used up to 24% for the optimum growth performance of juvenile black seabream.

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