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A Microbial Fermentation of Soybean and Cottonseed Meal Increases Antioxidant Activity and Gossypol Detoxification in Diets for Nile tilapia, *Oreochromis niloticus*

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Abstract

We report the beneficial effects of a microbial fermentation process of plant protein sources. To reduce anti-nutritional factors, soybean meal (SM) and cottonseed meal (CM) were fermented with *Aspergillus oryzae*, a probiotic microorganism, and a 12-wk feeding study was conducted to evaluate the supplemental effects of the fermented soybean meal (FSM) or fermented cottonseed meal (FCM) in diets for juvenile Nile tilapia. Growth performance and hematological parameters were not influenced by the fermentation process, while the highest inclusion of both FSM and FCM lowered feed utilization efficiency. The microbial fermentation significantly increased dietary total polyphenols and consequently led to higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities in both diets and fish tissue. Dietary and liver gossypol concentrations were significantly decreased by the fermentation process of CM. The findings suggest that microbial fermentation of SM or CM with *A. oryzae* can enhance antioxidant activities in diets and fish, and effectively reduce or degrade the toxicity of gossypol present in CM. A microbial fermentation could make plant protein sources more useful and functional in feeds for fish.

Replacement of fish meal is one of the most important issues in the aquaculture industry because of continuously increasing demand, unstable supply, and high price of fish meal. Soybean meal (SM) is one of the most nutritious plant protein sources. It has been widely used as a cost-effective feed ingredient for many fish species because of its high protein content, relatively well-balanced amino acid profile, reasonable price, and steady supply (Storebakken et al. 2000). Cottonseed meal (CM) has long been used in feeds for both terrestrial animals (Colin-Negrete et al. 1996) and fish (Barros et al. 2002; Lee 2002) because of its high protein content and lower cost than both fish meal and SM. Several studies have shown promising results using CM in aquafeed formulations for several fish species (Lee 2002; Gatlin et al. 2007) including tilapia (Mbahinzireki et al. 2001; Yue and Zhou 2008).

However, presence of anti-nutritional factors, such as protease inhibitors, tannins, oligosaccharide, and phytate in plant protein sources has led to limited use in fish feeds (NRC 1993; Masumoto et al. 2001; Guimaraes et al. 2008). In addition, CM contains gossypol, a yellow pigment gland, which is toxic to fish (Lee 2002; Yildirim et al. 2003) and terrestrial animals (Henry et al. 2001). Modification of the plant proteins including heat and enzyme treatments have been applied to eliminate or inactivate the anti-nutritional factors, consequently increasing the use of the plant proteins in diets for fish (Masumoto et al. 2001; Pham et al. 2007; Lim and Lee 2009).

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Fermentation has long been used to prepare traditional soybean foods in Far East Asia that are commonly known as "Dou-Bian-Jiang" in China, "Miso and Natto" in Japan, and "Duen-Jang" in Korea. It has been one of the most promising techniques to destroy or decrease the anti-nutritional factors present in plant proteins and thereby improve their nutritive values. Yigzaw et al. (2001) reported that fermentation of legumes is potentially an important method that could improve the nutritive value and decrease certain anti-nutritional factors, such as phytic acids, protease inhibitors, and flatulence factors. Zhang et al. (2006, 2007) also found, through *in vitro* studies, that microbial fermentation could greatly decrease free gossypol concentration in CM.

Aspergillus oryzae, a predominant fungus in Meju, the popular Korean fermented soybean and main ingredient for Duen-Jang, can produce several enzymes such as phytases and α-amylase (Fujita et al. 2003; Hong et al. 2004; Rahardjo et al. 2005). *A. oryzae* is the main functional microorganism being used as a fermentation starter in producing commercial Meju using whole soybean (Jung et al. 2006). It is also known to enhance antioxidant activity of soybean after fermentation (Lin et al. 2006). We recently reported that fermentation of SM with *A. oryzae* could increase antioxidant activities in diets and nonspecific immune responses of parrot fish (Kim et al. 2009).

We hypothesized that the fermentation process degrades anti-nutritional factors in plant protein sources, such as SM and CM in fish feeds. Therefore, the aim of this study was to evaluate the effects of dietary incorporation of the fermented SM or CM on growth performance, feed utilization and antioxidant capacities, focusing on gossypol detoxification of CM in diets for juvenile Nile tilapia, *Oreochromis niloticus*.

