

# Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs<sup>1</sup>

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**ABSTRACT:** An experiment was conducted to determine the apparent (AID) and standardized (SID) ileal digestibility of CP and AA in weanling pigs of 4 soybean products, fish meal, and casein. The 4 soybean products were conventional dehulled soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), and enzyme-treated soybean meal (ESBM). Seven weanling barrows (initial BW: 10.9 ± 2.3 kg) were individually fitted with a T-cannula in the distal ileum. The barrows were allotted to a 7 × 7 Latin square design with 7 diets and seven 7-d periods. Six cornstarch-based diets were prepared using each of the protein sources as the sole source of CP and AA. An N-free diet was used to measure basal endogenous losses

of CP and AA. Results showed that except for Lys, the AID and SID of AA in FSBM was not different from SBM, and with a few exceptions, the AID and SID of most AA in SBM, FSBM, and ESBM were not different from each other and from the AID and SID of AA in fish meal. Likewise, the AID and SID of AA in ESBM and SPI were not different, but the AID and SID of most AA in SPI were greater ( $P < 0.05$ ) than in SBM and FSBM. The AID and SID of most AA in SPI were not different from the AID and SID of AA in casein. In conclusion, FSBM and ESBM had similar SID of most AA as SBM, but SPI has the greatest SID of AA among the 4 soybean products. Casein had the greatest SID of AA among the protein sources studied.

**Key words:** amino acid, digestibility, enzyme-treated soybean meal, fermented soybean meal, pig, soy protein isolate

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## INTRODUCTION

Proper heat treatment of raw soybeans is necessary to inactivate antinutritional factors such as trypsin inhibitors (TI) and lectins to make them suitable for consumption (Baker, 2000). Soybean meal (SBM) usually has low concentrations of TI (Lallès, 2000) because it has undergone heating during the process of fat extraction (Baker, 2000). However, when SBM is fed to young pigs, digestive disturbances are sometimes observed. The presence of indigestible carbohydrate complexes (Li et al., 1990), antigenic soy proteins (Li et al., 1990), and residual TI (Lallès, 2000) may contribute to this problem. Therefore, fish meal or milk proteins are usually used as protein sources in pig starter diets, and only limited quantities of SBM are included in these

diets. Soy protein isolate (SPI) consists of defatted soy flakes that are processed to remove the heat-stable oligosaccharides and antigens (Zhu et al., 1998; Cromwell, 2000), and SPI is, therefore, better tolerated by young pigs than regular SBM (Li et al., 1991; Zhu et al., 1998).

Recently, fermented soybean meal (FSBM) and enzyme-treated soybean meal (ESBM) were introduced to the US feed market. Both of these soy products are believed to have a decreased concentration of TI and other antinutritional factors and a greater concentration of CP and AA than conventional SBM. There is, however, limited information about the digestibility of AA in FSBM and ESBM, and it is not known how the values for the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of AA in FSBM and ESBM compare with the AID and SID values of AA in animal proteins and other soy products. The objective of this experiment, therefore, was to test the hypothesis that the AID and SID of AA in FSBM and ESBM is greater than in conventional SBM and similar to the AID and SID of AA in SPI, fish meal, and casein.

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## MATERIALS AND METHODS

The animal part of the experiment was conducted at South Dakota State University, Brookings, and the protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University.

### *Animals, Housing, and Experimental Design*

Seven weanling barrows that were the offspring of SP-1 boars mated to Line 13 sows (Ausgene Intl. Inc., Gridley, IL) were individually equipped with a T-cannula in the distal ileum using the method described by Stein et al. (1998). After surgery, pigs were transferred to individual pens (1.2 × 1.8 m) in a temperature-controlled room (22°C) where they were allowed to recover for 10 d. A standard corn-SBM nursery diet (20% CP) was provided on an ad libitum basis during this time. Pigs were then allotted to a 7 × 7 Latin square design with pigs and 7-d periods comprising the rows and columns, respectively. Pigs had an average BW of 10.9 ± 2.3 kg at the start of period 1 and an average BW of 22.2 ± 6.3 kg at the start of period 7.

### *Ingredients, Diets, and Feeding*

The test ingredients consisted of SBM, SPI, FSBM, ESBM, fish meal, and casein (Tables 1 and 2). The conventional SBM was a commercial source of dehulled SBM (South Dakota Soybean Processors, Volga). The SPI (Ardex AF, Archer Daniels Midland, Decatur, IL) is the proteinaceous fraction of SBM (Baker, 2000) that is produced by solubilizing the protein at neutral or slightly alkaline pH and precipitating the extract by acidification to obtain the protein isolate (Berk, 1992). The FSBM (Pepsoygen, Genbiotech Co. Ltd., Seoul, South Korea) is produced by fermentation of SBM with *Aspergillus oryzae* GB-107 (Hong et al., 2004). The FSBM contains reduced amounts of TI and the partial digestion of large peptides by proteases secreted by *A. oryzae* during fermentation may reduce the number of large peptides (>60 kDa) and increase the concentrations of small peptides (<20 kDa) in the product (Hong et al., 2004). The ESBM (HP-300, Hamlet Protein, Horsens, Denmark) is produced by treating dehulled SBM with a proprietary mixture of enzymes, which results in a SBM with a reduced concentration of oligosaccharides and antinutritional factors compared with conventional SBM (Jiang et al., 2006). Because of posttreatment heating of ESBM, it is believed that all residual enzymes are inactivated and that no enzymes are active in the finished product. The fish meal used was prepared mainly from menhaden (Menhaden Select, Omega Protein, Houston, TX). The fish meal is produced when menhaden fish are steamed, strained, and pressed to remove oil and other liquids, and the remaining cake is then dried to produce the fish meal

(FAO, 1986). The casein that was used (Acid Casein, Erie Foods Intl., Erie, IL) is obtained by acid coagulation of defatted milk (Kellems and Church, 2002). Casein usually has a superior nutrient digestibility compared with soy proteins when fed to young pigs (Veum and Odle, 2001).

