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Inclusion of Distillers Dried Grain as Partial Replacement of Wheat Flour and Soybean Meal in the Diet of Juvenile Abalone *Haliotis discus hannai*

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Abstract

An 8-week feeding experiment was conducted to determine the influence of dietary distillers dried grain (DDG) on the growth and body composition of juvenile abalone *Haliotis discus hannai*. Five diets were formulated to contain 0% (DDG0), 15% (DDG15), 30% (DDG30), 45% (DDG45), and 60% (DDG60) DDG, and three replicate groups of abalone (average body weight: 3.6 ± 0.21 g) were fed one of the experimental diets at a feeding rate of 5% body weight per day once daily (17:00 h) for 8 weeks. Survival, shell length, and shell width of juvenile abalone were not affected by dietary DDG levels ($P > 0.05$). Weight gains of juvenile abalone fed DDG15 and DDG30 diets were not different compared to DDG0, but abalone fed DDG45 and DDG60 diets gained less weight than those fed DDG0 ($P < 0.05$). Soft body weight/body weight ratio of juvenile abalone fed the DDG60 diet was lower than that of those fed the DDG0 diet ($P < 0.05$), but proximate composition of the soft body was not affected by dietary DDG levels ($P > 0.05$). The results of this experiment suggest that DDG is a good replacement for wheat flour and soybean meal, and can be used up to 30% in the diet to maintain the growth performance of the juvenile abalone.

Key words: Abalone, *Haliotis discus hannai*, Distillers dried grain,Wheat flour, Soybean meal

Introduction

Successful operations associated with the aquaculture industry are growing rapidly. Abalone *Haliotis discus hannai* is one of the most commercially important shellfish species in East Asia, especially Korea, Japan, and China. Culture techniques of this species have been developed since 1970, and its aquaculture production has rapidly increased with demand for human consumption (Cho, 2010). The utilization of pelleted feed has been demonstrated to be convenient and cost-effective in commercial abalone production (Britz et al., 1994; Lee, 2004), but the species is characterized by naturally slow and heterogeneous growth rates (Bautista-Teruel et al., 2003). The poor growth rate of abalone has generated the need for more research into optimal nutrition for successful cultures (Fleming et al., 1996), and nutritional demands must be met to produce more efficient cultures (Lee, 2004). Recently, abalone cultures have become increasingly dependent on formulated feeds because of constantly limited supplies of harvested seaweed (Green et al., 2011). A great demand exists for identifying cost-effective ingredients to produce a diet that can maintain abalone growth (Lee et al., 2004), and the replacement of soybean meal and wheat flour using more economical ingredients may be beneficial for minimizing feed costs.

Distillers dried grain (DDG) is a cereal by-product that is fermented and distilled to create alcoholic beverages (Hertrampf and Piedad-Pascual, 2000). DDG is a useful feed ingredient in the livestock industry because of its high nutri-

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tional value and low cost, and it is currently mainly being tested for use as a possible energy source in ruminant diet programs (Jacob et al., 2008). However, due to the enhanced availability and potential cost–benefit of DDG, incorporating DDG into aquafeed may present a large economic value. DDG has already been tested as a possible alternative protein and/or energy source associated with aquafeed (Chevanan et al., 2010; Wu et al., 1996), and it is less expensive than traditional ingredients such as soybean meal and wheat flour (Rahman et al., 2013a, 2013b). Seo et al. (2011) reported that rice-based DDG contains a high protein content and well-balanced amino acid profile, and may be useful for producing low-cost feed for juvenile olive flounder (Rahman et al., 2013a) and sea cucumber (Choi et al., 2013). Therefore, the objective of the this study was to investigate the effects of dietary DDG as an alternative feed ingredient (compared to traditional soybean meal and wheat flour) on the growth and body composition of juvenile abalone.

Materials and Methods

Experimental diets

The essential amino acid and proximate compositions of major ingredients used in the experimental diets are presented in Table 1. The ingredients and chemical composition of the experimental diets are given in Table 2. Five isonitrogenous and isocaloric experimental diets were formulated to contain 0%, 15%, 30%, 45%, and 60% DDG (designated DDG0, DDG15, DDG30, DDG45, and DDG60, respectively). Fish meal was used as the primary protein source, and fish oil was the lipid source. The DDG used in this study was produced by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeast during the manufacturing process of Makgeolli, a traditional Korean alcoholic beverage. DDG produced from Gangneung Makgeolli factory (Gangneung, Korea) was dried at 60ºC for 24 h and finely ground prior to incorporation in the experimental diets. All ingredients were thoroughly mixed with 30% distilled water, and pellets were prepared using a moist pelleting machine in the laboratory. The pellets were dried at room temperature for 48 h and ground into desirable particle sizes. All diets were stored at –30ºC until experimental use.

