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# Concentrations of digestible and metabolizable energy and amino acid digestibility by growing pigs may be reduced by autoclaving soybean meal

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ABSTRACT

Two experiments were conducted to test the hypothesis that both the degree of heating and the time heat is applied will affect standardized ileal digestibility (SID) of amino acids (AA), and concentrations of digestible energy (DE) and metabolizable energy (ME) in soybean meal (SBM) fed to growing pigs. One source of SBM was divided into 9 batches and used in 2 experiments. One batch was not autoclaved and considered the control. Two batches were autoclaved at 110 °C for 15 or 30 min and 6 batches were autoclaved at 150 °C for 3, 6, 9, 12, 15, or 18 min. In Exp. 1, a corn-based diet and 9 diets based on corn and each source of SBM were fed to 20 barrows (43.6 kg) that were used in a replicated 10 × 4 Youden square design. Urine and fecal samples were collected for 5 days after 7 days of adaptation. In Exp. 2, nine cornstarch-based diets included 400 g/kg of each of the 9 sources of SBM. An N-free diet was also used. Ten growing barrows (36.8 kg) with a T-cannula installed in the distal ileum were allotted to a 10 × 7 Youden square design with 10 diets and 7 periods. Ileal digesta were collected on d 6 and 7 of each 7-d period. Results from the experiments indicated that the ATTD of GE, and the DE and ME, the SID of crude protein (CP) and all AA were less ($P < 0.001$) if SBM was autoclaved at 150 °C than at 110 °C. The ATTD of GE, the DE and ME, and the SID of CP and AA were not affected by increasing duration of autoclaving at 110 °C, but there were linear reductions ($P < 0.001$) in ATTD of GE, in DE and ME, and in SID of all AA if duration of autoclaving was increased at 150 °C. These observations demonstrate that over-heating of SBM not only results in reduced SID of AA, but ATTD of GE will also be impaired with a subsequent reduction in concentrations of DE and ME.

## 1. Introduction

Heating improves the nutritional value of soybeans and soybean meal (SBM) because it inactivates the trypsin inhibitors and other anti-nutritional factors that may be present in raw soybeans (González-Vega et al., 2011; Almeida et al., 2014a). As a consequence, all soybean products need to be heat treated prior to being fed to monogastric animals. In commercial production of SBM the heat

*Abbreviations:* AA, amino acids; AEE, acid-hydrolyzed ether extract; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; CP, crude protein; DE, digestible energy; DM, dry matter; GE, gross energy; ME, metabolizable energy; SBM, soybean meal; SID, standardized ileal digestibility

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treatment happens via the de-solventizing toasting process that follows the hexane extraction and in the production of soybean expellers, heat is usually applied via extrusion. However, overheating may have a negative impact on the stability of nutrients, especially amino acids (AA), because Maillard reactions may occur if heat and moisture are applied to ingredients containing AA and a reducing sugar (Almeida et al., 2014b). During processing, polyphenols and fat and their oxidation products may also react with AA (Hurrell and Carpenter, 1981). Consequently, these reactions result in a decrease in the concentration and digestibility of AA, and Lys is the AA that is most susceptible to the Maillard reaction (Pahm et al., 2008; González-Vega et al., 2011; Almeida et al., 2014b).

Overheating of a feed ingredient may also increase analyzed concentrations of acid detergent fiber (ADF) and neutral detergent acid (NDF) presumably because some of the sugar-AA complexes and advanced Mailard reaction products are analyzed as ADF and NDF (Almeida et al., 2014a). If sugars, other carbohydrates, and AA are made indigestible by excess heating, it is possible that energy digestibility will also be reduced. However, no information about how heating may influence energy digestibility and concentrations of DE and ME of soybean meal has been reported. Therefore, the objective of this experiment was to test the hypothesis that the degree of heating and the time that heat is applied will affect concentration of digestible energy (DE), metabolizble energy (ME), and the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA in SBM fed to growing pigs.

