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Effect of Fermented Food Garbage in Diet on Growth and Body Composition of Juvenile Flounder (*Paralichthys olivaceus*)

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Food garbage fermented with microbial starter was formulated to diet for the growth of juvenile flounder (*Paralichthys olivaceus*). Two replicate groups of fish, an average weight of 4.0 g, were fed the four isocaloric (19.5 MJ/kg diet) diets with different fermented food garbage levels (0, 5, 10 and 15%) for 45 days. Survival, feed efficiency, hepatosomatic index and protein efficiency ratio of fish were not affected by dietary fermented food garbage level ($P>0.05$). Weight gain of fish fed the diets with 5, 10 and 15% fermented food garbage was significantly higher than that of fish fed the control diet ($P<0.05$). Condition factor of fish fed the diet with 10% fermented food garbage was significantly higher than that of fish fed the control diet ($P<0.05$). Daily feed intake of fish fed the diets with 5 and 15% fermented food garbage was significantly higher than the control diet ($P<0.05$). Proximate composition of whole body and plasma glucose concentration were not affected by dietary fermented food garbage level ($P>0.05$). These findings indicate that fermented food garbage could be utilized as a feed ingredient for juvenile flounder.

Key words: *Paralichthys olivaceus*, Flounder, Diet, Fermented food garbage

Introduction

Aquaculture production of flounder (*Paralichthys olivaceus*) has been increased since the last decade in Korea owing to the developing technology for larval production. However, the use of moist pellet primarily made of raw fish (e.g. frozen horse mackerel) as feed for flounder aquaculture causes many problems including unstable supply of raw fish, increased storage cost, and heavy self-pollution resulted from the thawing of raw fish to make moist pellet and leaching of nutrients from moist pellet when fed to fish. Therefore it is essential to employ practical formulated feeds which can support not only sustainable productivity but also environment-friendly production in flounder farming.

Several studies on nutrition (Lee et al., 2000a, 2002, 2003; Kim et al., 2002) and utilization of some plant and animal protein source as a substitute for fish meal (Sato and Kikuchi, 1997; Yamamoto et al., 1998; Kikuchi, 1999; Kim et al., 2000) have been conducted for flounder diets. The use of plant and animal by-products to replace traditional protein sources such as fish meal in aquaculture diets has become common in recent years. The garbage by-products from human food occupy about 20-30% of total food consumed in Korea. However, this by-product has not been widely used, despite its nutritive value, due to rapid putrefaction and rancidity. Recently, environmental pollution by the food waste is one of the most important facing issues. One possible solution for such a problem is the re-use of food garbage as dietary ingredient for agriculture or aquaculture, although the development of effective techniques allowing the utilization of the food garbage is prerequisite. This study was aimed to investigate the potential usefulness of fermented food garbage as a energy source in formulated diet for juvenile flounder.

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Materials and Methods

Starter strains and food garbage

The microbial consortia (starter strains) for fer-

menting the food garbage were consisted of *Bacillus* sp. NK112, *Bacillus* sp. NK204, *Bacillus* sp. B4, and *Alcaligenes* sp. NK306. The microbial consortia were grown on a solid medium of defatted rice bran. The optimal condition for the starter production was determined by total viable cell counts. The microbial consortia were cultured in lactose broth at 30℃ for 48 hrs by shaking at 150 rpm. And then 3 L of culture broth was inoculated to 10 kg of defatted rice bran, mixed very well, and cultured on conditions of initial temperature, 30℃; pH, 7.0; and water content, 35%. Oxygen was supplied by turning over the culture media at which internal temperature was at ≥60℃. The microbial consortia were finally produced by drying in the shade if its temperature kept at constant after 4-5 day culture. Total count of viable cell in the final microbial consortia was 3.0×10^{10} CFU/g. The food garbage was obtained from private restaurants (Seoul, Korea). The production procedure of fermented food garbage was shown in Table 1.

