



Ileal Digestibility of Amino Acids in Conventional and Low-Kunitz Soybean Products Fed to Weanling Pigs*

K. P. Goebel and H. H. Stein**

Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA

ABSTRACT : An experiment was conducted to determine the standardized ileal digestibility (SID) of amino acids (AA) in four sources of full-fat soybeans (FFSB) and in one source of soybean meal (SBM). The FFSB had different concentrations of trypsin inhibitor units (TIU) and included two sources of conventional FFSB, and two sources of a soybean variety that was selected for a reduced concentration of the Kunitz trypsin inhibitor. The conventional FFSB was either low temperature-processed (LT-FFSB-CV; 37.7% CP, 35.4 TIU/mg) or high temperature-processed (HT-FFSB-CV; 40.5% CP, 4.4 TIU/mg). The low-Kunitz FFSB was also either low temperature-processed (LT-FFSB-LK; 36.2% CP, 23.5 TIU/mg) or high temperature-processed HT-FFSB-LK; (38.2% CP, 4.0 TIU/mg). The SBM contained 47.5% CP and 3.20 TIU/mg. Twelve weanling barrows (initial BW: 11.1±1.3 kg) were fitted with a T-cannula in the distal ileum. Pigs were allotted to a replicated 6×6 Latin square design with six diets and six periods per square. Five diets were prepared using each of the soybean sources as the only source of AA in the diet. An N-free diet was also included in the experiment to measure basal endogenous losses of AA. The two low temperature-processed FFSB had lower ($p<0.05$) AID and SID values for all indispensable AA than the two high temperature-processed FFSB and SBM. The SID values for all indispensable AA except Trp were greater ($p<0.05$) in LT-FFSB-LK than in LT-FFSB-CV, but the SID of AA in HT-FFSB-CV and HT-FFSB-LK were not different. The SID of AA in SBM were not different from the SID in HT-FFSB-CV and in HT-FFSB-LK. Results of this experiment show that a reduction of the TIU from 35.4 to 23.5 TIU/mg will improve the SID of AA, but this reduction is not sufficient to completely ameliorate the negative impact of trypsin inhibitors. Results also show that the SID of AA in high temperature-processed FFSB is similar to that in de-hulled SBM. (**Key Words** : Amino Acid Digestibility, Low Kunitz Soybeans, Full-fat Soybean, Trypsin Inhibitor, Pig)

INTRODUCTION

Trypsin inhibitors are the most important antinutritional factors in raw soybeans. Kunitz and Bowman-Birk are the two major types of trypsin inhibitors in soybeans (Rackis, 1972), but the Kunitz trypsin inhibitor is of particular interest because it is heat labile, whereas the Bowman-Birk inhibitor exhibits a considerable resistance to heat treatment (Clemente et al., 2007). After isolation and characterization of the Kunitz trypsin inhibitor (Kunitz, 1947a, b), it was demonstrated that this inhibitor results in decreased protein digestibility in pigs due to a reduction in the activity of trypsin, chymotrypsin, and other pancreatic enzymes (Yen et al., 1977).

Heat treatment of soybean products inactivates the Kunitz inhibitor (Liener and Kakade, 1980) and heat treatment of soybean products is, therefore, routinely done before soybean products are used in diets fed to swine. The apparent ileal digestibility (AID) of amino acids (AA) in soybeans is also improved with heat treatment by approximately 15 percentage units (Herkelman et al., 1992), but there is no information about the effect of trypsin inhibitors on the standardized ileal digestibility (SID) of AA in soybean meal (SBM). Because of the negative impact of the Kunitz trypsin inhibitor on protein digestibility, plant breeders have tried to select varieties of soybeans with a low concentration of the Kunitz inhibitor (Clark and Hymowitz, 1972). Previous research has demonstrated that growth performance in pigs is improved if unheated varieties of low-Kunitz soybeans instead of unheated conventional soybeans are fed to pigs (Yen et al., 1974; Cook et al., 1988; Palacios et al., 2004). However, in all of these experiments, pigs fed the low-Kunitz soybeans had performance that was lower than pigs fed heat-treated

* This manuscript is based on research supported by Schillinger Genetics Inc., Des Moines, IA, USA and from the USDA-SBIR program.

** Corresponding Author: Hans H. Stein. Tel: +1-217-333-0013, Fax: +1-217-333-7088, E-mail: hstein@illinois.edu

Received November 18, 2009; Accepted April 7, 2010

soybeans. Schillinger Genetics (Des Moines, IA, USA) has recently selected a new variety of low-Kunitz soybeans, but there are no data on the nutritional quality of this variety of soybeans.

