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Evaluating Nutritional Quality of Single Stage- and Two Stage-fermented Soybean Meal

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ABSTRACT : This study investigated the nutritional quality of soybean meal (SBM) fermented by *Aspergillus* ($FSBM_A$) and/or followed by *Lactobacillus* fermentation ($FSBM_{A+L}$). Both fermented products significantly improved protein utilization of SBM with higher trichloroacetic acid (TCA) soluble true protein content, *in vitro* protein digestibility and available lysine content, especially in $FSBM_{A+L}$. Moreover, $FSBM_{A+L}$ produced a huge amount of lactic acid resulting in lower pH as compared to the unfermented SBM or soybean protein concentrate (SPC) ($p<0.05$). $FSBM_A$ and $FSBM_{A+L}$ raised 4.14% and 9.04% of essential amino acids and 5.38% and 9.37% of non-essential amino acids content, respectively. The α-galactoside linkage oligosaccharides such as raffinose and stachyose content in $FSBM_A$ and $FSBM_{A+L}$ decreased significantly. The results of soluble protein fractions and distribution showed that the ratio of small protein fractions (<16 kDa) were 42.6% and 63.5% for $FSBM_A$ and $FSBM_{A+L}$, respectively, as compared to 7.2% for SBM, where the ratio of large size fractions (>55 kDa, mainly β-conglycinin) decreased to 9.4%, 5.4% and increased to 38.8%, respectively. There were no significant differences in ileal protein digestibility regardless of treatment groups. SPC inclusion in the diet showed a better protein digestibility than the SBM diet. In summary, soybean meal fermented by *Aspergillus*, especially through the consequent *Lactobacillus* fermentation, could increase the nutritional value as compared with unfermented SBM and is compatible with SPC. (**Key Words :** Fermented Soybean Meal, Nutritional Quality, Amino Acid, Oligosaccharides, Pig)

INTRODUCTION

Soybean meal contains an excellent amino acids profile and has been widely used as an indispensable major protein source in animal industries. Soybean protein also contains anti-nutritive compounds (ANCs) including trypsin inhibitor, lectin, goigotrens, saponins, phytate, flatulence producing oligosaccharides and allergenic soybean protein (Friedman and Brandon, 2001). Only the portion of heat-labile ANCs in SBM can be partially destroyed through roasting and extrusion, however, ninety percent protein residues in soy protein isolate are belonging to the two heat-stable globulins, glycinin and conglycinin, and are the allergen for the hypersensitivity and commonly associated with villous atrophy and malabsorption in weaning pig after ingestion of intact soybean proteins (Pluske et al., 1997). Due to the heat tolerance of ANCs contained in SBM some nutritionists suggest a very limited amount or no SBM to be used in piglets diet immediately after weaning. Many processing products have been developed to eliminate ANCs and to improve SBM feeding value including isolation of soy protein or microbial inoculation through fermentation. Soy protein concentrate (SPC) is a typical commercial product of low-allergenic protein (Hancock et al., 1990), while Tempe, Miso and Natto are fermented soybean products of *Rhizopus*, *Bacillus* and *Aspergillus*, which also contain low ANFs (Samanya and Yamauchi, 2002; Nout and Kiers, 2005). Piglets fed with *Aspergillus* fermented SBM diet increased weight gains and dramatically improved feed conversion (Hong et al., 2004). *Lactobacillus* fermentation technology has been widely applied in dairy, plant and silage preparation for years and is used recently in fermentation feed with the viable *Lactobacillus* play as probiotics resource for the improvement of piglet intestinal health. The purposes of this study are to investigate the applicability of the two-stage process of *Aspergillus* and *Lactobacillus* fermentation with SBM, and to evaluate the nutritional value of fermented SBM by analysis the quality and distribution of the *in vitro* hydrolyzed protein.

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MATERIALS AND METHODS

Commercial processed soybean meal, *Aspergillus* fermented soybean meal ($FSBM_A$), *Aspergillus* and *Lactobacillus* fermented soybean meal ($FSBM_{A+L}$) and Soy protein concentrate (SPC, Soycomil®) were used for this study.

Preparation of fermented soybean meal

Aspergillus oryzae and *Lactobacillus casei* used in this study were obtained from the Bioresource collection and Research Center (BCRC), Hsunchu, Taiwan. The *A. oryzae* strain used in this study is a traditional starter has been widely used in fermented food industry due to its capability in secretion of α-amylase and protease. The *Lact. casei* strain behave high growth rate and organic acid producing capability. The fermentation process was initiated by soaking SBM in water to achieve 40-45% moisture content. The soaked SBM was then inoculated with *Aspergillus oryzae* (10^8/g) at a rate of 20 g/kg SBM after which the mixture was fermented in a bed-packed incubator for 24 h at 30°C (single stage fermentation). Following the completion of single stage fermentation, the initial fermented mixture was reconstituted with water to contain 58-60% moisture content and inoculated with *Lactobacillus casei* (10^9/g) at a rate of 4 g/kg SBM. This mixture was allowed to ferment further under anaerobic conditions for 16 h at 37°C (two stage fermentation). After fermentation, the fermented SBM was dried at 55-60°C to a moisture content around 10%. Both dried SBM fermented with *Aspergillus* ($FSBM_A$) only and fermented with *Aspergillus* and *Lactobacillus* ($FSBM_{A+L}$) were ground to 0.25 mm in particle size for analysis.

