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Effects of Dietary Cheongkukjang on Liver Superoxide Dismutase Activity of Parrotfish *Oplegnathus fasciatus*

Minh Anh Pham and Kyeong-Jun Lee*
Department of Marine Life Science, Cheju National University, Jeju 690-756, Korea

A four-week feeding trial was conducted to investigate the effects of dietary soybean meal (SBM) and powdered Cheongkukjang (CKJ) on non-specific immune responses of parrotfish *Oplegnathus fasciatus*. Three isonitrogenous (42 % crude protein) and isocaloric (17.1 MJ/kg) diets were formulated to replace fish meal by 0, 25% SBM or 25% CKJ (designated as FM, 25SBM and 25CKJ, respectively). Ninety fish (initial body weight 122 g) were randomly allotted into nine 150 L tanks. One of the three experimental diets was fed to triplicate groups of fish for 4 weeks. After the feeding trial, no differences were observed in growth performances and feed utilization among fish groups. Liver superoxide dismutase activity of the fish fed CKJ containing diet was significantly higher than that of the control groups. DPPH radical scavenging and Fe^{2+}-chelating activities of the experimental diets containing SBM or powdered CKJ were significantly higher than that of the control diet. The results of the present study suggest that dietary inclusion of powdered 25CKJ significantly increased liver superoxide dismutase activity and did not affect the growth performances, feed utilization, morphological parameters, as well as hematological values of parrotfish.

Keywords: Parrotfish, *Opegnathus fasciatus*, Cheongkukjang, Soybean meal, Feeds

Introduction

Cheongkukjang (CKJ), a traditional Korean fermented soybean with rice straw, has been reported to have unique flavor, antimicrobial, anticarcinogenic, antioxidant activities, and high nutritional compositions (Kim et al., 1998; Kim et al., 1999; Youn et al., 2001; Kim et al., 2004; Lee et al., 2005; Mine et al., 2005). Recently, several studies reported that use of dietary fermented vegetable products could enhance non-specific immune responses and disease resistances in fish (Ashida et al., 2002; Ashida and Okimasu, 2005; Ashida et al., 2006). Use of natural fermented products has become more attractive since consumers are seriously paying concerns on quality and safety of aquaculture products. No information is available on the use of CKJ in diets for fish.

Parrotfish *Oplegnathus fasciatus* is one of the most important aquaculture fish species in Korea. However, outbreak of diseases has been reported as a cause of severe loss in captive parrotfish and decelerated the aquaculture development of this species. Jung and Oh (2000) reported that high mortalities of net-caged parrotfish were observed in southern coastal areas of Korea peninsula by a disease occurred in all developmental stages of fish including 3-year-old marketable fish.

Therefore, the aim of the present study was to evaluate the effect of dietary powdered Cheongkukjang on growth performances and immune responses of growing-out parrotfish.

Materials and Methods

Experimental diets

Three experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (42%) and gross energy (17.1 MJ/kg). The energy value of the experimental diets was estimated on the basis of mammalian physiological fuel value, i.e., 16.7 KJ g^{-1} protein or carbohydrate and 37.7 KJ g^{-1} lipid (Lee and Putman, 1973). The dietary formulation and proximate compositions are presented in Table 1. For the control diet (FM), white fish meal was used as major dietary protein source. For diet 25SBM and 25CKJ, fish meal in the control diet was replaced by 25% soybean meal (SBM) and 25% Cheongkukjang (CKJ), respectively. In diets containing SBM or CKJ, methionine and lysine were supplemented to meet their requirement of fish. The CKJ powder was purchased in the market, and it was a product processed by a tra-

*Corresponding author: kjlee@cheju.ac.kr

Table 1. Formulation, proximate composition of the experimental diets (% DM)