Six diets were formulated to contain each protein ingredient as the only protein and AA source (Tables 3 and 4). An N-free diet was also formulated to determine the basal endogenous losses of CP and AA (Stein et al., 2007). Chromic oxide (0.40%) was included in all diets as an inert marker. The 6 protein-containing diets were formulated to contain 12% CP to avoid including excessive amounts of SBM in the diets. Inclusion of increased amounts of SBM in diets fed to weanling pigs may cause allergic reactions because of antigens in the SBM (Li et al. 1990), which potentially could have influenced the results for the diet containing the conventional SBM. Although reduced concentrations of CP in experimental diets may reduce calculated values for AID of AA (Fan et al., 1994), dietary CP concentration does not influence values for SID of AA (Stein et al., 2007).

Pigs were allowed ad libitum access to water throughout the experiment. Although growing pigs used in digestibility experiments often are fed only 2 daily meals that provide 3 times the energy requirement for maintenance (Cervantes-Pahm and Stein, 2008; Baker and Stein, 2009), weanling pigs may not be able to consume sufficient feed in 2 daily meals. If the daily feed intake is reduced below 3 times the energy requirement for maintenance, values for SID of AA are increased (Moter and Stein, 2004). Pigs used in the present experiment were, therefore, allowed ad libitum access to their diets because digestibility values for AA obtained in pigs allowed ad libitum access to feed is similar to values obtained in pigs fed 3 times the energy requirement for maintenance (Chastanet et al., 2007).

### *Data and Sample Collection*

The BW of each pig was recorded at the beginning of the experiment and at the end of each period. Pigs were allowed to adapt to their diet during the initial 5 d of each period. On d 6 and 7, ileal digesta were collected for 8 h. A 225-mL plastic bag was attached to the cannula barrel using a cable tie, and digesta flowing into the bag were collected. Bags were removed every 30 min and replaced with a new one. Digesta were immediately stored at -20°C to prevent bacterial degradation of the AA in the digesta. At the completion of each experimental period, all the remaining feed in the feeders was removed and pigs were given access to a new diet.

Ileal samples obtained over the 2-d collection period were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. A sample of each diet and of each of the protein ingredients was

**Table 1.** Analyzed nutrient composition of soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), enzyme-treated soybean meal (ESBM), fish meal, and casein, as-fed basis

Item	Ingredient					
	SBM	SPI	FSBM	ESBM	Fish meal	Casein
DM, %	89.32	94.10	91.33	91.48	91.71	91.12
CP, %	45.07	77.89	53.74	54.40	63.52	87.33
Ether extract, %	1.07	1.24	0.80	1.13	8.73	0.09
Crude fiber, %	2.78	0.28	3.31	3.75	0.07	0.00
Ca, %	0.26	0.17	0.29	0.35	5.21	0.00
P, %	0.67	0.72	0.82	0.74	2.88	0.69
Indispensable AA, %						
Arg	3.06	5.55	3.50	3.75	3.56	3.24
His	1.13	1.96	1.30	1.35	1.39	2.64
Ile	1.89	3.64	2.48	2.31	2.33	4.61
Leu	3.37	5.94	4.09	3.98	4.10	8.30
Lys	2.77	4.78	3.11	3.06	4.50	7.02
Met	0.63	1.03	0.76	0.71	1.59	2.54
Phe	2.23	3.91	2.71	2.74	2.41	4.61
Thr	1.71	2.76	1.98	2.02	2.32	3.51
Trp	0.62	1.03	0.67	0.69	0.54	1.07
Val	1.96	3.84	2.69	2.40	2.83	5.98
Dispensable AA, %						
Ala	1.86	3.13	2.29	2.25	3.70	2.58
Asp	4.80	8.49	5.67	5.71	5.12	6.01
Cys	0.67	0.97	0.77	0.76	0.50	0.32
Glu	7.48	13.55	8.56	8.75	7.22	18.33
Gly	1.77	3.10	2.23	2.26	4.50	1.58
Pro	2.08	3.70	2.45	2.46	2.75	9.15
Ser	1.97	3.23	2.24	2.35	1.99	4.03
Tyr	1.67	2.81	1.97	2.03	1.88	5.11
Total AA	41.67	73.42	49.47	49.58	53.23	90.63

collected as well. Digesta samples were lyophilized and finely ground before chemical analysis.

