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Effects of Dietary Inclusion of Red Ginseng Byproduct on Growth, Body Composition, Serum Chemistry, and Lysozyme Activity in Juvenile Olive Flounder (*Paralichthys olivaceus*)

In-Cheol Choi[1], Kyoung Tae Kim[1], In Chul Bang[2], Mun-Gyeong Kwon[3], Jong Ha Lee[3], Bae Ik Lee[3] and Sung Hwoan Cho[1]*

[1]*Division of Marine Environment and BioScience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791, Korea*
[2]*Department of Marine Biotechnology, Soonchunhyang University, Asan 336-745, Korea*
[3]*National Fisheries Research & Development Institute, Busan 619-705, Korea*

This study examined the effects of the dietary inclusion of various concentrations of red ginseng byproduct (RB) and a mixture containing red ginseng byproduct, garlic extract, yeast and filler (CR) on the growth, body composition, serum chemistry, and lysozyme activity of juvenile olive flounder (*Paralichthys olivaceus*). Juvenile fish (n=630) weighing 5.0 g were randomly distributed into 21 180 L flow-through tanks (30 fish/tank). Seven experimental diets were prepared in triplicate: a control diet without additive, and diets containing 0.5, 1 and 2% concentrations of RB (RB-0.5, RB-1, RB-2) and CR (CR-0.5, CR-1, CR-2) at the expense of wheat flour. After an 8-week feeding trial, serum chemistry and lysozyme activity of fish were measured. Mean weight gain was significantly higher in fish fed the control diet than in fish fed the RB and CR diets. The dietary inclusion of RB and CR reduced feed utilization. Mean serum glucose and triglyceride (TG) levels were higher in fish fed the control diet than in fish fed the other diets. Mean glutamate pyruvate transaminase (GPT) levels of fish fed the control and RB-2 diets were higher than those of fish fed the RB-0.5, RB-1, CR-1, and CR-2 diets. Mean lysozyme activity levels of fish fed the RB-0.5 and RB-1 diets were higher than those of fish fed the control and CR diets. The results of this study indicate that red ginseng byproduct may be utilized as an immunostimulant rather than as a growth promoter for juvenile olive flounder. Dietary inclusion of 0.5% red ginseng byproduct effectively improved serum glucose, GPT, TG, and lysozyme activity of the fish in this study.

Key words: Olive flounder (*Paralichthys olivaceus*), Red ginseng byproduct, Lysozyme activity, Serum chemistry

Introduction

Olive flounder (*Paralichthys olivaceus*) is a commercially important marine fish species in the aquaculture of eastern Asia, including Korea, Japan, and China. Many feeding trials have been performed to determine the dietary and nutrient requirements (Lee et al., 2000a, 2002; Kim and Lee, 2004), optimum feeding frequency/ratio (Lee et al., 1999; Cho et al., 2006b), alternative animal and/or plant dietary protein sources (Sato and Kikuchi, 1997; Kikuchi, 1999), and availability of dietary additives (Kim et al., 1998, 2002a, 2006a, b; Pham et al., 2006; Cha et al., 2008; Seo et al., 2009) for the olive flounder.

Although many additives have been investigated, few have been used successfully in commercial flounder farms. These include dietary herb mixtures (obosan) and chitin solution (chitosan). The frequent and year-round occurrence of disease outbreaks and unfavorable environmental conditions, such as cold water mass, reduce fish production. Further research to identify dietary additives that effectively improve disease resistance and/or immune response among fish is thus necessary.

The use of natural dietary additives for fish has several advantages, including the maintenance of

*Corresponding author: chosunh@hhu.ac.kr

safety standards for human consumption. Ginseng (*Panax ginseng* CA Meyer) is a traditional Asian herbal medicine. Steamed fresh ginseng root, called red ginseng (Cho et al., 2006c), contains ginseng saponins and various ginsenosides that have healing effects on burn wounds (Kimura et al., 2006) and have been shown to exert antioxidant (Bae and Kim, 1998), antitumor (Lee et al., 2000b), anticancer (Fishbein et al., 2009), and anti-inflammatory (Bae et al., 2008) effects. These components have also produced antithrombotic and antiplatelet (Jin et al., 2007), and antistress activities that aid in the prevention of disease (Kaneko, 2004). Red ginseng has prevented thrombus and atheroma formation in individuals with hypercholesterolemia (Hwang et al., 2008).