Materials and Methods

Fermentation of SM and CM

Solvent extracted SM and CM were fermented by a process similar to the preparation of Meju with the following modifications (Kim et al. 2009). Briefly, SM and CM were finely ground and hydrated with distilled water at a ratio of 1:1.5 for 30 min. The materials were cooked for 30 min at 100 C, cooled and dried at room temperature. Subsequently, the materials were inoculated with 3% *A. oryzae* spores (Jeil Bio Tech. Co. Ltd., Hwaseong, South Korea) on a w/w basis. The inoculated materials were then made into a brick shape (3 cm × 15 cm × 10 cm) and incubated at 28 C for 48 h until a yellow layer formed. The fermented soybean meal (FSM) and cottonseed meal (FCM) were dried again at room temperature (25 C) for 48 h and ground prior to their inclusion into the experimental diets. The major difference between Meju and FSM is the ingredients, whole soybean versus defatted SM, respectively, and the duration of the fermentation. Proximate compositions of non-treated and fermented SM or CM determined by the standard methods of AOAC (1995) were, on a dry matter basis, as follows: moisture (7.6, 15.6, 8.9, and 16.4%); crude protein (50.4, 52.5, 48.0, and 50.2%); crude lipid (2.7, 2.6, 3.4, and 3.4%); and ash (6.0, 6.8, 7.0, and 7.5%) in SM, FSM, CM, and FCM, respectively. All analyses were performed in triplicate.

Experimental Diets

The dietary formulations and proximate compositions are presented in Table 1. Six experimental diets (designated by FSCM0, FSM50, FSM100, FCM50, FCM100, and FSCM100, respectively) were formulated to be isonitrogenous (36% CP) and isocaloric (20 MJ/kg DM). Diet FSCM0 containing 16% SM and 16% CM was used as the control diet. In diets FSM50 or FSM100, 50% or 100% SM in the control diet was replaced by FSM. In diets FCM50 or FCM100, 50% or 100% CM was replaced by FCM. In diet FSCM100, SM and CM in the control diet were completely replaced by FSM and FCM. All the dry ingredients were thoroughly mixed with 30% distilled water, extruded through a meat chopper (SMC-12, Kuposlice, Busan, South Korea) with a 3.0-mm die, freeze-dried at −40 C for 12 h and stored at −20 C until use.

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

Ingredients	Diets					
	FSCM0	FSM50	FSM100	FCM50	FCM100	FSCM100
White fish meal	20	20	20	20	20	20
Soybean meal[1]	16	8	—	16	16	—
Fermented soybean meal	—	8	16	—	—	16
Cottonseed meal[2]	16	16	16	8	—	—
Fermented cottonseed meal	—	—	—	8	16	16
Others[3]	48	48	48	48	48	48
Chemical analyses (% DM)[4]						
Moisture	6.8	7.1	6.3	6.5	7.0	6.9
Protein	36.2	37.0	37.2	36.1	36.3	36.6
Lipid	9.2	8.7	9.4	9.1	9.1	9.3
Ash	5.6	5.6	5.3	5.3	5.6	5.5
Gross energy, MJ/kg[5]	20.0	19.9	20.2	20.2	20.1	20.4

[1]Soybean meal was purchased from Woosung Feed Co. Ltd., Daejeon, South Korea.

[2]Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, TN, USA.

[3]Others (% DM): corn gluten meal, 6.0; starch, 23.0; dextrin, 7.0; yeast, 2.0; mineral mix, 1.0 (Lim and Lee 2009); vitamin mix, 1.0 (Lim and Lee 2009); Squid liver oil, 8.0.

[4]Values are means from duplicate sample of experimental diets.

[5]Gross energy of experimental diets was calculated according to gross energy values 5.64 kcal/g protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g fat (NRC 1993).

Feeding Trial and Sample Collection

Juvenile Nile tilapia obtained from a private hatchery (Kunsan, South Korea) were fed a commercial diet (CP, 32%; lipid, 5.6%) for a 2-wk acclimation period at Kunsan National University, Kunsan, South Korea. At the beginning of the feeding trial, 360 fish (initial body weight, 13.5 ± 0.01 g/fish) were randomly distributed into 60-L glass rectangular tanks with 20 fish per tank. The tanks in line with a semi-recirculation system were supplied at a flow rate of 1.5 L/min with continuous aeration. Water quality parameters were monitored daily. During the feeding trial, water temperature ranged from 25 to 28 C, dissolved oxygen ranged from 6.29 to 7.32 mg/L and ammonia–nitrogen was lower than 0.05 mg/L. Triplicate groups of fish were fed each diet in excess (twice a day, 0900 and 1700 h) for 12 wk. Uneaten feed was collected 30 min after feeding, dried, and reweighed to determine feed intake and feed conversion ratio. Growth rate of fish was measured every 2 wk. Feeding was stopped 24 h prior to weighing. At the end of the feeding trial, three fish per tank (nine fish per dietary treatment) were randomly sampled and anesthetized with MS-222 solution (200 mg/L) for blood analyses. Blood samples were taken from the caudal vein with heparinized syringes. Liver from three fish per tank were removed and stored at −80 C for analyses of antioxidant capacity, total polyphenol, and gossypol enantiomers. Experimental protocols followed the guidelines of the Animal Care and Use Committee of Jeju National University.

