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Amino acid digestibility in heated soybean meal fed to growing pigs[1]

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ABSTRACT: Heat treatment of soybean meal (SBM) is necessary to reduce the concentration of trypsin inhibitors, but excessive heat treatment may reduce AA concentration and digestibility because AA can be destroyed by the Maillard reaction. The objective of this experiment was to determine the effects of heat treatment of SBM on apparent ileal digestibility and standardized ileal digestibility (SID) of AA by growing pigs. A source of conventional dehulled SBM (48.5% CP) was divided into 4 batches. One batch was not additionally heated, 1 batch was autoclaved at 125°C for 15 min, 1 batch was autoclaved at 125°C for 30 min, and 1 batch was oven-dried at 125°C for 30 min. Four SBM-cornstarch diets were formulated, and each of the 4 batches of SBM was used as the sole source of dietary AA in 1 diet. A N-free diet was used to estimate basal endogenous losses of AA. Ten growing barrows with an initial BW of 25.3 ± 2.0 kg were individually fitted with a T-cannula in the distal ileum. Pigs were allotted to treatments in a replicated 5 × 5 balanced Latin square design with 5 diets and 5 periods. Each period lasted 7 d, and ileal digesta were collected on d 6 and 7 of each period. Results of the experiment indicated that the apparent ileal digestibility and SID of CP and all AA decreased linearly ($P < 0.01$) as the time of autoclaving increased from 0 to 30 min. The concentration of furosine and the color of samples of SBM indicated that autoclaving resulted in a Maillard reaction in the SBM. However, oven drying at 125°C for 30 min did not change ($P > 0.10$) the SID of CP and AA in the SBM or the furosine concentration, and the color in the oven-dried sample indicated that this sample was not heat damaged. In conclusion, the digestibility of all AA in autoclaved SBM is linearly reduced as the autoclaving time increases from 0 to 30 min. The reason for these changes is most likely that autoclaving at 125°C results in Maillard reactions in SBM.

Key words: amino acid, digestibility, heat, Maillard reaction, pig, soybean meal

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INTRODUCTION

Raw soybeans contain antinutritional factors, such as protease inhibitors, that reduce animal performance (Baker, 2000). Heating improves the nutritional value of soybeans and soybean meal (**SBM**) because it denatures the native protein structure and destroys trypsin inhibitors and other antinutritional factors that may be present in raw soybeans (Liener, 1994; Purushotham et al., 2007; Goebel and Stein, 2011). However, excessive heat treatment may result in the destruction of AA and the formation of Maillard reaction products that are biologically unavailable (Ford, 1973; Pahm et al., 2008). Maillard reaction involves condensation of the NH_2 group of an AA with a reducing sugar, and among all AA, Lys is the most susceptible to the Maillard reaction because it contains an exposed ε-NH_2 group that reacts with the carbonyl group of a reducing sugar (Pahm et al., 2008). The condensation product that is formed during this reaction is converted into Schiff bases, and a deoxyketosyl compound is formed after Amadori rearrangement. The deoxyketosyl compound is the major form of blocked Lys after the early Maillard reaction, and it is biologically unavailable (Hurrell and Carpenter, 1981). Further heating of the SBM may produce advanced Maillard reaction products, such as premelanoidins, that can also react with other AA, which may also make these AA unavailable (Ford, 1973; Hurrell, 1990).

Thus, the degree and type of heat treatment can affect the apparent ileal digestibility (**AID**) of AA in SBM (Chang et al., 1987). However, no information is available on how the heating time influences the AID of AA in SBM, and to our knowledge, no information is available on how the heat treatment of SBM affects the standardized ileal digestibility (**SID**) of AA. There-

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fore, the objective of this experiment was to test the hypothesis that the type of heat and the time that heat is applied will affect the AID and SID of AA in SBM by growing pigs.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. All pigs used in the experiment were Landrace (3/4) × Large White (1/4) crossbred barrows (Genetiporc, Alexandria, MN).