## 2. Materials and methods

Two experiments were conducted and the Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for both experiments. Both experiments were conducted at the Swine Research Center at the University of Illinois at Urbana-Champaign, USA. In both experiments, castrates that were the offspring of Line 359 males mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used. The SBM used in both experiments originated from one single source (ADM Ölmühle Hamburg AG, Hamburg, Germany), which was divided into 9 batches. One batch was not autoclaved and served as the control. Two batches were autoclaved for 15 or 30 min at 110 °C and 6 batches were autoclaved for 3, 6, 9, 12, 15,

**Table 1**
Analyzed nutrient composition of autoclaved soybean meal[1].

| Item[2] | SBM[3] | Autoclaving | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 110 °C | | 150 °C | | | | | |
| Duration (min): | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 |
| Gross energy, MJ/kg | 17.0 | 17.4 | 17.3 | 17.1 | 17.3 | 17.4 | 17.5 | 17.3 | 17.7 |
| Dry matter (DM), g/kg | 879 | 892 | 892 | 886 | 888 | 894 | 894 | 887 | 896 |
| Crude protein, g/kg | 474.4 | 476.4 | 479.8 | 478.1 | 474.5 | 476.6 | 467.5 | 479.5 | 481.4 |
| Acid-hydrolyzed ether extract, g/kg | 9.6 | 10.1 | 11.6 | 13.1 | 10.1 | 11.4 | 14.1 | 13.5 | 11.6 |
| Insoluble dietary fiber, g/kg | 180.0 | 163.0 | 159.0 | 108.0 | 95.0 | 87.0 | 78.0 | 72.0 | 83.0 |
| Soluble dietary fiber, g/kg | 8.0 | 7.0 | 13.0 | 59.0 | 85.0 | 98.0 | 97.0 | 102.0 | 106.0 |
| Total dietary fiber, g/kg | 188.0 | 170.0 | 172.0 | 167.0 | 180.0 | 185.0 | 175.0 | 174 | 189.0 |
| Ash, g/kg | 64.7 | 67 | 69.2 | 71 | 66.5 | 71.1 | 73.2 | 72.3 | 73.4 |
| Carbohydrates, g/kg | | | | | | | | | |
| Glucose | – | – | – | – | 0.6 | 0.8 | 0.9 | 1.2 | 1.4 |
| Sucrose | 73.4 | 71.5 | 68.8 | 66.9 | 63.4 | 55.7 | 46.8 | 40.1 | 31.0 |
| Fructose | 0.6 | 0.8 | 1.0 | 2.4 | 3.2 | 4.0 | 4.7 | 5.7 | 6.3 |
| Stachyose | 56.6 | 56.4 | 54.9 | 50.3 | 48.3 | 42.7 | 37.6 | 32.7 | 25.5 |
| Raffinose | 16.0 | 15.4 | 15.8 | 14.5 | 13.8 | 12.0 | 10.4 | 9.0 | 6.9 |
| Indispensable amino acids, g/kg | | | | | | | | | |
| Arg | 34.4 | 35.1 | 34.6 | 32.0 | 30.2 | 29.0 | 27.9 | 25.6 | 23.5 |
| His | 12.0 | 12.2 | 12.1 | 11.8 | 12.5 | 12.7 | 12.8 | 12.8 | 12.6 |
| Ile | 20.5 | 20.8 | 20.7 | 21.1 | 21.2 | 21.6 | 21.4 | 21.6 | 21.4 |
| Leu | 35.2 | 36.1 | 36.0 | 36.0 | 36.0 | 36.5 | 36.5 | 36.7 | 36.2 |
| Lys | 28.6 | 28.9 | 28.5 | 26.0 | 24.1 | 22.7 | 21.8 | 20.1 | 19.1 |
| Met | 6.0 | 6.1 | 6.1 | 6.0 | 6.0 | 6.1 | 6.2 | 6.1 | 6.0 |
| Phe | 23.5 | 24.3 | 24.3 | 24.0 | 23.5 | 24.0 | 23.9 | 24.0 | 23.7 |
| Thr | 17.4 | 18.0 | 17.8 | 17.7 | 18.0 | 18.4 | 18.3 | 18.3 | 17.9 |
| Trp | 6.3 | 6.5 | 6.4 | 6.4 | 6.2 | 6.3 | 6.2 | 6.2 | 6.1 |
| Val | 21.5 | 21.9 | 21.8 | 21.8 | 22.0 | 22.1 | 22.1 | 22.4 | 22.0 |
| Dispensable amino acids, g/kg | | | | | | | | | |
| Ala | 19.7 | 20.2 | 20.2 | 19.8 | 20.2 | 20.8 | 20.7 | 20.9 | 20.7 |
| Asp | 52.2 | 53.5 | 53.1 | 51.4 | 52.8 | 53.7 | 53.5 | 53.0 | 52.3 |
| Cys | 6.7 | 6.7 | 6.5 | 5.4 | 4.6 | 4.3 | 4.1 | 3.7 | 3.4 |
| Glu | 82.8 | 85.3 | 85.1 | 83.2 | 83.8 | 85.8 | 86.1 | 86.6 | 85.5 |
| Gly | 19.4 | 19.7 | 19.7 | 19.3 | 19.8 | 20.2 | 20.2 | 20.2 | 20.2 |
| Pro | 21.3 | 21.5 | 22.1 | 21.3 | 22.6 | 22.9 | 24.2 | 24.0 | 24.1 |
| Ser | 23.1 | 23.7 | 23.6 | 22.9 | 23.3 | 23.6 | 23.7 | 23.4 | 23.1 |
| Lys:CP, g/kg | 60.3 | 60.7 | 59.5 | 54.4 | 50.6 | 47.6 | 46.6 | 41.9 | 39.7 |