Experimental diets

The experimental diets (Table 2) were formulated to satisfy the protein and lipid requirements of flounder, based on the previous study (Lee et al., 2000a). The four experimental diets containing 0, 5, 10 and 15% fermented food garbage levels were prepared. Fish meal (produced by steam dry method, Han Chang Fish Meal Co., Busan, Korea) and casein (Serva, Feinbiochemica GmbH & Co. Heidelberg, Germany) were used as the primary protein sources. Fermented food garbage in the diets increased mainly at the expense of wheat flour. The energy level of the diets was designed to be isocaloric (19.5 MJ/kg

Table 1. Production procedure of fermented food garbage

	Step I	Step II	Step III	Step IV
Food garbage	Storage for 3 hrs with wheat and microbial consortia	Fermentation for 5 hrs at 55℃	Aging for 14 hrs at room temperature	Transfer

Table 2. Ingredients and proximate composition of the experimental diets

	Fermented food garbage (%)			
	0	5	10	15
Ingredients (g/100 g)				
Pollack fish meal[1]	65.0	64.0	63.0	62.0
Casein, vitamin-free[2]	5.0	5.0	5.0	5.0
Wheat flour	17.0	13.0	9.0	5.0
Fermented food garbage[3] (DM basis)	0.0	5.0	10.0	15.0
Squid liver oil[4]	2.0	2.0	2.0	2.0
Vitamin premix[5]	2.5	2.5	2.5	2.5
Mineral premix[5]	3.0	3.0	3.0	3.0
Carboxymethyl cellulose[6]	5.0	5.0	5.0	5.0
Choline salt[6]	0.5	0.5	0.5	0.5
Proximate analysis (%, dry matter basis)				
Crude protein	53.2	53.1	53.7	54.7
Crude lipid	8.1	8.5	8.5	9.2
Crude fiber	1.3	1.3	1.3	1.3
Ash	18.3	18.2	17.9	17.7
N-free extract[7]	19.2	19.0	18.7	17.1
Gross energy (MJ/kg)	19.2	19.3	19.5	20.0

[1]Crude protein: 69% DM; Crude lipid: 9% DM.
[2]Serva, Feinbiochemica GmbH & Co. Heidelberg, Germany.
[3]Crude protein: 19% DM; Crude lipid: 14% DM.
[4]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[5]Same as Lee et al. (2000a).
[6]Sigma Chemical, St. Louis, MO, USA.
[7]Calculated by difference (100-crude protein-crude lipid-crude fiber-ash).

diet). Ingredients of the experimental diets were mechanically mixed with water at the ratio of 100 g ingredient mixture to 35-40 g water and pressure-pelleted. The experimental diets as moist pellets were stored at -30℃ until used.

Fish and feeding trial

Juvenile flounder (*Paralichthys olivaceus*) were purchased from a private fish hatchery (Gangneung, Korea). They were fed a commercial feed containing 50% protein for two weeks while being acclimated to the experimental conditions. Juvenile flounder (an average body weight of 4 g) were randomly allocated into 8 green circular fiberglass reinforced plastic tanks (90 cmΦ, 100 cm depth) with 25 fish to each tank. Two replicate groups of fish were hand-fed to visual satiety two times daily at 09:00 h and 17:00 h for 45 days according to the result described by Lee et al. (2000b). Pellet size was adjusted and appropriate sized pellet was fed as the fish grew. Filtered seawater (34±0.2‰) was supplied at a flow rate of 5 L/min to each tank. Water temperature was maintained at 18.8±2.1℃, and photoperiod was left at natural condition during the feeding trail. Fish in each tank were collectively weighed on the day of initiation and termination of the experiment after they were starved for 24 h and anesthetized with MS-222 (Tricaine methanesulfonate, Sigma, USA) at the concentration of 100 ppm.

Sample collection and chemical analysis

At the end of the feeding trial, blood samples were obtained from the caudal vein of 6 fish from each tank by using heparinized syringes after they were starved for 24 h and anesthetized with MS-222 at the concentration of 100 ppm. Blood plasma was collected after centrifugation (7500 rpm for 5 min) and stored at -70℃ as separate aliquots for analysis of glucose The fish were randomly sampled at the beginning (20 fish) and the end (15 fish from each tank) of the feeding trial and stored at -70℃ for subsequent proximate analysis of whole body.

After growth feeding trial, another experiment was carried out to investigate the changes of plasma glucose of flounder. Three replicate groups (50 fish to each tank) of juvenile flounder averaging 15 g were adapted to control and 15% fermented food garbage diets for two weeks and then, after being fasted for 48 h, fed the experimental diets. Blood samples were collected just before (time 0) and 1, 3, 5, 8, 11, 16, 24 and 48 h after feeding. Blood plasma was stored into freezer at -75℃ as separate aliquots for analysis of glucose.