Analyses

All fish in each tank were group-weighed and counted after the feeding trial to compute growth performance and feed utilization efficiency. Hematocrit was determined for three individual fish per tank by a microhematocrit technique (Brown 1980). Plasma total glucose, triacylglycerol, cholesterol, and total protein were determined in the same three fish by an automated blood analyzer (SLIM, SEAC Inc., Florence, Italy). Proximate compositions of the feed ingredients and diets were analyzed by the methods of AOAC (1995). Amino acid compositions of the experimental diets, SM, FSM, CM, and FCM were analyzed using an automatic amino acid analyzer (Biochrom 30, Biochrom Ltd, Cambridge, UK). Gossypol concentrations in diets and

livers (nine fish per treatment) were determined by high-performance liquid chromatography according to the method described by Lee and Dabrowski (2002). Antioxidant activities in diets and liver were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay described by Sandoval et al. (2002). Approximately 2 g of diets or whole livers were homogenized (X-120, Germany) in aqueous methanol (80%) and homogenates were centrifuged at 2600*g* at 4 C for 10 min and filtered through 0.45-μm syringe filters (Whatman Inc., Clifton, NJ, USA) prior to the assay. One hundred microliters 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 was measured at 517 nm after 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 = $100 \times [(A_0 - A_{10})/A_0]$, where A_0 and A_{10} are the absorbance of sample at 0 and 10 min, respectively. Total polyphenolic compounds in diets and livers were measured by the 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.). One half milliliters of filtered extract was added to 2.5 mL of Folin-Ciocalteu reagent (0.2 N, Sigma, St. Louis, MO, USA) and incubated 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 of mixture was measured at 760 nm using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The results were expressed in grams of gallic acid per kilogram of dry diet.

Statistical Analysis

All diets were assigned to tanks by a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among treatments, the mean differences were tested with Duncan's multiple range test. Statistical significance was determined by setting the aggregate type I error at 5% ($P < 0.05$) for each set of comparisons. Data are presented as means ± SD. Percentage data were arcsine transformed before statistical analysis.

Results and Discussion

Microbial fermentation with *A. oryzae* slightly altered the compositions of SM and CM. The fermentation process increased crude protein content in SM and CM by 4.2 and 4.6%, respectively, and ash content by 13.3 and 7.1%, respectively. Lipid content was not influenced by the fermentation process. These nutritional changes were previously noticed in SM (Hong et al. 2004; Song et al. 2008) and CM (Zhang et al. 2006) by fermentation processes. The increased crude protein after fermentation was probably because of the growth of microorganisms which synthesize cellular protein, enzymes, or other cellular components.

During the 12-wk feeding trial, the fish readily accepted all diets and no mortality was observed. Weight gain and specific growth rate were not influenced by the microbial fermentation; however, fish fed FSCM100 diet exhibited significantly poorer feed conversion ratio and protein efficiency ratio than those fish fed the control diet (Table 2). In our previous study, we also found a decreasing trend in growth and feed utilization efficiency of parrot fish fed FSM or fermented whole soybean by *A. oryzae* although it was not significant (Kim et al. 2009).

In this study, all the dietary essential amino acid levels met the requirements for Nile tilapia (Santiago and Lovell 1988). Microbial fermentation with *A. oryzae* increased most of amino acids in both SM and CM while it decreased lysine by 19 and 3% and threonine by 16 and 18% in SM and CM, respectively. In addition, the dietary lysine and threonine, the commonly limiting essential amino acids in plant protein sources, were decreased by dietary incorporation of FSM and FCM (Table 3). Thus, the

TABLE 2. *Growth performance and feed utilization efficiency of juvenile tilapia fed the experimental diets for 12 wk.*[1]

Diets	FSCM0	FSM50	FSM100	FCM50	FCM100	FSCM100
IBW[2]	13.5 ± 0.07	13.5 ± 0.06	13.5 ± 0.04	13.5 ± 0.03	13.5 ± 0.06	13.5 ± 0.05
WG (%)[3]	397 ± 25.3	415 ± 24.4	374 ± 51.4	408 ± 33.6	387 ± 70.7	373 ± 37.0
SGR (%)[4]	1.91 ± 0.06	1.95 ± 0.06	1.85 ± 0.15	1.93 ± 0.08	1.88 ± 0.17	1.85 ± 0.09
FCR[5]	1.12 ± 0.09[a]	1.25 ± 0.02[ab]	1.36 ± 0.17[ab]	1.25 ± 0.06[ab]	1.29 ± 0.11[ab]	1.49 ± 0.20[b]
PER[6]	2.37 ± 0.19[a]	2.11 ± 0.03[ab]	1.96 ± 0.26[ab]	2.11 ± 0.10[ab]	2.05 ± 0.19[ab]	1.80 ± 0.24[b]

[1]Means of triplicate groups ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

[2]IBW (g/fish): initial body wet weight.

[3]WG (%): weight gain (%) = 100 × (final mean body weight − initial mean body weight)/initial body weight.

[4]SGR (%/day): specific growth rate = 100 × ($\log_e$ final body weight − $\log_e$ initial body weight)/days.

[5]FCR: feed conversion ratio = dry feed fed (g)/wet weight gain (g).