[1] All values except DM were adjusted to 880 g/kg of DM.
[2] Corn used in Exp 1, was analyzed to contain 869 g/kg of DM, and 16.1 MJ/kg of gross energy.
[3] Conventional soybean meal that was not autoclaved.

**Table 2**
Composition of experimental diets containing corn or corn and soybean meal, as-fed basis, Exp. 1.

| Item, g/kg | Diet | |
|---|---|---|
| | Basal | Soybean meal |
| Corn | 970.0 | 712.5 |
| Soybean meal | – | 260.0 |
| Ground limestone | 8.0 | 10.0 |
| Monocalcium phosphate | 15.0 | 10.5 |
| Sodium chloride | 4.0 | 4.0 |
| Vitamin-mineral premix[1] | 3.0 | 3.0 |
| Analyzed values | | |
| Gross energy, MJ/kg | 15.6 | 15.8[2] |
| Dry matter | 870 | 870[3] |

[1] Provided the following quantities of vitamins and micro minerals per kg of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin $D_3$ as cholecalciferol, 2,208 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin $B_{12}$, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[2] The gross energy value is for the diet containing the control soybean meal that was not autoclaved. Gross energy in diets containing soybean meal that was autoclaved at 110 °C (15 or 30 min) and 150 °C (3, 6, 9, 12, 15, or 18 min) was 15.9, 15.9, 15.8, 15.9, 16.0, 16.0, 16.0, and 16.0 MJ/kg, respectively.

[3] Dry matter value is for the diet containing the control soybean meal that was not autoclaved.

or 18 min at 150 °C (Table 1). These times and temperatures were chosen to mimic the temperatures that are usually used in commercial production of SBM during the de-solventizing and drying-cooling processes.

Autoclaving of the different batches of SBM was accomplished using a belt-autoclave (Hydrothermische Kochanlage, Amandus Kahl GmbH & Co, Reinbek, Germany), which continuously processed the material. The autoclave has a double valve for inlet and a double valve for outlet, so that pressure and temperature can be maintained, although new product is continuously entering and processed material is continuously leaving the autoclave. The layer inside the autoclave was 5–7 cm thick. Before entering the autoclave, SBM was heated to approximately 100 °C by steam conditioning. After autoclaving, the SBM entered a belt dryer for drying as the moisture content was increased during autoclaving to 190–230 g/kg, but after drying, the SBM had 90 to 100 g/kg moisture and a temperature of approximately 60 °C. Following the belt dryer, SBM was milled to homogenize the material, and it then entered a classical belt dryer/cooler for final product preparation.

### 2.1. Exp. 1. Energy digestibility and concentrations of DE and ME

Experiment 1 was designed to determine the effect of autoclaving on digestibility of energy and concentrations of DE and ME in SBM. A corn-based basal diet, and 9 diets containing corn and each source of SBM were formulated (Table 2). Vitamins and minerals were included in all diets to meet or exceed requirements for growing pigs (NRC, 2012). Twenty castrates (initial body weight: 43.6 ± 2.23 kg) were randomly allotted to the 10 diets in a replicated 10 × 4 Youden square design (Federer and Raghavarao, 1975) with 10 diets and 4 periods. There were 2 pigs per diet in each period for a total of 8 replicate pigs per diet. Pigs were placed in metabolism crates that were equipped with a feeder and a nipple drinker, fully slatted floors, a screen floor, and urine trays.

The amount of feed supplied daily to the pigs was calculated as 3 times the maintenance energy requirement (i.e., 0.82 MJ/kg × body weight $^{\cdot,60}$; NRC, 2012) and divided into 2 equal meals that were fed at 0800 and 1600 h. Diets were provided in a meal form and water was available at all times.