Dry matter, crude protein and amino acids

Dry matter and crude protein were determined by the method of AOAC (1980). Determination of total amino acids was carried out by acid hydrolysis, derivatization and HPLC quantification using the method of Blackburn (1978).

Protein solubility in KOH

Protein solubility in KOH was determined by the procedure described in Parsons et al. (1991). Approximately 1.5 g of sample was placed in a 250 ml beaker and 75 ml of 0.2% KOH solution was added and the mixture was stirred for 20 min at 22°C. The liquid (50 ml) was then centrifuged at 1,250×g for 10 min. The supernatant was collected and the nitrogen content was determined by the Kjedahl method (AOAC, 1980). The protein solubility was then calculated as a percentage of the total in the original SBM sample.

Trichloroacetic acid (TCA) soluble protein content

TCA soluble protein content was measured the portion of protein soluble in TCA which contain polypeptide of less than 10 amino acids linkage and free amino acids. The protein extracted from SBM (the method described in section 3) added 10% TCA, the unsolidfied protein was then removed by centrifugation. The concentration of free amino groups in TCA soluble protein was determined with the ninhydrin reaction by spectrophotometer (Sarin et al., 1981).

Available lysine

The method for determination of available lysine in proteins by their reactions with 2,4,6-trinitrobenzenesulphonic acid (TNP) was according to Hall et al. (1973). The absorbance of the yellow solution of TNP-lysine was measured at 415 nm and pure DL-lysine monohydrochloride was used as a standard.

pH and lactic acid determination

The pH value of the samples was measured using a pH meter in mixing 10 g sample with 10 ml water. The lactic acid concentration was determined by HPLC according to the method of Marsili et al. (1983).

Oligosaccharides

The oligosaccharides composition analyzed by high performance liquid chromatography (HPLC) according to the method of Delente and Ladenburg (1972). Sugars were extracted from soybean meal using water, mixture of 9% $Ba(OH)_2$:10% $ZnSO_4$ (1:1) and choloroform. Extract was concentrated under vacuum, made to 200 μl with de-ionized water. The extract was filtered with 0.22 μm filters and stored at a cold room temperature of 0°C. Ten μl of samples were analyzed by HPLC. Quantification was against authentic external standards of the sugars detected.

Soluble protein fractions and distribution

The soluble soybean protein in SBM, FSBM and SPC were extracted by the method of Faurobert (1997). One hundred miligram sample mixed thoroughly with 400 μl of protein extraction solution (50 mM Na_2CO_3, 100 mM NaCl, 0.05% Triton X-100, 0.05% Tween-20, 1 mM PMSF, 1% β-mecaptoethanol) for 10 min. The slurry was centrifuged at 7,000×g for 30 min. The supernatant was collected and the protein concentration was measured by the method of Bradford (1976) using BSA as the standard.

Soluble protein was fractionated by a Tricine-SDS-PAGE system according to the method described by Schagger (2006). The electrophoresis system was based on 10% polyacrylamide separating gels containing 0.1% SDS in Tris- tricine buffer. About 30 μg of extracted soluble protein was loading for each well and the sample was separated at 50-60 mV for 5 h. The protein markers (Invitrogen, USA) with the following molecular weights

include Myosin (210.0 kDa), Bovine serum albumin (78.0 kDa), Glutamic dehydrogenase (55.0 kDa), Alcohol dehydrogenase (45.0 kDa), Carbonic anhydrase (34.0 kDa), Myoglobin (23.0 kDa), Lysozyme (16.0 kDa), Aprotinin (7.0 kDa) and Insulin B chain (4.0 kDa). After electrophoresis, the gel was stained for 30 min using Coomassie Brilliant Blue R-250 and de-stained with acetic acid. Images of the gels were captured with densitometers (Astra 2200, UMAX®) and analysis with software (Gel pro 4.5v, Media Cybernetics®) to calculate relative percentage of each protein band in test samples.