The extraction of red ginseng creates byproducts that have potential as an immunostimulant additive to aquafeed. The combined dietary administration of ginseng, garlic extract, aloe powder, and kelp powder improved weight gain in juvenile and adult olive flounder (Heo and Yun, 1995). Garlic *(Allium sativum*) also has antimicrobial, antibacterial, anti-inflamma-tory, and antioxidant effects (Wilson and Demmig-Adams, 2007; Yang, 2007).

No study examining the application of red ginseng in fisheries has been published to date. In this study, the effects of the dietary administration of various concentrations of red ginseng byproduct and a mixture containing red ginseng byproduct, garlic extract, yeast, and filler on the growth, body composition, serum chemistry, and lysozyme activity of juvenile olive flounder were determined.

Materials and Methods

Experimental conditions

Juvenile olive flounder were purchased from a private hatchery (Taean, Chungcheongnam Do, Korea), transferred to the laboratory, and acclimated for 2 weeks before the initiation of the feeding trial. During the acclimation period, the fish were fed a commercially available extruded pellet food (54% crude protein, 11% crude lipid) twice a day. Six hundred thirty juvenile fish weighing 5.0 g were randomly distributed into 21 180 L flow-through tanks (30 fish/tank; water volume: 150 L; flow rate: 6.8 L/min). Sand-filtered natural seawater and aeration were supplied to each tank. The water temperature ranged from 18.4°C to 24.1°C (mean±SD: 21.5±1.71°C), and the photoperiod followed natural conditions.

Preparation of red ginseng byproduct for the experimental diets

Red ginseng byproduct (RB; 16.4% crude protein, 1.1% crude lipid, 5.9% ash; Korea Ginseng Corp., Daejeon, Korea) and a 5:1:2:2 mixture of red ginseng byproduct, garlic extract, yeast, and filler (CR; 18.9% crude protein, 2.3% crude lipid, 13.2% ash) were used as dietary additives. The following seven experimental diets with various concentrations of RB and CR were prepared in triplicate: a control diet without additive; diets with 0.5%, 1%, and 2% concentrations of RB (RB-0.5, RB-1, RB-2); and diets with 0.5%, 1%, and 2% concentrations of CR (CR-0.5, CR-1, CR-2) at the expense of wheat flour (Table 1). Fishmeal, dehulled soybean meal, and corn gluten meal were used as protein sources in the experimental diets. Wheat flour and squid liver and soybean oils were used as the carbohydrate and lipid sources, respectively. All experimental diets were prepared to satisfy the dietary nutrient requirements of olive flounder (Lee et al., 2000a, 2002). The ingredients were mixed with water at a ratio of 3:1 and pelletized with a pellet extruder. The pellets were then dried at room temperature overnight and stored at -20°C until use. All fish were hand fed to apparent satiation twice a day (09:00, 17:00) for 8 weeks.

Chemical analysis of the experimental diets and fish

Proximate analysis was conducted on the experimental diets and on 10 fish sacrificed at the beginning of the trial and five fish from each tank sacrificed at the end of the 8 week feeding trial. Crude protein was determined by the Kjeldahl method (Kjeltec 2100 distillation unit; Foss Tecator, Hoganas, Sweden), crude lipid was ascertained using an ether-extraction method (Soxtec TM 2043; Foss Tecator), moisture was measured after oven drying at 105°C for 24 h, fiber was determined using an automatic analyzer (Fibertec; Foss Tecator), and ash was measured using a muffle furnace at 550°C for 4 h. All methods were performed according to the standards of the Association of Official Analytical Chemists (AOAC, 1990).

Chemical analysis of blood

At the end of the 8 week feeding trial, blood samples were collected from the caudal veins of three randomly chosen fish from each tank. Fish were starved for 24 h prior to bleeding. Serum was collected after centrifugation (6,000 rpm, 5 min), pooled by tank, and stored at -70°C as separate aliquots. Serum samples were analyzed using an

Table 1. Ingredients and chemical composition (%, DM basis) of the experimental diets

	Experimental diets						
	Con	RB-0.5	RB-1	RB-2	CR-0.5	CR-1	CR-2
Ingredients (%)							
Fishmeal	55	55	55	55	55	55	55
Soybean meal	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Corn gluten	5	5	5	5	5	5	5
Wheat flour	25	24.5	24	23	24.5	24	23
Red ginseng byproduct[1]		0.5	1	2			
Mixture of red ginseng byproduct[2]					0.5	1	2
Squid liver oil	2	2	2	2	2	2	2
Soybean oil	2	2	2	2	2	2	2
Vitamin premix[3]	1	1	1	1	1	1	1
Mineral premix[4]	1	1	1	1	1	1	1
Choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient (%, DM)							
Dry matter	82.7	89.1	90.4	89.2	89.8	89.6	86.8
Crude protein	51.5	52.8	53.0	51.9	53.4	53.7	53.6
Crude lipid	8.9	10.8	10.7	11.0	10.6	10.3	10.8
Ash	8.4	9.3	9.3	9.2	9.5	9.4	9.9
GE[4] (kcal/g diet)	4.5	4.4	4.4	4.4	4.3	4.3	4.3