Pigs were fed experimental diets for 14 days. The initial 7 days were considered the adaption period to the diet. Fecal markers were fed on day 8 (5 g/kg chromic oxide) and day 13 (5 g/kg ferric oxide), and fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at −20 °C immediately after collection. Urine collections started on day 8 at 1000 h and ceased on day 13 at 1000 h. Urine buckets were placed under the metabolism crates to permit total collection. Buckets were emptied each morning and a preservative of 50 mL of 6 *N* HCL was added to each bucket when they were emptied. The collected urine was weighed and a 10 % subsample was stored at −20 °C.

After completing sample collections, urine samples were thawed and mixed within animal and diet, and a subsample was collected and lyophilized before analysis for gross energy (GE) as described by Kim et al. (2009). Fecal samples were dried at 65 °C in a forced-air oven and ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific Swedesboro, NJ, USA) before analysis. Diets and ingredient samples (Table 1 and 2) were analyzed for dry matter (DM; Method 930.15; AOAC Int., 2007) and GE was

determined using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL, USA). The concentration of CP in diet and ingredient samples was analyzed by combustion (Method 999.03; AOAC Int., 2007) using a Rapid N cube (Elementar Americas Inc, Mt. Laurel, NJ, USA), and AA were analyzed as previously described (Almeida et al., 2014a).

Fecal and urine samples were analyzed for GE as described for diets and ingredients. Ingredient samples were also analyzed for insoluble and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom$^{TDF}$ Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of soluble dietary fiber and insoluble dietary fiber. Total fat was analyzed in ingredient samples as acid-hydrolyzed ether extract (AEE). Samples were hydrolyzed using 3 *N* HCl (Ankom$^{HCl}$, Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Ankom$^{XT15}$, Ankom Technology, Macedon, NY, USA). These samples were also analyzed for dry ash (method 942.05; AOAC Int., 2007), and monosaccharides and oligosaccharides were analyzed as described by Cervantes-Pahm and Stein (2010).

Energy values that were determined from the excretion of GE in the feces and urine were subtracted from the intake of GE to calculate DE and ME for each diet using the direct procedure (Adeola, 2001). The DE and ME in the corn diet were divided by 0.970 (inclusion level of corn) to calculate the DE and ME in corn. The contributions of DE and ME from corn to the diets containing corn and SBM were then calculated and subtracted from the total DE and ME of these diets, and the concentrations of DE and ME in each batch of SBM were calculated by difference (Adeola, 2001). The DE and ME in each batch of SBM were calculated on an as-fed basis as well as on a DM basis. The apparent total tract digestibility (ATTD) of GE was also calculated for all diets, and also for each batch of SBM using the difference procedure (Adeola, 2001).

Outliers and homogeneity of the variances among treatments were tested using the UNIVARIATE procedure (SAS-Institute Inc, 2016). Data were analyzed using the Mixed procedure of SAS with the default setting for the covariance structure. The model included diet as fixed effect and period and animal were considered random effects (Wang and Goonewardene, 2004). The experimental unit was the pig. Mean values were calculated using the LSMeans statement. A contrast statement was used to determine effects of autoclaving temperature (i.e., control vs. 110 °C; 110 vs. 150 °C) on ATTD of GE and DE and ME. Contrast statements were also used to determine effects of duration of autoclaving (i.e., linear effect at 110 °C and linear and quadratic effects at 150 °C). An α-value of 0.05 was used to assess significance among means and results were considered a tendency at $0.05 < P < 0.10$.

### 2.2. Exp. 2. AA digestibility

The 9 batches of SBM used in Exp. 1 were also used in in Exp. 2. Ten castrates (36.8 ± 1.2 kg) were equipped with a T-cannula in the distal ileum according to procedures adapted from Stein et al. (1998). Pigs were allotted to a 10 × 7 Youden square design with 10 diets and 7 periods to determine the AID and the SID of CP and AA in each source of SBM. Pigs were housed individually in pens (1.2 × 1.5 m) in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen.

Ten diets were formulated (Tables 3 and 4). Nine diets were based on cornstarch and each source of SBM, and the last diet was a N-free diet that was used to estimate basal endogenous losses of CP and AA. Vitamins and minerals were included in all diets to meet or exceed current requirements for 25–50 kg growing pigs (NRC, 2012) and chromic oxide (4 g/kg) was included in all diets as an