Animals and Housing

Ten growing barrows with an initial BW of 25.3 ± 2.0 kg were used. Pigs were surgically fitted individually with a T-cannula in the distal ileum, using procedures adapted from Stein et al. (1998). Animals were randomly allotted to treatments in a replicated 5 × 5 Latin square design, using a spreadsheet-based program to balance potential residual effects (Kim and Stein, 2009). There were 5 diets and 5 periods in each square. Pigs were individually housed in pens (1.2 × 1.5 m) in an environmentally controlled room. Each pen was equipped with a feeder and a nipple drinker and had a fully slatted Tri-Bar steel floor.

Diets and Feeding

Conventional, dehulled SBM (Zeeland Farm Services, Zeeland, MI) was divided into 4 batches that were not heated, autoclaved at 125°C for 15 min, autoclaved at 125°C for 30 min, or oven-dried at 125°C for 30 min (Table 1). Five diets were prepared (Tables 2 and 3). The 4 batches of SBM were each used in a SBM- and cornstarch-based diet as the sole source of AA, and a N-free diet was also formulated to estimate basal endogenous losses of CP and AA. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates for growing pigs (NRC, 1998), and all diets contained 0.4% chromic oxide as an indigestible marker.

Feed was provided at 3 times the maintenance energy requirement (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998), and equal meals were provided at 0800 and 1700 h. The feed allowance for each pig was adjusted at the beginning of each period when the BW of each pig was

Table 1. Chemical composition of soybean meal after heat treatment

	Soybean meal			
Item[1]	Not heated	Autoclaved at 125°C for 15 min	Autoclaved at 125°C for 30 min	Oven dried at 125°C for 30 min
DM, %	88.1	87.6	86.2	88.8
Ash, %	5.91	5.87	5.85	5.97
AEE, %	1.44	1.21	1.35	1.31
CP, %	48.5	49.2	48.3	49.1
Furosine, %	0.015	0.023	0.026	0.016
Lys:CP ratio,[2] %	6.29	5.75	5.57	6.25
L*	77.7	61.8	52.5	77.4
a*	3.4	10.0	12.5	2.9
b*	30.4	29.1	30.5	30.9
Indispensable AA, %				
Arg	3.64	3.53	3.40	3.68
His	1.31	1.30	1.28	1.32
Ile	2.22	2.26	2.23	2.24
Leu	3.74	3.78	3.75	3.79
Lys	3.05	2.83	2.69	3.07
Met	0.66	0.68	0.66	0.68
Phe	2.49	2.51	2.48	2.52
Thr	1.89	1.90	1.90	1.92
Trp	0.65	0.65	0.65	0.67
Val	2.34	2.39	2.32	2.37
All indispensable AA	21.99	21.83	21.36	22.26
Dispensable AA, %				
Ala	2.12	2.14	2.12	2.15
Asp	5.57	5.61	5.54	5.64
Cys	0.71	0.65	0.62	0.73
Glu	8.95	9.03	8.91	9.08
Gly	2.06	2.07	2.05	2.09
Pro	2.44	2.51	2.51	2.50
Ser	2.44	2.44	2.44	2.48
All dispensable AA	24.29	24.45	24.19	24.67

[1]AEE = acid-hydrolyzed ether extract; L* = lightness; a* = redness; b* = yellowness.

[2]The Lys:CP ratio was calculated by expressing the concentration of Lys in each sample as a percentage of the concentration of CP (Stein et al., 2009).

Table 2. Ingredient composition of experimental diets, as-fed basis

	Soybean meal diet				
Ingredient, %	Not heated	Autoclaved at 125°C for 15 min	Autoclaved at 125°C for 30 min	Oven dried at 125°C for 30 min	N-free diet
Soybean meal (48% CP)	40.00	40.00	40.00	40.00	—
Soybean oil	3.00	3.00	3.00	3.00	4.00
Solka Floc[1]	—	—	—	—	4.00
Monocalcium phosphate	1.10	1.10	1.10	1.10	1.50
Ground limestone	0.75	0.75	0.75	0.75	0.80
Sucrose	15.00	15.00	15.00	15.00	15.00
Chromic oxide	0.40	0.40	0.40	0.40	0.40
Cornstarch	39.05	39.05	39.05	39.05	73.10
Magnesium oxide	—	—	—	—	0.10
Potassium carbonate	—	—	—	—	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30	0.30

[1]Fiber Sales and Development Corp., Urbana, OH.