[1]Red ginseng byproduct (16.4% crude protein, 1.1% crude lipid and 5.9% ash) and [2]Mixture of red ginseng byproduct (18.9% crude protein, 2.3% crude lipid and 13.2% ash) composed of red ginseng byproduct, garlic extract, yeast and filler at the ratio of 5:1:2:2 were supplied from Korea Ginseng Corp. (Daejeon, Korea).
[3]Vitamin and [4]Mineral premix were same as Cho et al. (2007)'s study.
[4]Gross energy (GE) (kcal/g diet) was determined by bomb calorimeter (6100 Compensated Jacket Calorimeter, Parr Instrument Company, IL, USA).

automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II; Johnson and Johnson Clinical Diagnostics, Inc., New York, NY, USA) to determine total protein, glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), cholesterol, and triglyceride (TG).

Lysozyme activity assay

Turbidimetric assay for lysozyme was carried out according to Parry et al. (1965). Briefly, test serum (100 μL) was added to 2 mL of a *Micrococcus lysodeikticus* suspension (0.2 mg/mL; Sigma Chemicals, MO, USA) in a sodium phosphate buffer (0.05 M, pH 6.2). The reaction was carried out at 25°C, and absorbance at 530 nm was measured with a spectrophotometer after 0.5 and 4.5 min. One lysozyme activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001/min.

Challenge test

Ten externally normal fish were chosen from each tank at the end of the 8-week feeding trial and determined to be free of bacterial infection. A reference pathogenic strain of *Edwardsiella tarda* was used for the challenge test. A culture suspension of *E. tarda* was grown on agar for 24 h, collected, washed, and suspended in a sterile 0.85% saline solution. The bacteria were then counted. The fish were artificially infected through intraperitoneal injections of 0.1 mL pathogenic *E. tarda* culture suspension containing 4×10^8 cells/mL and monitored for 96 h. Dead fish were removed every 3 h for the first 24 h, every 6 h for the second 24 h, and every 12 h for the final 48 h. The water temperature ranged from 22.0°C to 23.3°C.

Statistical analysis

Differences among means of dietary treatment effects were analyzed with one-way analysis of variance (ANOVA) and Duncan's multiple range test (Duncan, 1955) using SAS software (ver. 9.1; SAS Institute, Cary, NC, USA).

Results and Discussion

Survival ranged from 94.4% to 100% and did not differ significantly among the experimental diets ($P>0.05$; Table 2). However, average weight gain was significantly higher in juvenile olive flounders fed control diet than those in fish fed RB and CR diets ($P<0.05$). Average specific growth rate (SGR) of juvenile olive flounder fed control diet was significantly higher than those of fish fed RB-0.5, RB-2, CR-0.5, CR-1, and CR-2 diets ($P<0.05$), but did not differ significantly from those of fish fed RB-1 diet. The

Table 2. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of juvenile olive flounder fed the experimental diets with various concentrations of red ginseng byproduct and mixture of red ginseng byproduct for 8 weeks

Diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1]
Con	5.0 ± 0.01	33.9 ± 0.51	100 ± 0.00	28.8 ± 0.52[a]	3.60 ± 0.031[a]
RB-0.5	5.0 ± 0.01	28.4 ± 1.40	96.7 ± 0.00	23.4 ± 1.39[b]	3.28 ± 0.087[b]
RB-1	4.9 ± 0.01	30.3 ± 0.46	100 ± 0.00	25.4 ± 0.45[b]	3.42 ± 0.027[ab]
RB-2	5.0 ± 0.01	28.4 ± 1.59	94.4 ± 2.22	23.4 ± 1.59[b]	3.27 ± 0.105[b]
CR-0.5	5.0 ± 0.04	29.0 ± 0.10	97.8 ± 2.22	24.0 ± 0.10[b]	3.33 ± 0.016[b]
CR-1	5.0 ± 0.02	28.4 ± 1.47	97.8 ± 1.11	23.5 ± 1.45[b]	3.28 ± 0.090[b]
CR-2	5.0 ± 0.01	28.7 ± 0.68	96.7 ± 0.00	23.7 ± 0.69[b]	3.29 ± 0.048[b]

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).
[1]SGR = (Ln final weight of fish - Ln initial weight of fish) × 100/days of feeding trial.