In vitro protein digestibility

The protocol of the *in vitro* digestibility was according to the method of Babinszky et al. (1990). One gram of sample (feedstuffs) was incubated at 39°C for two hours in a 50 ml beaker with 10 ml pepsin/0.1 M HCl. After incubation, 2 ml of $NaHCO_3$ (110 mg/ml) was added to buffering the solution, then re-incubated in 25 ml of artificial pancreatin solution (4 g pancreatin (Sigma®), 2 g amylase (Sigma®), 40 mg pancreatic lipase (Sigma®) and 80mg bile salts (Sigma®) in 1 L of potassium phosphate buffer, pH 5.8) for 6 h at 40°C. The solution was added ten millimeter 5% of TCA and then filtered through filter paper (Whatman No. 1). The amount of crude protein in the TCA-insoluble residue was determined, and calculated the protein digestibility.

In vivo nutrient digestibility

The animal feeding protocol and care was approved by the Animal Care and Use Committee of National Chung-Hsing University. Four ileum cannulated Landrace castrated male pig weighing 60 kg were used in a Latin 4×4 Latin square experimental design in this trial. All pigs were housed in individual cages and fed one of four experimental diets during each replication period. Diets were formulated with SBM, $FSBM_A$, $FSBM_{A+L}$ or SPC into an iso-nitrogenous diet with 17% crude protein and 3,200 Kcal ME according to the NRC (1998) nutrient requirements for pig. Table 1 presents the experimental diet composition. A 3 g/kg Cr_2O_3 was included as indicator for the digestibility measurements. Feed was provided *ad libitum* during the 7 days preliminary period and followed by 80% of the *ad libitum* consumption to maintain a constant feed consumption in the subsequent 4-day period for collection the digesta from cannula. Digesta was collected four times per day and then mixed for analysis of protein and Cr_2O_3 to calculate the ileum digestibility. The Cr_2O_3 in feeds or digesta was determined calorimetrically with a spectrophotometer (U-2001, HITACHI, Japan) according to the methods described by Williams et al. (1962).

Statistical analysis

All results were statistically analyzed by using one-way analysis of variance (ANOVA), and significant difference among treatments were determined with Duncan's multiple range test using the Statistical Analysis System software package (version 6.1; SAS, 1999). The differences were considered to significant at $p<0.05$.

RESULTS AND DISCUSSION

Protein characteristics of FSBM

The concentration of crude protein, KOH protein

Table 1. Composition of experimental diet for *in vivo* feeding trial (g/kg)

Ingredients	SBM[1]	FSBM	SPC
Corn	659.0	698.0	747.0
Soybean meal, 44% CP	252.0		
FSBM		215.0	
SPC			164.0
Wheat bran	31.0	30.0	30.0
Soybean oil	19.5	17.8	19.6
Sodium chloride	5.0	5.0	5.0
Limestone, ground	18.0	18.0	18.9
Monocalcium phosphate	13.5	14.2	13.5
Vitamin premix[2]	0.5	0.5	0.5
Mineral premix[3]	1.5	1.5	1.5
Calculate value (g/kg)			
Crude protein	170.0	170.0	170.0
Ether extract	49.8	49.8	49.8

[1] SBM = Soybean meal; FSBM = Fermented soybean meal with *Aspergillus or Aspergillus+Lactobacillus*; SPC = Soybean protein concentrate.

[2] Supplied per kg of diet: Vit. A, 15,000 IU; Vit. D_3 3,000 IU; Vit. E 30 mg; Vit. K_3 4 mg ; Riboflavin 8 mg; Pyridoxine 5 mg; Vit. B_{12} mcg; Ca-pantothenate 19 mg; Niacin 50 mg; Folic acid 1.5 mg; Folic acid 1.5 mg; Biotin 60 mcg.

[3] Supplied per kg of diet: Co ($CoCO_3$) 0.255 mg; Cu ($CuSO_4 \cdot 5\ H_2O$) 10.8 mg; Fe ($FeSO_4 \cdot H_2O$) 90 mg; Zn (ZnO) 68.4 mg; Mn ($MnSO_4 \cdot H_2O$) 90 mg; Se (Na_2SeO_3) 0.18 mg.

Table 2. The nutrient quality of SBM, fermented SBM and SPC

Items	SBM[1]	$FSBM_A$	$FSBM_{A+L}$	SPC	SEM
Crude protein (%)	43.09±0.78[2c]	47.78±2.99[b]	47.50±0.61[b]	61.87±1.24[a]	0.978
KOH protein solubility (%)	83.58±2.86[a]	74.69±6.07[b]	76.32±1.08[b]	75.87±3.32[b]	2.186
TCA soluble protein (μmole/g)	65.26±8.02[c]	110.33±14.01[b]	1,010.32±64.83[a]	70.26±5.03[c]	15.211
Available lysine (%)	3.02±0.06[b]	3.22±0.30[b]	4.18±0.13[a]	4.26±0.08[a]	0.123
In vitro protein digestibility (%)	87.50±2.61[b]	85.89±1.20[b]	92.23±2.51[a]	87.63±1.68[b]	3.412
pH	6.98±0.19[a]	6.33±0.07[b]	4.5±0.06[c]	6.76±0.20[a]	0.084
Lactic acid (μmole/g)	0[c]	3.69±3.86[b]	140.25±20.34[a]	0[c]	5.979