**Table 3**
Composition of experimental diets containing soybean meal (SBM), as-fed basis, Exp. 2.

| | Diet | |
|---|---|---|
| Item, g/kg | SBM | N-free |
| Soybean meal | 400.0 | – |
| Cornstarch | 390.5 | 731.0 |
| Sucrose | 150.0 | 150.0 |
| Solka floc[1] | – | 40.0 |
| Soybean oil | 30.0 | 40.0 |
| Ground limestone | 7.5 | 8.0 |
| Monocalcium phosphate | 11.0 | 15.0 |
| Magnesium oxide | – | 1.0 |
| Potassium carbonate | – | 4.0 |
| Sodium chloride | 4.0 | 4.0 |
| Chromic oxide | 4.0 | 4.0 |
| Vitamin-mineral premix[2] | 3.0 | 3.0 |

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

[2] Provided the following quantities of vitamins and micro minerals per kg of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin $D_3$ as cholecalciferol, 2,208 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin $B_{12}$, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

**Table 4**
Analyzed nutrient composition of diets containing soybean meal and in the N-free diet, as-fed basis, Exp. 2.

| Item, g/kg | | Autoclaving 110 °C | | 150 °C | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Duration (min): | SBM[1] | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | N-free |
| Dry matter | 927 | 927 | 928 | 925 | 923 | 926 | 926 | 925 | 928 | 920 |
| Crude protein | 209 | 207 | 206 | 187 | 218 | 220 | 216 | 226 | 215 | 11 |
| Indispensable amino acids | | | | | | | | | | |
| Arg | 14.7 | 14.5 | 13.8 | 12.4 | 13.0 | 12.9 | 11.7 | 11.0 | 9.9 | 0.1 |
| His | 5.0 | 5.1 | 4.9 | 4.6 | 5.0 | 5.3 | 5.0 | 5.1 | 4.9 | < 0.1 |
| Ile | 9.3 | 9.3 | 8.9 | 8.6 | 9.5 | 10.0 | 9.6 | 9.9 | 9.5 | < 0.1 |
| Leu | 15.5 | 15.4 | 14.8 | 14.4 | 15.8 | 16.6 | 15.9 | 16.5 | 15.8 | 0.3 |
| Lys | 12.4 | 12.4 | 11.7 | 10.2 | 10.5 | 10.3 | 9.3 | 8.8 | 8.1 | < 0.2 |
| Met | 2.6 | 2.5 | 2.4 | 2.2 | 2.3 | 2.4 | 2.3 | 2.3 | 2.2 | < 0.1 |
| Phe | 10.7 | 10.5 | 10.1 | 9.7 | 10.8 | 11.2 | 10.7 | 11.0 | 10.6 | 0.2 |
| Thr | 7.9 | 7.8 | 7.5 | 7.2 | 7.9 | 8.2 | 7.9 | 8.1 | 7.9 | < 0.1 |
| Trp | 2.5 | 2.6 | 2.6 | 2.4 | 2.7 | 2.7 | 2.6 | 2.7 | 2.7 | < 0.2 |
| Val | 9.6 | 9.6 | 9.2 | 9.0 | 9.9 | 10.4 | 9.9 | 10.2 | 9.9 | < 0.2 |
| Dispensable amino acids | | | | | | | | | | |
| Ala | 8.8 | 8.7 | 8.4 | 8.1 | 8.9 | 9.4 | 9.0 | 9.4 | 9.0 | 0.2 |
| Asp | 23.2 | 22.9 | 22.2 | 21.3 | 23.1 | 24.1 | 23.1 | 23.8 | 22.7 | 0.2 |
| Cys | 2.9 | 2.8 | 2.7 | 2.1 | 2.0 | 1.9 | 1.6 | 1.5 | 1.3 | < 0.1 |
| Glu | 37.3 | 37.0 | 35.6 | 34.2 | 37.4 | 39.2 | 37.5 | 39.0 | 37.3 | 0.4 |
| Gly | 8.7 | 8.5 | 8.2 | 7.9 | 8.6 | 9.1 | 8.7 | 9.1 | 8.7 | 0.1 |
| Pro | 10.4 | 10.3 | 10.4 | 9.5 | 10.5 | 11.5 | 10.7 | 11.1 | 10.6 | 0.2 |
| Ser | 10.1 | 10.0 | 9.6 | 9.3 | 10.1 | 10.3 | 9.9 | 10.3 | 10.0 | 0.1 |

[1] Conventional SBM that was not autoclaved.

indigestible marker. Because dietary fat may influence AA digestibility (Cervantes-Pahm and Stein, 2008), 30 g/kg of soybean oil was added to each diet containing SBM so simulate a normal commercial diet.