Ingredients	Diets		
	FM	25SBM	25CKJ
White fish meal	52.0	39.0	39.0
Soybean meal	0.0	20.0	0.0
Cheongkukjang[1)]	0.0	0.0	21.8
Corn gluten meal	8.2	7.4	8.6
Starch	24.8	17.4	19.4
Yeast	2.0	2.0	2.0
Mineral mix[2)]	1.0	1.0	1.0
Vitamin mix[3)]	1.0	1.0	1.0
Squid liver oil	8.0	8.7	4.2
Lysine	0.0	0.4	0.4
Methionine	0.0	0.2	0.2
Monocalcium phosphate	0.0	0.6	0.6
Cellulose	3.0	2.3	1.8
Proximate composition			
Dry matter	96.3	96.9	96.2
Protein	42.1	43.6	42.9
Lipid	10.5	11.3	11.1
Ash	8.7	8.7	8.3
Gross energy, MJ/kg DM	17.1	17.1	17.1

[1)]Choengkukjang was purchased in the market.
[2)]Mineral premix (g/kg): $MgSO_4 \cdot 7H_2O$, 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; $CuCl_2$, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[3)]Vitamin premix (g/kg): L-ascorbic acid, 121.2; 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-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

ditional fermentation of soybean. The proximate compositions of major protein sources used in this study are given in Table 2. All dry ingredients were thoroughly mixed with 30% distilled water. Pellets were extruded through the meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size and dried with freeze drier (Operon FDT-8605, Korea) for 24 h. The pellets were crushed into desirable particle sizes (0.4~2.0 mm) and stored at -20°C until use.

Fish and feeding trial

Parrotfish were transported from a private hatchery in Jeju Island to Marine and Environmental Research Institute, Cheju National University. Fish were fed with a commercial diet for 4 weeks in a 1000 L tank. Ninety fish (initial body weight 122 ± 2.0 g) were randomly distributed into nine 150 L tanks (10 fish/tank) in a flow through system supplied with sand filtered seawater at flow rate of 3 L/min. One of the three experimental diets was fed to triplicate groups of fish at feeding rate of 3.5% body weight, twice a day (8:00 and 18:00), 7 days a week, for 4 weeks. The growth of fish was measured at the end of feeding trial. Feeding was stopped 24 h prior to weighing.

Growth performance and feed utilization

At the beginning and the end of feeding trial, all fish were weighed and counted for weight gain, feed conversion ratio, protein efficiency ratio, specific growth rate and survival calculation. Three fish from each tank (9 fish/diet) were randomly sampled and stored at -20°C for muscle proximate compositions analysis. Analyses of crude protein, moisture and ash were performed using the standard procedures (AOAC, 1995). Lipid was determined using Soxhlet System (SH6, Korea).

Morphological parameters

The total length, body weight, liver weight and gonad weight of 9 fish/diet (3 fish/tank) were individually measured. Condition factor (CF; 100 x [fish weight (g)/fish length (cm)^3], hepato somatic index (HSI; 100 x liver weight/body weight), and gonad somatic index (GSI; 100 x gonad weight/body weight) were calculated.

Hematological parameters

At the end of feeding trial, 3 fish/tank (9 fish/diet) were anesthetized in tricaine methanesulfonate (MS-222) solution (100 ppm). Blood was taken from caudal veins using non-heparinsed syringes. Hematocrit was measured using microhematocrit technique. The remained blood samples were used for nitro blue tetrazolium (NBT), serum cholesterol and triglyceride. Serum cholesterol and triglyceride

Table 2. Proximate composition of main ingredients used in the experimental diets

Ingredients	Moisture (%)	Protein (% DM)	Lipid (% DM)	NFE (% DM)[1)]	Ash (% DM)
White fish meal	8.72	68.33	8.56	9.04	14.07
Soybean meal	11.68	46.91	2.52	32.35	6.54
Corn gluten meal	9.50	61.70	1.03	26.59	1.18
Cheongkukjang	6.51	41.26	23.13	24.31	4.79

[1)]Nitrogen Free Extracts = 100 - (%Moisture + %Protein + %Lipid + %Ash).

were measured using a Photometer CH100 Plus (Calenzano, Firenze, Italy).