The objective of this experiment was, therefore, to test the hypothesis that low-Kunitz soybeans from Schillinger Genetics Inc. have greater AID and SID of crude protein (CP) and AA than conventional soybeans with normal concentrations of trypsin inhibitors and that heat treatment of low-Kunitz soybeans is not necessary to maximize AA digestibility.

MATERIALS AND METHODS

Animals, housing, and experimental design

The experimental protocol for this experiment was

reviewed and approved by the Animal Care and Use Committee at the University of Illinois. Twelve growing barrows (initial BW: 11.1±1.3 kg) were allotted to a replicated 6×6 Latin square design with 6 diets and 6 periods balanced for potential residual effects using the Balanced Latin Square Designer (Kim and Stein, 2009). Each pig was surgically equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Pigs were housed in individual pens (1.8×2.7 m) in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen.

Diets and feeding

Five sources of soybean products were used in the experiment (Table 1). The control source was a conventional dehulled SBM containing 47.5% CP. Two

Table 1. Chemical composition of soybean products produced from low temperature-processed conventional full fat soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and in conventional soybean meal (SBM), as-is basis

Item	Ingredient				
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM
Dry matter (%)	90.43	88.33	95.77	94.02	87.50
Crude protein (%)	37.70	36.17	40.45	38.19	47.47
Crude fat (%)	19.86	20.72	21.22	20.60	1.48
Crude fiber (%)	4.50	4.40	3.80	4.20	3.50
Ash (%)	4.79	5.28	5.23	5.72	6.06
Trypsin inhibitor (TIU/mg) ¹	35.40	23.50	4.40	4.00	3.20
Sucrose (%)	5.91	6.09	6.02	5.91	7.05
Stachyose (%)	4.74	4.46	4.56	4.84	4.61
Raffinose (%)	0.62	0.96	1.00	0.64	0.93
Indispensable amino acids (%)					
Arg	2.98	2.87	3.21	2.91	3.56
His	1.08	1.07	1.17	1.09	1.25
Ile	1.90	1.80	2.13	1.90	2.25
Leu	3.10	3.02	3.47	3.14	3.76
Lys	2.74	2.63	2.90	2.67	3.14
Met	0.61	0.60	0.64	0.61	0.68
Phe	2.04	1.98	2.24	2.02	2.48
Thr	1.65	1.61	1.75	1.59	1.83
Trp	0.57	0.59	0.72	0.68	0.69
Val	1.99	1.89	2.23	2.02	2.36
Dispensable amino acids (%)					
Ala	1.82	1.75	1.99	1.79	2.07
Asp	4.68	4.53	5.12	4.63	5.40
Cys	0.59	0.63	0.62	0.59	0.65
Glu	7.74	7.29	8.06	7.39	8.54
Gly	1.83	1.71	1.95	1.79	2.00
Pro	1.96	1.92	2.14	1.96	2.36
Ser	1.96	1.96	2.08	1.88	2.10
Tyr	1.50	1.47	1.63	1.49	1.70

¹ TIU = Trypsin inhibitor units.

sources of full-fat soybeans (FFSB) were also used. One of these FFSB was selected for a low concentration of Kunitz trypsin inhibitors and the other source was a conventional FFSB with normal activity of trypsin inhibitors. After isolation and characterization of the Kunitz trypsin inhibitor (Kunitz, 1947a, b), it was demonstrated that this inhibitor results in decreased protein digestion in pigs (Yen et al., 1977). Therefore, a newly developed variety with a low concentration of the Kunitz trypsin inhibitor was used. The two varieties of soybeans were grown in northeastern Indiana and were planted May 20, 2008, and harvested October 15, 2008. The growing season began very wet and cool and finished with very dry conditions. The conventional and low-Kunitz sources of soybeans were used as low temperature-processed FFSB (LT-FFSB-CV and LT-FFSB-LK) and high temperature-processed FFSB (HT-FFSB-CV and HT-FFSB-LK, respectively). The LT-FFSB-LK and LT-FFSB-CV were both dehulled and cracked through a Roskamp Double Roller Mill (Roskamp Champion, Waterloo, IA) at an ambient temperature of 21°C. The high temperature-processed FFSB were processed using the Insta-Pro model 600 autogenous extruder (Insta-Pro International, Des Moines, IA) with a 0.8 cm die operating at a rate of 182 kg per h. The extrusion temperature was 154°C for HT-FFSB-CV and 143°C for HT-FFSB-LK. The low temperature-processed soybeans and the extrudate from both high temperature processed FFSB sources were ground to 1.18 mm through a Bauer mill in the Ag Bioprocess Laboratory at the University of Illinois.