[1] SBM = Soybean meal; $FSBM_A$ = Fermented soybean meal with *Aspergillus*; $FSBM_{A+L}$ = Fermented soybean meal with *Aspergillus+Lactobacillus*.
[2] Values are the mean of four replications and standard deviation.
[a, b, c, d, e] Means in the same row without the same superscripts are significantly different ($p<0.05$).

solubility, TCA soluble protein, available lysine, *in vitro* protein digestibility, pH and lactic acid in regular SBM, $FSBM_A$, $FSBM_{A+L}$ and SPC were summarized in Table 2. Both of $FSBM_A$ and $FSBM_{A+L}$ contain the same crude protein concentration and are 4.5% higher than the untreated raw SBM. The result of protein concentration increase by fermentation agrees with Hong et al. (2004) on their 48 h *A. oryzae* fermented soybean meal. This protein increase may be attributed to the carbohydrate and protein in the SBM been used for microbial growth during fermentation. In addition, the KOH protein solubility of both $FSBM_A$ and $FSBM_{A+L}$ and SPC product was lower than the untreated raw SBM. This might be due to the application of heat during product drying process which decreases protein solubility. Soybean protein KOH solubility has been widely used to detect soybean protein quality caused by over processing (Araba and Dale, 1990). Protein solubility in KOH was also a good index of *in vivo* soybean protein quality for both pigs and chickens; where the feed efficiency was significantly decreased when protein KOH solubility is below 66% (Parsons et al., 1991). In this study, both of fermented SBM and SPC product obtained lower KOH protein solubility than SBM; however the solubilities in all tested SBM products were still above 74%. The $FSBM_{A+L}$ showed higher TCA soluble protein content and much faster rate in the *in vitro* protein digestion than SBM, $FSBM_A$ and SPC. The TCA soluble protein means the amount of digestible soybean protein soluble in TCA. Low (1980) showed that a half of the dietary protein leaving stomach is usually in the peptides form with a large proportion being soluble in TCA, i.e. peptides having ten or fewer amino acids. The results in this study showed that TCA soluble protein content in fermented SBM was significantly higher than unfermented SBM. Moreover, SBM processing through fermentation had a higher *in vitro* protein digestibility and available lysine content, especially when it is by combined microbial strain, i.e., $FSBM_{A+L}$, significantly improve protein utilization.

Change in pH and lactic acid concentration

$FSBM_{A+L}$ contained large amount of lactic acid with lower pH value compared to the other SBM products ($p<0.05$) (Table 2), reflected that *Lactobacillus* use galactose or sucrose as resources to proliferate and produce high volume of lactic acid in the second stage SBM fermentation. High lactic acid concentration of $FSBM_{A+L}$ reflected fast *Lactobacillus* growth in the second stage fermentation since lactic acid concentration and ATP, are indicators of microbial activity in the fermented diet. Lactic acid has been used as a flavor enhancer in food industry. Lactic acid supplemented dry compound diets also improved daily gain and feed efficiency in pig (Mikkelsen and Jensen, 2004). Therefore, lactic acid level may influence dietary palatability and consequently feed intake in animal. In this study, $FSBM_A$ contained higher lactic acid concentration with lower pH value compared to SBM, but significant higher pH and lower lactic acid than in the $FSBM_{A+L}$. These results may attributed to the *Aspergillus* which secreted protease and proceed proteolysis of protein and amino acid to buffer the pH value during SBM fermentation (Feng et al., 2007) and produced no organic acid in this stage. This may explain why pH value in $FSBM_A$ did not significant decrease as in $FSBM_{A+L}$.

The amino acid profile

Table 3 presents the amino acid profile of raw and fermented SBM. Since both of fermented FSBM increased crude protein content, the amino acid content of both FSBM increased and is reflected from the content of essential amino acid (EAA) and non-essential amino acid (NEAA). The EAA of $FSBM_A$ and $FSBM_{A+L}$ raised 4.14% and 9.04%, where NEAA content of $FSBM_A$ and $FSBM_{A+L}$ increased 5.38% and 9.37%, respectively, as compared with SBM. Although, Hong et al. (2004) reported that fermentation of soybean meal had no effect on the EAA concentration, but dramatically raised the concentrations of Gly, Glu and Asp. However, Frias et al. (2008) observed an increase of NEAA and EAA in fermented SBM through inoculation of