### Chemical Analysis

Samples were analyzed for nutrients according to procedures from AOAC International (2007). All pro-

tein sources were analyzed for DM (method 930.15), CP (method 990.03), ether extract (method 920.39), crude fiber (method 978.10), Ca (method 968.08), and P (method 964.06). Amino acids were analyzed in all samples on an AA analyzer (model No. L8800, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine

**Table 2.** Biochemical characteristics of soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), and enzyme-treated soybean meal (ESBM)<sup>1</sup>

Item	Ingredient			
	SBM	SPI	FSBM	ESBM
Peptide size distribution, g/100 g of CP				
≥60 kDa	27.00	23.59	29.21	28.87
20 to 60 kDa	43.16	41.55	42.68	37.51
≤20 kDa	29.84	34.86	28.11	33.62
Carbohydrate, g/100 g				
Glucose	ND	ND	0.36	0.49
Sucrose	7.81	ND	ND	ND
Maltose	ND	ND	ND	ND
Fructose	0.63	ND	0.70	1.11
Stachyose	5.17	0.14	ND	0.71
Raffinose	1.08	0.05	ND	0.16
Antigenic protein				
Glycinin, mg/kg	$2.3 \times 10^4$	46.0	$2.6 \times 10^4$	$5.3 \times 10^3$
β-Conglycinin, mg/kg	$1.5 \times 10^4$	4.0	$7.4 \times 10^3$	1.0
Trypsin inhibitor, TIU/mg	4.00	7.20	<1.00	2.10

<sup>1</sup>ND = none detectable; TIU = trypsin inhibitor units.

**Table 3.** Ingredient composition (as-fed basis) of experimental diets containing soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), enzyme-treated soybean meal (ESBM), fish meal, and casein

Ingredient, %	Diet						
	SBM	SPI	FSBM	ESBM	Fish meal	Casein	N-free
SBM	30.00	—	—	—	—	—	—
SPI	—	17.20	—	—	—	—	—
FSBM	—	—	30.00	—	—	—	—
ESBM	—	—	—	21.50	—	—	—
Fish meal	—	—	—	—	20.00	—	—
Casein	—	—	—	—	—	13.00	—
SBM oil	2.00	2.00	2.00	2.00	2.00	2.00	4.00
Cornstarch	55.32	67.82	55.32	63.77	67.02	72.42	68.42
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00	20.00
Solka flocc <sup>1</sup>	—	—	—	—	—	—	4.00
Ground limestone	0.75	0.90	0.75	0.80	—	0.90	0.90
Monocalcium phosphate	0.95	1.10	0.95	0.95	—	0.70	1.20
Magnesium oxide	—	—	—	—	—	—	0.10
Potassium carbonate	—	—	—	—	—	—	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix <sup>2</sup>	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Micro mineral premix <sup>3</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<sup>1</sup>Fiber Sales and Development Corp., Urbana, OH.

<sup>2</sup>Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 6,594 IU as vitamin A acetate; vitamin D<sub>3</sub>, 989 IU as D-activated animal sterol; vitamin E, 33 IU as  $\alpha$ -tocopherol acetate; vitamin K<sub>3</sub>, 2.6 mg as menadione dimethylpyrimidinol bisulfite; thiamine, 2.0 mg as thiamine mononitrate; riboflavin, 5.9 mg; pyridoxine, 2.0 mg as pyridoxine hydrochloride; vitamin B<sub>12</sub>, 0.026 mg; D-pantothenic acid, 20 mg as calcium pantothenate; niacin, 33 mg; folic acid, 0.66 mg; and biotin, 0.1 mg.

<sup>3</sup>Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

**Table 4.** Analyzed nutrient composition (as-fed basis) of experimental diets containing soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), enzyme-treated soybean meal (ESBM), fish meal, and casein

Item	Diet						
	SBM	SPI	FSBM	ESBM	Fish meal	Casein	N-free
DM, %	91.22	91.55	92.60	92.01	94.67	95.25	91.94
CP, %	12.20	13.62	15.01	11.67	13.26	11.80	0.35
Indispensable AA, %							
Arg	0.83	0.96	1.05	0.79	0.70	0.41	—
His	0.34	0.34	0.42	0.29	0.30	0.34	—
Ile	0.57	0.66	0.77	0.55	0.49	0.62	—
Leu	0.94	1.08	1.26	0.88	0.85	1.10	0.01
Lys	0.79	0.87	0.94	0.69	0.93	0.94	—
Met	0.15	0.20	0.24	0.18	0.34	0.34	—
Phe	0.63	0.72	0.82	0.60	0.49	0.62	0.01
Thr	0.45	0.49	0.59	0.42	0.47	0.46	—
Trp	0.19	0.19	0.21	0.17	0.14	0.16	0.04
Val	0.63	0.71	0.84	0.59	0.59	0.80	0.01
Dispensable AA, %							
Ala	0.53	0.58	0.72	0.51	0.78	0.36	0.01
Asp	1.35	1.55	1.75	1.27	1.06	0.81	0.01
Cys	0.20	0.18	0.24	0.17	0.11	0.07	—
Glu	2.21	2.62	2.83	2.07	1.59	2.61	0.01
Gly	0.51	0.57	0.70	0.47	0.94	0.22	—
Pro	0.57	0.65	0.75	0.54	0.57	1.17	0.01
Ser	0.51	0.60	0.67	0.48	0.41	0.56	—
Tyr	0.31	0.35	0.46	0.30	0.25	0.51	0.01
Total AA, %	11.71	13.32	15.26	10.97	11.01	12.10	0.08

as the internal standard. Samples were hydrolyzed before analysis with 6 N HCl for 24 h at 110°C (method 982.30 E[a]). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E[b]). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E[c]).

