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Apparent total tract digestibility and ileal digestibility of dry matter, nitrogen, energy and amino acids in conventional, *Bacillus subtilis*-fermented and enzyme-treated soybean meal fed to weanling pigs

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ABSTRACT: Apparent total tract digestibility (ATTD), apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of dry matter, nitrogen, energy and amino acids were evaluated in six weanling barrows [(Landrace × Yorkshire) × Duroc] fed soybean meal diet (SBM), fermented soybean meal A diet with 50% solubility in potassium hydroxide (FSMA), fermented soybean meal B diet with 60% solubility in potassium hydroxide (FSMB), fermented soybean meal C diet with 70% solubility in potassium hydroxide (FSMC), enzyme-treated soybean meal (ETSM) and nitrogen-free diet. Pigs having body weights of 8.99 ± 0.40 kg were surgically equipped with T-cannulas of approximately 15 cm prior to the ileo-cecal junction and randomly allotted to one of five dietary treatments and a nitrogen-free diet in 6 × 6 Latin squares. With regards to the ATTD of essential amino acids, the ATTD of arginine, isoleucine, lysine, methionine and total essential amino acids was greater in pigs fed FSMB, FSMC, and ETSM diets than in SBM and FSMA diets. The ATTD of leucine, phenylalanine, threonine and valine in the FSMC diet was non-significant compared with the values of ATTD in FSMB and ETSM diets. With respect to non-essential amino acids the ATTD of aspartic acid, cysteine, glutamic acid, proline, tyrosine and total amino acids was greater in pigs fed the FSMC diet compared with the SBM diet and not different from the values of pigs fed the ETSM diet. The AID of dry matter, nitrogen and energy in the FSMC diet was greater ($P < 0.05$) than in pigs fed SBM and FSMA diets but was equal to the values of FSMB and ETSM diets. With regards to the AID of essential amino acids, the AID of histidine, lysine, methionine and threonine was greater in pigs fed FSMB, FSMC and ETSM diets than in SBM and FSMA diets ($P < 0.05$). The AID of isoleucine was greater in pigs fed FSMB and ETSM diets than the FSMA diet. The AID of arginine, phenylalanine and valine was greater in pigs fed the FSMC diet compared with the SBM diet. With respect to non-essential amino acids the AID of aspartic acid, glutamic acid, glycine, tyrosine and total amino acids was the same as in pigs fed FSMB, FSMC, ETSM diets and greater ($P < 0.05$) than in pigs fed SBM and FSMA diets. The SID of all essential amino acids in pigs fed the FSMC diet was greater than for the FSMA diet. In the case of non-essential amino acids, the SID of all amino acids except for cysteine was greatest in pigs fed FSMB, FSMC and ETSM treatments. In conclusion, dietary supplementation of FSMB, FSMC and/or ETSM diets can improve the ATTD, AID and SID of dry matter, nitrogen, energy, total amino acids and most of the essential amino acids in weanling pigs.

Keywords: growth performance; fermented soybean meal; anti-nutritional factors; antigens; lectins; trypsin inhibitors; *Bacillus subtilis*; T-cannula; surgery

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List of abbreviations

AID = apparent ileal digestibility, **ATTD** = apparent total tract digestibility, **ETSM** = enzyme-treated soybean meal, **FSMA** = fermented soybean meal A diet, **FSMB** = fermented soybean meal B diet, **FSMC** = fermented soybean meal C diet, **SBM** = soybean meal diet, **SID** = standardised ileal digestibility

The inclusion of high-quality protein sources in weaned pig diets is a common practice in the swine industry. Due to the high cost and disease-associated risk factors linked with animal protein sources fed to weaned pigs, several studies have been conducted to evaluate alternative plant protein sources (Li et al. 2003). Conventional soybean meal is the most important vegetable protein source fed to pigs because it contains an excellent balance of essential amino acids. However, it also contains anti-nutritional factors such as antigens, lectins, trypsin inhibitors, and oligosaccharides that decrease nutrient availability and affect the growth performance of young pigs (Li et al. 1991; Hong et al. 2004; Zhang et al. 2013). Therefore, most processed soy proteins that are widely used as feed are heat-treated (Hancock et al. 1990), extruded (Burnham et al. 2000), purified (Hancock et al. 1989) or defatted (Jones et al. 1989) to decrease the concentrations of anti-nutritional factors. Fermentation and enzyme treatment also reduces the concentration of trypsin inhibitors in soybean meal (SBM), but increases the concentration of crude protein, amino acids and fat (Zhu et al. 1998; Min et al. 2009; Upadhaya and Kim 2015). Weaned pigs provided with diets enhanced with fermented soy protein had higher dry matter, nitrogen and energy digestibility, and exhibited increased average daily gain compared to those that were provided with untreated soybean meal diets (Yoo et al. 2009; Yan et al. 2011; Wang et al. 2014).