Six diets were prepared (Tables 2 and 3). Five of the diets contained one of the soybean sources and starch, sugar, and oil. The last diet was an N-free diet that was used to calculate basal endogenous losses of CP and AA. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 1998). All diets were supplemented with 0.4% chromic oxide as an indigestible marker.

All pigs were fed at a daily level of three times the maintenance energy requirement (106 kcal of ME per kg^{0.75}; NRC, 1998). The daily allotment of feed was provided at 0700 h. Water was available at all times throughout the experiment.

Data recording and sample collection

Pig body weights were recorded at the beginning and at the end of each period and the amount of feed supplied each day was recorded. The initial four d of each period was considered an adaptation period to the diet. Ileal digesta were collected for 8 h on d 5 and 6. The cannulas were opened and a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and stored at -20°C to prevent bacterial degradation of the AA in the digesta.

Chemical analysis

At the conclusion of the experiment, ileal samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. A sample of each diet

Table 2. Ingredient composition of experimental diets containing low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and conventional soybean meal (SBM), as-is basis

Ingredient (%)	Diet					
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	N-Free
Soybean product	50.00	50.00	50.00	50.00	40.00	-
Cornstarch	43.05	33.05	33.05	33.05	33.05	67.85
Soybean oil	3.60	3.60	3.60	3.60	3.60	4.00
Sugar	10.00	10.00	10.00	10.00	10.00	20.00
Solka flocc ^a	-	-	-	-	-	4.00
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.85
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.70
Magnesium oxide	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix ²	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00

¹ Fiber Sales and Development Corp., Urbana, OH, USA.

² The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D₃, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3. Chemical composition of experimental diets containing low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and conventional soybean meal (SBM), as-is basis

Item	Diet					
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	N-Free
Dry matter (%)	90.40	90.31	93.59	93.20	90.57	91.02
Crude protein (%)	15.99	17.95	21.42	20.0	25.5	1.95
Indispensable amino acids (%)						
Arg	0.94	1.27	1.44	1.30	1.87	0.11
His	0.36	0.49	0.53	0.51	0.68	0.04
Ile	0.64	0.86	0.96	0.89	1.23	0.09
Leu	1.08	1.42	1.59	1.47	2.01	0.14
Lys	0.87	1.18	1.30	1.21	1.63	0.10
Met	0.19	0.26	0.27	0.29	0.37	0.02
Phe	0.68	0.92	1.05	0.96	1.33	0.08
Thr	0.54	0.70	0.80	0.73	0.97	0.06
Trp	0.18	0.22	0.30	0.29	0.34	0.03
Val	0.69	0.93	1.03	0.96	1.29	0.09
Dispensable amino acids (%)						
Ala	0.63	0.81	0.91	0.85	1.13	0.08
Asp	1.52	2.05	2.32	2.14	2.94	0.19
Cys	0.19	0.25	0.29	0.26	0.34	0.02
Glu	2.41	3.21	3.59	3.39	4.53	0.32
Gly	0.60	0.78	0.88	0.82	1.09	0.07
Pro	0.69	0.97	1.00	0.94	1.30	0.08
Ser	0.64	0.76	0.90	0.82	1.09	0.07
Tyr	0.44	0.61	0.68	0.60	0.85	0.04

and of each of the soybean products was collected as well. Digesta samples were lyophilized and finely ground prior to chemical analysis. All samples were analyzed for DM (method 930.15; AOAC Int., 2007) and CP (method 990.03; AOAC Int., 2007). Chromium concentrations of diets and ileal digesta were determined using an inductive coupled plasma atomic emission spectrometric method (method 990.08; AOAC Int., 2007). Samples were prepared for analysis using nitric acid-perchloric acid (method 968.088D; AOAC Int., 2007). Amino acids were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110°C (method 982.30; AOAC, 2006). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C. Sucrose, raffinose, and stachyose were analyzed in the 5 sources of SBM using HPLC (method 982.14; AOAC Int., 2007). The concentration of total fat in these samples was measured after HCl hydrolysis, followed by petroleum ether extraction (method 954.02; AOAC, 2007),

and the concentration of crude fiber was analyzed using the Ankom method (procedure Ba 6a-05; AOCS; 2006). Trypsin inhibitor concentrations were also analyzed in each source of SBM (method Ba 12-75; AOCS; 2006).