Crude protein content was determined by Kjeldahl method using Auto Kjeldahl System (Buchi B-324/435/412, Switzerland), crude lipid content by ether-extraction method, moisture content by a dry oven (105℃ for 24 hours), crude fiber content by an automatic analyzer (Fibertec, Tecator, Hoganas, Sweden), and ash content by a furnace muffler (550℃ for 4 hours). Gross energy contents were analyzed using an adiabatic bomb calorimeter (Parr, Moline, IL, USA) and liver glycogen was measured by enzymatic method using amyloglucosidase (Fluka, EC 3.2.1.3) as described by Murat and Serfaty (1974). The content of glucose in the plasma were analyzed by using commercial clinical investigation kits (Wako Pure Chemical Industries, Ltd., Japan) according to manufacturer's recommendations.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using the SPSS program Version 7.5 (SPSS Inc., Michigan Avenue, Chicago, IL, USA). Significant differences ($P<0.05$) among mean were determined by Duncan's multiple range test (Duncan, 1955). The data are presented as mean±SEM of two replicate groups.

Results and Discussion

The growth performance of fish fed the experimental diets containing different fermented food garbage levels for 45 days are presented in Table 3. Survival, feed efficiency, hepatosomatic index and protein efficiency ratio of fish were not affected by dietary fermented food garbage level ($P>0.05$). Weight gain of fish fed the diets containing 5, 10 and 15% fermented food garbage were significantly higher than that of fish fed the control diet ($P<0.05$). Condition factor of fish fed the 10% fermented food garbage diet was significantly higher than that of fish fed the control diet ($P<0.05$). Daily feed intake and protein intake of fish fed the diets containing 5 and 15% fermented food garbage were significantly higher than those of fish fed the control diet ($P<0.05$). Daily lipid intake of fish fed the diets containing 5, 10 and 15% fermented food garbage were significantly higher than the control diet ($P<0.05$).

Higher feed intake of flounder fed the diets containing fermented food garbage was probably due to an increase of digestibility or attractive substances

Table 3. Growth performance of juvenile flounder (*Paralichthys olivaceus*) fed the diets containing various fermented food garbage levels for 45 days[1]

	Fermented food garbage (%)			
	0	5	10	15
Initial wt. (g/fish)	4.1±0.15	3.9±0.31	4.0±0.13	4.2±0.06
Survival (%)	78±18.0	94±2.0	82±14.0	98±2.0
Weight gain (%)[2]	167±27.3[a]	271±7.4[b]	266±23.9[b]	274±21.0[b]
Feed efficiency (%)[3]	90±3.6	96±4.2	94±2.4	94±0.1
Condition factor[4]	0.98±0.02[a]	1.03±0.01[ab]	1.09±0.04[b]	1.05±0.01[ab]
Hapatosomatic index[5]	1.35±0.05	1.35±0.14	1.41±0.13	1.37±0.01
Daily feed intake (%)[6]	2.02±0.11[a]	2.59±0.10[b]	2.43±0.15[ab]	2.72±0.11[b]
Daily protein intake (%)[6]	1.08±0.05[a]	1.46±0.05[b]	1.31±0.08[ab]	1.49±0.06[b]
Daily lipid intake (%)[6]	0.16±0.01[a]	0.22±0.01[b]	0.21±0.01[b]	0.25±0.01[b]
Protein efficiency ratio[7]	1.68±0.07	1.71±0.08	1.76±0.04	1.71±0.00

[1]Values (mean±SEM of two replications) in the same row not sharing a common superscript are significantly different ($P<0.05$).
[2](Final body weight − initial body weight)×100/initial body weight.
[3]Body wet weight gain×100/feed intake (DM basis).
[4]Body weight×100/total body length (cm)[3].
[5]Liver weight×100/body weight.
[6]Feed (protein or lipid) intake×100/[(initial fish wt.+final fish wt.+dead fish wt.)/2×days fed].
[7]Body wet weight gain/protein intake.