Bacillus subtilis-fermented (FSMA, FSMB and FSMC) and enzyme-treated soybean meal (ETSM) were recently introduced to the Korean feed industry and market. These soy products are believed to reduce the concentrations of trypsin inhibitors, oligosaccharides, and other anti-nutritional components that are present in SBM. The objective of this experiment was to evaluate the hypothesis that the apparent total tract digestibility (ATTD), apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of different nutrients were greater through the fermentation by *Bacillus subtilis* and enzymatic treatment compared to conventional SBM in weanling pigs.

MATERIAL AND METHODS

Animals, experimental design, diets, feedings and housing. All animals received human care as outlined in the Guide for the Care and Use of Experimental Animals (Dankook University, Animal Care Committee). Six [(Duroc × Yorkshire) × Landrace)] weanling barrows with average body weights of 8.99 ± 0.40 kg were surgically equipped with simple T-cannulas approximately 15 cm in front of the ileo-cecal junction according to procedures adapted from Sauer et al. (1983) and were administered one of six dietary treatments in 6 × 6 Latin squares with pigs and periods as the blocking criteria. Dietary treatments included: (1) soybean meal diet, (2) fermented soybean meal A diet with 50% solubility in potassium hydroxide, (3) fermented soybean meal B diet with 60% solubility in potassium hydroxide, (4) fermented soybean meal C diet with 70% solubility in potassium hydroxide, (5) enzyme-treated soybean meal and (6) nitrogen-free diet. Diets were formulated to meet or exceed the requirements suggested by the NRC (2012; Table 1). The pigs were fasted for 16–20 h prior to surgery. Anaesthesia was induced using Stresnil™ (Janssen Pharmaceutica, Belgium) and Virbac Zoletil 50 injections (Virbac Laboratory, France). After surgery, pigs were transferred to individual pens (1.2 × 0.6 m) in a temperature-controlled room (28 °C) where they were allowed to recover for 12 days. Pre- and post-operative care was as previously described in Li et al. (1994). The daily feed allowance was 0.05 × body weight (kg)$^{0.9}$ as proposed by (Armstrong and Mitchell 1955; Upadhaya and Kim 2015). The daily feed was provided as two meals at 12 h intervals (8:00 h and 20:00 h), and water was provided *ad libitum.* Chromic oxide (Cr_2O_3, 2 g/kg) was added to the diet as an indigestible marker to allow digestibility determinations (Fenton and Fenton 1979). Five diets were formulated to contain each protein ingredient as the only protein and amino acids source. A nitrogen-free diet was also formulated to determine the basal endogenous losses of crude protein and amino acids (Tables 1 and 2).

Preparation of soybean meal. The test ingredients consisted of SBM, FSMA, FSMB, FSMC and

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Table 1. Ingredient composition of experimental diets (as-fed basis)

Ingredients (%)	Diet					
	SBM	FSMA	FSMB	FSMC	ETSM	nitrogen-free
Soybean meal (48%)	42.00	–	–	–	–	–
FSMA	–	36.00	–	–	–	–
FSMB	–	–	36.00	–	–	–
FSMC	–	–	–	36.00	–	–
ETSM	–	–	–	–	36.00	–
Cornstarch	30.11	39.01	39.01	39.01	38.95	75.70
Soybean oil	4.00	1.00	1.00	1.00	1.00	–
Sugar	15.00	15.00	15.00	15.00	15.00	15.00
Solka-Floc‡	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	0.51	0.39	0.39	0.39	0.45	0.60
Dicalcium phosphate	1.68	1.90	1.90	1.90	1.90	2.00
Chromic oxide	0.20	0.20	0.20	0.20	0.20	0.20
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix*	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix†	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00

*Provided per kg of complete diet: 11 025 IU vitamin A, 1103 IU vitamin D_3, 44 IU vitamin E, 4.4 mg vitamin K, 8.3 mg riboflavin, 50 mg niacin, 4 mg thiamine, 29 mg D-pantothenic, 166 mg choline, 33 μg vitamin B_{12}

†Provided per kg of complete diet: 12 mg Cu (as $CuSO_4 \cdot 5H_2O$), 85 mg Zn (as $ZnSO_4$), 8 mg Mn (as MnO_2), 0.28 mg I (as KI), 0.15 mg Se (as $Na_2SeO_3 \cdot 5H_2O$)