Protein separation in the 4 soybean products was performed by SDS-PAGE according to the method of Laemmli (1970) using an electrophoresis system (Bio Rad Criterion, Bio-Rad, Hercules, CA). Proteins (40 µg) were applied to a 12% Criterion XT precast gel (Bio-Rad) and separated at 150 V for 60 min. After electrophoresis, the gel was stained with Coomassie Blue (Sigma-Aldrich, St. Louis, MO). Molecular weights were estimated by comparing the bands with known standards (Mark 12, Invitrogen, Carlsbad, CA), and protein quantification was performed with a scanner (Typhoon 9400, GE Healthcare, Fairfield, CT) using software [Image Quant (R) software, GE Healthcare]. The bands were classified as large ( $\geq 60$  kDa), medium (20 to 60 kDa), and small peptides ( $\leq 20$  kDa). The amount of each peptide in each class was expressed as a percentage of total protein in a sample.

Concentrations of glycinin and  $\beta$ -conglycinin in all soybean products were measured using an ELISA procedure (Van Biert and Hessing, 1993). The sugar profile of these samples was determined using a HPLC with an autosampler (Alcott, Norcross, GA), a pump (Waters 510, Milford, MA), a column (Dionex CarboPac PA1, Sunnyvale, CA), and a pulsed amperometric detector (Dionex) based on the procedure of Rocklin et al. (1998). Results were compared with known standards for glucose, sucrose, maltose, and fructose (Chem Service, West Chester, PA) and known standards for stachyose and raffinose (Sigma-Aldrich) to determine concentrations of monosaccharides, disaccharides, and oligosaccharides. The TI concentration was also determined (method Ba 12-75; AOCS, 1998).

All diets and ileal digesta samples were also analyzed for DM, CP, and AA as described for the ingredients. Chromium concentrations of diets and ileal digesta were determined according to the procedure of Fenton and Fenton (1979).

### Calculations and Statistical Analysis

Values for AID and SID were calculated according to Stein et al. (2007). Data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure of SAS. Outliers were determined as values that deviated from the treatment mean by more than 1.5 times the interquartile range (Devore and Peck, 1993). Two outliers were identified and removed from the data set (one outlier was a pig fed the SBM diet and the other was a pig fed the SPI diet). 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. Means were calculated using the LS Means statement in SAS, and whenever differences were detected, treatment means were separated using the PDIF option of SAS. The pig was the experimental unit for all analyses, and an  $\alpha$ -value of 0.05 was used to assess significance among treatments.

## RESULTS

### Chemical Characteristics of Ingredients

The concentration of most nutrients in FSBM was similar to the concentration of nutrients in ESBM (Table 1). Fish meal contained more CP, ether extract, Ca, and P than FSBM and ESBM, but SPI contained more DM, CP, and AA than the other soybean products and fish meal. Casein contained the greatest amounts of CP and most AA, whereas SBM contained the least amounts of CP and most AA compared with the other ingredients.

Peptide size distribution did not differ between SBM and FSBM, and the concentration of small peptides ( $\leq 20$  kDa) was less in these 2 soybean products than in ESBM and SPI (Table 2). Fermented soybean meal did not contain stachyose and raffinose, and the concentration of these oligosaccharides was also reduced in ESBM and SPI, but SBM contained 5.17% stachyose and 1.08% raffinose (as-fed basis). Soybean meal also contained 7.81% sucrose (as-fed basis), whereas no sucrose was present in the other 3 soybean products. The concentration of glycinin was less in SPI than in all other soy products, and ESBM contained less glycinin than SBM and FSBM. The concentration of  $\beta$ -conglycinin was least in SPI and ESBM, but the concentration of  $\beta$ -conglycinin in FSBM was close to the concentration in SBM. The concentration of TI was <1.00, 2.10, 4.00, and 7.20 units per mg in FSBM, ESBM, SBM, and SPI, respectively.

### AID

The AID of DM was greater ( $P < 0.05$ ) in casein (83.2%) than in all other ingredients, and the AID of DM in fish meal (76.1%) was greater ( $P < 0.05$ ) than in all the soybean products (Table 5). Soy protein isolate had the greatest ( $P < 0.05$ ) AID of DM (72.0%) among the soybean products, but no differences were observed among ESBM (61.3%), FSBM (61.0%), and SBM (59.9%).