Individual pig weights were recorded at the beginning of each period and the amount of feed supplied each day was also recorded. Diets were provided in a meal form. Pigs were fed at a daily level of 3.4-times the estimated maintenance requirement for energy, and the daily allotment of feed was provided at 0700 h each day. Water was available at all times throughout the experiment. Each experimental period lasted 7 days. The initial 5 days of each period was considered an adaption period to the diet whereas ileal digesta were collected for 8 h on day 6 and 7. A 225-mL plastic bag was attached to the cannula barrel using a zip tie, and digesta that flowed into the bag were collected. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and digesta were immediately stored at −20 °C to prevent bacterial degradation of AA in the digesta.

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for chemical analyses. Samples of each diet and of each source of SBM were collected as well. All ileal digesta samples were lyophilized and finely ground using a coffee grinder prior to chemical analyses. All samples of ileal digesta and diets were analyzed in duplicate for DM, CP, and AA as described for Exp. 1, and chromium was determined after nitric acid-perchloric acid wet ash sample preparation (Method 990.08, AOAC Int., 2007).

Values for AID, basal endogenous losses, and SID of CP and AA were calculated (Stein et al. (2007)). Because SBM was the only AA containing ingredient in each diet, the AID and SID values obtained for each diet also represent the AID and SID of CP and AA in each batch of SBM. The SID of CP and each AA was multiplied by the concentration of CP or the corresponding AA (as-fed basis) in the ingredients to calculate the concentration of standardized ileal digestible CP and AA for each ingredient (Liu et al., 2016).

Outliers and homogeneity of the variances among treatments were tested and data were analyzed using the Mixed procedure of SAS with a covariance structure as explained for Exp. 1 (SAS-Institute Inc, 2016). The experimental unit was the pig. Contrast statements were used to determine effects of different autoclaving temperatures and effects of duration of autoclaving on AID and SID of CP and AA as described for Exp. 1.

## 3. Results

### 3.1. Exp. 1. Energy digestibility and concentrations of DE and ME

Analyzed values for GE in diets were in agreement with values calculated from the corn and SBM that were included in the diets. Calculated values for the Lys:CP ratio were greater than 60 g/kg for the control SBM and the SBM that was autoclaved at 110 °C for 15 min, but for all other sources of SBM, a ratio that was less than 60 g/kg was calculated. There were no differences in ATTD of GE between the diet containing the control SBM and diets containing SBM that was autoclaved at 110 °C (Table 5). However, the ATTD of DM and GE, and DE and ME concentrations in the diets, were less ($P < 0.001$) if SBM was autoclaved at 150 ºC than at 110 ºC. At 110 ºC, there were no linear effects of duration of autoclaving on GE digestibility and metabolizability. In contrast, linear decreases ($P < 0.001$) in ATTD of DM and GE, and in DE and ME of the diets were observed with increasing duration of autoclaving at 150 ºC. Likewise, on a DM basis, the DE and ME in SBM were less ($P < 0.001$) when SBM was autoclaved at 150 ºC than at 110 ºC (Table 6).

**Table 5**
Energy digestibility and concentrations of digestible energy (DE) and metabolizable energy (ME) in the corn–based basal diet and diets containing corn and soybean meal (SBM)[1], as-fed basis, Exp. 1.

| Item | Basal | Soybean meal | | | | | | | | | | Contrast *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SBM[3] | Autoclaving temperature | | | | | | | | | | | | | |
| | | | 110 °C | | 150 °C | | | | | | | SBM vs. | 110 °C vs. | | At 150 °C | |
| Duration (min): | | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110 °C | 150 °C | At 110 °C Linear | Linear | Quadratic |
| Feed intake, kg/day | 1.77 | 1.83 | 1.88 | 1.90 | 1.93 | 1.84 | 1.87 | 1.86 | 1.86 | 1.78 | 0.090 | 0.209 | 0.282 | 0.198 | 0.155 | 0.131 |
| GE[2] intake, MJ/day | 27.7 | 29.0 | 29.9 | 30.2 | 30.5 | 29.3 | 29.9 | 29.7 | 29.7 | 28.6 | 1.427 | 0.119 | 0.344 | 0.118 | 0.431 | 0.096 |
| Fecal GE output, MJ/day | 3.7 | 3.7 | 4.0 | 3.9 | 4.4 | 4.3 | 4.9 | 5.2 | 5.9 | 5.5 | 0.268 | 0.357 | < 0.001 | 0.480 | < .0001 | 0.304 |
| ATTD[2] of DM[2] | 0.88 | 0.88 | 0.88 | 0.88 | 0.87 | 0.86 | 0.85 | 0.84 | 0.82 | 0.83 | 0.007 | 0.861 | < 0.001 | 0.971 | < .0001 | 0.854 |
| ATTD of GE | 0.87 | 0.87 | 0.87 | 0.87 | 0.86 | 0.85 | 0.84 | 0.83 | 0.80 | 0.81 | 0.007 | 0.852 | < 0.001 | 0.938 | < .0001 | 0.987 |
| DE in diet, MJ/kg | 13.5 | 13.7 | 13.8 | 13.8 | 13.5 | 13.5 | 13.4 | 13.2 | 12.8 | 12.9 | 0.117 | 0.423 | < 0.001 | 0.420 | < .0001 | 0.591 |
| Urine GE output, MJ/day | 0.5 | 1.0 | 0.9 | 1.0 | 1.0 | 1.1 | 1.1 | 1.3 | 1.2 | 1.2 | 0.100 | 0.894 | 0.019 | 0.660 | 0.019 | 0.485 |
| ME in diet, MJ/kg | 13.2 | 13.2 | 13.3 | 13.3 | 13.0 | 13.0 | 12.8 | 12.5 | 12.2 | 12.2 | 0.138 | 0.392 | < 0.001 | 0.565 | < .0001 | 0.634 |