‡Fibre source

ETSM. The conventional SBM was a commercial source of de-hulled SBM. The fermented soybean proteins evaluated in this study were composed of three types of FSM (FSMA, FSMB and FSMC) fermented by *Bacillus subtilis* with 50%, 60% and 70% solubility in potassium hydroxide (CJ Global Food and Bio Company, Seoul, Korea). Briefly, dried soybean meals were soaked in distilled water for 60 min to maintain a 35% moisture concentration. Hydrated soybean meals were then cooked in a steam tank at 60–70 °C for 1 h, after which they were cooled to room temperature for 1 h, inoculated with *Bacillus subtilis*, mixed, and fermented in a bed-packed incubator for 48 h at 37 °C. Finally, the fermented soybean meals were dried at 50–60 °C to a moisture concentration of approximately 10% and then ground in a hammer mill. Potassium hydroxide (KOH) solubility was evaluated according to the method described by (Araba and Dale 1990). ETSM ground soya protein product was provided by the Alltech Co. Korea. ETSM is produced by treating de-hulled SBM with a proprietary mixture of enzymes, which results in a SBM with a reduced concentration of oligosaccharides and anti-nutritional factors compared with conventional SBM (Jiang et al. 2006). Because of the post-treatment heating of ETSM, it is believed that all residual enzymes are inactivated and that no enzymes are active in the finished product.

Experimental and laboratory analysis. The body weight was determined at the start of experimental period. Each experimental period consisted of four days of diet adaptation followed by one day of collection of faeces and one day of collection of ileal digesta, respectively. Faeces were collected from 08:00 h on Day 5 to 08:00 h on Day 6. Ileal digesta were collected for a total of 24 h, from 08:00 h on Day 7 to 08:00 h on Day 8. The pigs were trained to consume their ration in 15 min or less; the amount of feed offered was limited to the feed intake of the pig that consumed the least to ensure that all pigs had the same daily consumption. The AID for dry matter, nitrogen, and energy were determined using Cr_2O_3 as an indigestible marker. Pigs were fed diets containing Cr_2O_3 for four days prior to the collection day, and fresh fae-

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cal samples were obtained once daily from the three piglets on the 5th and 6th days of the experiment. Ileal digesta were collected in plastic bags tied to the barrel of the cannula. The bags were removed and replaced as soon as they were filled with digesta; no bag remained attached to the cannula longer than 20 min. All the faecal samples, as well as ileal digesta were stored in a freezer at −20 °C pending analysis. Before chemical analysis, faecal samples and ileal digesta were thawed and dried at 70 °C for 72 h and subsequently ground to pass through a 1 mm screen. Samples were analysed for nutrients according to procedures from AOAC (2007). All protein sources were analysed for dry matter (930.15), crude protein (990.03), ether extract (920.39), crude fibre (978.10), calcium (Ca, 968.08), and phosphorous (P, 964.06). Amino acids (excluding tryptophan) were analysed using dansylation (Beckman Instruments Inc., Fullerton, CA) and HPLC after acid hydrolysis for 24 h in 6M HCl. All diets, faecal and ileal digesta samples were also analysed for dry matter, crude protein, and amino acids as described for the ingredients. Chromium levels were determined using UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan). Nitrogen was determined using a Kjectec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was analysed using an oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, USA). Values for AID and SID of nutrients and amino acids were calculated according to the method described by Stein et al. (2007).

Statistical analysis. Data were analysed using the Proc Mixed procedure (SAS Inst. Inc., Cary, USA). Homogeneity of the variances among treatments was confirmed using the Univariate procedure of SAS (2008). An ANOVA was conducted with diet as the fixed effect and pig and period as random effects. There were, however, no effects of pig or period. The pig was the experimental unit for all

Table 2. Analysed nutrient composition (as-fed basis) of experimental diets containing soybean meal (SBM), fermented soybean meal A (FSMA), fermented soybean meal B (FSMB), fermented soybean meal C (FSMC), and enzyme-treated soybean meal (ETSM)

Item (%)	Diet					
	SBM	FSMA	FSMB	FSMC	ETSM	nitrogen-free
Dry matter	91.25	92.61	92.65	92.67	92.05	91.89
Crude protein	12.21	13.05	13.12	13.13	11.68	0.34
Essential amino acids						
Arginine	0.85	1.04	1.09	1.08	0.80	–
Histidine	0.35	0.42	0.45	0.51	0.30	–
Isoleucine	0.58	0.78	0.78	0.77	0.55	–
Leucine	0.95	1.25	1.26	1.28	0.89	0.01
Lysine	0.80	0.95	0.95	0.96	0.70	–
Methionine	0.15	0.25	0.24	0.21	0.18	–
Phenylalanine	0.65	0.81	0.81	0.82	0.60	0.01
Threonine	0.45	0.60	0.60	0.62	0.41	–
Valine	0.63	0.85	0.85	0.81	0.60	0.01
Non-essential amino acids						
Alanine	0.54	0.73	0.74	0.73	0.52	0.01
Aspartic acid	1.35	1.76	1.76	1.78	1.28	0.01
Cysteine	0.21	0.25	0.25	0.26	0.18	–
Glutamic acid	2.21	2.84	2.86	2.89	2.08	0.01
Glycine	0.52	0.71	0.72	0.72	0.47	–
Proline	0.57	0.75	0.76	0.75	0.55	0.01
Serine	0.52	0.68	0.69	0.69	0.67	–
Tyrosine	0.32	0.47	0.49	0.50	0.31	0.01
Total amino acids	11.65	15.12	15.30	15.38	11.09	0.08