Calculations and statistical analysis

The AID and SID of CP and AA in the five diets containing FFSB or SBM were calculated (Stein et al., 2007). Because the soybean products were the only feed ingredients contributing CP and AA in each of the diets, these digestibility values also represented the digestibility values for each of the soybean products.

The Proc UNIVARIATE procedure (SAS Institute Inc., Cary, NC) was used to identify outliers and one outlier was removed within the LT-FFSB-CV treatment. Data were analyzed using the Proc GLM procedure of SAS. The initial model included diet, period, and animal, but period and animal were not significant, and thus, removed from the final model. Orthogonal contrasts were used to determine effects of the variety of FFSB, the thermal treatment, and their interaction. Treatment means were separated using the PDIFF option with Tukey's adjustment. The mean separation output was converted to letter groupings using a SAS macro program (Saxton, 1998). The pig was the

experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among treatments.

RESULTS

The CP and AA concentrations were greater in SBM than in any of the FFSB sources (Table 1). However, the concentration of crude fat was lower in SBM than in the other soybean products, but the concentration of sucrose was not different among the soybean products. The TIU concentration in LT-FFSB-CV (35.4 TIU/mg) was greater than in LT-FFSB-LK (23.5 TIU/mg), but HT-FFSB-CV and HT-FFSB-LK had TIU concentrations of only 4.4 and 4.0 TIU/mg, and SBM contained 3.2 TIU/mg.

The AID of CP and all AA except Pro in HT-FFSB-CV, HT-FFSB-LK, and SBM was greater ($p < 0.05$) than the AID of AA in LT-FFSB-CV and LT-FFSB-LK (Table 4). The AID of CP and all AA except Trp in LT-FFSB-CV was less

($p < 0.05$) than in LT-FFSB-LK, but no differences in the AID of CP and AA among HT-FFSB-CV, HT-FFSB-LK, and SBM were observed. The AID for CP and all AA was greater ($p < 0.05$) in HT-FFSB-LK and HT-FFSB-CV than in LT-FFSB-LK and in LT-FFSB-CV and the AID of all AA except Trp and Pro were greater ($p < 0.05$) in the 2 low-Kunitz meals than in the two conventional meals.

No differences in SID for CP and AA were observed among HT-FFSB-CV, HT-FFSB-LK, and SBM, but all of these soybean products had SID values for CP and all AA except Pro that were greater ($p < 0.05$) than the SID of CP and AA in LT-FFSB-LK and LT-FFSB-CV (Table 5). The SID of CP and all AA except Trp in LT-FFSB-LK was greater ($p < 0.05$) than in LT-FFSB-CV. The SID for CP and all AA except Trp and Pro in HT-FFSB-LK and LT-FFSB-LK was greater ($p < 0.05$) than in HT-FFSB-CV and LT-FFSB-CV, and the SID of CP and all AA in HT-FFSB-LK and HT-FFSB-CV were greater ($p < 0.05$) than in LT-FFSB-

Table 4. Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and in soybean meal (SBM)¹