The oxidative radical production by neutrophils during respiratory burst was measured by the (NBT) assay as a method described by Anderson and Siwicki (1995) with some modification (Kumari and Sahoo, 2005). Briefly, blood and 0.2% NBT were mixed in equal proportion (1:1), incubated for 30 min at room temperature, then 50 μL was taken out and dispensed in glass tubes. Then, 1 mL of dimethyl formamide (Sigma) was added and centrifuged at 2000 x g for 5 min. Finally, the optical density of supernatant was measured at 540 nm. Dimethyl formamide was used as the blank.

Serum lysozyme

A turbidometric method described by Swain et al. (2007) was used to measure serum lysozyme activity in fish fed the experimental diets. *Micrococcus lysodeikticus* concentration of 0.2 mg/mL (in 0.02 M sodium citrate buffer, pH 5.5) was added to serum sample at 10:1 ratio. Absorbance was measured at 450 nm immediately after adding *M. lysodeikticus* suspension. Final absorbance was measured after incubating for 1 h at 25°C. Lyophilized hen egg white lyzosyme (HEWL) was used as standard. Serum lysozyme values are expressed as μg/mL equivalent of HEWL activity.

Antioxidant activity

Antioxidant activity of the experimental diets and fish liver was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay described by Brand-Williams (1995) with some modifications. Two g of diets (3 replicates/diet) were homogenized in 20 mL aqueous methanol (80%) and kept at room temperature for 10 min. The homogenates were centrifuged (5000 rpm) at 4°C for 10 min and filtered through 0.45 μm syringe filters (Whatman Inc., Clifton, NJ) prior to the assay. Whole liver of 3 bled fish per tank (9 fish/diet) were homogenized in the aqueous methanol (80%) at a ratio of 1:4 (whole liver: aqueous methanol) for 1 min using a homogenizer (X-120, Germany). The homogenate was centrifuged (5000 rpm) at 4°C for 10 min. The supernatant was filtered through a 0.45 μm syringe filter. One hundred μL of filtered extract was pipetted into a 1.5 mL cuvette, then 900 μL of DPPH methanolic solution (100 μM) was added to obtain a final volume of 1 mL. The absorbance of the mixture was measured at 517 nm with 1 min intervals for 10 min by a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The antioxidant activity of the extract against the DPPH radicals was calculated as percent inhibition. Percent inhibition $= [(A_0-A_s)/A_0] \times 100$, where A_0 and A_s are the absorbance of sample at 0 and S min, respectively.

Total polyphenol analysis

Total polyphenol compounds in the experimental diets were measured by a colorimetric method described by Skerget et al. (2005). Briefly, 1 g of diets was extracted with 250 mL methanol for 2 h at 40°C. The solution was cooled and filtered through a 0.45 μm syringe filter (Whatman Inc., Clifton, NJ). To 0.5 mL filtered extract, 2.5 mL of Folin-Ciocalteu reagent (0.2 N, Sigma) was added and kept for 5 min at room temperature, then 2 mL of Na_2CO_3 solution (75 g/L) was added. The mixture was incubated for 5 min at 50°C and cooled. The absorbance was measured at 760 nm using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The results were expressed in gram of gallic acid per kilogram of dry diet.

Measurement of reducing, Fe^{2+}-chelating, and superoxide anion scavenging activities

Dietary sample (2 g) was finely ground and extracted in 20 mL methanol (80%, v/v) for 12 h with three replicates. The extract was filtered through a 0.45 μm syringe filter (Whatman Inc., Clifton, NJ) prior to assays.

Reducing activity of the experimental diets was measured as a method described by Oyaizu (1986). Filtered extract (0.3 mL) was mixed with 0.3 mL of 1.0% potassium ferricyanide and 0.3 mL sodium phosphate buffer (0.2 M, pH 6.6). The mixture was incubated at 50°C for 20 min. After cooling, 10% trichloroacetic acid (0.3 mL) was added and centrifuged at 6000 rpm for 10 min at 4°C. The supernatant (0.6 mL) was mixed with 0.1% ferric chloride solution (0.12 mL) and deionized water (0.6 mL). The mixture was incubated at room temperature for 10 min and the absorbance was measured at 700 nm using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA).