Experimental animal and feeding experiment

Juvenile abalone were produced at the Gangwon Province Marine Culture Experimental Station (Korea) and acclimated to a laboratory flow-through aquarium system for 2 weeks. During this conditioning period, abalones were fed a commercial feed before starting the feeding trial. Following the acclimation period, juvenile abalones (average body weight, 3.6 ± 0.21 g) were randomly allocated to 50-L rectangular plastic tanks (40 L of water each) in a seawater flow-through system at a density of 20 individuals per tank. Three replicate groups of abalone were fed one of the five experimental diets at a feeding rate of 5% body weight per day every other day (17:00 h) for 8 weeks. Uneaten feed in each aquarium was removed by siphoning prior to daily feeding. Photoperiod was maintained at the natural condition, and aeration was provided during feeding periods. Water was supplied at a flow rate of 1 L/min and maintained at 19.4 ± 2.16ºC. At the initiation and the termination of the experiment, abalones in each aquarium were collectively weighed using an electric balance following a 24 h starvation period.

Table 1. Proximate composition and essential amino acid (% in protein) of the ingredient of experimental diets

	Ingredients			
	Fish meal	Soybean meal	Wheat flour	Distillers dried grain (DDG)*
Proximate composition (% dry matter)				
Dry matter	95.8	89.2	89.3	97.0
Crude protein	75.3	55.2	19.3	21.5
Crude lipid	8.8	1.6	3.9	4.5
Ash	14.7	6.8	2.2	0.9
Essential amino acid composition (% protein)				
Arg	6.7	7.6	5.7	5.9
His	2.3	2.8	2.9	2.4
Ile	4.5	3.3	2.3	4.0
Leu	8.3	7.5	6.0	8.2
Lys	8.8	6.5	3.7	3.2
Met + Cys	5.1	2.4	2.8	4.3
Phe + Tyr	8.1	8.2	6.8	9.2
Thr	4.8	4.4	3.5	4.4
Val	4.5	3.3	3.2	4.9

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

Sample collection and chemical methods

All surviving abalone at the end of the feeding experiment were sampled after a 24 h starvation period and stored at –25ºC for proximate analysis. Proximate composition of the soft body of abalones was analyzed according to standard methods (AOAC, 1995). Crude protein content was determined using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Moisture content was measured by drying in an oven at 105ºC for 6 h. Crude lipid content was determined by ether-extraction in a Soxhlet extractor (SER 148; VELP Scientifica, Milano, Italy), and ash content was determined using a muffle furnace at 600ºC for 4 h. Amino acid compositions of the experimental diets were analyzed with acid hydrolysis with 6 N HCL (reflux for 23 h at 110ºC) using an automatic amino acids analyzer (Hitachi, Tokyo, Japan).

Statistical analysis

Differences among groups were subjected to one-way analysis of variance (ANOVA) using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Significant differences ($P < 0.05$) among the means were determined using a Duncan`s multiple range test (Duncan, 1955). The data are presented as the mean ± standard error (SE) of three replicate groups.

Table 2. Ingredient and chemical composition of the experimental diets

	Diets				
	DDG0	DDG15	DDG30	DDG45	DDG60
Ingredients (%)					
Fish meal	10.0	10.0	10.0	10.0	10.0
Soybean meal	30.0	25.0	20.0	15.0	10.0
Wheat flour	45.2	34.9	24.6	14.3	4.0
Distillers dried grain*		15.0	30.0	45.0	60.0
Fish oil	2.0	2.0	2.0	2.0	2.0
Na alginate	10.0	10.0	10.0	10.0	10.0
L-lysine HCL		0.3	0.6	0.9	1.2
Vitamin premix†	1.0	1.0	1.0	1.0	1.0
Mineral premix‡	1.0	1.0	1.0	1.0	1.0
Choline salt (50%)	0.8	0.8	0.8	0.8	0.8
Proximate composition (%, dry matter basis)					
Dry matter	85.3	82.1	83.7	82.7	87.3
Crude protein	32.9	32.0	30.4	29.3	29.1
Crude lipid	2.8	2.5	3.4	3.5	3.6
Ash	8.1	7.8	7.7	7.1	6.8

DDG, distillers dried grain.

*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 premix): L-ascorbic acid, 200; 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.

‡Mineral premix contained the following ingredients (g/kg premix): NaCl, 10; $MgSO_4 \cdot 7H_2O$, 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.

Table 3. Growth performance of juvenile abalone fed the experimental diets for 8 weeks*

	Diets				
	DDG0	DDG15	DDG30	DDG45	DDG60
Survival (%)	96 ± 1.7^{ns}	98 ± 1.7	97 ± 1.7	98 ± 1.7	97 ± 1.7
Weight gain (%)†	76.2 ± 0.90^{b}	69.7 ± 2.55^{b}	61.2 ± 6.33^{ab}	53.1 ± 1.74^{a}	51.7 ± 7.49^{a}
Shell length (cm)	3.79 ± 0.23^{ns}	3.66 ± 0.37	3.50 ± 2.64	3.51 ± 1.43	3.54 ± 1.33
Shell width (cm)	2.54 ± 0.77^{ns}	2.45 ± 0.45	2.27 ± 3.10	2.35 ± 1.40	2.37 ± 1.37
Soft body weight/ body weight	0.64 ± 0.003^{a}	0.65 ± 0.006^{ab}	0.63 ± 0.009^{ab}	0.64 ± 0.003^{ab}	0.62 ± 0.003^{b}

DDG, distillers dried grain; ns, not significant ($P > 0.05$).