[6]PER (g/g): protein efficiency ratio = wet weight gain/total protein fed.

TABLE 3. *Amino acid composition of SM, FSM, CM, FCM, and the experimental diets (g/100 g dry matter).*

Amino acid	SM	FSM	CM	FCM	FSCM0	FSM50	FSM100	FCM50	FCM100	FSCM100
Essential amino acid										
Arginine	3.43	3.45	3.26	3.68	2.78	2.83	2.83	2.86	2.77	2.79
Histidine	1.46	1.70	1.41	1.40	1.53	1.56	1.60	1.49	1.47	1.45
Isoleucine	2.18	2.53	1.70	1.82	1.46	1.58	1.57	1.55	1.53	1.53
Leucine	3.74	4.22	3.26	3.11	2.68	2.73	2.83	2.83	2.74	2.81
Lysine	3.53	2.96	2.52	2.45	2.75	2.42	2.28	2.42	2.30	2.21
Methionine	0.66	0.63	0.55	0.60	0.46	0.54	0.55	0.54	0.64	0.62
Phenylalanine	2.55	3.34	2.92	3.24	1.69	1.76	1.78	1.68	1.73	1.78
Threonine	2.10	1.80	1.81	1.53	1.56	1.48	1.37	1.41	1.35	1.31
Tryptophane	1.71	1.89	1.43	1.58	0.53	0.58	0.58	0.55	0.53	0.53
Valine	2.48	2.89	2.46	2.44	1.80	1.97	1.97	1.93	1.92	1.99
Nonessential amino acid										
Alanine	2.33	2.59	2.22	2.21	2.11	2.09	2.06	2.03	2.06	2.01
Aspartic acid	5.92	5.84	4.90	4.80	3.50	3.47	3.42	3.45	3.40	3.38
Cystine	0.84	0.94	0.80	0.82	0.41	0.46	0.48	0.41	0.49	0.44
Glutamic acid	8.94	9.76	10.90	10.81	5.61	6.02	6.00	6.00	5.81	5.88
Glycine	2.23	2.40	2.25	2.26	1.94	1.91	1.94	1.91	1.98	2.07
Serine	2.83	3.02	2.46	2.43	1.64	1.66	1.65	1.70	1.66	1.66

reduction of some essential amino acid such as lysine and threonine in the diets may be one of the leading causes of the decreased feed utilization and protein efficiency ratio of fish fed the highest levels of fermented products in this study. Some essential amino acid decrease by fermentation processes was previously reported. Song et al. (2008) found that when defatted SM was fermented with different microorganisms (*Lactobacillus plantarum*, *Bifidobacterium lactis* or *L. plantarum*), most amino acids were significantly increased while methionine and cysteine were decreased. In our previous study (not published), fermentation with different microorganisms (*Saccharomyces cerevisiae*, *Pediococcus pentosaceus* and *Bacillus subtilis*) resulted in decreased levels of lysine and methionine in defatted SM. In contrast, Hong et al. (2004) reported that fermentation of defatted SM treated with *A. oryzae* did not affect the essential amino acid concentration. Therefore, the effects of microbial fermentation on amino acid composition in plant protein sources might differ depending on fermentation process parameters such as microorganism, substrate, moisture content, pH, incubation temperature and period, or inoculum level. The reduction in specific essential amino acids by the fermentation process remains for further study.

TABLE 4. *Blood parameters of juvenile tilapia fed the experimental diets for 12 wk.*[1]

Diets	FSCM0	FSM50	FSM100	FCM50	FCM100	FSCM100
Hematocrit (%)	32.0 ± 2.5	31.8 ± 3.4	32.2 ± 2.4	32.8 ± 2.3	30.1 ± 4.7	32.0 ± 2.4
Plasma glucose (mg/dL)	61.1 ± 9.5	73.0 ± 6.9	67.5 ± 9.8	70.5 ± 13.5	63.6 ± 17.2	67.6 ± 5.1
Plasma triacyglycerol (mg/dL)	5.0 ± 1.8	4.3 ± 1.1	4.9 ± 0.8	4.4 ± 0.8	3.7 ± 1.2	4.7 ± 1.4
Plasma cholesterol (mg/dL)	230 ± 78.2	218 ± 25.3	242 ± 22.4	263 ± 4.4	207 ± 31.5	224 ± 19.9
Plasma total protein (mg/dL)	11.8 ± 0.7	11.9 ± 0.5	12.0 ± 0.5	12.0 ± 0.5	11.5 ± 0.2	12.0 ± 0.4

[1]Means of triplicate groups ± SD. Values are not significantly different ($P < 0.05$).

Blood parameters such as hematocrit, plasma glucose, plasma cholesterol, plasma triacyglycerol, and plasma total protein were not influenced by the fermentation process (Table 4). Kim et al. (2009) reported that hematocrit and hemoglobin of parrot fish were not affected by the fermentation of dietary SM and suggested further studies to investigate the effects of dietary incorporation of fermented plant proteins on hematological parameters for fish.