Table 3. Amino acids profile of various soybean meal products

Amino acids	SBM[1]	$FSBM_A$	$FSBM_{A+L}$	SPC	SEM
Crude protein	44.81±0.06[2c]	46.76±1.29[bc]	48.95±1.34[b]	65±0.47[a]	0.68
Asparagine	5.43±0.04[b]	5.67±0.42[b]	5.71±0.91[b]	7.7±0.83[a]	0.46
Threonine	1.83±0.06[b]	1.90±0.06[ab]	2.04±0.30[a]	2.73±0.51[a]	0.21
Serine	2.09±1.34[a]	2.28±0.17[a]	2.59±0.44[a]	3.38±0.95[a]	0.60
Glutamine	8.26±0.17[b]	8.62±0.86[b]	10.22±3.28[a]	11.73±0.44[a]	0.40
Proline	2.38±0.03[b]	2.48±0.10[b]	2.39±0.13[b]	3.38±0.35[a]	0.14
Glycine	1.71±0.49[a]	1.96±0.08[a]	2.37±0.58[a]	2.88±0.51[a]	0.33
Alanine	2.01±0.20[a]	2.10±0.06[a]	2.41±0.55[a]	2.85±0.28[a]	0.23
Valine	2.17±0.10[b]	2.25±0.04[b]	2.42±0.03[b]	3.38±0.42[a]	0.16
Methionine	0.66±0.21	0.68±0.17	0.75±0.01	0.91±0.03	0.10
Isoleucine	2.09±0.08[b]	2.18±0.03[b]	2.28±0.13[b]	3.19±0.51[a]	0.19
Leucine	3.58±0.10[b]	3.74±0.13[b]	4.11±0.33[b]	5.20±0.54[a]	0.23
Tyrosine	1.75±0.10[ab]	1.82±0.11[ab]	1.54±0.27[b]	2.5±0.54[a]	0.22
Phenylalanine	2.38±0.06[ab]	2.48±0.07[ab]	2.63±1.36[b]	3.45±0.47[a]	0.51
Histidine	1.26±0.04[b]	1.31±0.07[b]	1.17±0.04[b]	1.82±0.34[a]	0.12
Lysine	2.87±0.68[b]	3.00±0.13[b]	3.07±0.27[ab]	4.23±0.44[a]	0.30
Arginine	3.41±0.66[ab]	3.54±0.17[ab]	2.60±1.05[b]	4.94±0.76[a]	0.52
Total	43.88±0.55[b]	46.01±1.74[b]	48.3±3.97[b]	64.27±7.92[a]	3.20

[1] SBM = Soybean meal; $FSBM_A$ = Fermented soybean meal with *Aspergillus*.
$FSBM_{A+L}$ = Fermented soybean meal with *Aspergillus+Lactobacillus*; SPC = Soybean protein concentrate.
[2] Values are the mean of three replications and standard deviation.
[a, b, c] Means in the same row without the same superscripts are significantly different ($p<0.05$).

Lactobacillus or *Bacillus subtilis* while observed no raising effect of the NEAA content with *A. oryzae* fermentation. Increase in amino acid content after fermentation of other legumes in addition to SBM was also reported in kidney beans (Mibithi-Mwikya et al., 2000) and rice-bean mixture (Barampama and Simard, 1995). These amino acids concentration increases could be attributed to the simple protein constituents of bacteria mass and microbial metabolism that take place during fermentation of soy. SBM is the major lysine resources in diet, while not all of the total lysine presented in a protein is available to the animal, especially in heat processed protein sources. Our data showed higher lysine content in both fermented than in the unfermented SBM (Table 3) and did not agree with Hong et al. (2004). In addition, our data also showed that the available lysine of $FSBM_{A+L}$ and SPC both were higher than SBM and $FSBM_A$ (Table 2). The observed higher available lysine content in $FSBM_{A+L}$ might be explained by two possibilities. The first possibility was that Maillard reaction did not occur when $FSBM_{A+L}$ was dried at temperatures ranging from 50 to 60°C. The other possibility was that bound lysine found in regular SBM might be released by the two-stage fermentation. In fact, re-roast fermented SBM during dry processing increased palatability including smell and taste.