Table 4. Proximate analysis and (%) plasma glucose (mg/100 mL) of juvenile flounder (*Paralichthys olivaceus*) fed the diets containing various fermented food garbage levels for 45 days

	Fermented food garbage (%)			
	0	5	10	15
Whole body				
Moisture	75.1±0.7	75.6±0.7	75.9±0.9	76.0± 0.5
Crude protein	15.5±0.1	16.3±0.4	15.8±0.8	16.2± 0.8
Crude lipid	2.4±0.5	3.6±1.1	3.0±0.3	3.9± 0.1
Ash	4.2±0.1	4.0±0.7	4.1±0.5	3.9± 0.1
Liver				
Moisture	72.7±0.8	71.4±0.8	74.5±0.6	74.9± 0.2
Crude lipid	10.8±1.0	12.1±0.3	10.4±1.1	8.9± 1.5
Glycogen	2.6±0.3	2.9±0.5	2.8±0.8	2.8± 0.9
Plasma glucose	55.3±6.6	43.8±3.2	50.8±3.8	54.7±10.2

of fermented food garbage. Although nutrient digestibilities of ingredients used in this experimental diet were not investigated, the improvement of growth and feed efficiency of flounder fed the diets containing fermented food garbage is probably due to high digestibility of fermented food garbage by flounder. High nutrient digestibility and protein utilization have been reported in other fish fed the silage diet (Lapie and Bigueras-Benitez, 1992; Bairagi et al., 2002). However, more detail studies of its utilization are necessary for flounder.

Fish meal is an important ingredient for aquaculture diet. However the supply of high quality fish meal for aquaculture feeds has gradually decreased during the last decade, leading to an increased price in the product (Bimbo and Crowther, 1992; Tacon, 1997). Therefore, numerous studies have been carried out to investigate the potential of alternative and low-cost protein sources (Kaushik et al., 1995; El-Sayed, 1999; Bairagi et al., 2002), and mainly focused on utilization of available by-product protein sources to replace fish meal. On the other hand, because of low protein

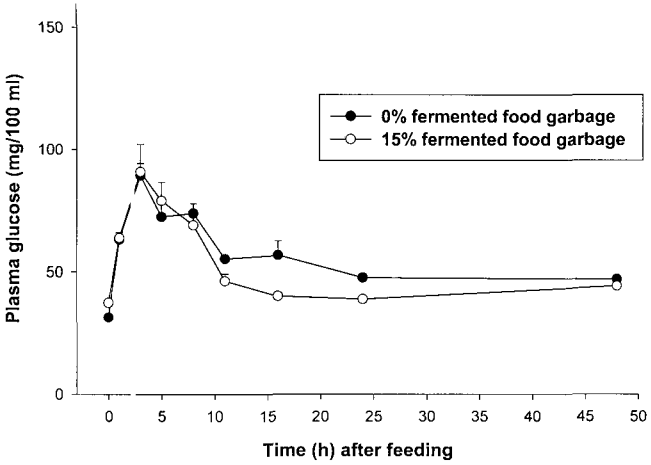

Fig. 1. Plasma glucose concentration of juvenile flounder (*Paralichthys olivaceus*) fed the diets containing 0 and 15% fermented food garbage levels.

content (about 20%) in the fermented food garbage, mainly wheat flour in the control diet was replaced with fermented food garbage in this study. Therefore, more detail study is needed to increase the utilization of fermented food garbage as a substitute for fish meal in the diet for flounder.

Proximate composition of whole body and liver of fish fed the diets containing different fermented food garbage levels are shown in Table 4. Moisture, protein, lipid, ash or glycogen contents of whole body or liver were not affected by dietary fermented food garbage level ($P>0.05$). Plasma glucose of fish at the end of the feeding trial was not affected by dietary fermented food garbage level ($P>0.05$).

Plasma glucose concentration of juvenile flounder after feeding of the experimental diets is shown in Fig. 1. Plasma glucose concentrations of fish fed the control diet and 15% fermented food garbage diet peaked at 5 h (89-90 mg/100 mL), then decreased to 24 h and 16 h, respectively, after feeding. The relatively prolonged glucose concentration of flounder fed the control diet with wheat flour compare to fermented food garbage diet indicates that carbohydrate in fermented food garbage was efficiently utilized.

The growth and feed utilization of juvenile flounder fed diets incorporating fermented food garbage were better than those of fish fed the control diet. It was concluded from the present study that fermented food garbage could be partially used as a feed ingredient for juvenile flounder up to 15% incorporation level. These results show that food garbage, which is one of environmental pollution source, can be made into silage acceptable for flounder farming.

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