The AID of CP in casein (81.3%) was greater ( $P < 0.05$ ) than in SBM (70.0%), FSBM (70.1%), and fish meal (70.8%), but not different from the values of 76.8 and 82.2% obtained for ESBM and SPI, respectively. The AID of Arg in casein was not different from that in SBM and fish meal, but this value was less ( $P < 0.05$ ) than the AID of Arg in FSBM, ESBM, and SPI. With



**Table 5.** Apparent ileal digestibility of DM, CP, and AA in soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), enzyme-treated soybean meal (ESBM), fish meal, and casein by weanling pigs<sup>1</sup>

Item	Ingredient						Pooled SEM	P-value
	SBM	SPI	FSBM	ESBM	Fish meal	Casein		
DM, %	59.9 <sup>d</sup>	72.0 <sup>c</sup>	61.0 <sup>d</sup>	61.3 <sup>d</sup>	76.1 <sup>b</sup>	83.2 <sup>a</sup>	0.9	0.001
CP, %	70.0 <sup>b</sup>	82.2 <sup>a</sup>	70.1 <sup>b</sup>	76.8 <sup>ab</sup>	70.8 <sup>b</sup>	81.3 <sup>a</sup>	2.9	0.002
Indispensable AA, %								
Arg	84.7 <sup>bc</sup>	92.8 <sup>a</sup>	87.5 <sup>b</sup>	90.4 <sup>a</sup>	85.0 <sup>bc</sup>	81.6 <sup>c</sup>	2.2	0.003
His	80.8 <sup>b</sup>	87.9 <sup>a</sup>	80.1 <sup>b</sup>	82.7 <sup>b</sup>	80.8 <sup>b</sup>	90.2 <sup>a</sup>	1.8	0.001
Ile	79.8 <sup>c</sup>	87.2 <sup>ab</sup>	81.9 <sup>c</sup>	84.4 <sup>bc</sup>	81.3 <sup>c</sup>	90.8 <sup>a</sup>	1.9	0.001
Leu	78.9 <sup>c</sup>	86.4 <sup>b</sup>	81.4 <sup>bc</sup>	83.7 <sup>bc</sup>	82.4 <sup>bc</sup>	91.9 <sup>a</sup>	1.9	0.001
Lys	79.9 <sup>c</sup>	88.8 <sup>ab</sup>	72.7 <sup>d</sup>	82.2 <sup>bc</sup>	82.9 <sup>bc</sup>	92.6 <sup>a</sup>	2.7	0.001
Met	80.1 <sup>c</sup>	87.8 <sup>b</sup>	83.7 <sup>bc</sup>	86.2 <sup>b</sup>	86.0 <sup>b</sup>	95.1 <sup>a</sup>	1.9	0.001
Phe	80.4 <sup>d</sup>	88.4 <sup>ab</sup>	82.5 <sup>cd</sup>	85.5 <sup>bc</sup>	79.3 <sup>d</sup>	91.0 <sup>a</sup>	1.7	0.001
Thr	67.5	76.5	69.0	72.5	74.7	78.4	3.1	0.076
Trp	82.2	84.3	79.1	82.1	81.8	86.0	1.7	0.071
Val	74.2 <sup>c</sup>	82.7 <sup>b</sup>	76.8 <sup>c</sup>	78.9 <sup>bc</sup>	78.1 <sup>bc</sup>	88.8 <sup>a</sup>	2.2	0.001
Mean	79.0 <sup>c</sup>	87.0 <sup>ab</sup>	79.7 <sup>c</sup>	83.3 <sup>bc</sup>	81.4 <sup>c</sup>	89.5 <sup>a</sup>	2.0	0.001
Dispensable AA, %								
Ala	68.9	80.3	72.9	77.3	80.6	76.4	3.3	0.070
Asp	77.2 <sup>bc</sup>	85.9 <sup>a</sup>	77.4 <sup>bc</sup>	82.4 <sup>ab</sup>	73.8 <sup>c</sup>	84.5 <sup>a</sup>	2.3	0.002
Cys	70.2 <sup>abc</sup>	76.2 <sup>a</sup>	61.5 <sup>bcd</sup>	73.7 <sup>ab</sup>	57.6 <sup>cd</sup>	54.7 <sup>d</sup>	5.0	0.010
Glu	80.9 <sup>b</sup>	91.2 <sup>a</sup>	80.9 <sup>b</sup>	89.3 <sup>a</sup>	80.8 <sup>b</sup>	92.5 <sup>a</sup>	2.6	0.001
Gly	38.6 <sup>b</sup>	64.2 <sup>a</sup>	50.4 <sup>ab</sup>	59.3 <sup>ab</sup>	66.0 <sup>a</sup>	-2.4 <sup>c</sup>	7.7	0.001
Pro	32.7	70.1	55.8	43.9	13.2	61.3	17.0	0.145
Ser	74.9 <sup>b</sup>	84.1 <sup>a</sup>	74.7 <sup>b</sup>	78.9 <sup>ab</sup>	74.0 <sup>b</sup>	83.4 <sup>a</sup>	2.3	0.002
Tyr	82.4 <sup>cd</sup>	88.2 <sup>ab</sup>	83.7 <sup>bc</sup>	86.0 <sup>bc</sup>	78.1 <sup>d</sup>	92.0 <sup>a</sup>	2.0	0.001
Mean	70.3 <sup>cd</sup>	83.8 <sup>a</sup>	73.6 <sup>bcd</sup>	78.7 <sup>abc</sup>	69.3 <sup>d</sup>	80.2 <sup>ab</sup>	3.4	0.013
All AA	74.4 <sup>b</sup>	85.3 <sup>a</sup>	76.5 <sup>b</sup>	80.9 <sup>ab</sup>	75.2 <sup>b</sup>	84.7 <sup>a</sup>	2.6	0.004

<sup>a-d</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Data are least squares means of 7 observations for all treatments except for SBM and SPI where only 6 observations were included.

the exception of the AID of His, Ile, Lys, and Phe in SPI, the AID of all indispensable AA in SBM, FSBM, ESBM, SPI, and fish meal were less ( $P < 0.05$ ) than in casein.

