FAS Fisheries and Aquatic Sciences

Original Article

Fish Aquat Sci 16(4), 279-283, 2013

Distillers Dried Grain from Makgeolli By-product Is Useful as a Dietary Ingredient for Growth of Juvenile Sea Cucumber *Apostichopus japonicus*

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Abstract

We tested the effects of various distillers dried grains (DDGs) in a formulated diet on growth and body composition of juvenile sea cucumber *Apostichopus japonicus*. DDG is a solid residue obtained by filtering an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts. Six isonitrogenous and isocaloric diets (DDG0, DDG-G, DDG-C, DDG-K, DDG-W, and DDG-P) were formulated to contain 20% DDG obtained from different Makgeolli factories. Juvenile sea cucumber (average weight, 2.1 ± 0.02 g) were randomly distributed in eighteen 50-L rectangular plastic tanks (40 L of water each) in a seawater flow-through system at a density of 50 juveniles/tank. Three replicate groups of sea cucumber were fed each of the six experimental diets at a feeding rate of 5% body weight per day for 22 weeks. At the end of the feeding experiment, survival and weight gain of the juvenile sea cucumber were not affected by the type of dietary DDG ($P > 0.05$), and the proximate and amino acid compositions of the whole body were not affected by dietary DDG diets ($P > 0.05$). These results indicate that rice-based DDG is a potential dietary ingredient that could be used at dietary concentrations of up to 20% for growth of juvenile sea cucumber.

Key words: Sea cucumber, Distillers dried grain, Growth, Body composition, *Apostichopus japonicus*

Introduction

The sea cucumber *Apostichopus japonicus* is a commercially beneficial aquaculture species in Asian markets because of its high demand as a health food (Seo and Lee, 2011). However, production of sea cucumber has declined due to constant over-exploitation and water pollution (Conand, 2004). Okorie et al. (2008) noted that sea cucumber are used as a remedy for alleviating internal and external wounds and it is thought to possess aphrodisiac and curative properties. Sea cucumber are produced worldwide and have long been exploited as a potential fishery asset in Russia, China, Japan, and Korea (Yuan et al., 2006). The demand for sea cucumber has increased rapidly in recent years, leading to severe overfishing.

Many studies have reported the nutrient requirements (Choi et al., 2009; Seo et al., 2011a, 2011b) and feed ingredients (Yuan et al., 2006; Liu et al., 2009; Slater et al., 2009) for growth of sea cucumber. Seo and Lee (2011) found that sea cucumber require relatively low dietary protein and lipid content for optimum growth. Based on the previous finding, we hypothesized that incorporating a vegetable ingredient such as distillers dried grain (DDG) into the diet might reduce feed costs (Seo et al., 2011a; Rahman et al., 2013). DDG is a cereal by-product of distillation processing and has great potential for use as an aquatic animal feed ingredient (Lim et al., 2007, 2009). Therefore, we conducted this study to investigate the effects of DDG as a promising dietary ingredient on the growth and body composition of juvenile sea cucumber.

http://dx.doi.org/10.5657/FAS.2013.0279

Received 24 July 2013; **Revised** 03 September 2013
Accepted 04 September 2013

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http://e-fas.org pISSN: 2234-1749 eISSN: 2234-1757

Materials and Methods

Experimental diets

The essential amino acid and proximate compositions of ingredients used in the experimental diets are presented in Table 1. Ingredients and nutrient contents of the experimental diets are presented in Table 2. Soybean meal and algae *Undaria pinnatifida* and *Sargassum thunbergii* powder were used as the primary protein source in the control diet. The experimental diets were formulated to contain 20% each of rice-based DDG obtained from different Makgeolli factories in place of *S. thunbergii* powder in the control diet. Fish oil and soybean oil were used as lipid sources. The DDG used was produced by filtering an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts. All ingredients were thoroughly mixed mechanically with water at a ratio of 7:3. The pellets were dried at room temperature for 48 h and ground into desirable particle sizes for the juvenile sea cucumber. All diets were stored at −30°C until use.

Experimental animal and feeding experiment

Juvenile sea cucumber were obtained from a local sea cucumber farm (Taean, Korea), and acclimated to the experimental condition for 2 weeks. Juvenile sea cucumber (average body weight, 2.1 ± 0.02 g) were randomly allocated to eighteen 50-L rectangular plastic tanks (40 L of water each) in a seawater flow-through system at a density of 50 animals/tank. Three replicate groups of sea cucumber were fed one of the six experimental diets at a feeding rate of 5% body weight per day every other day (17:00 h) for 22 weeks. Filtered seawater was continuously supplied at a flow rate of 1 L/min. Water temperature was maintained at 10.7 ± 1.07°C, and the photoperiod during the feeding trial followed natural conditions. Each tank was cleaned by siphoning every 2 days.