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Table 3. Analysed nutrient composition (as-fed basis) of soybean meal (SBM), fermented soybean meal A (FSMA), fermented soybean meal B (FSMB), fermented soybean meal C (FSMC), and enzyme-treated soybean meal (ETSM)

Item (%)	Diet				
	SBM	FSMA	FSMB	FSMC	ETSM
Dry matter	90.33	91.32	91.33	91.32	91.48
Crude protein	45.08	53.75	53.74	53.75	54.42
Ether extract	1.08	0.82	0.81	0.83	1.12
Crude fiber	2.78	3.35	3.36	3.34	3.81
Ca	0.26	0.28	0.29	0.28	0.36
P	0.67	0.82	0.85	0.86	0.75
Essential amino acids					
Arginine	3.05	3.53	3.52	3.56	3.76
Histidine	1.13	1.28	1.28	1.27	1.36
Isoleucine	1.90	2.45	2.46	2.47	2.32
Leucine	3.35	4.08	4.09	4.08	3.56
Lysine	2.76	3.20	3.29	3.28	3.05
Methionine	0.63	0.75	0.71	0.72	0.72
Phenylalanine	2.24	2.72	2.72	2.73	2.75
Threonine	1.72	1.99	1.98	1.99	2.01
Valine	1.95	2.68	2.68	2.68	2.39
Non-essential amino acids					
Alanine	1.86	2.30	2.31	2.32	2.26
Aspartic acid	4.81	5.56	5.58	5.59	5.72
Cysteine	0.68	0.78	0.78	0.74	0.75
Glutamic acid	7.48	8.56	8.59	8.60	8.76
Glycine	1.77	2.24	2.30	2.30	2.25
Proline	2.07	2.46	2.40	2.40	2.46
Serine	1.98	2.25	2.27	2.28	2.35
Tyrosine	1.65	1.97	1.97	1.97	2.03
Total amino acids	41.03	48.80	48.93	48.98	48.50

analyses. Duncan's multiple range test (Duncan 1955) was used to determine significant differences among treatments. Variability in the data was expressed as SEM and a $P < 0.05$ was considered statistically significant.

RESULTS

Nutrient composition of ingredients

The analysed nutrient composition of the experimental diets and test ingredients is shown in Tables 2 and 3. The concentration of nutrients and amino acids in fermented soybean meal (FSM; i.e. FSMA, FSMB and FSMC) was similar to the nutrients in ETSM. However, SMB contained lower concentrations of dry matter, crude fibre, Ca and P than FSM and ETSM. SBM contained the least amount of crude protein and amino acids among all the tested ingredients. The total amino acids in SBM were lower than FSM and ETSM. However, SBM contained higher ether extract compared with FSM.

ATTD

The ATTD is presented in Table 4. The ATTD for dry matter in FSMC and ETSM diets was greater ($P < 0.05$) than in pigs fed SBM and FSMA diets but not different from the values obtained for the FSMB diet. The ATTD of nitrogen in the FSMC diet was the same in pigs fed ETSM and FSMB diets but was greater than ($P < 0.05$) the ATTD of

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Table 4. Apparent total tract digestibility of dry matter, nitrogen, energy and amino acids in soybean meal (SBM), fermented soybean meal A (FSMA), fermented soybean meal B (FSMB), fermented soybean meal C (FSMC), and enzyme-treated soybean meal (ETSM) by weanling pigs*