Table 3. Daily feeding rate (DFR), feed efficiency ratio (FER), protein efficiency ratio (PER) and protein retention (PR) of juvenile olive flounder fed the experimental diets with various concentrations of red ginseng byproduct and mixture of red ginseng byproduct for 8 weeks

Diets	DFR[1]	FER[2]	PER[3]	PR[4]
Con	2.1 ± 0.06	1.33 ± 0.034[a]	2.57 ± 0.067[a]	44.5 ± 1.17[a]
RB-0.5	2.3 ± 0.01	1.10 ± 0.044[b]	2.08 ± 0.083[b]	32.8 ± 1.37[b]
RB-1	2.3 ± 0.10	1.20 ± 0.025[ab]	2.26 ± 0.047[b]	38.5 ± 0.76[ab]
RB-2	2.3 ± 0.01	1.09 ± 0.070[b]	2.11 ± 0.134[b]	35.5 ± 3.73[b]
CR-0.5	2.4 ± 0.06	1.15 ± 0.012[b]	2.15 ± 0.023[b]	34.6 ± 1.73[b]
CR-1	2.3 ± 0.05	1.14 ± 0.070[b]	2.12 ± 0.130[b]	36.0 ± 3.72[b]
CR-2	2.4 ± 0.08	1.14 ± 0.004[b]	2.13 ± 0.008[b]	36.4 ± 1.38[b]

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).
[1]Daily feeding rate (%, DFR) =Feed intake × 100/[(initial fish weight + final fish weight + dead fish weight)/2 × days of feeding].
[2]Feed efficiency ratio (FER) = Weight gain of fish/feed consumed.
[3]Protein efficiency ratio (PER) = Weight gain of fish/protein consumed.
[4]Protein retention (PR) = Protein gain of fish/protein consumed.

adverse effects of the RB and CR diets on weight gain and SGR of fish in this study indicate that the dietary inclusion of RB and CR could diminish the growth of fish. Another study found that the dietary inclusion of chitin and chitosan depressed growth in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*; Shiau and Yu, 1999). However, Heo and Yun (1995) reported that dietary administration of a mixture containing 1% ginseng powder, 0.5% garlic extract, 1% aloe powder, and 5% kelp powder improved weight gain in juvenile and adult olive flounder at commercial farms, but cautioned that the sole effect of ginseng on fish performance has not been clearly demonstrated. Several additive types, such as herbs (Kim et al., 1998), glucan (Kim et al., 2006b), *Chlorella ellipsoidea* powder (Kim et al., 2002a), onion powder (Cho et al., 2011), and wood vinegar (Lee et al., 2008), improved weight gain among olive flounder.

Average daily feeding rates (DFR) of juvenile olive flounder did not differ significantly among the experimental diets (Table 3), indicating that the inferior weight gain observed among fish fed the RB and CR diets was not due to decreased feed consumption. Average feed efficiency ratio (FER) and protein retention (PR) of olive flounder fed the control diet were significantly higher than those of fish fed RB-0.5, RB-2, and CR diets ($P<0.05$), but did not differ significantly from those of fish fed RB-1 diet. The poor weight gain and FER of fish fed RB and CR diets in this study indicate that the dietary inclusion of RB and CR likely reduced the digestion and absorption of dietary fat (Han et al., 2005; Karu et al., 2007) and increased energy expenditure, as observed among rats (Lee et al., 2009). Average protein efficiency ratio (PER) of fish fed control diet was significantly higher than that of fish fed the other diets ($P<0.05$). In contrast to the results of this study, administration of various additives has been shown to improve FER and/or PER and increase weight

Table 4. Chemical composition (%, wet weight basis) of the whole body excluding the liver and liver of olive flounder at the end of the 8-week feeding trial