The AID of most indispensable AA in FSBM was not different from the AID for AA in SBM, ESBM, and fish meal except for the AID of Lys, which was less ( $P < 0.05$ ) in FSBM than in the other protein sources. The AID of most of the indispensable AA in FSBM was also less ( $P < 0.05$ ) than the AID of AA in SPI. Except for the AID of His, which was less ( $P < 0.05$ ) in ESBM than in SPI, the AID for all AA in ESBM was not different from that of SPI.

### SID

The SID of CP in casein (96.7%) was greater ( $P < 0.05$ ) than in SBM (84.3%), FSBM (81.8%), and fish meal (84.4%), but not different from the SID of CP in ESBM (91.9%) and SPI (95.0%; Table 6). The SID for most AA in SPI was not different from the SID of AA in casein, but except for Arg, the SID for all indispensable AA in SBM, ESBM, FSBM, and fish meal were less ( $P < 0.05$ ) than in casein. The SID of Lys in FSBM was the least ( $P < 0.05$ ) among all the protein sources, but the SID for most other AA in FSBM was not different from the SID of AA in SBM and fish meal except for the SID of Thr and Trp, which were greater ( $P < 0.05$ )

in fish meal than in FSBM. With the exception of the SID of Phe, which was greater in ESBM than in FSBM, most of the remaining indispensable AA in FSBM was also not different from the SID of AA in ESBM, but these values were less ( $P < 0.05$ ) than in SPI.

The SID for all indispensable AA in ESBM was not different from the SID of AA in SPI and fish meal. With the exception of the SID of His, Phe, Asp, Glu, and Tyr, which were greater ( $P < 0.05$ ) in SPI than in fish meal, the SID of AA in SPI was not different from the SID of AA in fish meal and casein.

## DISCUSSION

### Composition and Chemical Characteristics of Ingredients

The concentrations of sucrose, stachyose, and raffinose in the conventional SBM were similar to the concentrations reported by Grieshop et al. (2003) and by Baker and Stein (2009). However, the concentrations of CP and AA in the conventional SBM was less than previous estimates (NRC, 1998; Baker and Stein, 2009), which may be a result of the SBM being produced in the northern part of the United States where the CP and AA concentration in SBM usually is less than in SBM produced in the central and southern part of the United States (Grieshop et al., 2003; Karr-Lilienthal

**Table 6.** Standardized ileal digestibility of CP and AA in soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), enzyme-treated soybean meal (ESBM), fish meal, and casein by weanling pigs<sup>1,2</sup>