Item (%)	Diet					SEM	*P*-value
	SBM	FSMA	FSMB	FSMC	ETSM		
Dry matter	85.84[bc]	84.24[c]	87.32[ab]	89.02[a]	88.86[a]	1.90	0.027
Nitrogen	83.43[b]	80.02[c]	87.86[a]	89.28[a]	88.84[a]	2.03	0.035
Energy	84.42[b]	86.83[ab]	87.52[ab]	88.19[a]	87.69[a]	2.02	0.041
Essential amino acids							
Arginine	86.91[b]	86.39[b]	92.93[a]	94.51[a]	93.15[a]	3.14	0.029
Histidine	88.31[c]	87.37[c]	90.81[b]	93.73[a]	91.65[ab]	4.85	0.019
Isoleucine	86.73[b]	84.85[c]	91.92[a]	92.73[a]	92.94[a]	3.63	0.029
Leucine	86.57[b]	85.50[b]	90.29[a]	88.20[ab]	88.94[ab]	1.10	0.009
Lysine	87.40[b]	85.16[c]	89.43[a]	89.22[a]	89.04[a]	2.51	0.005
Methionine	90.18[b]	88.98[b]	92.63[a]	92.71[a]	92.99[a]	2.68	0.032
Phenylalanine	86.70[b]	86.29[b]	89.01[a]	89.88[a]	88.12[ab]	0.65	< 0.001
Threonine	83.32[bc]	82.19[c]	85.21[ab]	87.31[a]	87.56[a]	2.89	0.042
Valine	91.76[ab]	89.43[b]	92.45[ab]	95.80[a]	93.01[ab]	1.73	0.021
Total	87.54[b]	86.24[c]	90.52[a]	91.56[a]	90.82[a]	2.40	0.008
Non-essential amino acids							
Alanine	87.07[bc]	85.85[c]	86.93[bc]	89.73[ab]	90.52[a]	1.04	0.027
Aspartic acid	82.99[bc]	81.94[c]	84.03[ab]	85.59[a]	84.40[ab]	0.62	< 0.001
Cysteine	72.83[b]	79.42[ab]	82.81[ab]	85.89[a]	85.31[a]	3.61	0.032
Glutamic acid	88.13[bc]	86.38[c]	89.98[ab]	90.66[a]	90.78[a]	0.72	< 0.001
Glycine	87.03	85.68	88.00	89.26	88.18	1.29	0.089
Proline	85.54[b]	85.76[b]	88.50[a]	88.59[a]	87.70[a]	0.59	0.006
Serine	85.10	84.71	86.63	87.84	86.70	1.02	0.097
Tyrosine	85.80[c]	87.52[bc]	88.74[ab]	90.35[a]	89.36[ab]	0.87	< 0.001
Total	84.31[b]	84.66[b]	86.95[a]	88.49[a]	87.87[a]	3.66	0.045
Total amino acids	85.93[c]	85.45[c]	88.74[b]	90.03[a]	89.35[ab]	3.39	0.039

[a–c]Means in the same row with different superscripts differ ($P < 0.05$)

*Data are least square means of six observations

nitrogen in SBM and FSMA diets. The ATTD of energy in the SBM diet was not different from that in FSMA and in FSMB diets, but this value was less than the ATTD of FSMC and ETSM diets. With regards to the ATTD of essential amino acids, the ATTD of arginine, isoleucine, lysine, methionine and total essential amino acids was greater in pigs fed FSMB, FSMC, and ETSM diets than in those fed SBM and FSMA diets. With the exception of ATTD of histidine in FSMC, the ATTD of leucine, phenylalanine, threonine and valine in the FSMC diet was not different from the values of ATTD in FSMB and ETSM diets. The ATTD of all essential amino acids in the FSMC diet was not different from the ATTD for amino acids in FSMB and ETSM diets except for the ATTD of histidine which was less in the FSMB diet. With respect to non-essential amino acids the ATTD of aspartic acid, cysteine, glutamic acid, proline, tyrosine and total amino acids was greater in pigs fed the FSMC diet compared with the SBM diet and not different from the values of pigs fed the ETSM diet.

AID

The AID is shown in Table 5. The AID of dry matter, nitrogen and energy in pigs fed the FSMC diet was greater ($P < 0.05$) than in pigs fed SBM and FSMA diets but was the same as the values of pigs fed FSMB and ETSM diets. With regards to the

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Table 5. Apparent ileal digestibility of dry matter, nitrogen, energy and amino acids in soybean meal (SBM), fermented soybean meal A (FSMA), fermented soybean meal B (FSMB), fermented soybean meal C (FSMC), and enzyme-treated soybean meal (ETSM) by weanling pigs*