[2]Supplied the following per kilogram of complete diet: vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamine, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 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.

recorded. Animals had access to water throughout the experiment.

Sample Collection

Each period lasted 7 d. The initial 5 d was a period of adaptation to the diet, and ileal digesta samples were collected for 8 h on d 6 and 7. A plastic bag was attached to the cannula barrel with a cable tie, 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 all samples were stored at −20°C to prevent bacterial degradation of AA in the digesta.

Table 3. Chemical composition of experimental diets, as-fed basis

	Soybean meal diet				
Item	Not heated	Autoclaved at 125°C for 15 min	Autoclaved at 125°C for 30 min	Oven dried at 125°C for 30 min	N-free diet
DM, %	89.8	89.9	89.3	90.9	90.4
Ash, %	4.76	4.81	4.97	4.81	3.03
AEE,[1] %	3.87	3.09	3.43	2.98	3.05
CP, %	21.9	21.0	21.3	21.7	0.3
Indispensable AA, %					
Arg	1.40	1.44	1.32	1.38	—
His	0.49	0.51	0.48	0.48	—
Ile	0.87	0.92	0.89	0.88	—
Leu	1.46	1.55	1.49	1.44	—
Lys	1.19	1.18	1.06	1.17	—
Met	0.26	0.26	0.24	0.26	—
Phe	0.96	1.02	0.97	0.95	—
Thr	0.75	0.80	0.77	0.73	—
Trp	0.25	0.26	0.24	0.24	<0.02
Val	0.90	0.95	0.92	0.92	—
All indispensable AA	8.53	8.89	8.38	8.45	—
Dispensable AA, %					
Ala	0.84	0.89	0.85	0.82	—
Asp	2.18	2.31	2.21	2.14	—
Cys	0.28	0.27	0.24	0.27	—
Glu	3.41	3.62	3.48	3.35	—
Gly	0.81	0.86	0.82	0.79	—
Pro	0.96	1.04	0.98	0.94	—
Ser	0.97	1.03	0.98	0.91	—
All dispensable AA	9.45	10.02	9.56	9.22	—

[1]AEE = acid-hydrolyzed ether extract.

Chemical Analysis

At the conclusion of the experiment, ileal samples were thawed at room temperature and mixed within animal and diet, and 2 subsamples were collected for chemical analysis. Ileal digesta samples were lyophilized and finely ground before chemical analysis.

The 4 SBM samples, all diets, and all digesta samples were analyzed for DM (method 930.15; AOAC International, 2007) and CP (method 990.03; AOAC International, 2007). Diets and SBM samples were also analyzed for ash (method 942.05; AOAC International, 2007) and acid-hydrolyzed ether extract (method 954.02; AOAC International, 2007). Amino acids were analyzed in all samples by using ninhydrin for post-column derivatization and norleucine as the internal standard (Hitachi Amino Acid Analyzer Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA). Before analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (method 982.30 E; AOAC International, 2007). Methionine and Cys were analyzed as Met sulfone and cysteic acid, respectively, after cold performic acid oxidation overnight before hydrolysis. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C. Cromium concentrations of the diets and ileal digesta samples were measured by using inductively coupled plasma atomic emission spectrometry (method 990.08; AOAC International, 2007) after nitric acid-perchloric acid wet ash sample preparation (method 968.088D; AOAC International, 2007).

The concentrations of furosine in the 4 batches of SBM were analyzed after 6 *N* HCl hydrolysis on a reversed-phase HPLC instrument with gradient mobile phases (0.1% trifluoroacetic acid in deionized water for mobile phase A and 0.1% trifluoroacetic acid in methanol for mobile phase B), and were detected by tandem mass spectrometry in the multiple-reaction monitoring operation mode. Quantification was performed based on an external calibration with 5 standards made of furosine dihydrochloride (NeoMPS, Neosystems Laboratory, Strasbourg, France).