Item	Ingredient						Pooled SEM	P-value
	SBM	SPI	FSBM	ESBM	Fish meal	Casein		
CP, %	84.3 <sup>b</sup>	95.0 <sup>a</sup>	81.8 <sup>b</sup>	91.9 <sup>a</sup>	84.4 <sup>b</sup>	96.7 <sup>a</sup>	2.9	0.001
Indispensable AA, %								
Arg	92.0	99.2	93.4	98.1	94.0	97.1	2.2	0.071
His	85.9 <sup>c</sup>	93.0 <sup>ab</sup>	84.4 <sup>c</sup>	88.8 <sup>bc</sup>	86.9 <sup>c</sup>	95.6 <sup>a</sup>	1.8	0.001
Ile	84.9 <sup>c</sup>	91.6 <sup>ab</sup>	85.7 <sup>c</sup>	89.7 <sup>bc</sup>	87.4 <sup>bc</sup>	95.6 <sup>a</sup>	1.9	0.001
Leu	84.0 <sup>c</sup>	90.9 <sup>b</sup>	85.3 <sup>c</sup>	89.2 <sup>bc</sup>	88.2 <sup>bc</sup>	96.4 <sup>a</sup>	1.9	0.001
Lys	85.0 <sup>c</sup>	93.5 <sup>ab</sup>	77.0 <sup>d</sup>	88.1 <sup>bc</sup>	87.4 <sup>bc</sup>	97.0 <sup>a</sup>	2.7	0.001
Met	87.0 <sup>c</sup>	93.2 <sup>ab</sup>	88.2 <sup>bc</sup>	92.1 <sup>bc</sup>	89.3 <sup>bc</sup>	98.4 <sup>a</sup>	1.9	0.001
Phe	86.3 <sup>d</sup>	93.6 <sup>ab</sup>	87.1 <sup>d</sup>	91.8 <sup>bc</sup>	87.2 <sup>cd</sup>	97.3 <sup>a</sup>	1.7	0.001
Thr	79.6 <sup>bc</sup>	87.8 <sup>ab</sup>	78.4 <sup>c</sup>	85.7 <sup>bc</sup>	86.8 <sup>ab</sup>	90.9 <sup>a</sup>	3.1	0.027
Trp	87.0 <sup>bc</sup>	89.1 <sup>ab</sup>	83.5 <sup>c</sup>	87.5 <sup>bc</sup>	88.6 <sup>ab</sup>	92.0 <sup>a</sup>	1.7	0.019
Val	84.0 <sup>c</sup>	91.4 <sup>ab</sup>	84.2 <sup>c</sup>	89.4 <sup>bc</sup>	88.9 <sup>bc</sup>	96.8 <sup>a</sup>	2.2	0.001
Mean	85.7 <sup>cd</sup>	92.9 <sup>ab</sup>	84.9 <sup>d</sup>	90.5 <sup>bc</sup>	88.6 <sup>bcd</sup>	96.1 <sup>a</sup>	2.0	0.001
Dispensable AA, %								
Ala	79.5 <sup>c</sup>	90.0 <sup>a</sup>	80.8 <sup>bc</sup>	88.5 <sup>ab</sup>	88.1 <sup>abc</sup>	92.8 <sup>a</sup>	3.3	0.015
Asp	82.3 <sup>bc</sup>	90.6 <sup>ab</sup>	81.6 <sup>c</sup>	88.1 <sup>b</sup>	80.9 <sup>c</sup>	93.9 <sup>a</sup>	2.3	0.001
Cys	79.9 <sup>ab</sup>	86.8 <sup>a</sup>	69.6 <sup>b</sup>	85.1 <sup>a</sup>	75.8 <sup>ab</sup>	84.4 <sup>a</sup>	5.0	0.001
Glu	84.9 <sup>b</sup>	94.5 <sup>a</sup>	84.0 <sup>b</sup>	93.5 <sup>a</sup>	86.5 <sup>b</sup>	95.9 <sup>a</sup>	2.6	0.002
Gly	70.6	93.0	74.1	94.4	84.1	75.2	7.7	0.128
Pro	130.7	156.3	131.4	148.2	114.9	111.1	17.0	0.251
Ser	84.6 <sup>cd</sup>	92.3 <sup>ab</sup>	82.1 <sup>d</sup>	89.2 <sup>abc</sup>	86.4 <sup>bcd</sup>	92.9 <sup>a</sup>	2.3	0.004
Tyr	88.1 <sup>bcd</sup>	93.3 <sup>ab</sup>	87.6 <sup>cd</sup>	92.0 <sup>abc</sup>	85.5 <sup>d</sup>	95.6 <sup>a</sup>	2.0	0.006
Mean	86.8 <sup>c</sup>	98.3 <sup>a</sup>	86.4 <sup>c</sup>	96.4 <sup>ab</sup>	88.0 <sup>bc</sup>	97.2 <sup>a</sup>	3.4	0.012
All AA	86.3 <sup>c</sup>	95.8 <sup>a</sup>	85.7 <sup>c</sup>	93.7 <sup>ab</sup>	88.3 <sup>bc</sup>	96.7 <sup>a</sup>	2.6	0.003

<sup>a-d</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Data are least squares means of 7 observations for all treatments except for SBM and SPI where only 6 observations were included.

<sup>2</sup>Standardized ileal digestibility values were calculated by correcting the values for apparent ileal digestibility for the basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DMI) as CP, 19.06; Arg, 0.67; His, 0.19; Ile, 0.32; Leu, 0.52; Lys, 0.44; Met, 0.12; Phe, 0.41; Thr, 0.60; Trp, 0.10; Val, 0.67; Ala, 0.62; Asp, 0.80; Cys, 0.21; Glu, 0.96; Gly, 1.79; Pro, 6.12; Ser, 0.54; and Tyr, 0.20.

et al., 2006). The AA composition of FSBM was also similar to that reported by Hong et al. (2004) and Yun et al. (2005). Fermented SBM contained more DM, CP, Ca, and P than SBM, which is consistent with observations from other experiments (Hong et al., 2004; Feng et al., 2007). However, the concentration of crude fiber was greater and the concentration of ether extract was less in FSBM than in SBM. These results are not consistent with previous data (Zamora and Veum, 1979, 1988; Feng et al., 2007). The SBM used in this experiment was obtained from a commercial source, and the chemical characteristics of SBM may vary among sources (Grieshop et al., 2003). The absence of sucrose, stachyose, and raffinose in FSBM may be attributed to the production of  $\alpha$ -galactosidase by *Aspergillus oryzae* during the fermentation process (Shankar and Mulimani, 2007). The disappearance of these saccharides is the main reason for the analyzed increase in the concentration of other nutrients in FSBM as compared with the conventional SBM.

The concentrations of CP in fish meal and in casein were similar to previously reported values, but the concentration of AA in fish meal was slightly less than previously reported (NRC, 1998; Kim and Easter, 2001). Likewise, the CP and AA composition of ESBM were similar to the values reported by Zhu et al. (1998).

The reduced concentration of sucrose, raffinose, and stachyose in ESBM compared with SBM indicates that sucrase and  $\alpha$ -galactosidase may have been included in the enzyme mixture used in the preparation of this meal. The disappearance of the resulting monosaccharides may be caused by fermentation or by posttreatment separations.

The CP and AA concentration in the SPI used in the present experiment were greater than NRC (1998) values. The use of new processing technology results in more efficient protein recovery (Koseoglu and Rhee, 1993), which may account for the greater AA concentration in the SPI used in this experiment compared with previous values (NRC, 1998). Fractionation of soy proteins during the production of SPI (Wolf, 1970) and the enzymatic cleavage of proteins in SBM to produce ESBM may have contributed to an increase in the concentration of small peptides in ESBM and SPI.