*Values (mean ± SE of three replications) in the same row not sharing a common superscript (e.g., a, b, ab, ns) are significantly different ($P < 0.05$).

†Weight gain (%) = (final fish weight – initial fish weight) × 100 / initial fish weight.

Table 4. Proximate composition (%) of the soft body of juvenile abalone fed the experimental diets for 8 weeks*

	Diets				
	DDG0	DDG15	DDG30	DDG45	DDG60
Moisture	75.4 ± 0.71^ns^	74.7 ± 0.57	74.5 ± 1.30	74.0 ± 0.68	74.8 ± 0.80
Crude protein	16.8 ± 0.88^ns^	17.1 ± 0.60	16.6 ± 1.54	16.0 ± 1.10	15.4 ± 0.30
Crude lipid	1.4 ± 0.44^ns^	1.4 ± 0.12	1.4 ± 0.53	1.4 ± 0.25	1.1 ± 0.07
Ash	2.2 ± 0.09^ns^	2.1 ± 0.02	2.3 ± 0.09	2.2 ± 0.20	2.3 ± 0.08

DDG, distillers dried grain; ns, not significant ($P > 0.05$).
*Values are presented as mean ± SE of three replications.

Results

The growth performances of juvenile abalone in each experimental diet group are presented in Table 3. Survival, shell length, and shell width were not affected by dietary DDG levels ($P > 0.05$). Weight gains of juvenile abalone fed DDG15 and DDG30 diets were not different compared to DDG0, but abalone fed DDG45 and DDG60 diets gained less weight than those fed DDG0 ($P < 0.05$). Soft body weight/body weight of juvenile abalone fed the DDG60 diet was lower than that of abalone fed the DDG0 diet ($P < 0.05$), but proximate composition of the soft body was not affected by dietary DDG levels ($P > 0.05$; Table 4).

Discussion

The results of this study indicate that dietary supplementation of DDG up to 30% did not affect the growth performance and body composition of juvenile abalone, which suggests that rice-based DDG can be an effective low-cost feed ingredient for this species, as has been demonstrated for juvenile olive flounder (Rahman et al., 2013a), black seabream (Rahman et al., 2013b), and juvenile sea cucumber (Choi et al., 2013). Similarly, when supplemented with fish meal, corn-based DDG can be included in juvenile Nile tilapia feed without incurring negative effects on growth performance (Wu et al., 1996; Schaeffer et al., 2009; Coyle et al., 2004). Previous studies (Tidwell et al., 1990; Robinson and Li, 2008; Li et al., 2010) have also demonstrated that corn-based DDG can be integrated into channel catfish diets without negative effects on growth performance, and is suitable to replace soybean meal and corn meal in hybrid catfish diets (Zhou et al., 2010). The effectiveness of a diet containing DDG on the growth of freshwater fishes is related to several factors such as improved digestibility (Randall and Drew, 2010) and decreased exposure to anti-nutritional factors (Borgeson et al., 2006). In our study, weight gain of juvenile abalone fed diets containing up to 30% DDG did not differ from the control group. Although growth performance tended to decrease in groups fed high-DDG diets, dietary treatments had no effect on the soft body proximate composition of abalone at the end of the experiment. Poor palatability and unknown post-fermentation anti-nutritional factors may have been responsible for low growth in the high-DDG diet groups.

The experimental feed used in this study appears to contain sufficient protein, carbohydrate, and essential amino acid contents to meet abalone nutritional requirements. Uki et al. (1985) identified diets containing many protein sources such as soybean meal that promoted good growth in abalone. Mai et al. (1995) reported that high levels of dietary carbohydrates can be utilized easily by abalone to satisfy their energy requirements, and Lee et al. (1998) suggested that abalone can utilize carbohydrates more efficiently than lipids as an energy source. Considering these results, dietary supplementation using DDG may provide not only sufficient protein, but also energy for body growth and maintenance of juvenile abalone.

We found that replacing wheat flour and soybean meal with DDG may be a useful solution for producing more economical abalone feed. The use of rice-based DDG may provide the feed producer with greater flexibility in formulating a nutritious diet at the lowest possible cost by reducing the dependence on wheat flour and soybean meal. The results of this experiment suggest that DDG is a good substitute for plant materials such as wheat flour and soybean meal, and can be used up to 30% in feed to maintain the growth performance of juvenile abalone.

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