The color of each batch of SBM was determined by measuring L* (lightness), a* (redness), and b* yellowness) values (Hunter Lab Miniscan XE Plus apparaturs, Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA) with illuminant D65 and a 10° observer. The spectrocolorimeter was calibrated using a black and a white tile, as described by Holmer et al. (2009).

Calculations and Statistical Analysis

The Lys:CP ratio for each batch of SBM was calculated by expressing the concentration of Lys in the sample as a percentage of the CP in the sample. Values for AID and SID of each batch of SBM were also calculated (Stein et al., 2007). The UNIVARIATE procedure (SAS Inst. Inc., Cary, NC) was used to identify outliers, but no outliers were removed from the data. Data were analyzed using the MIXED procedure of SAS. The model included diet as the fixed effect and period and animal as random effects. Means were separated using the PDIFF option with the Tukey adjustment. The means separation output was converted to letter groupings by using an SAS macro program (Saxton, 1998). Orthogonal polynomial contrasts were used to test for linear and quadratic effects of autoclaving time on AID and SID values. The animal was the experimental unit for all calculations, and an α level of 0.05 was used to assess differences among means.

RESULTS

The concentrations of DM, ash, and CP were similar in all 4 samples of SBM regardless of the form of heat treatment to which the meal was subjected (Table 1). However, the concentrations of Arg, Lys, and Cys were 3.64, 3.05, and 0.71% in the unheated sample, but only 3.53, 2.83, and 0.65% in the sample that was autoclaved for 15 min and 3.40, 2.69, and 0.62% in the sample that was autoclaved for 30 min. The Lys:CP ratio was 6.29% in the unheated sample but was 5.75 and 5.57% in the samples that were autoclaved for 15 and 30 min, respectively. The L^* values were 77.7, 61.8, and 52.5, and the a^* values were 3.4, 10.0, and 12.5 in the SBM that was unheated, autoclaved for 15 min, and autoclaved for 30 min, respectively. These changes indicated that autoclaving resulted in darker and redder colors, which could also be observed by visual assessment of the samples (Figure 1). Values for Lys, Cys, furosine, Lys:CP ratio, and L^* and a^* that were measured in the oven-dried sample were close to the values measured in the sample that was not heated.

All 4 diets had similar concentrations of DM, ash, and CP (Table 3). The concentrations of Lys and Cys were, however, only 1.06 and 0.24% in the diet containing the SBM that was autoclaved for 30 min, whereas the concentration of Lys was between 1.17 and 1.19% and the concentration of Cys was between 0.27 or 0.28% in the other diets.

The AID and SID of CP and all AA in SBM decreased linearly ($P < 0.01$) as the autoclaving time increased from 0 to 30 min (Tables 4 and 5). The greatest reductions were observed for Lys and Asp. Although the AID and SID for most AA were numerically reduced in oven-dried SBM compared with nonheated SBM, none of these reductions were statistically significant.

DISCUSSION

The nutrient composition of the unheated SBM agrees with published values (NRC, 1998; Cervantes-Pahm and Stein, 2008; Baker and Stein, 2009). The reductions in the concentration of Lys and Cys in autoclaved SBM that were observed in this experiment support the data by Fontaine et al. (2007), who reported that the concentrations of Lys and Cys in autoclaved soy products and distillers dried grains with solubles (**DDGS**) were reduced compared with samples that were not