Sample collection and chemical methods

All sea cucumber surviving at the end of the feeding experiment were collectively weighed and sampled after 24 h of starvation, freeze dried, and ground with a mortar and pestle. Proximate composition of the experimental diets and whole body of the sea cucumber were analyzed according to standard methods (Association of Official Analytical Chemists, 1995). Crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Moisture content was measured after drying in a 105°C oven for 6 h. Crude lipid was determined by the ether-extraction method, and ash content was determined with a muffle furnace at 600°C for 4 h. Amino acid composition in the experimental diets and whole body of sea cucumber were analyzed using an automatic amino acid analyzer (L-8800; column, ion exchange; injection pump pressure, 0-19.6 Mpa; flow rate, 0.05-0.99 mL/min; column oven, electrothermal cooling [30-70°C]; reaction unit, reaction column [135°C and 50°C]; photometer: wavelengths 570 and 440 nm; Hitachi, Tokyo, Japan).

Statistical analysis

Data were subjected to one-way analysis of variance using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). Signifi-

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

	Fish meal	Soybean meal	Wheat flour	Distillers dried grain (DDG)*				
				G	C	K	W	P
Proximate composition (% dry matter)								
Dry matter	95.8	89.2	89.3	97.0	94.3	92.9	93.7	92.6
Crude protein	75.3	55.2	19.3	21.5	4.8	23.3	48.0	31.3
Crude lipid	8.8	1.6	3.9	4.5	0.2	5.8	14.6	2.7
Ash	14.7	6.8	2.2	0.9	0.2	1.5	1.1	6.9
Essential amino acid composition (% in protein)								
Arg	6.7	7.6	5.7	5.9	6.6	6.6	7.6	6.5
His	2.3	2.8	2.9	2.4	3.4	2.8	2.3	2.4
Ile	4.5	3.3	2.3	4.0	3.6	3.6	3.5	3.9
Leu	8.3	7.5	6.0	8.2	6.7	7.2	8.2	8.2
Lys	8.8	6.5	3.7	3.2	2.9	3.3	3.1	2.1
Met + Cys	5.1	2.4	2.8	4.3	4.5	5.5	4.9	4.6
Phe + Tyr	8.1	8.2	6.8	9.2	8.5	8.5	9.7	9.2
Thr	4.8	4.4	3.5	4.4	4.1	4.4	3.8	4.5
Val	4.5	3.3	3.2	4.9	5.0	5.0	4.5	5.4
Total	53.1	46.0	36.9	46.5	45.4	46.9	47.6	46.7

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

cant differences ($P < 0.05$) among the means were determined using Duncan's multiple range test. Data are presented as mean ± standard error of three replicate groups.

Results and Discussion

The growth performance of juvenile sea cucumber fed the experimental diets containing dietary DDG are presented in Table 3. Survival and weight gain of juvenile sea cucumber were not affected by dietary DDG ($P > 0.05$). The compositions of proximate and essential amino acid of whole body in juvenile sea cucumber fed the experimental diets are presented in Table 4. Proximate and amino acid compositions of the whole body in juvenile sea cucumber were not affected by dietary DDG ($P > 0.05$).

No differences in growth and body composition of sea cucumber in this study by dietary DDG indicate that the rice-based DDG used in this study is considered to be a candidate as a feed ingredient for juvenile sea cucumber. Many authors have found satisfactory growth performance in tilapia when fed a diet containing corn-based DDG (Wu et al., 1996, 1997; Coyle et al., 2004; Schaeffer et al., 2009). Robinson and Li (2008) demonstrated that up to 30% corn-based DDG can be integrated into the channel catfish diet without negative effects on growth performance. Furthermore, dietary corn-based DDG may improve palatability in sunshine bass (Thompson et al., 2008). In the present study, weight gain in sea cucumber

Table 2. Ingredient and composition of the experimental diets

	Diets					
	DDG0	DDG-G	DDG-C	DDG-K	DDG-W	DDG-P
Ingredients (%)						
Soybean meal	33	28	35	28	13	23
Wheat flour	7	12	5	12	27	17
Undaria pinnatifida	35	35	35	35	35	35
Sargassum thunbergii	20	-	-	-	-	-
Distillers dried grain*-G	-	20	-	-	-	-
Distillers dried grain-C	-	-	20	-	-	-
Distillers dried grain-K	-	-	-	20	-	-
Distillers dried grain-W	-	-	-	-	20	-
Distillers dried grain-P	-	-	-	-	-	20
Beer yeast	3	3	3	3	3	3
Vitamin premix†	1	1	1	1	1	1
Mineral premix‡	1	1	1	1	1	1
Proximate composition (% dry matter basis)						
Dry matter	93.1	94.6	92.8	93.4	92.5	93.4
Crude protein	30.1	30.0	29.1	29.8	30.6	31.1
Crude lipid	0.9	2.1	1.6	0.6	2.5	4.6
Ash	20.5	13.0	13.2	13.1	14.0	12.3
Essential amino acid composition (% in protein)						
Arg	6.8	6.8	7.0	6.9	6.8	7.1
His	2.4	2.4	2.4	2.3	2.4	2.5
Ile	4.6	4.5	4.4	4.3	4.4	4.7
Leu	8.3	8.3	8.2	8.3	8.2	8.4
Lys	5.8	5.4	5.7	4.6	5.0	5.6
Met + Cys	1.7	2.5	2.2	2.9	2.4	2.6
Phe + Tyr	8.3	8.5	8.4	8.7	8.4	8.7
Thr	4.6	4.4	4.5	4.2	4.5	4.5
Val	5.1	5.2	4.9	5.3	5.2	5.4
Total	47.6	48.0	47.8	47.5	47.2	49.3

DDG, distillers dried grain.

*Residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts produced from Makgeolli factory in 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 sea cucumber (initial average body weight, 2.1 ± 0.02 g) fed the experimental diets for 22 weeks*

	Diets					
	DDG0	DDG-G	DDG-C	DDG-K	DDG-W	DDG-P
Survival (%)	63 ± 11.8ns	58 ± 15.3	78 ± 4.2	69 ± 2.9	80 ± 2.0	81 ± 0.7
Weight gain (%)†	279 ± 49.4ns	292 ± 67.1	268 ± 46.4	211 ± 20.6	162 ± 58	147 ± 28.1

ns, not significant (*P* > 0.05).
*Values are presented as mean ± SE of three replications.
†(Final weight − initial weight) × 100/initial weight.

Table 4. Proximate (%) and essential amino acid composition of the whole body in juvenile sea cucumber fed the experimental diets for 22 weeks*

	Diets					
	DDG0	DDG-G	DDG-C	DDG-K	DDG-W	DDG-P
Proximate composition (%, dry matter basis)						
Crude protein	38 ± 0.5ns	41 ± 2.3	38 ± 0.2	37 ± 1.2	38 ± 1.2	38 ± 0.3
Crude lipid	3.7 ± 0.18ns	2.6 ± 0.40	2.6 ± 0.71	3.2 ± 0.25	3.1 ± 0.70	3.3 ± 0.41
Ash	41 ± 0.9ns	42 ± 1.1	42 ± 0.5	42 ± 0.6	42 ± 0.2	40 ± 0.8
Essential amino acids (% in protein)						
Arg	7.2 ± 0.15 ns	7.4 ± 0.10	7.2 ± 0.06	7.2 ± 0.12	7.3 ± 0.50	7.3 ± 0.07
His	1.8 ± 0.03 ns	1.7 ± 0.07	1.8 ± 0.03	1.8 ± 0.01	1.8 ± 0.05	1.7 ± 0.09
Ile	3.3 ± 0.22 ns	3.2 ± 0.26	3.1 ± 0.33	3.5 ± 0.12	3.2 ± 0.15	3.2 ± 0.18
Leu	6.4 ± 0.09 ns	6.1 ± 0.18	6.2 ± 0.15	6.4 ± 0.06	6.2 ± 0.10	6.1 ± 0.15
Lys	5.9 ± 0.06 ns	5.5 ± 0.13	5.7 ± 0.12	5.2 ± 0.66	5.7 ± 0.10	5.7 ± 0.21
Met + Cys	4.1 ± 0.01 ns	3.9 ± 0.10	3.9 ± 0.12	3.9 ± 0.03	4.1 ± 0.05	3.9 ± 0.03
Phe + Tyr	7.1 ± 0.09 ns	6.8 ± 0.15	6.9 ± 0.12	7.1 ± 0.03	7.0 ± 0.10	6.7 ± 0.15
Thr	6.0 ± 0.07 ns	6.8 ± 0.53	6.3 ± 0.44	6.0 ± 0.06	6.0 ± 0.45	6.3 ± 0.19
Val	3.8 ± 0.17 ns	3.8 ± 0.20	3.7 ± 0.26	4.0 ± 0.09	3.8 ± 0.10	3.7 ± 0.19

ns, not significant (*P* > 0.05).
*Values are presented as mean ± SE of three replications.

fed diets containing DDG was not different from that of the control group. This is thought to be due to the improved digestibility and removal of anti-nutritional factors in the DDG by fermentation. Fermentation of legumes is an important process that can develop nutritive value and reduce specific anti-nutritional factors such as phytic acids, protease inhibitors, and flatulence factors (Yigzaw et al., 2001). Including fermented soybean meal using *Aspergillus oryzae* improved protein and carbohydrate digestibility in a yellowtail diet (Shimeno et al., 1993). Many studies have reported that fermented plant ingredients at the proper level may be good nutrient sources for fish (Sun et al., 2007; Rahman et al., 2013) and shrimp (Molina-Poveda and Morales, 2004).

The results of our study suggest that rice-based DDG is a good dietary ingredient and could be used at concentrations up to 20% in diet for growth of juvenile sea cucumber.

Acknowledgements

This research was supported by the Fishery Commercialization Technology Development Program (110077-3) funded by Ministry of Oceans and Fisheries in Korea.

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