Interestingly, the gossypol concentrations in diets and livers were affected by the fermentation process of CM. Total and (+) or (−) gossypol enantiomer concentrations in both diets and livers significantly decreased by microbial fermentation of CM indicating of destruction or inactivation of gossypol present in CM (Fig. 1A, B). Gossypol has been the main constraint limiting the use of CM for animals and fish feeds (Lee 2002). To increase the use of cottonseed products in fish feeds, the adverse effects of gossypol on the nutritional value of diets and growth or reproductive performance has been extensively studied and reported (Robinson 1991; Dabrowski et al. 2000; Yildirim et al. 2004; Lee et al. 2006). Results of the present study clearly demonstrate that the microbial fermentation of CM can inactivate or reduce the toxicity of dietary gossypol to fish. Gossypol detoxification by microbial fermentation was previously reported (Zhang et al. 2006, 2007). An *in vitro* test indicated that microbial fermentation could greatly decrease free gossypol concentrations in CM although the effectiveness depends on the species of microorganism used (Zhang et al. 2006). The study evaluated six fungi strains including *A. oryzae* and concluded that *Candida tropicalis* was the most effective among the six strains in detoxifying free gossypol. The decrease in gossypol concentrations, both in diets and fish livers, seemed to be due to the binding of free gossypol to amino acids produced by microorganism or the microorganism itself which contains several exoenzymes that could degrade gossypol molecules (Brock et al. 1994; Zhang et al. 2006).

Dietary total polyphenol concentrations significantly increased by dietary incorporation of FSM and FCM. The highest dietary polyphenol concentration was observed in FSCM100 diet (Fig. 2A). However, the polyphenol concentration was not significantly increased in fish liver by the dietary incorporation of FSM or FCM, although it was slightly higher in fish fed FSM100 and FSCM100 diets than in fish fed the control diet (Fig. 2B). Polyphenolic compounds are plant-derived antioxidants that possess radical scavenging properties (Lopes et al. 1999). SM and CM containing various amounts of polyphenolic compounds have been shown to possess antioxidant capacities. The increased dietary total polyphenols after fermentation in this study are in agreement with results reported in other studies (Vattem and Shetty 2002; Randhir et al. 2004). They suggested that β-glucosidase produced from microorganisms catalyzed the release of aglycones from the substrate and thereby increased their polyphenol concentration.

Dietary DPPH radical scavenging activities were significantly higher in FSM100, FCM100, and FSCM100 diets compared to the control diet. The highest dietary DPPH radical scavenging activity was observed in FSCM100 diet (Fig. 2C). Liver DPPH radical scavenging

