



## Effects of Dietary Supplementation of a Meju, Fermented Soybean Meal, and *Aspergillus oryzae* for Juvenile Parrot Fish (*Oplegnathus fasciatus*)\*

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**ABSTRACT :** In this study, dietary supplementations of Korean Meju, fermented soybean meal (F-SBM) by *Aspergillus oryzae*, and *A. oryzae* itself were evaluated on growth performance, feed utilization, immune responses and phosphorus availability in juvenile parrot fish, a marine aquaculture fish species. Four isonitrogenous and isocaloric diets were formulated to contain 8% soybean meal (control diet), 4% Meju (50% soybean meal was replaced by Meju), 4% F-SBM (50% soybean meal was replaced by F-SBM), or 0.08% *A. oryzae* itself. One of the four experimental diets was fed to triplicate groups of fish for 8 weeks. At the end of the feeding trial, no significant differences were found in growth performances and feed utilization. Red blood cell counts in the fish fed the *A. oryzae* diet were significantly higher than that of fish fed the control diet. The antioxidant activity in Meju diet was significantly higher than that of the control and *A. oryzae* diets. Fish fed Meju and F-SBM diets showed numerically higher antioxidant activity of serum compared to that of fish fed the control diet, even though it was not significant. Liver superoxide dismutase activity of fish fed the test diets was significantly higher than that of fish fed the control diet. The apparent digestibility coefficients of protein of fish fed all the diets were not significantly different. Phosphorus absorption was numerically increased in fish fed F-SBM and *A. oryzae* diets compared to that of fish fed the control diet. This study indicates that the fermentation process of soybean meal does not impair growth performance and feed utilization in parrot fish. The fermentation process could enhance the availability of phosphorus in soybean meal and non-specific immune responses of parrot fish. (**Key Words :** Meju, Fermentation, Parrot Fish, *Aspergillus oryzae*, Immune Responses, Phosphorus)

### INTRODUCTION

One of the current problems in Korean aquaculture is the fact that farmers use a large quantity of antibiotics to prevent cultured fish from bacterial diseases. Moreover, residual of antibiotics in aquaculture products are being seriously concerned by consumers. Many researches have been conducted to search natural feed supplements, such as *Spirulina pacifica*, *Hizikia fusiformis*, Natto and

Cheongkukjang (Kim et al., 2006; Pham et al., 2006; Pham and Lee, 2007; Fujiwara et al., 2008) for a substitute of antibiotic. Administration of fermented products, which can enhance non-specific immune responses and disease resistances of the cultured fish, might be a promising solution to avoid the use of antibiotic in aquaculture industry. Fermented foods derived from soybean have been placed as important parts of diets in Korea and Japan. Recently, several studies reported that the use of fermented vegetable products could enhance non-specific immune responses, growth performances, feed efficiency and digestibility of nutrients in fish as well as terrestrial animal (Ashida and Okimasu, 2005; Cho et al., 2007; Kim et al., 2007; Yang et al., 2007; Min et al., 2009). However, no information is available on the use of fermented products in diets for fish.

Meju, a Korean traditional fermented soybean, has been reported to have high antioxidant activity (Cheigh et al., 1993; Park and Jung, 2005). It possesses a beneficial mold *Aspergillus oryzae* which has been reported to produce

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**Table 1.** Formulation and proximate composition of the experimental diets (% dry matter)

Ingredients	Diets			
	Control	Meju	F-SBM	<i>A. oryzae</i>
White fish meal <sup>1</sup>	48.0	48.0	48.0	48.0
Soybean meal <sup>1</sup>	8.0	4.0	4.0	8.0
Meju <sup>7</sup>	0.0	4.0	0.0	0.0
F-SBM <sup>8</sup>	0.0	0.0	4.0	0.0
<i>Aspergillus oryzae</i>	0.0	0.0	0.0	0.08
Cottonseed meal <sup>2</sup>	8.0	8.0	8.0	8.0
Corn gluten meal <sup>1</sup>	8.0	8.0	8.0	8.0
Potato starch <sup>1</sup>	13.0	13.0	13.0	13.0
Mineral mix <sup>3</sup>	1.0	1.0	1.0	1.0
Vitamin mix <sup>3</sup>	1.0	1.0	1.0	1.0
Squid liver oil <sup>4</sup>	12.0	12.0	12.0	12.0
Carboxyl methyl cellulose <sup>5</sup>	1.0	1.0	1.0	0.92
Proximate composition				
Dry matter (%)	94.1	94.3	93.5	94.9
Protein (% DM)	50.5	50.6	51.8	50.3
Lipid (% DM)	17.0	17.2	16.6	16.9
Ash (% DM)	9.6	9.6	9.7	9.4
Gross energy (MJ/kg DM) <sup>6</sup>	18.1	18.2	18.1	18.1

<sup>1</sup> Provided by Suhyp Feed Co. Ltd., Uiryeong, Korea.

<sup>2</sup> Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, Tennessee 38108, USA.

<sup>3</sup> Same as Kim and Lee (2003). <sup>4</sup> Squid liver oil was purchased from E-Wha oil Co. Ltd., Busan, Korea.