Fe^{2+}-chelating activity of the experimental diets was measured according to Decker and Welch (1990). The reaction mixture containing dietary extract (1.0 mL), 3.7 mL methanol, 2 mM $FeCl_2$ (0.1 mL), and 5 mM ferrozine (0.2 mL) was incubated at room temperature for 10 min. The absorbance of mixture was measured at 562 nm. The chelating effect of dietary extract was calculated as follows: Chelating effect (%) = 100 x (1- absorbance sample/absorbance control).

Superoxide anion radical scavenging activity of the experimental diets was evaluated using a method described by

Nagai et al. (2001). The system contained 1.2 mL of 0.05 M sodium carbonate buffer (pH 10.5), 0.1 mL of 3 mM xanthine, 0.1 mL of 3 mM ethylenediamine tetraacetic acid disodium salt (EDTA), 0.1 mL of 0.15% bovine serum albumin, 0.1 mL of 0.75 mM nitroblue tetrazolium (NBT) and 0.1 mL dietary extract. After incubation at 25°C for 10 min, the reaction was initiated by adding 0.1 mL of 6 mU xanthine oxidase and kept at 25°C for 20 min. The reaction was stopped by adding 0.1 mL of 6 mM CuCl. The absorbance of the mixture was measured at 560 nm.

Measurement of liver thiobarbituric acid reactive substances (TBARS)

Liver TBARS in fish fed the experimental diets was measured according to method of Burk et al. (1980) and modified by Tocher et al. (2002). Liver (30 mg) was homogenized in 1.5 mL of 20% (w/v) trichloroacetic acid (TCA) containing 0.05 mL of 1% butylated hydrotoluene (BHT) in ethanol. To the homogenate, 2.95 mL of freshly prepared 10 mM thiobarbituric acid was added. The mixture was vortexed in a glass tube and heated at 100°C for 10 min. Protein was removed by centrifugation of 12,000 x g. Absorbance of supernatant was measured at 532 nm. The concentration of TBARS, expressed as μM TBARS/g liver, was calculated using the extinction coefficient 0.156 μM^{-1} cm^{-1}.

Measurement of liver superoxide dismutase

Fish liver was homogenized in 9 volumes of 20 mM phosphate buffer pH 7.4, 1 mM EDTA and 0.1% Triton X-100. The homogenate was centrifuged at 10,000 rpm (4°C, 10 min) to remove debris. The resultant supernatants were used for superoxide dimustase assay as method of Ukeda et al. (1999). Into 2.4 mL of a 50 mM sodium carbonate buffer (pH 10.2), 0.1 mL of 3 mM xanthine, 3mM EDTA, 0.75 mM NBT, 15% bovine serum albumin and 0.1 mL supernatant were added. The reaction was initiated by adding 0.1 mL of 100 mU/mL xanthine oxidase. The absorbance of mixture was measured at 560 nm after incubation at 25°C for 25 min.

Statistical analysis

Data were subjected to one-way ANOVA in SPSS version 11.0. The significant differences between group means were compared using Duncan's multiple test. Data presented are means ± SD. The percentage data of weight gain and specific growth rate were arcsine transformed before the ANOVA analysis. Differences were considered significant at $P < 0.05$.

Results

Higher antioxidant activities were found in the diets containing soybean (SBM) and Cheongkukjang (CKJ) compared to that in the control diet (Fig. 1). Growth performances and feed utilization of fish fed the experimental diets are presented in Table 3. After 4 weeks of feeding trial, no significant differences were observed in final body weight, weight gain, specific growth rate, protein efficiency ratio, feed conversion ratio and feed intake among fish groups fed all the experimental diets. No mortality occurred during feeding trial. The growth performances do not seem to be affected by the supplementation of SBM and CKJ in this study, although the period of feeding trial was short. Muscle compositions did not differ among fish groups fed the experimental diets, including the control diet (Table 4). Condition factor, visceral somatic index, hepato somatic index and gonad somatic index did not differ in all fish groups fed the experimental diets (Table 5). Hematocrit (%), NBT activity (OD, 540 nm), serum cholesterol, serum triglyceride, and liver DPPH radical scavenging activity were not significantly different among the treatments (Table 6). However, dietary Fe^{2+}-chelating and superoxide radical scavenging activities increased by the supplementation of SBM and CKJ (Table 7). The reducing activity of the diets only increased by SBM supplementation. Interestingly, liver superoxide dismutase activity increased significantly by dietary CKJ after four weeks of feeding trial (Fig. 2). The liver of fish fed the diets containing SBM or CKJ also had a decreasing trend in lipid peroxidation (expressed as μM TBARS/g liver) even though it was not significant (Fig. 3).