Item (%)	Diet					SEM	*P*-value
	SBM	FSMA	FSMB	FSMC	ETSM		
Apparent ileal digestibility							
Dry matter	78.45[b]	76.53[b]	82.40[a]	82.71[a]	82.59[a]	2.12	0.035
Nitrogen	79.33[b]	76.09[c]	82.54[a]	83.24[a]	82.95[a]	2.11	0.027
Energy	76.77[b]	75.10[b]	81.26[a]	82.27[a]	81.68[a]	2.20	0.005
Essential amino acids							
Arginine	80.04[b]	79.38[b]	83.44[ab]	84.41[a]	81.02[ab]	1.31	0.047
Histidine	78.39[b]	77.29[b]	80.74[a]	80.63[a]	80.69[a]	0.52	< 0.001
Isoleucine	80.62[bc]	79.59[c]	83.73[a]	82.76[ab]	83.26[a]	1.76	0.002
Leucine	80.51[ab]	78.43[b]	81.42[a]	81.48[a]	80.53[ab]	0.77	0.049
Lysine	76.29[b]	74.73[c]	78.15[a]	78.52[a]	77.92[a]	1.49	< 0.001
Methionine	82.50[ab]	80.39[b]	84.54[a]	84.83[a]	84.64[a]	0.77	0.001
Phenylalanine	80.83[b]	79.61[b]	82.33[ab]	84.01[a]	83.86[a]	0.91	0.007
Threonine	74.14[b]	73.24[b]	76.54[a]	76.94[a]	77.17[a]	1.54	< 0.001
Valine	80.53[bc]	78.48[c]	83.18[ab]	84.80[a]	84.60[a]	1.18	0.002
Total	79.32[b]	77.90[c]	81.56[a]	82.04[a]	81.52[a]	1.21	< 0.001
Non-essential amino acids							
Alanine	73.78[b]	74.44[ab]	75.84[ab]	76.66[a]	76.71[a]	0.73	0.026
Aspartic acid	77.21[b]	76.54[b]	78.91[a]	79.36[a]	79.69[a]	1.57	0.001
Cysteine	74.75[b]	76.72[b]	83.48[a]	83.88[a]	78.20[ab]	1.90	0.005
Glutamic acid	78.61[b]	77.36[b]	80.90[a]	81.15[a]	81.70[a]	0.62	< 0.001
Glycine	69.78[b]	71.30[b]	73.64[a]	74.24[a]	73.60[a]	0.56	< 0.001
Proline	79.61[ab]	79.25[b]	80.19[a]	80.37[a]	80.42[a]	1.31	0.045
Serine	80.62[ab]	79.31[b]	81.76[a]	82.51[a]	82.02[a]	0.73	0.030
Tyrosine	82.44[b]	82.27[b]	87.17[a]	88.71[a]	87.95[a]	0.81	< 0.001
Total	77.10[b]	77.15[b]	80.24[a]	80.86[a]	80.04[a]	1.40	< 0.001
Total amino acids	77.83[b]	75.53[c]	80.90[a]	81.45[a]	80.78[a]	1.23	< 0.001

[a–c]Means in the same row with different superscripts differ ($P < 0.05$)

*Data are least square means of six observations

AID of essential amino acids, the AID of histidine, lysine and threonine was greater in pigs fed FSMB, FSMC, and ETSM diets than those fed SBM and FSMA diets ($P < 0.05$). The AID of isoleucine was greater in pigs fed FSMB and ETSM diets than in pigs fed SBM and FSMA diets. The AID of arginine, phenylalanine and valine in pigs fed the FSMC diet was greater than in pigs fed SBM and FSMA diets. The AID of lysine and total amino acids in FSMA pigs was lower ($P < 0.05$) compared with values from pigs fed FSMC and ETSM diets. With respect to non-essential amino acids the AID of aspartic acid, glutamic acid, glycine, tyrosine and total amino acids was the same in pigs fed FSMB, FSMC, ETSM diets and greater ($P < 0.05$) than in pigs fed SBM and FSMA diets.

SID

The SID of crude protein in pigs fed FSMB, FSMC, and ETSM diets was greater ($P < 0.05$) than in pigs fed SBM and FSMA diets (Table 6). The SID of all essential amino acids in pigs fed the FSMC diet was greater than in pigs fed the FSMA diet and was the same as in pigs fed the FSMB and

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Table 6. Standardised ileal digestibility (SID) of crude protein and amino acids in soybean meal (SBM), fermented soybean meal A (FSMA), fermented soybean meal B (FSMB), fermented soybean meal C (FSMC), and enzyme-treated soybean meal (ETSM) by weanling pigs*