Treatments	Whole body excluding liver			
	Moisture	Crude protein	Crude lipid	Ash
Con	69.5 ± 0.95[b]	17.2 ± 0.25	3.6 ± 0.10	3.7 ± 0.13
RB-0.5	76.0 ± 0.35[a]	15.9 ± 0.32	3.2 ± 0.14	3.3 ± 0.10
RB-1	74.5 ± 0.98[a]	17.1 ± 0.56	3.4 ± 0.09	3.4 ± 0.12
RB-2	75.0 ± 0.95[a]	16.8 ± 0.56	3.3 ± 0.20	3.4 ± 0.23
CR-0.5	75.6 ± 1.12[a]	16.2 ± 0.72	3.4 ± 0.25	3.4 ± 0.19
CR-1	75.4 ± 1.01[a]	16.9 ± 0.60	3.4 ± 0.30	3.4 ± 0.12
CR-2	74.8 ± 0.97[a]	17.0 ± 0.58	3.5 ± 0.13	3.7 ± 0.20
	Liver			
	Moisture	Crude protein	Crude lipid	
Con	66.7 ± 1.88	10.3 ± 0.20	17.9 ± 1.38	
RB-0.5	65.8 ± 0.67	9.8 ± 0.15	17.9 ± 0.18	
RB-1	66.7 ± 0.95	10.0 ± 0.29	16.9 ± 0.29	
RB-2	64.1 ± 0.58	10.1 ± 0.38	17.4 ± 0.56	
CR-0.5	65.4 ± 1.19	10.1 ± 0.39	16.6 ± 0.60	
CR-1	65.7 ± 1.54	10.1 ± 0.32	16.5 ± 0.57	
CR-2	65.5 ± 1.14	9.9 ± 0.14	17.4 ± 0.39	

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

Table 5. Serum chemical composition in juvenile olive flounder at the end of the 8-week feeding trial

Diets	Total protein (g/dL)	Glucose (mg/dL)	GOT (IU/L)	GPT (IU/L)	Cholesterol (mg/dL)	TG (mg/dL)
Con	3.1 ± 0.12	74.0 ± 7.23[a]	28.7 ± 4.06	13.7 ± 2.96[a]	190 ± 15.8	459 ± 98.4[a]
RB-0.5	2.7 ± 0.09	30.3 ± 6.96[b]	19.3 ± 1.45	2.3 ± 0.67[b]	118 ± 19.5	83 ± 7.2[b]
RB-1	2.8 ± 0.30	41.3 ± 11.3[b]	19.0 ± 1.73	4.0 ± 1.53[b]	151 ± 22.7	106 ± 12.7[b]
RB-2	3.1 ± 0.12	41.7 ± 6.57[b]	27.7 ± 5.36	13.7 ± 4.10[a]	173 ± 11.7	110 ± 8.8[b]
CR-0.5	2.5 ± 0.38	30.7 ± 4.37[b]	22.7 ± 5.78	9.3 ± 1.76[ab]	153 ± 16.5	151 ± 17.9[b]
CR-1	2.6 ± 0.33	38.7 ± 3.71[b]	20.0 ± 2.65	4.0 ± 1.53[b]	149 ± 26.7	93 ± 21.5[b]
CR-2	2.3 ± 0.25	34.0 ± 6.43[b]	19.7 ± 7.67	2.3 ± 0.33[b]	117 ± 10.6	98 ± 3.0[b]

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

gain among fish (Kim et al., 1998, 2002a; Lee et al., 2008).

Chemical composition of the whole bodies of fish (excluding the liver) did not differ significantly among the experimental diets, except for the moisture content, which was significantly lower among fish fed the control diet than among fish fed the other diets ($P<0.05$; Table 4). Similarly, previous studies have not found the dietary inclusion of additives to affect the proximate composition of olive flounder (Park et al., 2003; Cho et al., 2011).

Average serum total protein, GOT, and cholesterol levels of juvenile olive flounder did not differ significantly among the experimental diets in this study (Table 5). However, average glucose level of olive flounder fed control diet was significantly higher than those of fish fed the other diets ($P<0.05$), likely due to the ginsenosides in the red ginseng byproduct. Similarly, the administration of red ginseng (Shon et al., 2004) and fermented red ginseng extracts (Kim et al., 2009) containing ginsenosides lowered the serum glucose levels of streptozotocin-induced diabetic rats. Average GPT level of olive flounder fed control and RB-2 diets was significantly higher than those of fish fed RB-0.5, RB-1, CR-1, and CR-2 diets ($P<0.05$), but did not differ significantly from those of fish fed CR-0.5 diet. In contrast, Kim et al. (2009) reported that red ginseng fermented with mixed *Lactobacillus plantarum* and *Saccharomyces cerevisiae* at the ratio of 1:1 lowered both GOT and GPT among diabetic rats. Average TG of olive flounder fed control diet was significantly higher than those of fish fed the other diets ($P<0.05$), in agreement with the results of the other studies (Shon et al., 2004; Kim et al., 2009) showing that dietary inclusion of red ginseng lowered the serum TG of rats. Administration of red ginseng has also been shown to lower serum TG in humans (Kim et al., 2002b).