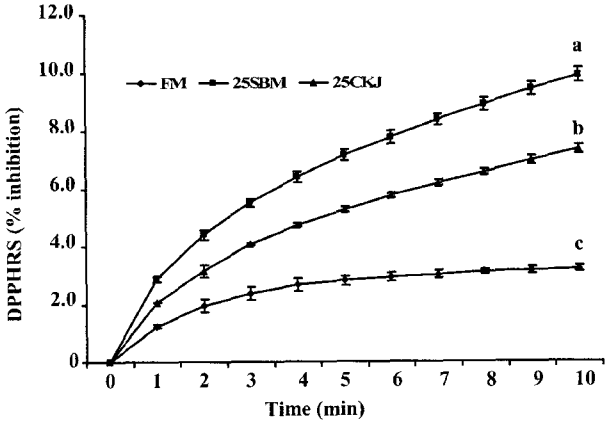

Fig. 1. DPPH radical scavenging activity (DPPHRS) in the experimental diets.

Table 3. Growth performance and feed utilization of parrotfish *O. fasciatus* fed the experimental diets for 4 weeks

Diets	FM	25SBM	25CKJ
Initial body weight, g	122.4 ± 0.5	123.0 ± 0.6	122.5 ± 0.9
Final body weight, g	141.0 ± 0.2	143.8 ± 1.2	142.0 ± 1.3
Weight gain (WG)[1], %	15.2 ± 0.6	17.0 ± 1.3	15.9 ± 0.6
Specific growth rate (SGR)[2], %	0.51 ± 0.0	0.56 ± 0.0	0.53 ± 0.0
Protein efficiency ratio (PER)[3]	1.17 ± 0.04	1.26 ± 0.09	1.20 ± 0.04
Feed conversion ratio (FCR)[4]	2.03 ± 0.07	1.89 ± 0.09	1.95 ± 0.07
Feed intake (FI)[5], g/g body weight	0.27 ± 0.0	0.26 ± 0.0	0.27 ± 0.0
Survival, %	100	100	100

Values presented are means ± SD. Value in the same row having different superscripts is significantly different ($P<0.05$).
[1]WG (%) = 100 x (final mean body weight - initial mean body weight)/initial mean body weight.
[2]SGR (%) = [(ln final body weight - ln initial body weight)/days] x 100.
[3]PER = wet weight gain/ total protein given.
[4]FCR = dry feed fed/wet weight gain.
[5]FI (g/g BW) = dry feed consumed/body weight.

Table 4. Muscle composition of parrotfish, *O. fasciatus* fed the experimental diets for 4 weeks

Diets	FM	25SBM	25CKJ
Moisture content, %	73.8 ± 0.2	74.7 ± 0.0	73.8 ± 0.0
Protein	20.5 ± 0.7	19.6 ± 1.2	20.6 ± 0.4
Lipid, % DM	4.4 ± 0.2	4.5 ± 0.4	4.3 ± 0.1
Ash, % DM	1.3 ± 0.0	1.2 ± 0.1	1.3 ± 0.2

Values presented are means ± SD. Value in the same row having different superscripts is significantly different ($P<0.05$).

Table 5. Morphological parameters of parrotfish, *O. fasciatus* fed the experimental diets for 4 weeks

Diets	FM	25SBM	25CKJ
Condition factor	2.6 ± 0.0	2.6 ± 0.2	2.6 ± 0.0
Viscera somatic index	13.4 ± 0.8	13.2 ± 1.3	12.0 ± 0.8
Gonad somatic index	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.01
Hepato somatic index	3.4 ± 0.2	3.3 ± 0.4	3.2 ± 0.9

Values presented are means ± SD. Value in the same row having different superscripts is significantly different ($P<0.05$).