Item	Diet					SEM	p-values ^{2,3}		
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM		Overall	LT vs. HT	CV vs. LK
CP	49.4 ^c	59.6 ^b	81.10 ^a	81.22 ^a	81.90 ^a	2.29	<0.001	<0.001	0.023
Indispensable AA (%)									
Arg	57.9 ^c	74.1 ^b	91.4 ^a	92.3 ^a	93.7 ^a	1.51	<0.001	<0.001	<0.001
His	56.6 ^c	71.3 ^b	86.9 ^a	88.4 ^a	89.3 ^a	1.79	<0.001	<0.001	<0.001
Ile	48.2 ^c	63.1 ^b	84.9 ^a	87.3 ^a	88.7 ^a	2.00	<0.001	<0.001	<0.001
Leu	47.1 ^c	61.3 ^b	84.2 ^a	86.3 ^a	88.0 ^a	2.13	<0.001	<0.001	<0.001
Lys	49.3 ^c	65.0 ^b	85.1 ^a	86.6 ^a	86.3 ^a	2.22	<0.001	<0.001	<0.001
Met	52.5 ^c	67.4 ^b	86.3 ^a	89.8 ^a	90.8 ^a	1.94	<0.001	<0.001	<0.001
Phe	50.1 ^c	64.7 ^b	86.5 ^a	87.9 ^a	88.8 ^a	1.94	<0.001	<0.001	<0.001
Thr	45.2 ^c	57.9 ^b	78.6 ^a	80.0 ^a	82.1 ^a	2.57	<0.001	<0.001	0.005
Trp	59.7 ^b	65.9 ^b	87.3 ^a	88.5 ^a	87.7 ^a	2.23	<0.001	<0.001	0.079
Val	44.8 ^c	60.1 ^b	81.2 ^a	83.2 ^a	85.3 ^a	2.36	<0.001	<0.001	<0.001
Mean	50.2 ^c	64.8 ^b	85.3 ^a	87.0 ^a	88.2 ^a	1.95	<0.001	<0.001	<0.001
Dispensable AA (%)									
Ala	44.3 ^c	58.2 ^b	80.8 ^a	82.7 ^a	82.9 ^a	2.48	<0.001	<0.001	0.001
Asp	53.3 ^c	66.4 ^b	84.8 ^a	86.1 ^a	85.5 ^a	1.84	<0.001	<0.001	<0.001
Cys	29.5 ^c	44.0 ^b	76.6 ^a	77.3 ^a	76.7 ^a	2.95	<0.001	<0.001	0.009
Glu	61.6 ^c	73.5 ^b	88.2 ^a	88.2 ^a	84.0 ^a	1.92	<0.001	<0.001	0.002
Gly	32.4 ^c	51.2 ^b	75.1 ^a	74.2 ^a	76.1 ^a	3.08	<0.001	<0.001	0.003
Pro	3.2 ^c	38.7 ^{bc}	72.4 ^{ab}	70.0 ^{ab}	79.8 ^a	9.57	<0.001	<0.001	0.072
Ser	49.1 ^c	58.8 ^b	83.5 ^a	85.0 ^a	87.1 ^a	2.12	<0.001	<0.001	0.007
Tyr	49.1 ^c	65.1 ^b	84.9 ^a	85.7 ^a	87.4 ^a	2.14	<0.001	<0.001	<0.001
Mean	47.4 ^c	62.7 ^b	83.3 ^a	83.7 ^a	83.5 ^a	2.12	<0.001	<0.001	<0.001

^{a-c} Means within a row lacking a common superscript letter are different ($p < 0.05$).

¹ Each least squares mean represents 12 observations.

² p-values: Overall = The p-value for the comparison of diets containing all 5 ingredients; LT vs. HT = Comparison of the two low temperature-processed full-fat meals and the two high temperature-processed meals; CV vs. LK = Comparison of the two conventional soybeans and the two low-Kunitz soybeans.

³ The interaction of the effect of processing temperature on variety of soybean were significant ($p < 0.05$) for CP and all AA except for Trp.

Table 5. Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in low temperature processed conventional soybeans (LT-FFSB-CV), low temperature processed low-Kunitz soybeans (LT-FFSB-LK), high temperature processed conventional soybeans (HT-FFSB-CV), high temperature processed low-Kunitz soybeans (HT-FFSB-LK), and in soybean meal (SBM)^{1,2}