Reduced serum glucose levels, GPT, and TG of olive flounder fed RB and CR diets in this study indicate that RB and CR dietary additives may improve the quality of fish. Various additives have shown different effects on serum criteria in olive flounder; administration of *C. ellipsoidea* (Kim et al., 2002a), green tea (Cho et al., 2007), and herbs (Lee et al., 1998) lowered serum cholesterol, low-density lipoprotein cholesterol, and GOT and GPT, respectively.

Average lysozyme activity level of olive flounder fed RB-0.5 and RB-1 diets were significantly higher than those of fish fed control and CR diets ($P<0.05$), but did not differ significantly from that of fish fed the RB-2 diet (Fig. 1). Average lysozyme activity level of olive flounder fed RB-2 diet was significantly higher than those of fish fed control and CR-2 diets ($P<0.05$). Higher lysozyme activity level of fish fed RB diets compared with fish fed control diet in this study indicates that RB acted effectively as an immunostimulant. Saponins in red ginseng extract have been found to induce apoptosis and decrease telomerase activity in human leukemia cells (Park et al., 2009) and to induce burn wound healing in mice (Kimura et al., 2006). The inhibitory effects of red ginseng against malon-dialdehyde tetrabutylammonium (MDA) salt and superoxide, and its protective effect against tert-butyl hydroperoxide (t-BHP) cytotoxicity in normal hepatocytes have also been reported (Bae and Kim, 1998). Dietary inclusion of *Undaria* improved lysozyme activity in olive flounder (Park et al., 2003), carp (*Cyprinus carpio*), and Nile tilapia (*Oreochromis niloticus*) (Ardo et al., 2008; Yin et al., 2009).

Mortality was initially observed in all fish groups at 60 h after *E. tarda* infection, and exceeded 95% for all experimental diets at 96 h. Sakai (1999) demonstrated that immunostimulants could reduce aquaculture losses caused by disease, but that their effectiveness depended on the disease and the physical condition of the fish, and on the timing, dosage, and method of administration.

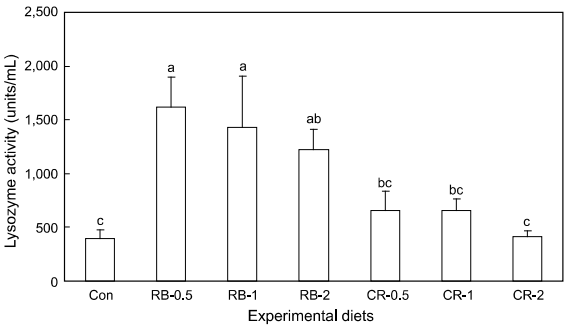

Fig. 1. Lysozyme activity (units/mL) of juvenile olive flounder fed the experimental diets containing the various concentrations of red ginseng byproduct (RB) and mixture of red ginseng byproduct (CR). Values (means of triplicate ± SE) with the same letter are not significantly different ($P > 0.05$).

Dietary administration of RB and CR diminished growth and feed utilization of olive flounder in this study, but improved lysozyme activity and some serum criteria. Such diets should thus be cautiously used. Red ginseng byproduct may be utilized as an immunostimulant rather than as a growth promoter for juvenile olive flounder. Dietary inclusion of 0.5% red ginseng byproduct effectively improved serum glucose, GPT, TG, and lysozyme activity among the fish in this study.

References

AOAC. 1990. Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Ardo L, Yin G, Xu P, Varadi L, Szigeti G, Jeney Z and Jeney G. 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistant against *Aeromonas hydrophila*. Aquaculture 275, 26-33.

Bae K and Kim S. 1998. Antioxidant effects of Korea ginseng *Radix*, Korea red ginseng *Radix* and saponin. Kor J Oriental Med Pathol 12, 72-81.

Bae E, Yoo Y, Lee I, Han MJ and Kim D. 2008. The anti-inflammatory effect of fermented red ginseng in experimental colitic mice. Food Agricult Immunol 19, 313-323.

Cha S, Lee J, Song C, Lee K and Jeon Y. 2008. Effects of chitosan-coated diet on improving water quality and innate immunity in the olive flounder, *Paralithchys olivaceus*. Aquaculture 278, 110-118.