<sup>5</sup> Aldrich-Sigma, St. Louis, MO, USA.

<sup>6</sup> Energy was estimated by using the values of 16.7 KJ/g protein or carbohydrate and 37.6 KJ/g fat for dietary ingredients (Garling and Wilson, 1976).

<sup>7</sup> Meju was purchased from Mun-Wha Meju (Daegu, Korea). <sup>8</sup> F-SBM was fermented with *Aspergillus oryzae* in our laboratory.

extra-cellular enzymes, such as phytase (Fujita et al., 2000; 2003a, b), cellulose-degrading enzyme (Yamane et al., 2002), proteinase (Kundu et al., 1968; Kundu and Manna, 1975), alpha-amylase (Kundu and Das, 1970) and carboxypeptidase (Blinkovshi et al., 1999). Esaki et al. (1999) reported that *A. oryzae* can synthesize high antioxidant compounds, such as 6-hydroxydaidzein, 8-hydroxydaidzein and 8-hydroxygenistein from soybean isoflavones. Particularly, phytase can release orthophosphate from phytic acid resulting in the improved phosphorus availability of feeds (Um et al., 2000; Debnath et al., 2005; Pham et al., 2008). It is expensive for Meju to be used directly in fish feeds. We propose that the fermentation of soybean meal which is used as one of the most important fish feed ingredients, could have many benefits in saving feed costs as well as gaining elevated immunity in fish.

Parrot fish (*Oplegnathus fasciatus*) is one of the emerging aquaculture species in Asian countries. Its high commercial value makes it a promising aquaculture species in the future. Therefore, we conducted a feeding trial to examine the effects of dietary supplementations of a Meju (fermented soybean), F-SBM (fermented soybean meal) and *A. oryzae* (a mold to be used in fermentation of traditional Meju) on growth performances, feed utilization, digestibility, phosphorus retention, non-specific immune responses and antioxidant capacity in juvenile parrot fish.

## MATERIALS AND METHODS

### Experimental diets

Experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (48.9%) and gross energy (18.1 MJ/kg). Four experimental diets (Table 1) were formulated to contain 8% soybean meal, 4% Meju (50% soybean meal was replaced by Meju), 4% F-SBM (50% soybean meal was replaced by fermented soybean meal), or 0.08% *A. oryzae* (designated as Control, Meju, F-SBM and *A. oryzae*, respectively). The dietary inclusion and replacement levels of SBM were selected based on a previous study (Pham, 2008). A commercial Meju was purchased from Munwha Meju Inc. (Daegu, Korea) and ground into fine powder by a grinding machine (MF10 basis, Germany). F-SBM was prepared by the same process in making Meju, a traditional Korean fermented soy paste, with some modification. Briefly, soybean meal was used instead of whole soybean in fermentation with *A. oryzae*. The fermented mixture was directly used in dietary formulation. *A. oryzae* was kindly provided from Jeil Bio Tech. Co. Ltd., Hwaseong, Korea. All dry ingredients were thoroughly mixed with 30% distilled water to become dough. Pellets were extruded through the meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size, dried at room temperature, crushed into desirable particles sizes (0.4-2.0 mm), and stored at -20°C until use. Four

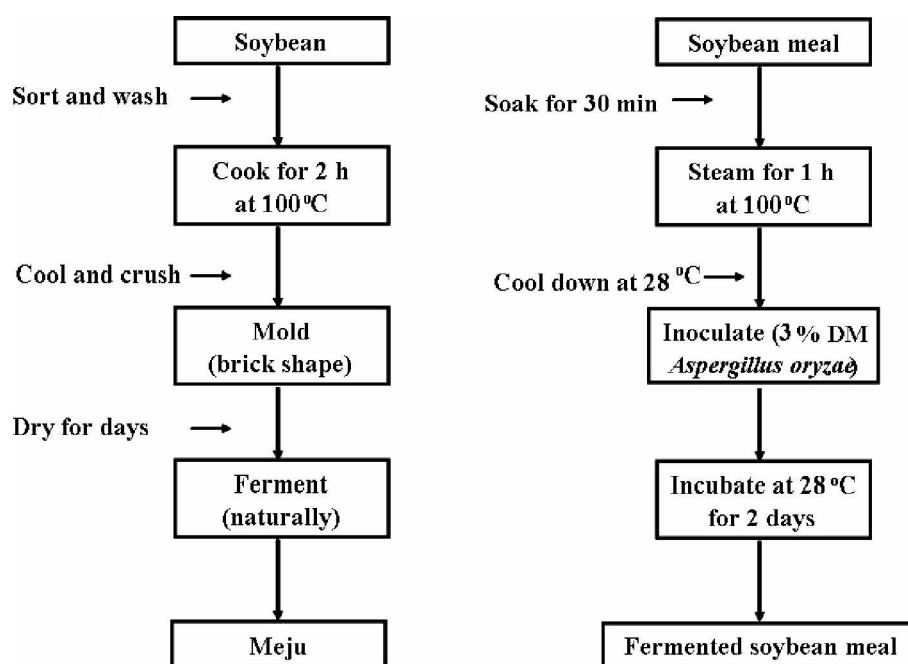


Figure 1. Fermentation processes for Meju and soybean meal (F-SBM).

experimental diets for digestibility test were made based on the same formulation of each diet with supplementation of 0.5% chromic oxide.