Item	Diet					SEM	p-values ^{3,4}		
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM		Overall	LT vs. HT	CV vs. LK
CP	65.2 ^b	70.2 ^b	90.5 ^a	91.2 ^a	89.5 ^a	2.16	<0.001	<0.001	0.218
Indispensable AA (%)									
Arg	64.6 ^c	79.0 ^b	95.9 ^a	97.2 ^a	97.1 ^a	1.62	<0.001	<0.001	<0.001
His	63.5 ^c	76.3 ^b	91.8 ^a	93.2 ^a	92.9 ^a	1.91	<0.001	<0.001	<0.001
Ile	55.3 ^c	68.4 ^b	89.8 ^a	92.3 ^a	92.4 ^a	2.14	<0.001	<0.001	<0.001
Leu	54.3 ^c	66.7 ^b	89.2 ^a	91.4 ^a	91.8 ^a	2.27	<0.001	<0.001	<0.001
Lys	57.5 ^c	71.0 ^b	90.7 ^a	92.5 ^a	90.6 ^a	2.38	<0.001	<0.001	<0.001
Met	58.7 ^c	71.9 ^b	90.8 ^a	93.8 ^a	94.0 ^a	2.07	<0.001	<0.001	<0.001
Phe	56.6 ^c	69.5 ^b	90.9 ^a	92.4 ^a	92.1 ^a	2.08	<0.001	<0.001	<0.001
Thr	56.4 ^c	66.5 ^b	86.4 ^a	88.0 ^a	88.3 ^a	2.74	<0.001	<0.001	0.022
Trp	66.9 ^b	71.9 ^b	91.8 ^a	92.8 ^a	91.6 ^a	2.38	<0.001	<0.001	0.176
Val	54.7 ^c	67.5 ^b	88.1 ^a	90.0 ^a	90.6 ^a	2.52	<0.001	<0.001	0.002
Mean	58.0 ^c	70.7 ^b	90.6 ^a	92.4 ^a	92.3 ^a	2.09	<0.001	<0.001	<0.001
Dispensable AA (%)									
Ala	54.6 ^c	66.3 ^b	88.2 ^a	90.3 ^a	88.7 ^a	2.66	<0.001	<0.001	0.006
Asp	60.2 ^c	71.5 ^b	89.5 ^a	91.2 ^a	89.1 ^a	1.98	<0.001	<0.001	<0.001
Cys	40.8 ^c	52.6 ^b	84.3 ^a	85.5 ^a	83.1 ^a	3.16	<0.001	<0.001	0.028
Glu	67.1 ^c	77.7 ^b	92.1 ^a	92.8 ^a	87.0 ^a	2.05	<0.001	<0.001	0.004
Gly	55.1 ^c	68.6 ^b	91.1 ^a	91.7 ^a	88.6 ^a	3.29	<0.001	<0.001	0.022
Pro	51.3 ^c	72.9 ^{bc}	106.7 ^{ab}	107.3 ^{ab}	105.3 ^a	10.25	<0.001	<0.001	0.237
Ser	58.1 ^c	66.4 ^b	90.2 ^a	91.8 ^a	92.4 ^a	2.26	<0.001	<0.001	0.02
Tyr	56.9 ^c	70.7 ^b	90.2 ^a	91.1 ^a	91.4 ^a	2.29	<0.001	<0.001	<0.001
Mean	59.9 ^c	72.1 ^b	92.0 ^a	93.2 ^a	90.2 ^a	2.27	<0.001	<0.001	0.002

^{a-c} Means within a row lacking a common superscript letter are different ($p < 0.05$).

¹ Each least squares mean represents 12 observations.

² 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 from pigs fed the N-free diet as (g/kg Dry Matter Intake): CP, 21.45; Arg, 0.70; His, 0.28; Ile, 0.51; Leu, 0.87; Lys, 0.74; Met, 0.13; Phe, 0.50; Thr, 0.68; Trp, 0.14; Val, 0.77; Ala, 0.72; Asp, 1.17; Cys, 0.24; Glu, 1.50; Gly, 1.47; Pro, 3.29; Ser, 0.64; Tyr, 0.39.

³ p-values: Overall = The p-value for the comparison of diets containing all 5 ingredients; LT vs. HT = Comparison of the two low temperature-processed full-fat meals and the two high temperature-processed meals; CV vs. LK = Comparison of the two conventional soybeans and the two low-Kunitz soybeans.

⁴ The interaction of the effect of processing temperature on variety of soybean were significant ($p < 0.05$) for all AA except for CP, Trp, Ala, and Pro.

LK and LT-FFSB-CV.

DISCUSSION

The two major types of trypsin inhibitors in legumes, particularly soybeans, are the Kunitz and Bowman-Birk trypsin inhibitors, which make up nearly 60% of the protein in soybeans (Losso, 2008). Bowman-Birk makes up approximately 40% of the trypsin inhibitors and Kunitz the remaining 60% (Liener, 1981). Similar to Kunitz, Bowman-Birk inhibitors reduce the activity of the proteolytic enzymes secreted by the pancreas. Heat treatment inactivates the Kunitz trypsin inhibitors in soybeans, thus allowing for increased AA digestibilities and improved growth performance (Herkelman et al., 1992; Palacios et al., 2004). However, the Bowman-Birk inhibitor loses no

activity if treated at a pH of two or heated to 100°C for ten minutes (Losso, 2008). The resistance of the Bowman-Birk inhibitor to highly acidic conditions and to heat treatment up to 100°C is due to the presence of disulfide bridges within the protein molecules (Losso, 2008). This structure will tightly bind up pancreatic proteases and cause severe inhibitory responses (Clemente et al., 2008). To inactivate the Bowman-Birk inhibitors, the temperature needs to exceed 100°C. During soybean extrusion, the temperature at the end of the extruder may range from approximately 143 to 166°C, which is effective in inactivating both Kunitz and Bowman-Birk inhibitors (Webster et al., 2003). The low TIU levels in both of the high temperature-processed FFSB used in the present study is, therefore, a result of the heat treatment these products had undergone. However, if the beans are processed under low temperatures, the Bowman-

Birk inhibitors are active.