[1] Each least squares mean represents 8 observations.
[2] GE, gross energy; ATTD, apparent total tract digestibility; DM, dry matter.
[3] Conventional SBM that was not autoclaved.

**Table 6**
Concentrations of gross energy (GE), digestible energy (DE), and metabolizable energy (ME) in 9 sources of soybean meal (SBM) fed to growing pigs[1], dry matter basis, Exp. 1.

| Item | SBM[2] | Autoclaving temperature | | | | | | | | Contrast *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 110 °C | | 150 °C | | | | | | SBM vs. | 110 °C vs. | At 110 °C | At 150 °C | |
| Duration (min): | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110 °C | 150 °C | Linear | Linear | Quadratic |
| Energy values, MJ/kg | | | | | | | | | | | | | | | |
| GE | 19.3 | 19.5 | 19.4 | 19.5 | 19.6 | 19.7 | 19.7 | 19.7 | 19.8 | – | – | – | – | – | – |
| DE | 16.7 | 16.7 | 16.9 | 15.8 | 15.7 | 14.9 | 14.0 | 12.5 | 12.7 | 0.577 | 0.853 | < 0.001 | 0.693 | < 0.001 | 0.571 |
| ME | 15.3 | 15.5 | 15.5 | 14.4 | 14.0 | 13.4 | 12.0 | 10.7 | 10.7 | 0.633 | 0.739 | < 0.001 | 0.811 | < 0.001 | 0.698 |
| Energy digestibility and metabolizability | | | | | | | | | | | | | | | |
| DE:GE ratio | 0.86 | 0.86 | 0.87 | 0.81 | 0.80 | 0.76 | 0.71 | 0.63 | 0.64 | 0.030 | 0.948 | < 0.001 | 0.828 | < 0.001 | 0.765 |
| ME:DE ratio | 0.92 | 0.93 | 0.91 | 0.91 | 0.89 | 0.89 | 0.86 | 0.85 | 0.85 | 0.016 | 0.663 | < 0.001 | 0.910 | < 0.001 | 0.951 |
| ME:GE ratio | 0.79 | 0.80 | 0.80 | 0.74 | 0.72 | 0.68 | 0.61 | 0.54 | 0.54 | 0.033 | 0.900 | < 0.001 | 0.928 | < 0.001 | 0.877 |

[1] Each least squares mean represents 8 observations.
[2] Conventional SBM that was not autoclaved.

**Table 7**
Apparent ileal digestibility (AID) of crude protein and amino acids (AA) in autoclaved soybean meal (SBM) fed to growing pigs[1], Exp 2.