#### Fermentation of soybean meal

Fermentation of soybean meal was applied as the preparation process of the Meju with some modification (Figure 1). Soybean meal was finely ground, soaked with water at a ratio of 1:3 for 40 min and steamed at 100°C for 1 h. After cooling at 28°C in an incubator (Mir-253, Sanyo, Japan), 3% *A. oryzae* was inoculated into the steamed soybean meal and incubated at 28°C for 48 h. The fermented soybean meal (F-SBM) was directly incorporated into the F-SBM diet.

#### Fish, facilities and feeding trial

Parrot fish juveniles were obtained from a private hatchery (Chang-Hae Fisheries, Jeju, Korea) in Jeju Island and transported to the Marine and Environmental Research Institute, Cheju National University, Jeju, Korea. The fish were conditioned to be adopted in the experimental facilities for 2 weeks with a commercial feed (Suhyup Feed Co. Ltd., Uiryeong, Korea). Two hundred and forty fish (initial body weight 22.0 g) were randomly distributed into twelve 100 L PVC tanks (20 fish per tank) in a flow through system supplied with sand filtered sea water at a flow rate of 3 L/min. The fish were fed at a rate of 4% body weight, twice a day (8:00 and 18:00), 7 days a week, for 8 weeks. The growth of fish was measured every two weeks and feeding rate was adjusted accordingly. Feeding was stopped

24 h prior to weighing.

#### Sample collection and analysis

At the beginning and the end of the feeding trial, all fish were weighed and counted to calculate percent body weight gain, feed conversion ratio, protein efficiency ratio, specific growth rate. Blood samples were obtained from the caudal vein of six fish from each tank (18 fish per treatment) using heparinized syringes after anesthetizing the fish with tricaine methanesulfonate (MS-222) at a concentration of 100 mg/L for hematocrit, hemoglobin, nitro blue tetrazolium (NBT) activity, and red blood and white blood cell counts. The blood cell counts were determined by a hemacytometer. Three fish from each tank were randomly sampled at the end of the feeding trial and stored at -70°C for the subsequent proximate analysis of the whole body.

#### Proximate analyses

Proximate compositions of all ingredient, experimental diets and whole body were analyzed by AOAC methods (1995). The fish samples were rigorously blended and chopped into crumble in a mixer (SFM-3500B, Sbhini, Hwaseong, Korea) and used for determination of whole body lipid, protein and ash contents. Lipid level of 2 g samples was determined using the Soxhlet Method with extraction in diethyl ether (Soxhlet Extraction System C-SH6, Korea). Protein content (N×6.25) was determined in the fish crumble using the Kjeldahl method (Kjeltec Analyzer Unit 2300, FOSS, Sweden). Ash content was determined after combustion of the fish crumble at 550°C

for 4 h. Nitrogen-free extract (NFE) was computed by subtracting the sum values of crude protein, lipid, and ash.

#### Hematological assay

Hematocrit was determined using microhematocrit technique (Brown, 1980). Blood was drawn into plastic capillary tubes and centrifuged at 12,000×g for 10 min in a micro-hematocrit centrifuge (VS-12000, Bucheon, Korea). Hemoglobin was determined as follows; Blood sample (without heparin) of 25 µl was diluted into 5 ml modified hemoglobin solution (composed of 0.7 g K<sub>3</sub>Fe(CN)<sub>6</sub> and 0.1 g KCN in 1 L double distilled water). Then, absorbance of the mixture was measured by a spectrophotometer (Genesys 10 UV, Rochester, NY, USA) at a wave length of 540 nm. Hemoglobin (Hb) was calculated using the formula; Hb = 0.146×F×OD, where F: dilution factor (201) and OD: optical density. Red blood cell (RBC) and white blood cell (WBC) were counted as described by Kumar et al. (2005). The blood cell counts were determined by hemacytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Konigshofen, Germany). The respiratory burst of blood phagocytic cells based on the superoxide anion (O<sub>2</sub><sup>-</sup>) production was measured by nitro blue tetrazolium (NBT) assay with a slight modification (Anderson et al., 1992; Stasiak and Bauman, 1996). A drop of whole blood was placed on cover slip and incubated in humidified chamber at room temperature for 30 min. Then, the cover slips were thoroughly rinsed with 0.85% saline solution and inverted onto a slide with drops of 0.2% NBT solution. The slides were continuously incubated for another 30 min and counted using a optical microscope. Four random fields of positive, dark blue stained cells (activated neutrophils) were observed for each cover slip at 400×.

#### Antioxidant activity assay

Antioxidant activity of the experimental diets and serum was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay described by Brand-Williams et al. (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 (5,000 rpm) at 4°C for 10 min and filtered through 0.45 µm syringe filters (Whatman Inc., Clifton, NJ) prior to the assay. One hundred µl serum from 3 fish per tank (9 fish per diet) 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 observed at wavelength of 517 nm with 1 min intervals for 10 min by a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The antioxidant capacity of the extract against the DPPH radicals was calculated as percent inhibition. Percent

inhibition =  $((A_0 - A_s)/A_0) \times 100$ , where A<sub>0</sub> and A<sub>s</sub> are the absorbance of sample at 0 and s min, respectively.