The reason the SID of most AA was greater in LT-FFSB-LK than in LT-FFSB-CV is that the concentration of TIU is reduced in the low-Kunitz beans compared with the conventional soybeans. Heat treatment improves the AID of AA in conventional FFSB fed to pigs (Herkelman et al., 1992), but the results of this experiment document that heat treatment also improves the AID and the SID of AA in low-Kunitz beans. This observation concurs with Kim et al. (1999) who reported that weanling pig performance was improved when low-Kunitz soybeans were processed at high temperatures. The reason for this observation is most likely that although the concentration of trypsin inhibitors was reduced in the low-Kunitz soybeans, the concentration was not nearly as low as it was in heat treated soybean products. Improved nutrient digestibility was also observed by Qin et al. (1996) who reported that by heat treating soybeans to reduce trypsin inhibitor concentrations, nutrient digestibility of soybeans was greatly improved. It, therefore, appears that additional efforts need to be placed on selecting soybean varieties that have lower concentrations of trypsin inhibitors than the low-Kunitz beans used in this experiment.

The AID and SID of AA were not different between the high temperature-processed FFSB and the conventional SBM. The differences in chemical composition between the 2 FFSB and SBM are due to processing. Unlike SBM, oil is not removed from FFSB, which is the reason that the conventional soybean meal has a lower concentration of fat than the FFSB. Previous research showed that the greater fat concentration in FFSB resulted in greater AA digestibility in FFSB than in non-dehulled SBM (Cervantes-Pahm and Stein, 2008). This may be due to a slower gastric and intestinal emptying, which gives the feed proteins increased exposure to proteolytic enzymes (Gentilcore et al., 2006). In contrast, AA digestibility is reduced if soy hulls are added to SBM (Dilger et al., 2004). In this experiment, a dehulled source of SBM and dehulled FFSB were used, and the AID and SID of AA in HT-FFSB-CV and HT-FFSB-LK were similar to the AID and SID in SBM. The AID values for AA in SBM that were measured in this experiment are similar to values previously reported (NRC, 1998). However, AID values for HT-FFSB-CV and HT-FFSB-LK were greater than those obtained by Herkelman et al. (1992). This may be due to processing differences between the heated soybean sources used in the different studies.

Values for basal endogenous losses of AA were measured in the present experiment, which allowed for the calculation of SID for all AA. To our knowledge, SID values have not previously been measured in low-Kunitz soybeans. However, the values obtained for the SID of AA

in HT-FFSB-CV and in SBM concur with the values reported by Cervantes-Pahm and Stein (2008), but they are greater than the values published by NRC (1998).

In conclusion, if conventional or low-Kunitz de-hulled FFSB are processed at 140 to 160°C, values for the SID of AA are similar to those for de-hulled SBM. Even though it is advantageous to use a low-Kunitz variety of soybeans compared with conventional beans, the SID of AA in the non-heated beans is lower than the SID of AA in beans processed at 140 to 160°C. Processing at these temperatures increase SID of AA in both soybean varieties and the removal of the Kunitz trypsin inhibitor increases the SID of AA only in soybeans that have not been heat-treated. Therefore, even if low Kunitz varieties of soybeans are used, they still need to be heat-treated to maximize AA digestibility.