Cho SH, Lee SM, Park BH, Ji SC, Kwon M, Kim Y, Lee JH, Park S and Han HK. 2006a. Effect of dietary inclusion of various sources of green tea on immune system and challenging test of juvenile olive flounder *Paralichthys olivaceus*. J Aquacult 19, 84-89.

Cho SH, Lee S, Park BH and Lee S. 2006b. Effect of feeding ratio on growth and body composition of juvenile olive flounder *Paralichthys olivaceus* fed extruded pellets during the summer season. Aquaculture 251, 78-84.

Cho WC, Chung WS, Lee Sk, Leung AW, Cheng CH and Yue KK. 2006c. Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipi-

demic efficacies in a streptozotocin-induced diabetic rats. Europ J Pharmacol 550, 173-179.

Cho SH, Lee S, Park BH, Ji SC, Lee JH, Bae J and Oh S. 2007. Effect of dietary inclusion of various sources of green tea on growth, body composition and blood chemistry of juvenile olive flounder *Paralichthys olivaceus*. Fish Physiol Biochem 33, 49-57.

Cho SH, Cho YJ, Lee S and Yoo J. 2011. Effects of putative growth or health enhancing dietary additives on juvenile olive flounder, *Paralichthys olivaceus*, performance. J World Aquacult Soc. (in press).

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

Fishbein AB, Wang C, Li X, Mehendale SR, Sun S, Aung HH and Yuan C. 2009. Asian ginseng enhances the anti-proliferative effect of 5-fluorouracil on human colorectal cancer: comparison between white and red ginseng. Arch Pharm Res 32, 505-513.

Han LK, Zheng ZY, Yoshikawa M, Okuda H, Kimura Y. 2005. Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicus* rhizomes. BMC Complement Altern Med 5, 9.

Heo G and Yun H. 1995. Efficacy of the ginseng powder-fortified crude drug to cultured flounders. J Vet Sci CNU 3, 65-70.

Hwang S, Seon DJ, Kim I, Kim D, Sohn S, Lee J and Kim S. 2008. Korean red ginseng attenuates hyper-cholesterolemia-enhanced platelet aggregation through suppression of diacylglycerol liberation in high-cholesterol-diet-fed rabbits. Phytother Res 22, 778-783.

Jin Y, Yu JY, Lee J, You S, Chung J, Noh J, Im J, Han X, Kim T, Shin K, Wee J and Yun Y. 2007. Antithrombotic and antiplatelet activities of Korean red ginseng extract. Basic Clin Pharmacol Toxicol 100, 170-175.

Kaneko H. 2004. Proof of the mysterious efficacy of ginseng: basic and clinical trials: clinical effects of medical ginseng, Korean red ginseng: specifically, its anti- stress action for prevention of disease. J Pharm Sci 95, 158-162.

Karu N, Reifen R and Karem Z. 2007. Weight gain reduction in mice fed *Panax ginseng* saponin, a pancreatic lipase inhibitor. J Agric Food Chem 55, 2824-2828.

Kikuchi K. 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 179, 3-11.

Kim DS, Kim JH, Jung CH, Lee SY, Lee SM and Moon YB. 1998. Utilization of obosan (dietary herbs) I. Effects on survival, growth, feed conversion ratio and condition factor in olive flounder, *Paralichthys olivaceus*. J Aquacult 11, 213-221.

Kim K, Bai S, Koo J, Wang X and Kim S. 2002a. Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile Japanese flounder *Paralichthys olivaceus*. J World Aquacult Soc 33, 425-431.

Kim S, Kim J, Kim H, Shin M, Park C, Park M and Yang J. 2002b. The effects of red ginseng product and combined exercise on blood lipids and body composition of obese women in their twenties. J Ginseng Res 26, 59-66.

Kim K and Lee S. 2004. Requirements of dietary n-3 highly unsaturated fatty acids for juvenile flounder (*Paralichthys olivaceus*). Aquaculture 229, 315-323.

Kim MJ, Kim MC, Kim T, Kim KU and Heo MS. 2006a. Effect of dietary supplementation of extracts of mushroom mycelium on survival and growth of juvenile flounder, *Paralichthys olivaceus*. J Aquacult 19, 231-235.

Kim Y, Kim K, Lee S, Park G, Okorie OE, Kang YJ and Bai SC. 2006b. Dietary β-1, 3 glucan on growth and immune responses in juvenile olive flounder, *Paralichthys olivaceus*. J Aquacult 19, 247-253.