#### 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 pellets. The resultant supernatants were used for superoxide dismutase assay by Ukeda et al. (1999). Into 2.4 ml of a 50 mM sodium carbonate buffer (pH 10.2), 0.1 mM of 3 mM xanthin, 3 mM EDTA, 0.75 mM NBT, 15% bovine serum albumin and 0.1 mM supernatant were added. The reaction was initiated by adding 0.1 mM of 100 mU/ml xanthin oxidase. The absorbance of the mixture was read at 560 nm after incubation at 25°C for 25 min.

#### Feces collection and apparent digestibility test

The indirect method described by Cho and Kaushik (1990) was used to calculate the apparent digestibility coefficient of protein and phosphorus with chromic oxide (0.5% in diets) as the inert indicator. Feces were collected with a modified fecal collection system for olive flounder (Yamamoto et al., 1998). After 8 weeks of the feeding trial, the remaining fish of each group (3 groups/treatment) were mixed into four respective groups (1 group/treatment) and then transferred to four 150 L fecal collection tanks. To collect the feces, all the fish were fed their respective diets containing 0.5% chromic oxide to satiation at 18:00 h and the feces were collected in the next morning at 08:00 h. The collected feces were immediately frozen at -20°C until analysis. The fecal collection was repeated 4 times as 4 replicates. Dietary and fecal protein was analysed using Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Sweden). Chromic oxide in feces and diets was determined according to the method described by Furukawa and Tsukahara (1966). Total phosphorus in diets and feces were measured using an inductively coupled plasma (ICP) emission spectrophotometer as described by Leske and Coon (1999).

#### 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±standard deviations (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

At the end of 8 week feeding trial, no significant differences were observed in weight gain, protein efficiency

**Table 2.** Growth performance of juvenile parrot fish fed the experimental diets for 8 weeks<sup>1</sup>

Parameters	Diets			
	Control	Meju	F-SBM	<i>A. oryzae</i>
Initial body weight (g)	22.0±0.04	21.9±0.31	22.1±0.03	22.0±0.01
Final body weight (g)	81.7±2.32	80.6±2.88	78.4±2.34	79.7±2.54
Protein efficiency ratio (PER) <sup>2</sup>	1.8±0.06	1.7±0.04	1.7±0.05	1.7±0.04
Feed conversion ratio (FCR) <sup>3</sup>	1.1±0.04	1.2±0.02	1.2±0.03	1.2±0.02
Survival (%)	100	100	100	100

<sup>1</sup> Values are presented as mean±SD. Value in the same row having different superscripts is significantly different ( $p < 0.05$ ).

<sup>2</sup> PER = Wet weight gain/total protein given. <sup>3</sup> FCR = Dry feed fed/wet weight gain.

**Table 3.** Blood parameters of juvenile parrot fish fed the experimental diets for 8 weeks<sup>1</sup>

Parameters	Diets			
	Control	Meju	F-SBM	<i>A. oryzae</i>
Hematocrits (%)	35.0±2.4	34.7±2.7	35.2±2.6	36.8±3.7
Hemoglobin (g/dl)	7.7±0.2	7.9±0.2	8.0±0.6	7.6±0.5
White blood cell (million cell/mm <sup>3</sup> )	0.13±0.01	0.14±0.01	0.13±0.02	0.12±0.01
Red blood cell (million cell/mm <sup>3</sup> )	3.7±0.38 <sup>a</sup>	3.9±0.39 <sup>a</sup>	4.4±0.48 <sup>a</sup>	5.1±0.42 <sup>b</sup>

<sup>1</sup> Values are presented as mean±SD. Value in the same row having different superscripts are significantly different ( $p < 0.05$ ).

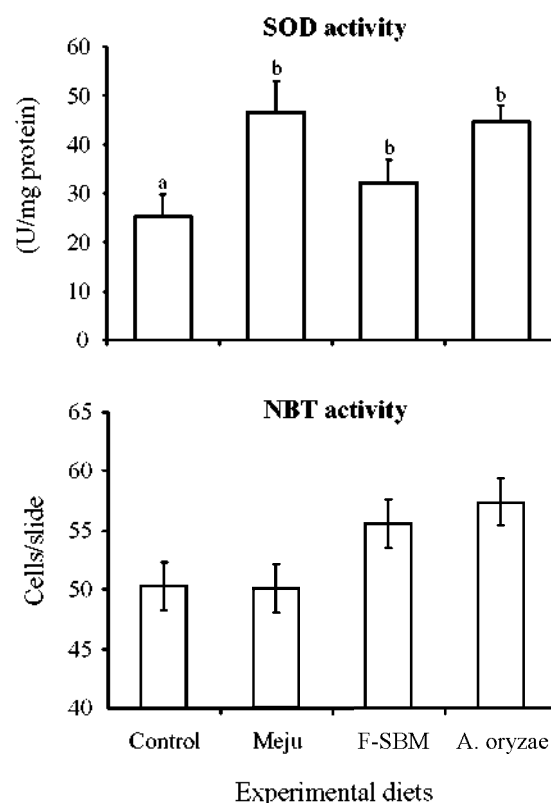
ratio and feed conversion ratio of parrot fish fed traditional Korean Meju, fermented soybean meal (F-SBM) by *A. oryzae* or *A. oryzae* itself (Table 2). No mortality occurred during the feeding trial.