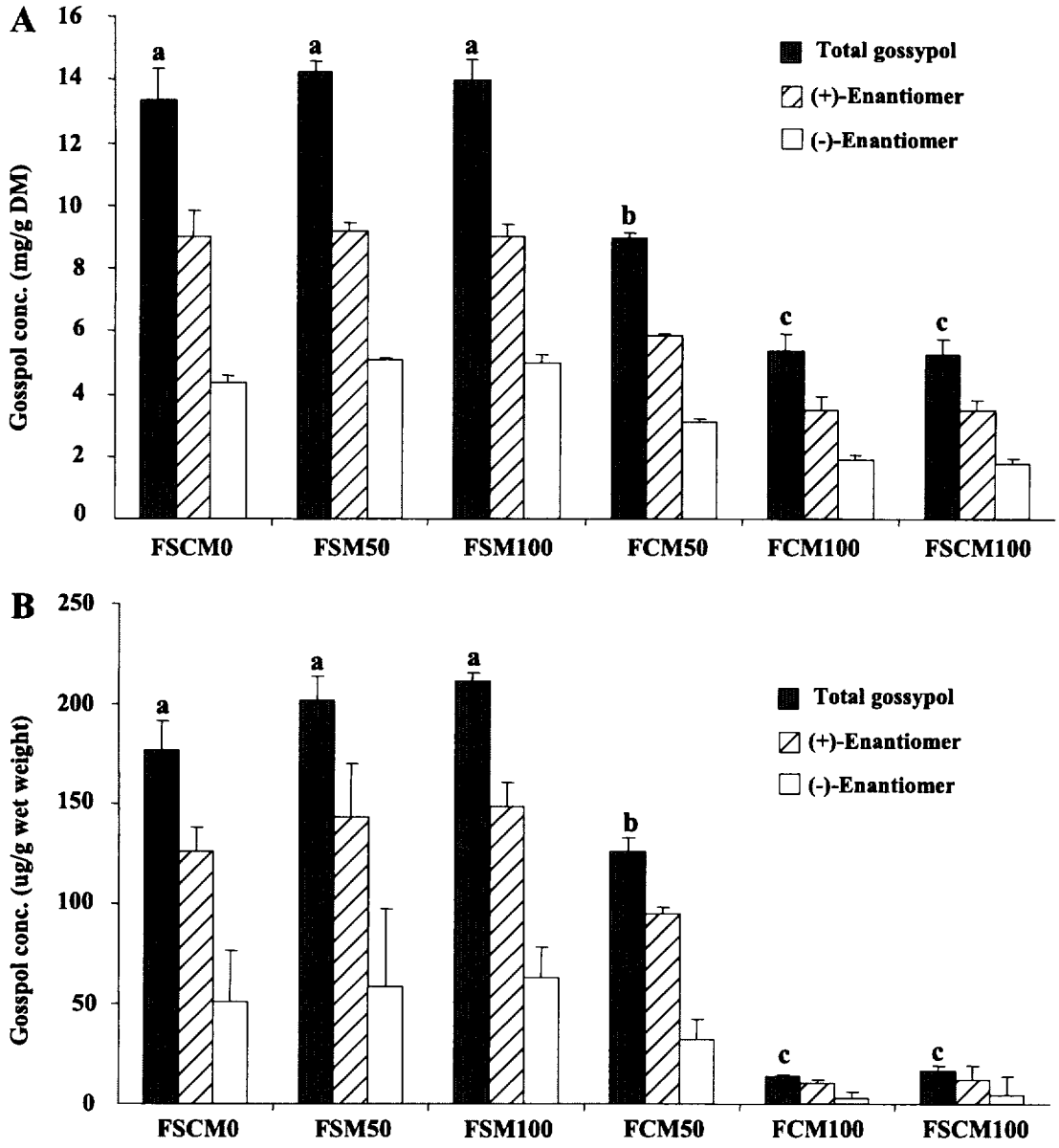

FIGURE 1. *Total and (+) and (−) enantiomers of gossypol in diets (A) and liver (B) of juvenile tilapia fed experimental diets for 12 wk. Values represent mean ± SE of nine fish from each of triplicate groups. Bars with different letters are significantly different* ($P < 0.05$).

activity was also significantly increased in fish fed FSM100 and FSCM100 diets compared to fish fed the control diet (Fig. 2D). Polyphenolic compounds have been demonstrated to exhibit scavenging capacities for free radicals (McCue and Shetty 2003). Thus, the higher antioxidant activities in diets containing high levels of fermented plant proteins could be related to their high total polyphenol concentrations. Skerget et al. (2005) reported that DPPH radical scavenging activities were linearly correlated with the concentration of polyphenol compounds. Pham and Lee (2007) also found that dietary supplementation of Chungkukjang, a traditional Korean fermented soybean powder which is different from Duen-Jang due to

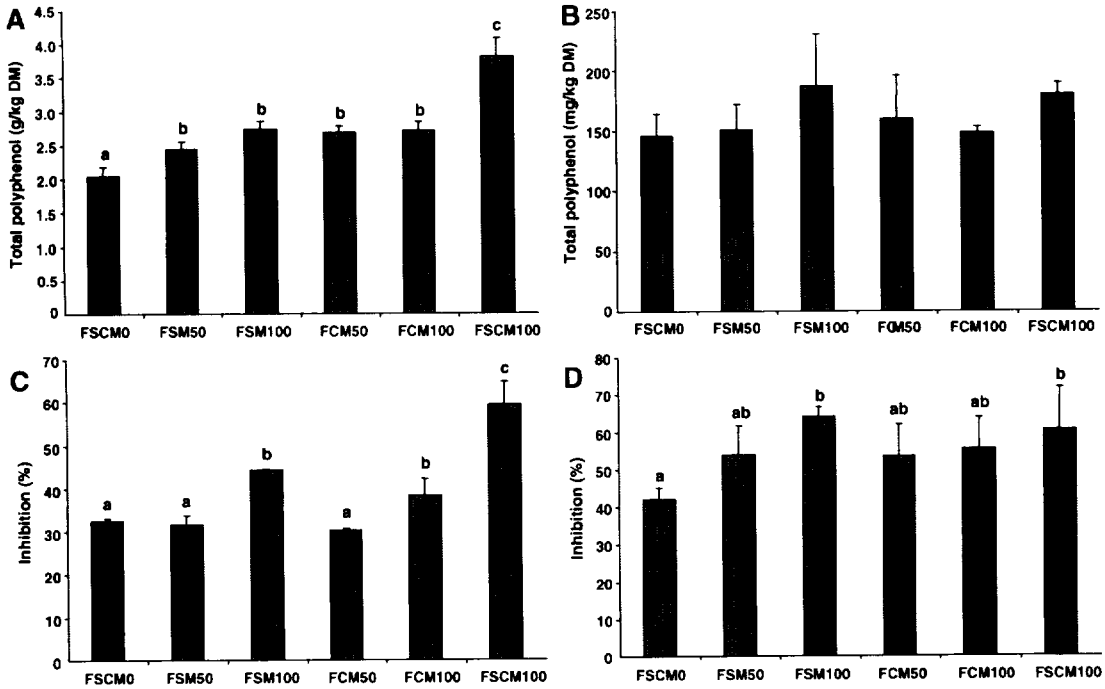

FIGURE 2. *Total polyphenolic compounds in diets (A) and liver (B) and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity in diets (C) and liver (D) of juvenile tilapia fed experimental diets for 12 wk. Values represent mean ± SE of nine fish from each of triplicate groups. Bars with different letters are significantly different (P < 0.05).*