Kim HJ, Seo MH, Lee EK, Cho HE, Choi YH, Lee KN and Chong MS. 2009. Effect of fermented red ginseng extracts on physiological activity and blood glucose level in streptozotocin induced diabetic rats. Kor J Oriental Physiol Pathol 23, 107-1094.

Kimura Y, Sumiyoshi M, Kawahira K and Sakanaka M. 2006. Effects of ginseng saponins isolated from red ginseng roots on burn wound healing in mice. British J Pharm 148, 860-870.

Lee KH, Lee YS, Kim JH and Kim DS. 1998. Utilization of obosan (dietary herbs) II. Muscle quality of olive flounder, *Paralichthys olivaceus* fed with diet containing obosan. J Aquacult 11, 319-325.

Lee S, Seo C and Cho Y. 1999. Growth of the juvenile olive flounder (*Paralichthys olivaceus*) fed the diets at different feeding frequencies. J Kor Fish Soc 32, 18-21.

Lee S, Cho SH, Kim KD. 2000a. Effects of dietary protein and energy levels on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). J World Aquacult Soc 31, 306-315.

Lee TH, Kim SH, Park JD and Kim DH. 2000b. Study on antitumor activity, antimetastatic effects of Hongsam-daibotangKaKambang. Kor J Oriental Med Pathol 12, 72-81.

Lee SM, Park CS and Bang IC. 2002. Dietary protein requirement of young Japanese flounder *Paralichthys olivaceus* fed isocaloric diets. Fish Sci 68, 158-164.

Lee S, Park GJ and Bai S. 2008. Effects of dietary wood vinegar supplementation on growth and immune responses of juvenile olive flounder, *Paralichthys olivaceus*. J Kor Fish Soc 41, 248-252.

Lee HJ, Lee Y, Park SK, Kang ES, Kim H, Lee YC, Choi

CS, Park SE, Ahn CW, Cha BS, Lee KW, Kim K, Lim SK and Lee HC. 2009. Korean red ginseng (*Panax ginseng*) improves insulin sensitivity and attenuates the development of diabetes in Otsuka Long-Evans Tokushima fatty rats. Metabolism Clinical Exp 58, 1170-1177.

Park SU, Kwon MG, Lee YH, Shin IS and Min SM. 2003. Effects of supplemental *Undaria*, obosan and wasabi in the experimental diets on growth, body composition, blood chemistry and non-specific immune response of juvenile flounder, *Paralichthys olivaceus*. J Aquacult 16, 210-215.

Park SE, Park C, Kim SH, Hossain MA, Kim MY, Chung HY, Son WS, Kim G, Choi YH and Kim ND. 2009. Korean red ginseng extract induces apoptosis and decreases telomerase activity in human leukemia cells. J Ethnopharm 121, 304-312.

Parry RM, Chandau RC and Shahani RM. 1965. A rapid and sensitive assay of muramidase. Proc Soc Exp Bio Med 119, 384-386.

Pham MA, Lee KJ, Lee BJ, Lim SJ, Kim SS, Lee YD, Heo MS and Lee KW. 2006. Effects of dietary *Hizikia fusiformis* on growth and immune responses in juvenile olive flounder (*Paralichthys olivaceus*). Asian-Aust J Anim Sci 19, 1769-1775.

Sakai M. 1999. Current research status of fish immunostimulants. Aquaculture 172, 63-92.

Sato T and Kikuchi K. 1997. Meatmeal as a protein source in the diet of juvenile Japanese flounder. Fish Sci 63, 77-880.

Seo J, Kim K, Shin I, Choi K and Lee S. 2009. Effect of supplemental dietary wasabi extract, chitosan and *Pophyra* on growth and body composition of juvenile flounder, *Paralichthys olivaceus*. Kor J Fish Aquati Sci 42, 257-261.

Shiau Y and Yu Y. 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 179, 439- 446.

Shon M, Choi S, Cho H and Sung N. 2004. Effects of cereal and red ginseng flour blood glucose and lipid level in streptozotocin-induced diabetic rats. J Korean Soc Food Sci Nutr 33, 1463-1468.

Wilson EA and Demmig-Adams B. 2007. Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onions. Nutr Food Sci 37, 178-183.

Yang ST. 2007. Antioxidant activity of extracts of aged black garlic on oxidation of human low density lipoprotein. J Life Sci 17, 1330-1336.

Yin G, Ardo L, Thompson KD, Adams A, Jeney Z and Jeney G. 2009. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. Fish Shell Immunol 26, 140-145.

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