Oligosaccharide concentration

Table 4 presents the oligosaccharide distribution. The

Table 4. Oligosaccharides distribution of soybean meal products

Item (%, W/W)	SBM[1]	$FSBM_A$	$FSBM_{A+L}$	SPC	SEM
Stachyose	6.39±0.29[2a]	0.42±0.00[c]	ND[3d]	1.93±0.12[b]	0.10
Raffinose	1.35±0.03[a]	0.24±0.06[c]	ND[d]	0.42±0.07[b]	0.02
Sucrose	8.13±0.43[a]	0.90±0.06[b]	0.27±0.02[c]	0.44±0.11[bc]	0.15
Glucose	0.16±0.04[c]	2.77±0.18[a]	2.62±0.31[a]	1.49±0.12[b]	0.11
Galactose	0.07±0.02[c]	1.32±0.21[a]	1.16±0.20[a]	0.88±0.16[b]	0.07
Fructose	2.40±0.41[b]	3.13±0.22[a]	2.52±0.22[ab]	1.11±0.13[c]	0.17

[1] SBM = Soybean meal; $FSBM_A$ = Fermented soybean meal with *Aspergillus*;
$FSBM_{A+L}$ = Fermented soybean meal with *Aspergillus+Lactobacillus*; SPC = Soybean protein concentrate.
[2] Values are the mean of four replications and standard deviation. [3] Non detectable.
[a-d] Mean within same row without same superscripts are significantly different ($p<0.05$).

tested SBM contained 2.06% raffinose, 5.18% stachyose, 7.48% sucrose as air dry basis agreed to the oligosaccharide results of Delente and Ladenburg (1972) (1.0%, 4.7% and 8.2%). The oligosaccharides content in $FSBM_A$ and $FSBM_{A+L}$ decreased significantly after fermentation, whereas the stachyose and sucrose of SPC also decreased as well. The *A. oryzae* strain used in this study characterized with α-amylase and protease secretion capability. In addition, *A. oryzae* inoculated SBM increased α-galactosidase activity with up to 30 h fermentation. Both raffinose and stachyose are α-galactoside linkage oligosaccharides and are generally degraded by α-galactosidase in the first fermentation stage, their intermediate products provide carbon source for *Lactobacillus* growth in the consequent fermentation. That stachyose and raffinose were not detected in $FSBM_{A+L}$ might be due to their degradation during the two-stage fermentation process. Likewise, the significant higher content of released glucose and galactose found in $FSBM_A$ and $FSBM_{A+L}$ as opposed to SBM or SPC suggested that both glucose and galactose released during fermentation might be used as carbon sources by *Lactobacillus casei* in our study reported herein. In addition, the amylase secreted from *Aspergillus* in the first step fermentation hydrolyzed SBM starch in the second step fermentation leading to a glucose increase.

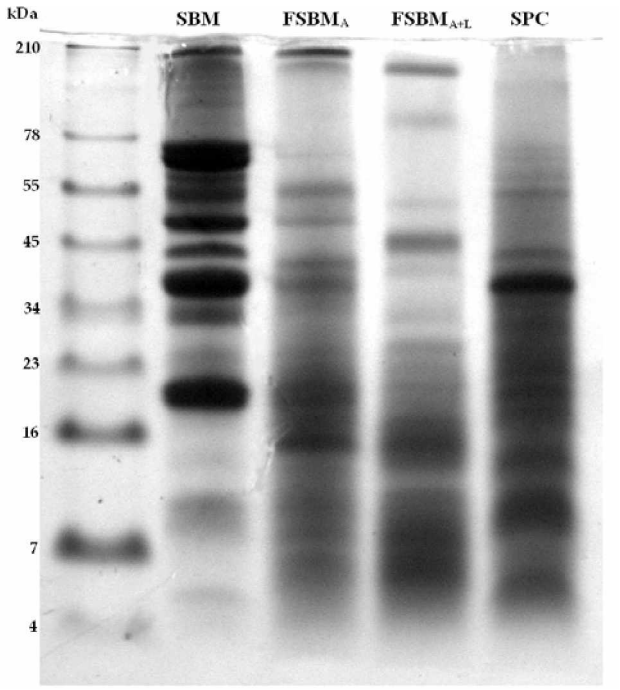

Figure 1. Tricine-SDS-PAGE of variant soybean products. Lane 1: molecular marker, Lane 2: SBM, Lane 3: $FSBM_A$ (Soybean meal fermented with *Aspergillus*), Lane 4: $FSBM_{A+L}$ (Soybean meal fermented with *Aspergillus* and *Lactobacillus*), Lane 5: SPC (Soybean protein concentrate).