different fermentation temperature and duration, resulted in higher dietary DPPH scavenging activities compared to nontreated SM-based diet. Soybean flavones and isoflavones are well known to have strong antioxidant capacities against free radicals and some effects on immunity of organisms (Birt et al. 2001). They reported that the bioavailability of flavones or isoflavones is influenced by their chemical form in the products (mostly glycoside conjugates), their hydrophobicity and susceptibility to degradation by microbial flora. However, fish cannot utilize soy isoflavones in glycoside conjugates which can only be hydrolyzed by sulfatase and glucuronic acid (Piskula and Terao 1998). It is believed that the presence of microorganism, *A. oryzae*, in the fermented products hydrolyze the isoflavone glycosides into isoflavone aglycones which can be utilized by fish. Therefore, this is considered to be the main reason for the increased dietary polyphenols and thereby improved DPPH scavenging activities, both in diets containing FSM or FCM and liver of fish fed the diets in this study.

In conclusion, the microbial fermentation process by *A. oryzae* could increase antioxidant activities both in diets containing FSM or FCM and fish fed these diets. It also reduces/degrades the gossypol in CM. These preliminary results of microbial fermentation provide insight into improving the quality of fish feeds by protecting polyunsaturated fatty acids from oxidation. Also, because of the reduction/degradation of gossypol, increased levels of CM could be used in fish feeds.

Acknowledgments

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Literature Cited

AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Barros, M. M., C. Lim, and P. H. Klesius. 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus punctatys*) to *Edwrdsiella ictaluri* challenge. Aquaculture 207:263–279.

Birt, D. F., S. Hendrich, and W. Q. Wang. 2001. Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacology & Therapeutics 90: 157–177.

Brock, T. D., M. T. Madigan, J. M. Martinko, and J. Parker. 1994. Biology of microorganisms. 7th edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Brown, B. A. 1980. Routine hematology procedures. Pages 71–112 *in* B. A. Brown, editor. Hematology, principles and procedures. Lea and Febiger, Philadelphia, Pennsylvania, USA.

Colin-Negrete, J., H. E. Kiesling, T. T. Rose, and J. F. Smith. 1996. Effects of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing Holstein heifers. Journal of Dairy Science 79:2016–2023.

Dabrowski, K., J. Rinchard, K. J. Lee, J. H. Blom, A. Ciereszko, and J. Ottobre. 2000. Effects of diets containing gossypol on reproductive capacity of rainbow trout (*Oncorhynchus mykiss*). Biology of Reproduction 62:227–234.

Fujita, J., Y. I. Yamane, H. Fukuda, Y. Kizaki, S. Wakabayashi, S. Shigeta, O. Suzuki, and K. Ono. 2003. Production and properties of phytase and acid phosphatase from a sake koji mold, *Aspergillus oryzae*. Journal of Bioscience and Bioengineering 95:348–353.

Gatlin III, D. M., F. T. Barrows, P. Brown, K. Dabrowski, T. G. Gaylord, R. W. Hardy, E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E. J. Souza, D. Stone, R. Wilson, and E. Wurtele. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38:551–579.

Guimaraes, I. G., L. E. Pezzato, and M. M. Barros. 2008. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 14:396–404.

Henry, M. H., G. M. Pesti, R. Bakalli, J. Lee, R. T. Toledo, R. R. Eitenmiller, and R. D. Phillips. 2001. The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine. Poultry Science 80:762–768.

Hong, K. J., C. H. Lee, and S. W. Kim. 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. Journal of Medicinal Food 7:430–435.

Jung, K. O., S. Y. Park, and K. Y. Park. 2006. Longer aging time increases the anticancer and antimetastatic properties of *Deonjang*. Nutrition 22:539–545.

Kim, S. S., G. B. Galaz, M. A. Pham, J. W. Jang, D. H. Oh, I. K. Yeo, and K. J. Lee. 2009. Effects of dietary supplementation of a Meju, fermented soybean meal, and *Aspergillus oryzae* for juvenile parrot fish (*Oplegnathus fasciatus*). Asian-Australasian Journal of Animal Sciences 22:849–856.

Lee, K. J. 2002. Nutritional and physiological studies of rainbow trout following utilization of cottonseed meal-based diets. Dissertation for the degree of Ph.D. Ohio State University, Columbus, Ohio, USA.

Lee, K. J. and K. Dabrowski. 2002. Gossypol and gossypolone enantiomers in tissues of rainbow trout fed low and high levels of dietary cottonseed meal. Journal of Agricultural and Food Chemistry 50:3056–3061.