Discussion

Growth performances of parrotfish fed the diets containing 25% CKJ or SBM as protein sources were comparable to that of the control diet (Table 3). Low growth rate was observed in this feeding study because of low water temperature (~15°C).

Table 6. Hematological parameters and liver DPPH in parrotfish, *O. fasciatus* fed the experimental diets for 4 weeks

Diets	FM	25SBM	25CKJ
Hematocrit (%)	39.9 ± 2.4	38.9 ± 4.8	40.5 ± 1.9
Blood NBT (OD, 540 nm)	1.01 ± 0.11	1.14 ± 0.11	1.02 ± 0.05
Serum lysozyme (μg/ml)	1.87 ± 0.21	1.46 ± 1.14	2.00 ± 0.14
Serum cholesterol (mg/dL)	185 ± 27	150 ± 13	179 ± 4
Serum triglyceride (mg/dL)	454 ± 120	508 ± 208	622 ± 250
Liver DPPH (%)	10.7 ± 1.7	11.5 ± 0.61	10.6 ± 1.9

Values presented are means ± SD. Value in the same row having different superscripts is significantly different ($P<0.05$).

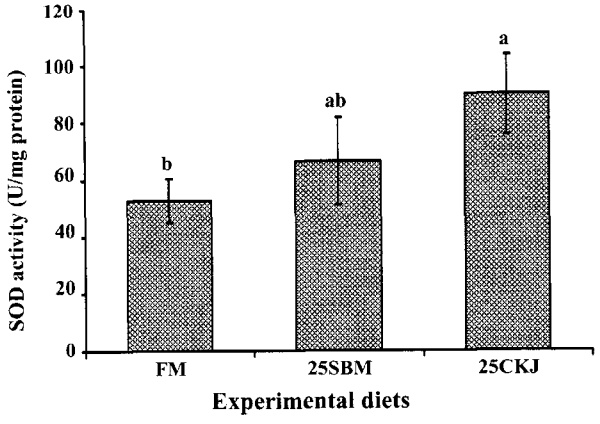

Fig. 2. Superoxide dismutase (SOD) activity in the liver of parrotfish, *Oplegnathus fasciatus* fed the experimental diets for 4 weeks.

Table 7. Polyphenol content, reducing activity, Fe^{2+} chelating and superoxide radical scavenging (SRS) activities in the experimental diets containing soybean meal or Cheongkukjang

Diets	FM	25-SBM	25-CKJ
Polyphenol (g/kg)	0.018 ± 0.006	0.018 ± 0.002	0.025 ± 0.014
Fe^{2+} chelating activity (%)	6.77 ± 0.38^b	9.42 ± 0.32^a	8.47 ± 0.63^b
Reducing activity (OD, 700nm)	1.014 ± 0.02^b	1.071 ± 0.01^a	1.014 ± 0.01^b
SRS (OD, 560 nm)	0.042 ± 0.002^a	0.052 ± 0.002^b	0.055 ± 0.003^b

Values presented are as means ± SD. Value in the same row having different superscripts is significantly different ($P<0.05$).

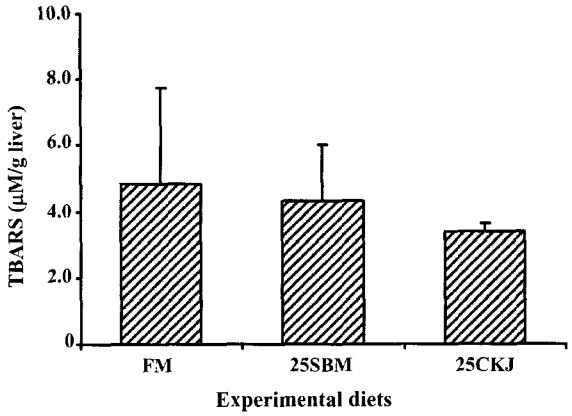

Fig. 3. Thiobarbituric acid reactive substance (TBARS) concentration in the liver of parrotfish, *O. fasciatus* fed the experimental diets for 4 weeks.