Soluble protein distribution profile

Figure 1 presents the Tricine-SDS-PAGE analysis of SBM, $FSBM_A$, $FSBM_{A+L}$ and SPC protein. The SDS-PAGE technique has originally been used to study soybean protein subfractions and to evaluate degradation rate of individual soybean meal protein subfractions in the rumen by densitometric measurement (Schwingel and Bates, 1996). The SDS-PAGE analysis is primary for resolution of the peptide with MW above 14 kDa. To resolute clearly the profile of protein subfractions with MW under 30 kDa, Tricine-SDS-PAGE (Schägger, 2006) was used to analysis fermented SBM in this study. Our result showed the soluble protein profile of SBM contain polypeptide and the subunits including β-conglycinin of α, α' and β, and glycinin of acidic and basic which were separated with estimated MW of 83, 72, 48.4, 38.9 and 18 kDa, respectively. These results of raw soybean protein profile are in agreement with those of previously reported (Feng et al., 2007). Figure 2 presents the ratio of each band in the different soluble protein profile among SBM, $FSBM_A$, $FSBM_{A+L}$ and SPC. The ratio of each band was determined by densitometric analysis after the major peaks in PAGE was identified. The protein subfractions were divided into three parts according to the MW. The protein bands ranged of 55 kDa and above were group as large size fractions, 16-55 kDa as medium-size fractions, and smaller than 16 kDa as small-size fractions. Our result showed that the ratio of small protein fraction in fermented SBM increased, 42.6% and 63.5% for $FSBM_A$ and $FSBM_{A+L}$ compared to 7.2% for SBM, where the ratios of large size fractions decreased; 9.4%, 5.4% and 38.8%, respectively. This showed that the large size fraction was significantly degraded during fermentation, especially the β-conglycinin component and is agreed with Hong (2004).

Schwingel and Bates (1996) indicated that soybean meal protein digested by *P. ruminicola* GA33 resulted in complete digestion of α and α' subunits of conglycinin within 4 h. The β-subunit of conglycinin and glycinin fraction was graded slowly and represented a large proportion of the protein remaining after 48 h of incubation. Feng et al. (2007) indicated that fermentation with *Bacillus subtilis* improved the nutritive value of SBM and reduced or eliminated part of important ANFs such as allergenic protein. Hong et al. (2004) also indicated that increasing amounts of small size protein fraction in fermented soybeans is due to partial digestion of large size fraction in soybeans by protease secreted by *Aspergillus* during fermentation. However, our observation showed only the β-coglycinin protein but not basic glycinin in the FSBM was significantly eliminated in this study. The resistance of glycinin to proteolytic attack may be attributed to the intermolecular disulfide bonds of glycinin that join its basic and acidic polypeptide (Badley et al., 1975). In addition, basic polypeptides are more hydrophobic and thus more