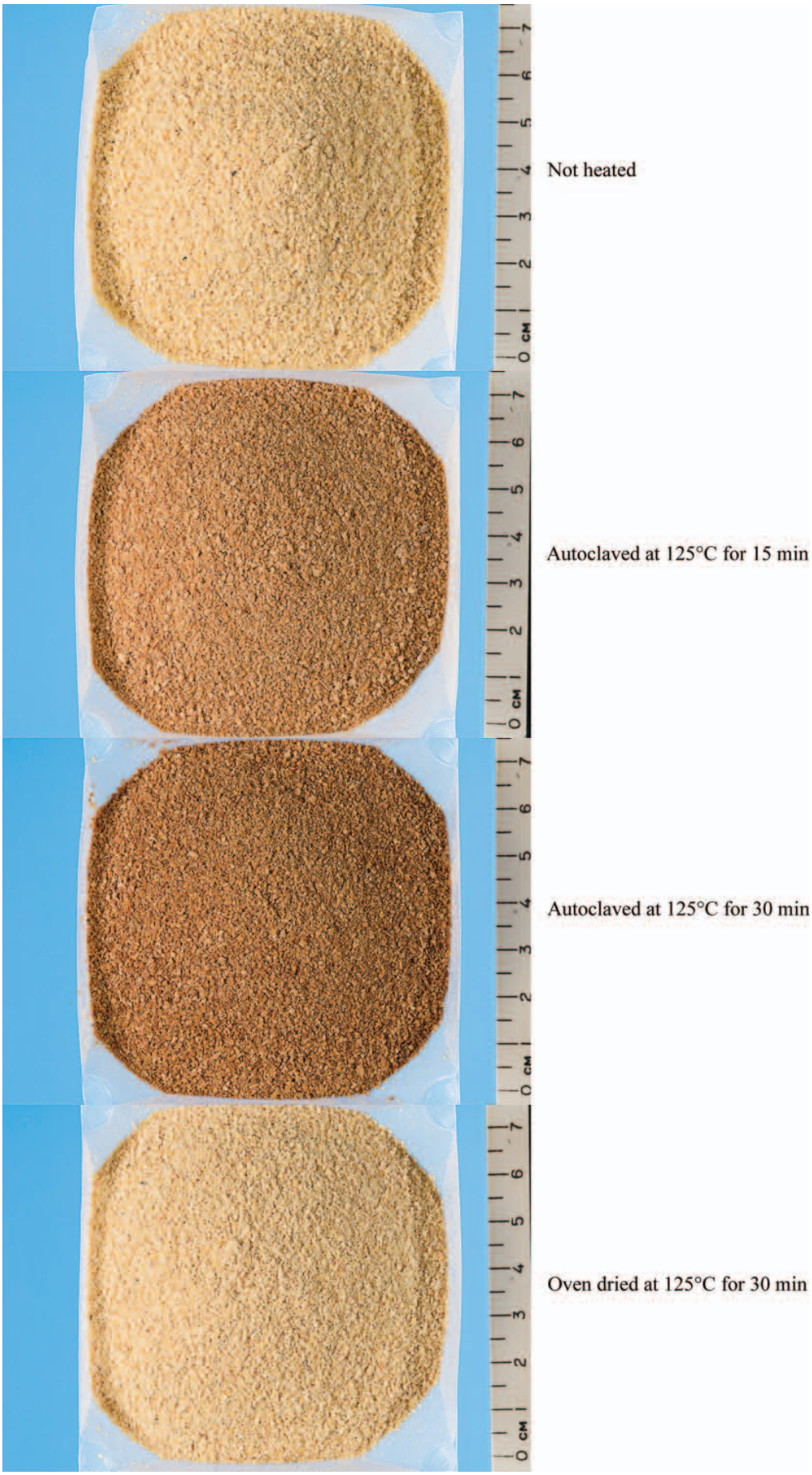

Figure 1. Soybean meal subjected to different thermal treatments.

Table 4. Apparent ileal digestibility by growing pigs of CP and AA in soybean meal subjected to different thermal treatments[1]