The concentration of Lys calculated as a percentage of CP are less in FSBM and ESBM than in SBM and SPI. The reason for this observation may be that these 2 meals could have been heat damaged during drying because heat damage may result in Maillard reactions that will destroy some of the Lys in the products (Stein et al., 2009). Maillard reactions will also result in reduced SID of Lys (Pahm et al., 2008; Stein et al., 2009),

and the reduced SID of Lys in FSBM may, therefore, support the hypothesis that this meal was heat damaged. However, the SID of Lys in ESBM was not different from the SID in SBM and SPI. It is, therefore, not likely that this meal was heat damaged, and it is not known why the concentration of Lys in ESBM is less than in SBM.

### *Ileal AA Digestibility*

The development of new processes such as fermentation or enzymatic treatment of SBM to remove antinutritional factors and make SBM more tolerable to young pigs creates opportunities for increased use of SBM in nursery diets. Removal of oligosaccharides in FSBM and the reduced concentrations of oligosaccharides in ESBM and SPI may be an advantage because the diarrhea associated with feeding SBM to weanling pigs may be caused by the oligosaccharides present in SBM (Liener, 2000; Liying et al., 2003). The absence of oligosaccharides and a reduced concentration of TI in FSBM, therefore, were expected to contribute to an increased digestibility of AA in FSBM when fed to young animals. Hong et al. (2004) showed that FSBM contained greater amounts of small peptides than SBM. The rate and extent of absorption of small peptides is greater than for free AA (Gilbert et al., 2008), and an increased concentration of small peptides is expected to improve the digestibility of FSBM. However, the FSBM and SBM that were used in the present experiment contained similar amounts of small peptides, which may explain the lack of a difference in the AID and SID of AA between FSBM and SBM.

Increased concentrations of the antigenic proteins glycinin and  $\beta$ -conglycinin may reduce villus height in the small intestine and decrease N digestibility in young pigs fed SBM (Li et al., 1991). Purified glycinin and  $\beta$ -conglycinin also reduce ADG and G:F in young pigs, but because  $\beta$ -conglycinin is less digestible than glycinin, the reduction in pig performance is greater by pigs fed  $\beta$ -conglycinin than glycinin (Zhao et al., 2008). The reduction in the concentration of  $\beta$ -conglycinin may, therefore, have contributed to the improved AID and SID of AA in SPI compared with SBM. This is consistent with the observation that  $\beta$ -conglycinin is the best predictor of N digestibility in soybean proteins (Lallès et al., 1996).

The presence of TI and oligosaccharides in soybean products reduce AA digestibility (Smiricky et al., 2002; Świąch et al., 2004), but despite the absence of oligosaccharides and a reduced concentration of TI in FSBM, no improvement in the SID of AA in FSBM compared with SBM was observed in the present experiment. Previously, Yang et al. (2007) reported that with the exception of Arg, Ile, Lys, Gly, and Pro, there are no differences in the SID of AA between FSBM and SBM. It has, however, also been reported that the AID of most AA in FSBM is greater than in SBM (Yun et al., 2005). It is not clear why the results for FSBM seem to

be inconsistent among experiments, but the least SID for all the indispensable AA in FSBM in the present experiment was obtained for Lys. This indicates that the batch of FSBM that was used in the present experiment may have been overheated. In addition, the concentration of glycinin and  $\beta$ -conglycinin in the FSBM that was used in this experiment was not different from the concentrations in SBM, which may also contribute to the lack of a difference in the digestibility of AA between FSBM and SBM.

The reduced concentration of oligosaccharides and antigens in ESBM indicates that it may be used in diets fed to weanling pigs because the problems that limit the inclusion of conventional SBM in these diets do not exist for ESBM. Research is, however, needed to verify this hypothesis.

The SID of AA in SPI was greater than in SBM for most indispensable AA and also greater than in fish meal for some AA. This observation is in agreement with data presented by Urbaityte et al. (2009) and indicates that SPI is an excellent protein source for weanling pigs.

The values for the AID of AA in casein that were measured in this experiment were less than the values reported by NRC (1998), but similar to the values reported by Walker et al. (1986) who also worked with weanling pigs. Milk protein is believed to be the ideal protein source for young pigs because of the excellent SID of AA and because no antinutritional factors are present in casein. The values for the SID of Lys and most other AA in SBM and fish meal were less than values reported by NRC (1998), but SID values for most ingredients measured in this experiment agree with SID values measured in weanling pigs (Urbaityte et al., 2009). We are not aware of experiments, in which SID values have been measured for the same ingredients in weanling and in growing pigs, but based on the fact that SID values obtained in this experiment and in the experiment by Urbaityte et al. (2009) are less than values reported in other experiments, it may be speculated that SID values in weanling pigs are less than in growing pigs.

### *Conclusions*

The SID of most AA in FSBM and ESBM is not greater than in conventional SBM, but FSBM and ESBM contain more digestible AA than SBM because of the greater concentration of AA in FSBM and ESBM than in SBM. The SID of most AA in FSBM and ESBM was not different from fish meal. The SID of most AA in SPI was similar to casein and greater than in conventional SBM and FSBM.

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