Item (%)	Diet					SEM	*P*-value
	SBM	FSMA	FSMB	FSMC	ETSM		
Crude protein	84.32[b]	81.13[c]	88.11[a]	89.25[a]	88.87[a]	0.87	<.0001
Essential amino acids							
Arginine	85.00[ab]	83.39[b]	87.19[ab]	87.84[a]	84.06[ab]	1.30	0.041
Histidine	84.19[a]	82.28[b]	85.61[a]	85.12[a]	84.52[a]	0.57	0.003
Isoleucine	84.23[bc]	83.63[c]	86.75[a]	86.78[a]	86.31[ab]	1.74	0.010
Leucine	83.45[ab]	81.23[b]	84.17[a]	84.27[a]	83.87[a]	0.78	0.040
Lysine	80.53[b]	78.65[c]	82.21[a]	82.49[a]	82.04[a]	1.42	< 0.001
Methionine	87.02[ab]	85.40[b]	88.65[a]	89.18[a]	88.75[a]	0.74	0.007
Phenylalanine	84.37[bc]	82.88[c]	86.11[ab]	86.91[a]	86.55[ab]	1.78	0.004
Threonine	80.83[ab]	79.08[b]	82.62[a]	83.08[a]	83.05[a]	0.97	0.026
Valine	85.76[ab]	84.55[b]	88.83[a]	89.10[a]	89.26[a]	1.24	0.033
Total	83.93[b]	82.34[c]	85.79[a]	86.09[a]	85.38[a]	0.27	< 0.001
Non-essential amino acids							
Alanine	81.87[b]	83.78[ab]	85.12[a]	85.51[a]	85.68[a]	0.75	0.006
Aspartic acid	81.17[b]	80.99[b]	82.82[a]	83.16[a]	83.52[a]	1.48	0.001
Cysteine	83.08[b]	84.01[b]	89.74[a]	90.20[a]	85.10[b]	1.60	0.008
Glutamic acid	83.00[b]	82.50[b]	85.14[a]	85.28[a]	85.92[a]	0.71	0.006
Glycine	83.76[b]	84.27[b]	87.91[a]	87.84[a]	87.36[a]	0.67	< 0.001
Proline	85.77[ab]	84.76[b]	86.56[a]	86.73[a]	87.29[a]	1.49	0.011
Serine	86.74[ab]	84.91[b]	87.50[a]	88.15[a]	87.87[a]	0.69	0.020
Tyrosine	87.49[b]	86.33[b]	91.45[a]	92.79[a]	92.32[a]	1.74	< 0.001
Total	84.11[b]	83.94[b]	87.03[a]	87.46[a]	86.88[a]	2.41	< 0.001
Total amino acids	83.15[b]	80.03[c]	86.41[a]	86.77[a]	86.13[a]	1.60	< 0.001

[a–c]Means in the same row with different superscripts differ ($P < 0.05$)

*Data are least square means of six observations

ETSM diets. With respect to non-essential amino acids, the SID of all amino acids except for alanine, proline and serine was greatest in pigs fed FSMB, FSMC and ETSM treatments.

DISCUSSION

In the current study, FSM and ETSM diets had higher dry matter, crude protein, crude fibre, Ca and P than the SBM diet. Essential amino acids, crude protein, ether extract, crude fibre, Ca and P of soybean meal were similar to our previous study (Wang et al. 2011; Upadhaya and Kim 2015). The SBM used in our experiment was obtained from a commercial source, and the chemical characteristics of SBM may vary among sources (Grieshop et al. 2003). The amino acids composition of FSM was similar to that reported by Yun et al. (2005). Fermented soybean meal contained more dry matter, crude protein, Ca, and P than SBM, which is consistent with observations from other experiments (Hong et al. 2004; Feng et al. 2007; Wang et al. 2014). However, we observed that the concentration of crude fibre was greater and the concentration of ether extract was less in FSM than in SBM. Our results are inconsistent with previous data (Zamora and Veum 1988; Feng et al. 2007; Yoo et al. 2009) which may be due to differing particle sizes and KOH solubility (Parsons et al. 1991). The crude protein and amino acids composition of ETSM are similar to the values reported by Zhu et al. (1998) and Cervantes-Pahm and Stein (2010).The concentration of lysine calculated as a percentage of crude

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protein was less in FSM and ETSM than in SBM. A possible reason for this observation may be that these meals were heat-damaged during drying; heat damage may result in Maillard reactions that will destroy some of the lysine in the products (Stein et al. 2009).

In our study, the ATTD of dry matter and nitrogen was significantly higher in FSMC and ETSM diets than in the SBM diet, which was similar to that reported by Min et al. (2009). Zhu et al. (1998) reported that the net availability and biological value of protein was improved when enzyme-treated soy protein was added to the pigs' diet. In addition, Min et al. (2004) suggested that diets that contained fermented soy protein or enzyme-treated soy protein during Phase 2 in the weaned pigs increased dry matter and nitrogen digestibility. These consistent results can be attributed to the improved quality of treated soybean meal and may be due to the reduced amount of trypsin inhibitors following the fermentation and enzymatic processing of soybean meal. This, in turn, might be correlated with improved nitrogen digestibility (Min et al. 2009; Zhang et al. 2013). The ATTD of essential and non-essential amino acids of FSMB and FSMC diets was similar to that in the ETSM diet. The ATTD of essential amino acids, except for valine in the ETSM diet, was superior compared to the SBM diet. The findings in the present study confirmed that FSMC and ETSM diets could significantly improve essential amino acid digestibility leading to a higher average daily growth than in basal group that we and others have reported previously (Zhu et al. 1998; Yun et al. 2005; Yoo et al. 2009; Upadhaya and Kim 2015). Therefore, we conclude that soybean meal fermented by *Bacillus subtilis* with 60 and 70% KOH solubility together with ETSM in the weanling pigs diet may improve the ATTD of dry matter, nitrogen, energy and most essential and non-essential amino acids.