	Soybean meal					*P*-value[2]		
Item	Not heated	Autoclaved at 125°C for 15 min	Autoclaved at 125°C for 30 min	Oven dried at 125°C for 30 min	SEM	Diet	Linear	Quadratic
CP, %	84.6[a]	80.0[ab]	75.3[b]	82.7[a]	1.5	<0.001	<0.001	0.987
Indispensable AA, %								
Arg	93.2[a]	90.9[a]	87.2[b]	92.7[a]	1.2	<0.001	<0.001	0.378
His	90.1[a]	87.5[a]	83.3[b]	88.2[a]	0.9	<0.001	<0.001	0.139
Ile	89.5[a]	88.3[a]	85.4[b]	88.5[a]	0.9	0.002	<0.001	0.227
Leu	89.3[a]	88.5[ab]	85.7[b]	87.8[ab]	1.0	0.013	<0.001	0.154
Lys	90.5[a]	86.8[b]	81.4[c]	88.8[ab]	1.2	<0.001	<0.001	0.311
Met	90.5[a]	88.4[a]	85.3[b]	89.6[a]	0.9	<0.001	<0.001	0.518
Phe	88.1[a]	87.7[a]	84.7[b]	86.6[ab]	0.9	0.019	<0.001	0.059
Thr	83.1[a]	81.3[ab]	77.5[b]	79.6[ab]	1.5	0.011	<0.001	0.244
Trp	85.7[a]	83.0[a]	78.4[b]	82.9[a]	1.4	0.001	<0.001	0.284
Val	87.0[a]	85.7[a]	82.4[b]	85.8[a]	1.1	0.004	<0.001	0.185
Mean	89.2[a]	87.4[a]	83.8[b]	87.7[a]	1.0	<0.001	<0.001	0.153
Dispensable AA, %								
Ala	83.4[a]	81.9[ab]	77.3[b]	81.1[ab]	1.6	0.026	<0.001	0.104
Asp	88.8[a]	84.2[b]	77.9[c]	86.5[ab]	1.2	<0.001	<0.001	0.266
Cys	82.9[a]	76.5[a]	67.2[b]	77.4[a]	2.1	<0.001	<0.001	0.244
Glu	90.7[a]	88.6[a]	84.7[b]	88.6[a]	1.0	<0.001	<0.001	0.208
Gly	73.7[a]	68.2[a]	56.8[b]	69.4[a]	3.7	0.003	<0.001	0.252
Pro	41.6[a]	31.2[a]	−3.0[b]	41.9[a]	14.4	0.004	0.002	0.258
Ser	87.3[a]	85.5[a]	81.4[b]	84.0[ab]	1.2	0.001	<0.001	0.100
Mean	82.6[a]	78.7[a]	70.3[b]	80.3[a]	2.3	<0.001	<0.001	0.151

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Each least squares means represents 10 observations.

[2]Linear and quadratic effects of time of autoclaving.

autoclaved. The reductions in Arg concentrations in the autoclaved samples of SBM are in agreement with data for wheat DDGS, for which heating also reduced the concentration of Arg (Cozannet et al., 2010). The concentrations of other AA were, however, not influenced by heat treatment, which is also in agreement with the results of Fontaine et al. (2007). Reductions in the concentrations of Lys and Cys in heat-treated protein sources have been observed in other experiments (Bjarnason and Carpenter, 1970; Shirley and Parsons, 2000; Karr-Lilienthal et al., 2005). These reductions may be due to advanced Maillard reactions, which is consistent with the development of the brown color in the autoclaved SBM. Lysine is the AA most affected by the Maillard reaction because of a reaction between the ε-NH_2 group of Lys and the carbonyl group of a reducing sugar. Losses in the concentration of Cys may be due to the formation of cross-linked compounds that are formed during an advanced Maillard reaction (Van Barneveld et al., 1994a; Gerrard, 2002).

The values for SID of most AA in the nonheated SBM agree with values reported previously (NRC, 1998; Cervantes-Pahm and Stein, 2008; Kim et al., 2009; Goebel and Stein, 2011). The reductions in the AID and SID of CP and AA in SBM as the time of autoclaving increased from 0 to 30 min are in agreement with the results of Moughan and Rutherfurd (1996), who reported that the digestibility of all AA decreased by autoclaving a diet based on lactose and casein at 121°C for 3.5 min. Martinez-Amezcua et al. (2007) also observed that autoclaving DDGS for 45 min at 120°C reduced AA digestibility in poultry.

During advanced Maillard reactions, some cross-linkages are established between Lys and other AA and polypeptide chains within the protein, which may reduce the efficiency of proteolytic enzymes (Moughan and Rutherfurd, 1996; Martinez-Amezcua et al., 2007). In advanced Maillard reactions, the formation of a deoxyketosyl compound continues toward the formation of brown pigments or melanoidins, which is likely the reason for the color change that was observed in autoclaved SBM. Melanoidins may also block absorption sites because of steric hindrances and may reduce the digestibility of AA and peptides (Hurrell, 1990), which may have contributed to the reduction in SID of all AA that was observed.