Lee, K. J., J. Rinchard, K. Dabrowski, I. Babiak, J. S. Ottobre, and J. E. Christensen. 2006. Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout: Three-year study. Animal Feed Science and Technology 126:93–106.

Lim, S. J. and K. J. Lee. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture 290:283–289.

Lin, C. H., Y. T. Wei, and C. C. Chou. 2006. Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. Food Microbiology 23: 628–633.

Lopes, G. K. B., H. M. Schulman, and M. Hermes-Lima. 1999. Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. Biochimica et Biophysica Acta. General Subjects 1472:142–152.

Masumoto, T., B. Tamura, and S. Shimeno. 2001. Effects of phytase on bioavailability of phosphorus in soybean meal-based diets for Japanese flounder, *Paralichthys olivaceus*. Fisheries Science 67:1075–1080.

Mbahinzireki, G. B., K. Dabrowski, K. J. Lee, D. El-Saidy, and E. R. Wisner. 2001. Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal-based diets in a recirculating system. Aquaculture Nutrition 7:189–200.

McCue, P. and K. Shetty. 2003. Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. Food Biotechnology 17:27–37.

NRC (National Research Council). 1993. Nutritional requirements of fish. National Academic Press, Washington, DC, USA.

Pham, M. A. and K. J. Lee. 2007. Effects of dietary Cheongkukjang on liver superoxide dismutase activity of parrot fish *Oplegnathus fasciatus*. Journal of Aquaculture 20:132–139.

Pham, M. A., K. J. Lee, S. J. Lim, and K. H. Park. 2007. Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder *Paralichthys olivaceus*. Fisheries Science 73:760–769.

Piskula, M. and J. Terao, 1998. Quercetin's solubility affects its accumulation in rat plasma after oral administration. Journal of Agricultural and Food Chemistry 46:4313–4317.

Rahardjo, Y. S. P., F. J. Weber, S. Haemers, J. Tramper, and A. Rinzema. 2005. Aerial mycelia of *Aspergillus oryzae* accelerate alpha-amylase production in a model solid-state fermentation system. Enzyme and Microbial Technology 36:900–902.

Randhir, R., D. Vattem, and K. Shetty. 2004. Solid-state bioconversion of fava bean by *Rhizopus oligosporus* for enrichment of phenolic antioxidants and L-DOPA. Innovative Food Science and Emerging Technologies 5:235–244.

Robinson, E. H. 1991. Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. Journal of Applied Aquaculture 1:1–14.

Sandoval, M., N. N. Okuhama, F. M. Angeles, V. V. Melchor, L. A. Condezo, J. Lao, and M. J. S. Miller. 2002. Antioxidant activity of the cruciferous vegetable Maca (*Lepidium meyenii*). Food Chemistry 79:207–213.

Santiago, C. B. and Lovell, R. T. 1988. Amino acid requirements for growth of Nile tilapia. Journal of Nutrition 118:1540–1546.

Skerget, M., P. Kotnik, M. Hadolin, A. R. Hras, M. Simonic, and Z. Knez. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89:191–198.

Song, Y. S., J. Frias, C. Martinez-Villaluenga, C. Vidal-Valdeverde, and E. Gonazalez de Mejia. 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. Food Chemistry 108:571–581.

Storebakken, T., S. Refstie, and B. Ruyter. 2000. Soy products as fat and protein sources in fish diets for intensive aquaculture. Pages 127–170 *in* J. K. Drackley, editor. Soy in animal nutrition. Federation of Animal Science Societies, Savoy, Illinois, USA.

Vattem, D. A. and K. Shetty. 2002. Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. Food Biotechnology 16:189–210.

Yigzaw, Y., L. Gorton, G. Akalu, and T. Solomon. 2001. Fermentation of teff (*Eragrostis tef*), grass pea (*Lathyrus sativus*) and their mixtures: Aspects of nutrition and food safety. Lathyrus Lathyrism Newsletters 2:8–10.

Yildirim, M., C. Lim, P. Wan, and P. H. Klesius. 2003. Growth performance and immune response of channel catfish (*Ictalurus punctatus*) fed diets containing graded levels of gossypol-acetic acid. Aquaculture 219:751–768.

Yildirim, M., C. Lim, P. Wan, and P. H. Klesius. 2004. Effect of natural free gossypol and gossy-acetic acid on growth performance and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. Aquaculture Nutrition 10:153–165.

Yue, Y. R. and Q. C. Zhou. 2008. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. Aquaculture 284:185–189.

Zhang, W. J., Z. R. Xu, S. H. Zhao, J. F. Jiang, Y. B. Wang, and X. H. Yan. 2006. Optimization of process parameters for reduction of gossypol levels in cottonseed meal by *Candida tropicalis* ZD-3 during solid substrate fermentation. Toxicon 48:221–226.

Zhang, W. J., Z. R. Xu, S. H. Zhao, J. Y. Sun, and X. Yang. 2007. Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal. Animal Feed Science and Technology 135:176–186.