Red blood cell counts in fish fed *A. oryzae* was significantly higher than that of fish fed the control diet containing 8% soybean meal (Table 3). However, no significant differences were found in hematocrit, hemoglobin and white blood cell counts.

Dietary DPPH radical scavenging activity was higher in the Meju diet compared to that of the control and *A. oryzae* diets although there was no significant difference in serum (Table 4). Interestingly, superoxide dismutase (SOD) activity in liver was significantly higher in fish fed the traditional Korean Meju, F-SBM and *A. oryzae* itself than that of the control diet (Figure 2). The phagocytic activity (NBT) was not significantly different among all the dietary groups. Whole body compositions did not differ among the fish groups fed the experimental diets (data not presented).

The apparent digestibility coefficients (ADC) of protein and phosphorus, and inorganic phosphorus retention of the fish fed the experimental diets were provided in Figure 3. The ADC of protein in the fish fed all the diets was not significantly different. However, phosphorus availability was numerically increased in the fish fed F-SBM and *A.*

*oryzae* diets compared to that of fish fed the control diet, even though it was not significantly different. Inorganic phosphorus retention in the plasma of the fish fed Meju, F-SBM or *A. oryzae* itself showed a similar trend found in

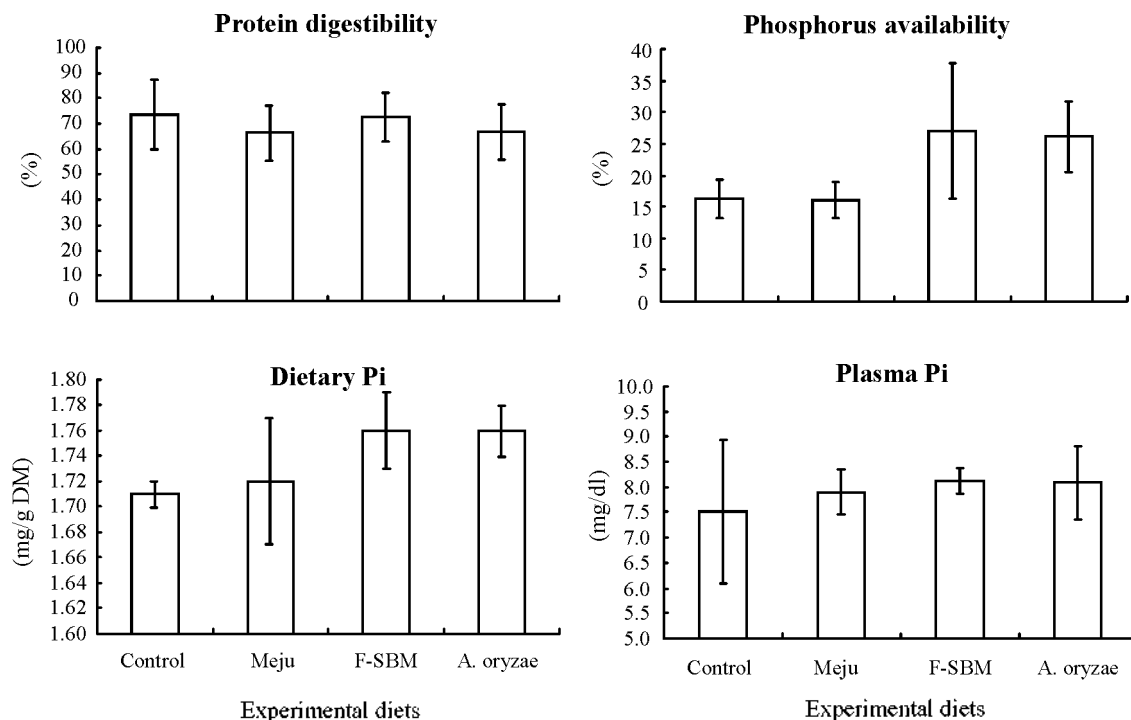


**Figure 2.** Superoxide dismutase (SOD) activity in the liver and the mean counts of activated neutrophils by nitro blue tetrazolium (NBT) of juvenile parrot fish fed the experimental diets for 8 weeks. Values are means of three replicates per treatment. Bars with different letters are significantly different ( $p < 0.05$ ).

**Table 4.** Percent antioxidant inhibition against DPPH radicals in the experimental diets and serum of fish fed the experimental diets for 8 weeks<sup>1</sup>

Parameters	Diets			
	Control	Meju	F-SBM	<i>A. oryzae</i>
Diets	41.6±2.4 <sup>a</sup>	45.3±2.7 <sup>b</sup>	42.6±2.6 <sup>ab</sup>	40.8±3.7 <sup>a</sup>
Serum	4.0±0.3	4.2±1.0	4.2±0.7	3.8±0.5

<sup>1</sup> Values are presented as mean±SD. Value in the same row having different superscripts are significantly different ( $p < 0.05$ ).