Reduced Lys digestibility by pigs or poultry resulting from Maillard reactions was reported previously for SBM (Chang et al., 1987; Fontaine et al., 2007; Boucher et al., 2009a), corn DDGS (Cromwell et al., 1993; Pahm et al., 2008; Boucher et al., 2009b), field peas (Van Barneveld et al., 1994a,b), meat and bone meal (Shirley and Parsons, 2000), and fish meal (Boucher et al., 2009b). The concentration and the digestibility

Table 5. Standardized ileal digestibility by growing pigs of CP and AA in soybean meal subjected to different thermal treatments[1,2]

	Soybean meal					*P*-value[3]		
Item	Not heated	Autoclaved at 125°C for 15 min	Autoclaved at 125°C for 30 min	Oven dried at 125°C for 30 min	SEM	Diet effect	Linear	Quadratic
CP, %	93.1[a]	88.8[a]	84.0[b]	91.4[a]	1.5	<0.001	<0.001	0.777
Indispensable AA, %								
Arg	98.4[a]	96.0[a]	92.6[b]	97.9[a]	1.2	<0.001	<0.001	0.584
His	93.6[a]	90.9[a]	86.9[b]	91.8[a]	0.9	<0.001	<0.001	0.225
Ile	92.3[a]	90.9[a]	88.1[b]	91.3[a]	0.9	0.002	<0.001	0.291
Leu	92.2[a]	91.2[ab]	88.6[b]	90.8[ab]	1.0	0.012	<0.001	0.212
Lys	93.0[a]	89.3[b]	84.2[c]	91.3[ab]	1.2	<0.001	<0.001	0.381
Met	93.2[a]	91.1[a]	88.3[b]	92.4[a]	0.9	<0.001	<0.001	0.619
Phe	91.7[a]	91.1[ab]	88.2[b]	90.3[ab]	0.9	0.021	<0.001	0.095
Thr	89.2[a]	87.1[ab]	83.5[b]	86.1[ab]	1.5	0.010	<0.001	0.385
Trp	90.9[a]	88.0[ab]	83.8[b]	88.4[a]	1.4	0.001	<0.001	0.452
Val	91.0[a]	89.5[a]	86.3[b]	89.8[a]	1.1	0.003	<0.001	0.257
Mean	93.0[a]	91.0[a]	87.6[b]	91.6[a]	1.0	<0.001	<0.001	0.239
Dispensable AA, %								
Ala	90.6[a]	88.7[ab]	84.4[b]	88.5[ab]	1.6	0.025	<0.001	0.193
Asp	91.7[a]	86.9[b]	80.7[c]	89.5[ab]	1.2	<0.001	<0.001	0.345
Cys	88.7[a]	82.6[a]	74.0[b]	83.5[a]	2.1	<0.001	<0.001	0.327
Glu	93.1[a]	90.9[a]	87.0[b]	91.0[a]	1.0	<0.001	<0.001	0.261
Gly	98.2[a]	91.2[ab]	80.9[b]	94.8[a]	3.7	0.002	<0.001	0.490
Pro	121.2[a]	104.8[ab]	74.5[b]	124.2[a]	14.4	0.002	0.001	0.506
Ser	92.1[a]	90.1[a]	86.2[b]	89.2[ab]	1.2	0.002	<0.001	0.180
Mean	95.6[a]	90.9[a]	83.1[b]	93.7[a]	2.3	<0.001	<0.001	0.292

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Each least squares means represents 10 observations.

[2]Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses. Basal endogenous losses were determined using pigs fed the N-free diet as (g/kg of DMI): CP, 20.7; Arg, 0.80; His, 0.19; Ile, 0.27; Leu, 0.47; Lys, 0.33; Met, 0.08; Phe, 0.39; Thr, 0.52; Trp, 0.14; Val, 0.40; Ala, 0.67; Asp, 0.70; Cys, 0.18; Glu, 0.89; Gly, 2.21; Pro, 8.51; and Ser, 0.52.