No significant differences were found in growth performance, muscle compositions, morphological parameters, hematocrit and serum cholesterol and triglyceride (Table 3, 4, 5, 6). It suggests that 25% dietary fishmeal protein can be replaced with SBM and/or CKJ with methionine and lysine in growing-out parrotfish (initial body weight 122 g).

Parrotfish has been considered as a potential candidate for intensive aquaculture. However, there have been few works for this species to date (Jung and Oh 2000; Wang et al., 2003; Lee et al., 2004; Nam et al., 2005; An et al., 2006; Cho et al., 2006; Choi et al., 2006; Makino et al., 2006; Oh et al., 2006; Tachibana et al., 1997). Outbreak of diseases has been announced to result in severe economic loss. Finding a method to prevent the outbreak of diseases is prioritized for aquaculture development of parrotfish. Unlike terrestrial animals, innate immune responses play more crucial important role in preventing diseases in fish (Ellis, 2001; Kollner et al., 2002; Magnadottir, 2006). The innate immune responses can be enhanced by dietary supplementation of immunostimulants, such as vitamin C (Ai et al., 2004; Lin and Shiau, 2005; Xie et al., 2006), vitamin E (Puang et al., 2004; Lin and Shiau, 2005), β-glucan (Kumari and Sahoo, 2006) and other components. Recently, use of natural products, such as fermented materials as an immunostimulant has been promoted in aquaculture because it is cheap, easy to treat, and rich in bioactive compounds (Ashida et al., 2002). CKJ has been reported to have many beneficial effects on antimicrobial, anticarcinogenic and antioxidant activities (Kim et al., 1998; Kim et al., 1999; Youn et al., 2001; Kim et al., 2004; Lee et al., 2005; Mine et al., 2005; Shon et al., 2007). However, in the present study, there were no clear effects of dietary supplementation of CKJ on serum lysozyme, respiratory burst and liver DPPH radical scavenging activities in parrotfish after 4 weeks of feeding trial. Contrastingly, several studies demonstrated that fermented vegetable products reduced oxidative stress, suppressed the lipid peroxidation, enhanced the antioxidant, and increased the phagocytic and lysozyme activities in Japanese flounder, *Paralichthys olivaceus* (Ashida et al., 2002; Ashida and Okimasu, 2005; Ashida et al., 2006). It was noted that there were some differences between the present study and the others. Firstly, different method in administration of the fermented products was used in the present study. In the other studies, only extractants of fermented products were used instead of whole fermented products. The condensed bioactive compounds in the extractant could increase their absorption, and thereby it could be more effective in enhancing the immune responses of the fish. Secondly, a big fluctuation of water temperature (from 27°C at the beginning to 15°C at the end of the present study) during feeding trial might have also affected the immune responses of fish in the present study. Kumari et al. (2006) reported that the seasonal variation of temperature remarkably had fluctuated innate immune parameters including serum myeloperoxidase, lysozyme, haemagglutination and alternative complement activities in Asian catfish.

However, the present study demonstrated that 25% dietary CKJ significantly increased superoxide dismutase (SOD) activity in the liver of fish (Fig. 2). The effects of dietary CKJ on liver antioxidant defense enzymes, such as SOD, could result in increased oxidant scavenging activities. Higher superoxide radical scavenging (SRS) activities in diets supplemented 25% CKJ (Table 7) might have increased the SOD activity in the liver of fish fed the CKJ containing diet. The elevation of liver SOD consequently inhibited the generation of TBARS (Fig. 3). It is well demonstrated that liver SOD and lipid peroxidation are influenced by dietary antioxidants. Ashida et al. (2002) reported that administration of fermented vegetable products (FVP) significantly inhibited the lipid peroxidation of erythrocytes induced by tert-butyl hydroperoxide *in vivo*.

In conclusion, the results of the present study suggest that dietary Choengkukjang significantly increased liver superoxide dismutase activity of growing-out parrotfish after four week feeding trial. To evaluate immunostimulatory effects of dietary Cheongkukjang in parrotfish, a long term feeding trial with dietary Cheongkukjang extract at optimum water temperature is recommended for further study.

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