**Figure 3.** Protein digestibility, phosphorus availability, dietary inorganic phosphorus, and plasma inorganic phosphorus of juvenile parrot fish after apparent digestibility test. Values are means of three replicates per treatment. Bars with different letters are significantly different ( $p < 0.05$ ).

phosphorus availability of the fish, and responded to the dietary inorganic phosphorus concentration.

## DISCUSSION

The growth and feed utilization of fish fed the experimental diets were not significantly different compared to that of fish fed the control diet. The survivals were 100% showing that the experimental fish grew well on the experimental diets during the feeding trial. The results in this study suggest that dietary supplementation of Meju, fermented soybean meal or *A. oryzae* itself do not affect the palatability and acceptability of the diets containing cottonseed and soybean meal, thereby do not impair the growth of juvenile parrot fish. It was elicited that dietary supplementation of Meju or fermented soybean meal up to 50% soybean meal does not exhibit any mycotoxic effects on the fish. Strong antimutagenicity of Meju against mycotoxic compounds including aflatoxin B1 has been reported (Park et al., 2003). The existence of beneficial micro-flora in Meju, particularly *Bacillus* strains, is very effective to inhibit the growth of other toxic fungi and eliminate the toxic substances. Petchkongkaew et al. (2008) reported that two *Bacillus* strains including *Bacillus licheniformis* and *B. subtilis* isolated from Thai fermented soybean were able to inhibit the growth of toxic fungi *Aspergillus flavus* and effectively degrade aflatoxin B1 and

ochratoxin A.

There has been no information on the effects of dietary *A. oryzae* itself on hematological parameters of fish. The increased red blood cell counts in the present study indicated that the *A. oryzae* is likely to be one of useful microorganisms (Wang et al., 2008) as probiotic in diets for fish. The white blood cell counts, however, were not affected by the fermentation process or *A. oryzae* itself. The mechanism for the increased red blood cell counts by the *A. oryzae* cannot be explained through the present study. Therefore, further studies on the effects of *A. oryzae* on hematological parameters in fish are recommended. Jung et al. (2006) reported that *A. oryzae* is the main functional microorganism and being used as fermentation starter in producing commercial Meju. *A. oryzae* is also known to enhance antioxidant activity of soybean after fermentation (Lin et al., 2006). The present study also showed that the supplementation of Meju can increase the dietary antioxidant activity (Table 4). However, *A. oryzae* itself did not increase the antioxidant activity of the diet.

The present study indicated that fermentation process of soybean meal could improve the quality of soybean meal resulting in the improved non-specific immune response of the fish by an increased SOD activity (Figure 2). Pham and Lee (2007) reported that dietary supplementation of Chungkukjang (traditional Korean fermented soybean with rice straw) showed higher dietary DPPH free radical

scavenging activities and liver SOD activity in growing parrot fish (122 g) compared to those of fish fed a soybean meal based control diet. It was also postulated that Chungkukjang had higher concentration of polyphenol compounds, particularly two fractions including aglycone and malonylglycoside isoflavone, and resulted in much stronger antioxidant activity than unfermented steamed soybeans. A higher hepatic SOD activity was also found in rats fed Chungkukjang supplemented diet than soy based control diet (Kwak et al., 2007). However, the mechanisms how the fermentation with the fungi and/or supplementation of fungi itself affect to the antioxidant activity and innate immunity of fish was not established in the present study.

Phytase initiates the release of phosphorus from phytate (myo-inositol hexakisphosphate), the storage form of phosphorus present in various seeds and grains. Many studies on phytase production have been carried out on fungi, especially those in the genus *Aspergillus spp.* due to their high production yields and their low-pH tolerance (Wodzinski et al., 1996). Phytase production was also studied in solid substrate fermentation with a fungal strain (*A. oryzae* AK9) normally used to produce soy sauce (Chantasartramee et al., 2005). It was postulated that the fungus would have exhibited the phytase activity since phytate is a major constituent of soybean phosphorus. Therefore, the increasing trends in phosphorus absorption of fish fed the F-SBM and/or *A. oryzae* diets (Figure 3) can be explained by the phytase activity produced by the fermentation process or *A. oryzae* or self.

In conclusion, the fermentation process of soybean meal with *Aspergillus oryzae* does not affect growth performances and feed utilization in parrot fish. We suggest that the fermentation process of soybean meal could enhance the absorption of phosphorus and non-specific immune responses in juvenile parrot fish.