REFERENCES

- AOAC Int. 2007. Official methods of analysis .18th ed. Rev. 2 (Ed. W. Howitz, and G. W. Latimer Jr.). AOAC Int., Gaithersburg, MD.
- AOCS. 2006. Official methods and recommended practices (5th Ed). Association of Oil Chemists Society. Urbana, IL.
- Cervantes-Pahm, S. K. and H. H. Stein. 2008. Effect of dietary soybean oil and soybean protein concentration on the concentration of digestible amino acids in soybean products fed to growing pigs. *J. Anim. Sci.* 86:1841-1849.
- Clark, R. W. and T. Hymowitz. 1972. Activity variation between and within two soybean trypsin inhibitor eletrophoretic forms. *Biochem. Genet.* 6:169-182.
- Clemente, A., E. Jimenez, M. C. Marin-Manzano and L. A. Rubio. 2008. Active Bowman-Birk inhibitors survive gastrointestinal digestion at the terminal ileum of pigs fed chickpea-based diets. *J. Sci. Food Agric.* 88:513-521.
- Cook, D. A., A. H. Jensen, J. R. Fraley and T. Hymowitz. 1988. Utilization by growing and finishing pigs of raw soybeans of low Kunitz trypsin inhibitor content. *J. Anim. Sci.* 66:1686-1691.
- Dilger, R. N., J. S. Sands, D. Ragland and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. *J. Anim. Sci.* 82:715-724.
- Gentilicore, D., R. Chaikomin, K. L. Jones, A. Rusco, C. Feinle-Bisset, J. M. Wishart, C. K. Rayner and M. Horitz. 2006. Effect of fat on gastric emptying and the glycemic, insulin, and incretin responses to a carbohydrate meal and Type 2 diabetes. *J. Clin. Endocrinol. Metab.* 91:2062-2067.
- Herkelman, K. L., G. L. Cromwell, T. S. Stahly, T. W. Pfeiffer and D. A. Knabe. 1992. Apparent digestibility of amino acids in raw and heated conventional and low-trypsin-inhibitor soybeans for pigs. *J. Anim. Sci.* 70:818-826.
- Kim, B. G. and H. H. Stein. 2009. A spreadsheet program for making a balanced latin square design. *Rev. Colomb. Cienc. Pecu.* 22:591-596.
- Kim, I. H., J. D. Hancock, D. B. Jones and P. G. Reddy. 1999. Extrusion processing of low-inhibitor soybeans improves

- growth performance of early-weaned pigs. *Asian-Aust. J. Anim. Sci.* 12:1251-1257.
- Kunitz, M. 1947a. Crystalline soybean trypsin inhibitor. *J. Gen. Physiol.* 30:291-310.
- Kunitz, M. 1947b. Isolation of a crystalline protein compound of trypsin and of soybean trypsin-inhibitor. *J. Gen. Physiol.* 30:311-320.
- Liener, I. E. 1981. Factors affecting the nutritional quality of soya products. *J. Am. Oil Chem. Soc.* 58:406-415.
- Liener, I. E. and M. L. Kakade. 1980. Protease inhibitors. In: *Toxic Constituents of plant foodstuffs* (Ed. I. E. Liener). pp 7-71. Academic Press, New York.
- Losso, J. N. 2008. The biochemical and functional food properties of the Bowman-Birk inhibitor. *Crit. Rev. Food Sci. Nutr.* 48:94-118.
- NRC. 1998. *Nutrient requirements of swine* (10th Ed.). National Academy Press, Washington DC.
- Palacios, M. F., R. A. Easter, K. T. Soltwedel, C. M. Parsons, M. W. Douglas, T. Hymowitz and J. E. Pettigrew. 2004. Effect of soybean variety and processing on growth performance of young chicks and pigs. *J. Anim. Sci.* 82:1108-1114.
- Qin, G., E. R. ter Elst, M. W. Bosch and A. F. B. van der Poel. 1996. Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility in piglets. *Anim. Feed Sci. Technol.* 57:313-324.
- Rackis, J. J. 1972. Biologically active components. Pages 158-202. In: *Soybeans: Chemistry and Technology* (Ed. A. K. Smith and S. J. Circle). Vol. I. Proteins. The AVI Publ. Co. Inc., Westport, CT.
- Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243-1246 in *Proc. 23rd SAS Users Group Intl.*, SAS Institute Inc., Cary, NC.
- Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. *J. Anim. Sci.* 85:172-180.
- Stein, H. H., C. F. Shipley and R. A. Easter. 1998. Technical Note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. *J. Anim. Sci.* 76:1433-1436.
- Webster, M. J., R. D. Goodband, M. D. Tokach, J. L. Nelssen, S. S. Dritz, J. C. Woodworth, M. De La Llata and N. W. Said. 2003. Evaluating processing temperature and feeding value of high temperature-processed-expelled soybean meal on nursery and finishing pig growth performance. *J. Anim. Sci.* 81:2032-2040.
- Yen, J. T., T. Hymowitz and A. H. Jensen. 1974. Effects of soybeans of different trypsin-inhibitor activities on performance of growing swine. *J. Anim. Sci.* 38:304-309.
- Yen, J. T., A. H. Jensen and J. Simon. 1977. Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in small intestinal juice of growing swine. *J. Nutr.* 107:156-165.