[3]Linear and quadratic effects of time of autoclaving.

of Lys in oven-dried DDGS were reduced at a drying temperature of 100°C (Martinez-Amezcua and Parsons, 2007; Pahm et al., 2008), but in the present experiment, oven drying at 125°C for 30 min had minimal effects on the SID of AA in SBM. The difference between DDGS and SBM in the effect of oven drying may be a result of the thermal stress that DDGS undergoes during production and the greater sugar concentration in DDGS than in SBM (Fontaine et al., 2007). Thus, it seems that the temperature at which a feed ingredient will be heat damaged depends not only on the type of heat that is applied and the length of heating, but also on the feed ingredient that is being heated.

Autoclaving is associated with pressure, moisture, and high temperature, whereas oven drying is only a thermal treatment. The greater effects of autoclaving on AID and SID of all AA than of oven drying are, therefore, probably due to the pressure and moisture that are associated with autoclaving. Amino acids are less stable at greater pressure (Qian et al., 1993), and the rate of reaction between the amino group of AA and glucose increases if the humidity is increased (Schwartz and Lea, 1952). The development of Maillard reactions depends on water activity, temperature, pH, time of heating, and the type and availability of the reactants (Rufián-Henares et al., 2009; Jaeger et al., 2010). Intermediate moisture content, temperatures above 50°C, and pH between 4 and 7 are favorable conditions for Maillard reactions (Ramíres-Jiménez et al., 2001).

Development of a brown color is indicative of the formation of advanced Maillard products (melanoidins), and a brown color was observed in the autoclaved SBM. Recently, it was reported that a brown color was associated with a reduced SID of Lys in wheat DDGS (Cozannet et al., 2010). However, oven-dried SBM had a lighter color, which indicates no development of advanced Maillard reaction products (Hurrell, 1990). It is likely that the moisture content was too low in the oven-dried product for developing Maillard reactions.

Furosine is a breakdown product that is produced as Amadori products are acid hydrolyzed, and it is believed that approximately 32% of all the blocked Lys in a feed ingredient will be converted to furosine during acid hydrolysis (Pahm et al., 2008). It is, therefore, possible to calculate the total concentration of blocked Lys in a feed ingredient if the concentration of furosine is measured (Pahm et al., 2008). The furosine procedure has been used extensively to measure heat damage in dried milk products (Campos Giménez et al., 2004), but this procedure has also been used successfully to

estimate the degree of heat damage in DDGS (Pahm et al., 2008; Cozannet et al., 2010). To our knowledge, there are no previous data on the concentration of furosine in SBM, but the current results indicate that the concentration of furosine in SBM increases as SBM is heat damaged. It is likely, based on this observation, that the furosine procedure may be used to estimate heat damage in SBM.

The degree of heat damage in a feed ingredient may also be estimated by calculating the Lys:CP ratio because the concentration of Lys, but not the concentration of CP, is reduced if samples are heat damaged (Stein et al., 2009). The values for the Lys:CP ratio in SBM decreased as the autoclaving time increased, whereas the Lys:CP ratio in the oven-dried SBM was similar to that in the unheated SBM. This observation further indicates that the autoclaved samples, but not the oven-dried sample, were heat damaged; therefore, the values for the Lys:CP ratio support the conclusions from the values for furosine and the color measurements. Reduced Lys:CP ratios have previously been reported for heat-damaged DDGS (Stein et al., 2009; Cozannet et al., 2010), but to our knowledge, this is the first time it has been shown that the Lys:CP ratio decreases as SBM is heat damaged. Thus, calculation of the Lys:CP ratio is a relatively quick method to estimate if a given source of SBM is heat damaged, and this procedure may potentially be used by the soybean crushing industry and by the feed industry to evaluate the quality of SBM.

In conclusion, autoclaving of SBM at 125°C, which applies both moisture and pressure to the sample, reduces concentrations of Arg, Lys, and Cys, and also reduces the AID and SID of AA. Increasing the autoclaving time increases the negative effects of autoclaving. In contrast, oven drying at 125°C for 30 min does not substantially reduce the concentration and digestibility of AA in SBM. Thus, heat treatment of SBM should be optimized to prevent reducing the digestibility of AA. Color measurements, analysis for furosine concentrations, and calculations of the Lys:CP ratio are procedures that may be used to assess the degree of heat damage in SBM.

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