## REFERENCES

- AOAC. 1995. Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists, Arlington, Virginia.
- Anderson, D. P., T. Moritomo and R. De Grooth. 1992. Neutrophil, glass-adherent, nitroblue tetrazolium assay gives early indication of immunization effectiveness in rainbow trout. *Vet. Immunol. Immunop.* 30:419-429.
- Ashida, T. and E. Okimasu. 2005. Immunostimulatory effects of fermented vegetable product on the non-specific immunity of Japanese flounder *Paralichthys olivaceus*. *Fisheries Sci.* 71:257-262.
- Blinkovsky, A. M., T. Byun, K. M. Brown and E. J. Golightly. 1999. Purification, characterization, and heterologous expression in *Fusarium venenatum* of a novel serine carboxypeptidase from *Aspergillus oryzae*. *Appl. Environ. Microb.* 65:3298-3303.
- Brand-Williams, W., M. E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* 28:25-30.
- Brown, B. A. 1980. Routine hematology procedures. In: *Hematology, principles and procedures* (Ed. B. A. Brown). Lea and Febiger, Philadelphia, PA. pp. 71-112.
- Chantasartramee, K., D. I. N. Ayuthaya, S. Intarareugsorn and S. Dharmsthiti. 2005. Phytase activity from *Aspergillus oryzae* AK9 cultivated on solid state soybean meal medium. *Process Biochem.* 40:2285-2289.
- Cheigh, H. S., J. S. Lee and C. Y. Lee. 1993. Antioxidative characteristics of melanoidin related products fractionated from fermented soybean sauce. *J. Kor. Soc. Food Sci. Nutr.* 22:570-575.
- Cho, C. Y. and S. J. Kaushik. 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nut. Diet.* 61:132-172.
- Cho, J. H., B. J. Min, Y. J. Chen, J. S. Yoo, Q. Wang, J. D. Kim and I. H. Kim. 2007. Evaluation of FSP (fermented soy protein) to replace soybean meal in weaned pigs: growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. *Asian-Aust. J. Anim. Sci.* 20(12):1874-1879.
- Debnath, D., N. P. Sahu, A. K. Pal, K. Brauak, S. Yengkokpam and S. C. Mukkerjee. 2005. Present scenario and future prospects of phytase in aquafeed-Review. *Asian-Aust. J. Anim. Sci.* 18(12):1800-1812.
- Esaki, H., S. Kawakishi, Y. Morimitsu and T. Osawa. 1999. New potent antioxidative 0-dihydroxyisoflavones in fermented Japanese soybean products. *Biosci. Biotech. Bioch.* 63:1627-1629.
- Furukawa, H. and H. Tsukahara. 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish fed. *Bull. Jpn. Soc. Sci. Fish.* 32:502-508.
- Fujita, J., N. Budda, M. Tujimoto, Y. I. Yamane, H. Fukada, S. Mikami and Y. Kizaki. 2000. Isolation and characterization of phytase isozymes produced by *Aspergillus oryzae*. *Biotechnol. Lett.* 22:1797-1802.
- Fujita, J., S. Shigeta, Y. I. Yamane, H. Fukada, Y. Kizaki, S. Wakabayashi and K. Ono. 2003a. Production of two types of phytases from *Aspergillus oryzae* during industrial Koji making. *J. Biosci. Bioeng.* 95:460-465.
- Fujita, J., Y. I. Yamane, H. Fukada, Y. Kizaki, S. Wakabayashi, S. Shigeta, O. Suzuki and K. Ono. 2003b. Production and properties of phytase and acid phosphatase from a Sake Koji mold, *Aspergillus oryzae*. *J. Biosci. Bioeng.* 95:348-353.
- Fujiwara, K., Y. Miyaguchi, A. Toyoda, Y. Nakamura, M. Yamazaki, K. Nakashima and H. Abe. 2008. Effect of fermented soybean "Natto" supplement on egg production and qualities. *Asian-Aust. J. Anim. Sci.* 21(11):1610-1615.
- Garling, D. L. Jr. and R. P. Wilson. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. *J. Nutr.* 106:1368-1375.
- Jung, K. O., S. Y. Park and K. Y. Park. 2006. Longer aging time increases the anticancer and antimetastatic properties of deonjang. *Nutr.* 22:539-545.
- Kim, S. S. and K. J. Lee. 2008. Effects of dietary kelp (*Ecklonia cava*) on growth and innate immunity in juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquac. Res.* 39:1687-1690.