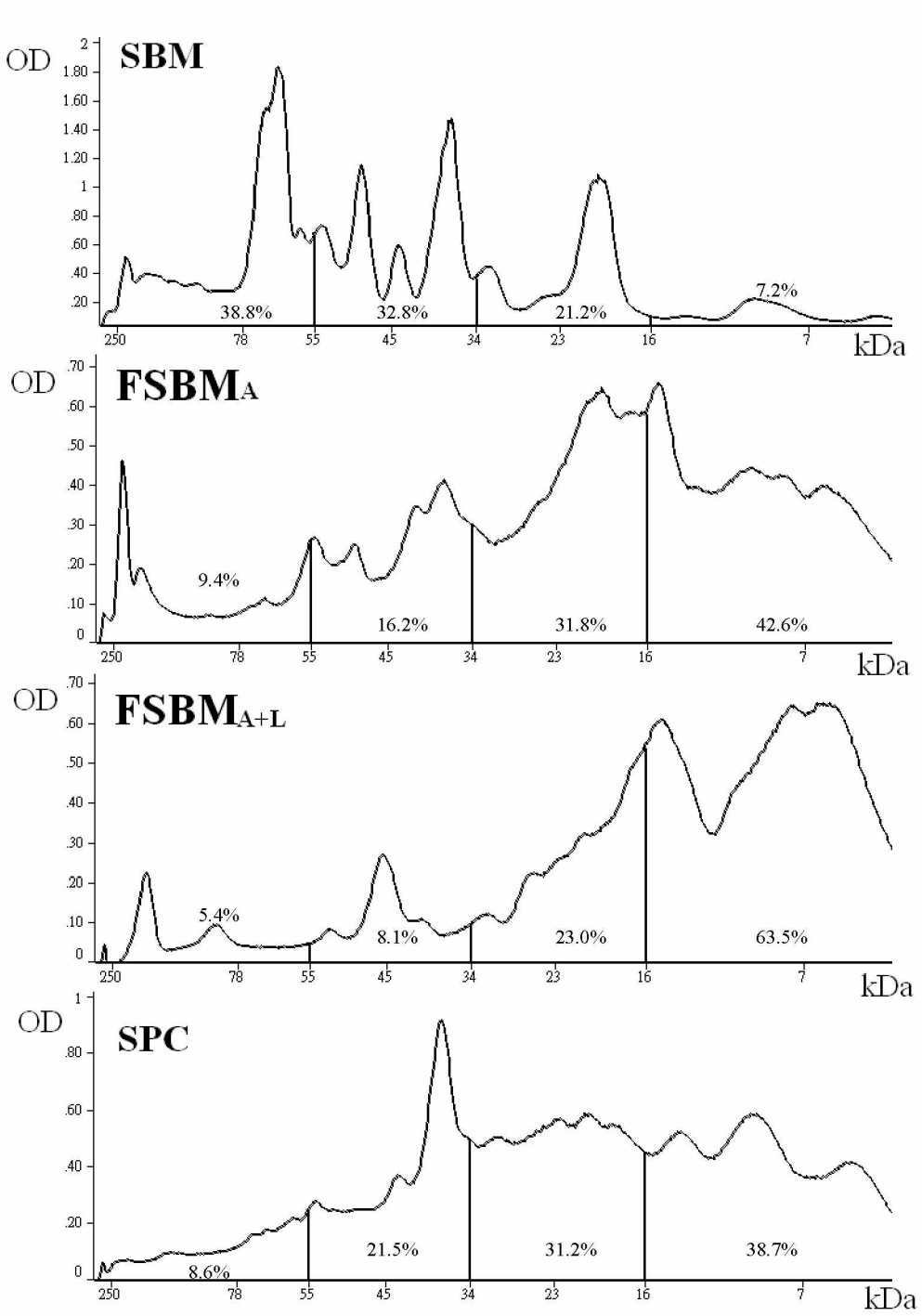

Figure 2. Densitometry profiles of the blue-stained soybean products on the electrophoresis gel. SBM = Soybean meal; $FSBM_A$ = Soybean meal fermented with *Aspergillus*; $FSBM_{A+L}$ = Soybean meal fermented with *Aspergillus* and *Lactobacillus*; SPC = Soybean protein concentrate.

compact and less accessible to enzymatic attack. Small protein fraction (<16 kDa) was represented result of the degraded large protein molecular. An increase in the amount of small protein fraction observed particularly in $FSBM_{A+L}$ between the two fermented SBM was a direct result of degrading the large protein molecule during fermentation. Similarly, an apparent increase in the small and medium protein fraction observed in SPC might be contributed by the breakdown of the intact protein molecule during industrial soybean processing. This protein degradation may lead to the increase of digestion and absorption rates of FSBM and SPC. In addition, it also eliminates the allergenic properties of soybean protein.

Table 5. Protein digestibility of diet inclusion of soybean meal for pig

Sample	*In vivo* (ileum)
SBM[1]	81.56±1.62[2b]
$FSBM_A$	83.54±3.45[ab]
$FSBM_{A+L}$	84.95±4.24[ab]
SPC	89.14±1.02[a]
SEM	1.67

[1] SBM = Soybean meal; $FSBM_A$ = Fermented soybean meal with *Aspergillus*; $FSBM_{A+L}$ = Fermented soybean meal with *Aspergillus*+ *Lactobacillus*; SPC = Soybean protein concentrate.

[2] Values are the mean of four replications and standard deviation.

[a, b, c] Means in the same column without the same superscripts are significantly different ($p<0.05$).

***In vivo* protein digestibility**

Table 5 presents the protein digestibility of pig fed with diet consist of SBM, FSBM or SPC as major protein source. The SPC inclusion diet showed a better ileum protein digestibility than SBM diet. Pigs fed either $FSBM_A$ or $FSBM_{A+L}$ improved ileal protein digestibility at a rate similar to those fed either SBM or SPC. Our result of fermented SBM agree with the report from Yang et al. (2007) who showed equivalent DM and N digestibility when pigs fed with 8% SPC or fermented soybean protein inclusion diet. Zamora and Veum (1988) also demonstrated the fungal fermented soybean had a slightly improvement in growth but not in protein efficiency as compared with unfermented controls in a neonatal pigs feeding trial. In this study, either SBM or with fermented was efficiently utilized by grower pigs, therefore, resulted in similar protein digestibility. However, FSBM showed higher protein digestibility than the unfermented SBM determined in *in vitro* assay. Hong et al. (2004) reported that the trypsin inhibitor of soybean meal was degraded about 84% throughout the *Aspergillus oryzae* fermentation. In the fermentation processing, *Aspergillus* release enzyme to soften the texture of soybean meal resulted in major biochemical changes leading to an increase in solubility and removal of ANFs. Generally, SPC is a good protein source for weaned piglet due to high dry matter and nitrogen digestibility. Since both of $FSBM_{A+L}$ and SPC obtained a similar protein digestibility in this study, $FSBM_{A+L}$ can therefore substitute SPC for protein source in piglet diet.

CONCLUSION

Fermented soybean meal through inoculation of *Aspergillus* or inoculated especially by *Lactobacillus* in the consequent fermentation, could increase the nutritional value compatible to SPC. Soybean protein with its antigenic protein was hydrolyzed and broken down into small peptides fractions via fermentation procedure, which is beneficial for protein utilization. The effectiveness of fermented SBM in piglet growth performance is still pending to be confirmed in the future.

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