Fermented foods are commonly consumed by humans in Southeast Asian countries but food fermentation and enzyme treatment remains limited with regard to its application in the animal production sector. Fermentation or enzymatic treatment of SBM to remove anti-nutritional factors and to make SBM more tolerable to young pigs creates opportunities for the increased use of SBM in weanling diets. Removal of oligosaccharides in fermented soybean (FSMA, FSMB, FSMC) and the low concentrations of oligosaccharides in ETSM may be an advantage because the diarrhoea associated with feeding SBM to weanling pigs may be caused by the oligosaccharides present in these diets (Liener 2000; Liying et al. 2003). Thus, the absence of oligosaccharides and low concentration of trypsin inhibitors in FSMA, FSMB and FSMC (Cervantes-Pahm and Stein 2010), were expected to contribute to an increased digestibility of amino acids in fermented soybean meal when fed to weanling pigs. Soy beans that were fermented showed increased digestibility and the absorbability was much higher than in cooked and autoclaved beans (Kiers et al. 2000). Hong et al. (2004) showed that FSMA, FSMB and FSMC contained greater amounts of small peptides than SBM and enzyme-treated soybean meal (ETSM).

The rate and extent of absorption of small peptides is greater in fermented soybean meal (FSMA, FSMB, FSMC) than for free amino acids (Gilbert et al. 2008), and the increased concentration of small peptides is expected to improve digestibility. Fermentation or enzymatic treatment of SBM leads to high concentrations of the antigenic proteins glycinin and β-conglycinin, which may reduce villus height in the small intestine and decrease nitrogen digestibility in young pigs fed SBM (Li et al. 1991). Purified glycinin and β-conglycinin also reduce average daily gain and growth efficiency in young pigs, but because β-conglycinin is less digestible than glycinin, the reduction in pig performance is greater in pigs fed β-conglycinin than glycinin (Zhao et al. 2008). It has been noted that the use of *Bacillus subtilis*-fermented soybeans was likely to increase feed utilisation because of the extensive protein hydrolysis, which produces readily available free amino acids and peptides (Steinkraus 1996; Sarkar et al. 1997; Upadhaya and Kim 2015). In our experiments, we observed that the SID of crude protein essential and non-essential amino acids was improved in FSMB, FSMC and ETSM diets compared to the SBM diet. Smiricky et al. (2002) and Swiech et al. (2004) reported that the presence of trypsin inhibitors and oligosaccharides in soybean reduces amino acids digestibility. Previously, Yang et al. (2007) reported that, with the exception of arginine, isoleucine, lysine, glycine, and proline, there are no differences in the SID of amino acids between fermented soybean meal (FSMA, FSMB, FSMC) and SBM. It has, however, also been reported that the AID of most amino acids in fermented soybean meal (FSMA, FSMB, FSMC) and in ETSM is greater than in SBM (Yun et al. 2005). The reason

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for the inconsistent results may lie in the different doses, bacteria, KOH solubility and enzymes used in each experiment. The SID of amino acids in FSMB, FSMC and ETSM was greatest among all soybean meals for all essential amino acids. The values for the AID of amino acids in FSMB, FSMC and ETSM diets that were measured in this experiment were also greater than for conventional SBM. We observed that the overall SID of crude protein of SBM, FSMA, ETSM and FSMC was 84.32, 81.13, 88.87 and 89.25%, respectively, which was similar to the study conducted by Cervantes-Pahm and Stein (2010). High protein digestibility ensures that the protein's content of essential amino acids is readily bioavailable (Urbaityte et al. 2009). Of equal importance is the fact that the volume of undigested protein transported to the colon is low, minimising opportunities for unfavourable gut microbes to develop (Upadhaya and Kim 2015). Thus, we can conclude that the AID and SID of amino acids may improve through supplementation of *Bacillus subtilis*-fermented soybean meal and enzyme-treated soybean meal in the diets of weanling pigs.

Results from the present experiments demonstrate that the AID, SID and ATTD of most amino acids in FSMB, FSMC and ETSM diets are similar to each other and greater than in the SBM diet. The overall protein digestibility of FSMB, FSMC and ETSM diets ensures the protein content of essential amino acids is readily bioavailable in weanling pigs. The ATTD of dry matter and nitrogen was higher in FSMB, FSMC and ETSM diets and the AID of dry matter, nitrogen and energy was higher in FSMB, FSMC and ETSM diets than in other diets.

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