- Kim, S. S., G. B. Galaz, K. J. Lee and Y. D. Lee. 2006. Effects of dietary supplementation of *Spirulina* and Astaxanthin for juvenile olive flounder, *Paralichthys olivaceus* in low temperature season. *J. Aquacult.* 19:57-63.
- Kim, Y. G., J. D. Lohakare, J. H. Yun, S. Heo and B. J. Chae. 2007. Effect of feeding levels of microbial fermented soy protein on the growth performance, nutrient digestibility and intestinal morphology in weaned piglets. *Asian-Aust. J. Anim. Sci.* 20(3):399-404.
- Kumar, S., N. P. Sahu, A. K. Pal, D. Choudhury, S. Yengkokpam and S. C. Mukherjee. 2005. Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *Labeo rohita* juveniles. *Fish Shellfish Immun.* 19:331-334.
- Kundu, A. K. and S. Das. 1970. Production of amylase in liquid culture by a strain of *Aspergillus oryzae*. *Appl. Environ. Microb.* 19:598-603.
- Kundu, A. K., S. Das, S. Manna and N. Pal. 1968. Extracellular proteinases of *Aspergillus oryzae*. *Appl. Microbiol.* 16:1799-1801.
- Kundu, A. K. and S. Manna. 1975. Purification and characterization of extracellular proteinases of *Aspergillus oryzae*. *Appl. Microbiol.* 30:507-513.
- Kwak, C. S., M. S. Lee and S. C. Park. 2007. Higher antioxidant properties of Chungkukjang, a fermented soybean paste, may be due to increased aglycone and malonylglycoside isoflavone during fermentation. *Nutr. Res.* 27:719-727.
- Leske, K. L. and O. O. Coon. 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poult. Sci.* 78:1151-1157.
- Lin, C. H., Y. T. Wei and C. C. Chou. 2006. Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. *Food Microbiol.* 23:628-633.
- Min, B. J., J. H. Cho, Y. J. Chen, H. J. Kim, J. S. Yoo, Q. Wang, I. H. Kim, W. T. Cho and S. S. Lee. 2009. Effects of replacing soy protein concentrate with fermented soy protein in starter diet on growth performance and ileal amino acid digestibility in weaned pigs. *Asian-Aust. J. Anim. Sci.* 22(1):99-106.
- Park, K. Y. and K. O. Jung. 2005. Fermentation soybean products as functional foods: functional properties of doenjang (fermented soybean paste). In: *Asian functional foods* (Ed. J. Shi, C. T. Ho and F. Shahidi). Boca Raton, CRC Press, USA, pp. 555-596.
- Park, K. Y., K. O. Jung, S. H. Rhee and Y. H. Choi. 2003. Antimutagenic effects of doenjang (Korean fermented soy paste) and its active compounds. *Mutat. Res.* 523-524:43-53.
- Petchkongkaew, A., P. Taillandier, P. Gasaluck and Q. Lebrhi. 2008. Isolation of *Bacillus Spp.* from Thai fermented soybean (Thua-nao): screening for aflatoxin B1 and ochratoxin A detoxification. *J. Appl. Microbiol.* 104:1495-1502.
- Pham, M. A. 2008. Nutritional and immunological studies on fermented soybean products in diets for marine fishes. Ph.D. Thesis, Cheju National University, Jeju, Korea.
- Pham, M. A. and K. J. Lee. 2007. Effects of dietary Cheongkukjang on liver superoxide dismutase activity of parrot fish *Oplegnathus fasciatus*. *J. Aquacult.* 20:132-139.
- Pham, M. A., K. J. Lee, B. J. Lee, S. J. Lim, S. S. Kim, Y. D. Lee, M. S. Heo and K. W. Lee. 2006. Effects of dietary *Hizikia fusiformis* on growth and immune responses in juvenile olive flounder (*Paralichthys olivaceus*). *Asian-Aust. J. Anim. Sci.* 19(12):1769-1775.
- Pham, M. A., K. J. Lee, T. M. Dang, S. J. Lim, G. Y. Ko, J. Eo and D. H. Oh. 2008. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*). *Asian-Aust. J. Anim. Sci.* 21(9):1367-1375.
- Stasiak, S. A. and P. C. Bauman. 1996. Neutrophil activity as a potential bioindicator for contaminant analysis. *Fish Shellfish Immun.* 6:537-539.
- Ukeda, H., D. Kawana, S. Maeda and M. Sawamura, 1999. Spectrophotometer assay for superoxide dismutase based on the reduction of highly water soluble tetrazolium salts by xanthin-xanthin oxidase. *Biosci. Biotech. Bioeng.* 63:485-488.
- Um, J. S., H. S. Lim, S. H. Anh and I. K. Park. 2000. Effects of microbial phytase supplementation to low phosphorus diets on the performance and utilization of nutrients in broiler chickens. *Asian-Aust. J. Anim. Sci.* 13(6):824-829.
- Wang, Y. B., J. R. Li and J. Lin. 2008. Probiotics in aquaculture: Challenges and outlook. *Aquaculture* 281:1-4.
- Wodzinski, R. J. and A. H. J. Ullah. 1996. Phytase. *Adv. Appl. Microbiol.* 42:263-302.
- Yamamoto, T., A. Akimoto, S. Kishi, T. Unuma and T. Akiyama. 1998. Apparent and true availabilities of amino acids from several protein sources for fingerlings rainbow trout, common carp, and red sea bream. *Fisheries Sci.* 64:448-458.
- Yamane, Y. I., J. Fujita, S. Izuwa, K. Fukuchi, R. I. Shimizu, A. Hiyoshi, H. Fukuda, S. Mikami, Y. Kizaki and S. Wakabayashi. 2002. Properties of cellulose-degrading enzymes and their contribution to material utilization and alcohol yield in sake mash fermentation. *J. Biosci. Bioeng.* 93:479-484.
- Yang, Y. X., Y. G. Kim, J. D. Lohakare, J. H. Yun, J. K. Lee, M. S. Kwon, J. I. Park, J. Y. Choi and B. J. Chae. 2007. Comparative efficacy of different soy protein sources on growth performance, nutrient digestibility and intestinal morphology in weaned pigs. *Asian-Aust. J. Anim. Sci.* 20(5):775-783.