| Item, g/kg | | Autoclaving temperature 110 °C | | 150 °C | | | | | | Contrast *P*-value SBM vs. | 110 °C vs. | At 110 °C | | At 150 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Duration (min): | SBM[2] | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110 °C | 150 °C | Linear | Quadratic | Linear | Quadratic |
| Crude protein | 0.84 | 0.83 | 0.84 | 0.74 | 0.72 | 0.75 | 0.72 | 0.70 | 0.63 | 0.017 | 0.569 | < 0.001 | 0.669 | 0.684 | < 0.001 | 0.741 |
| Indispensable AA | | | | | | | | | | | | | | | | |
| Arg | 0.94 | 0.93 | 0.93 | 0.89 | 0.87 | 0.88 | 0.86 | 0.82 | 0.79 | 0.011 | 0.678 | < 0.001 | 0.719 | 0.827 | < 0.001 | 0.651 |
| His | 0.91 | 0.91 | 0.91 | 0.84 | 0.83 | 0.84 | 0.82 | 0.82 | 0.76 | 0.011 | 0.744 | < 0.001 | 0.807 | 0.816 | < 0.001 | 0.769 |
| Ile | 0.90 | 0.90 | 0.90 | 0.84 | 0.84 | 0.84 | 0.82 | 0.83 | 0.77 | 0.013 | 0.997 | < 0.001 | 0.993 | 0.983 | < 0.001 | 0.988 |
| Leu | 0.90 | 0.90 | 0.90 | 0.84 | 0.85 | 0.85 | 0.84 | 0.85 | 0.80 | 0.012 | 0.965 | < 0.001 | 0.945 | 0.977 | < 0.001 | 0.906 |
| Lys | 0.93 | 0.92 | 0.91 | 0.82 | 0.78 | 0.78 | 0.73 | 0.72 | 0.59 | 0.019 | 0.486 | < 0.001 | 0.396 | 0.968 | < 0.001 | 0.797 |
| Met | 0.92 | 0.91 | 0.91 | 0.85 | 0.85 | 0.85 | 0.83 | 0.83 | 0.77 | 0.010 | 0.262 | < 0.001 | 0.334 | 0.550 | < 0.001 | 0.929 |
| Phe | 0.91 | 0.91 | 0.92 | 0.87 | 0.87 | 0.87 | 0.86 | 0.87 | 0.82 | 0.011 | 0.849 | < 0.001 | 0.771 | 0.915 | < 0.001 | 0.848 |
| Thr | 0.83 | 0.83 | 0.83 | 0.76 | 0.76 | 0.77 | 0.75 | 0.76 | 0.68 | 0.017 | 0.816 | < 0.001 | 0.928 | 0.761 | < 0.001 | 0.984 |
| Trp | 0.84 | 0.83 | 0.85 | 0.78 | 0.78 | 0.79 | 0.77 | 0.77 | 0.72 | 0.015 | 0.995 | < 0.001 | 0.648 | 0.464 | < 0.001 | 0.927 |
| Val | 0.87 | 0.87 | 0.87 | 0.81 | 0.81 | 0.82 | 0.80 | 0.81 | 0.75 | 0.014 | 0.878 | < 0.001 | 0.964 | 0.823 | < 0.001 | 0.854 |
| Dispensable AA | | | | | | | | | | | | | | | | |
| Ala | 0.85 | 0.84 | 0.83 | 0.76 | 0.76 | 0.78 | 0.75 | 0.75 | 0.69 | 0.018 | 0.352 | < 0.001 | 0.384 | 0.701 | < 0.001 | 0.752 |
| Asp | 0.88 | 0.87 | 0.86 | 0.74 | 0.69 | 0.71 | 0.67 | 0.68 | 0.58 | 0.020 | 0.412 | < 0.001 | 0.366 | 0.913 | < 0.001 | 0.016 |
| Cys | 0.82 | 0.80 | 0.80 | 0.65 | 0.62 | 0.61 | 0.54 | 0.54 | 0.35 | 0.032 | 0.511 | < 0.001 | 0.555 | 0.756 | < 0.001 | 0.879 |
| Glu | 0.91 | 0.91 | 0.91 | 0.84 | 0.83 | 0.83 | 0.81 | 0.81 | 0.74 | 0.012 | 0.587 | < 0.001 | 0.641 | 0.771 | < 0.001 | 0.987 |
| Gly | 0.76 | 0.73 | 0.73 | 0.61 | 0.56 | 0.65 | 0.59 | 0.55 | 0.49 | 0.030 | 0.298 | < 0.001 | 0.399 | 0.519 | < 0.001 | 0.366 |
| Pro | 0.62 | 0.59 | 0.52 | 0.37 | 0.09 | 0.49 | 0.31 | −0.02 | 0.11 | 0.128 | 0.536 | < 0.001 | 0.409 | 0.879 | < 0.001 | 0.528 |
| Ser | 0.87 | 0.87 | 0.87 | 0.81 | 0.79 | 0.80 | 0.78 | 0.78 | 0.72 | 0.013 | 0.934 | < 0.001 | 0.963 | 0.934 | < 0.001 | 0.773 |

[1] Each least squares mean represents 7 observations except for the SBM that was autoclaved at 110 °C for 15 min and the SBM that was autoclaved at 150 °C for 6 or 9 min (n = 6).
[2] Conventional SBM that was not autoclaved.

**Table 8**
Standardized ileal digestibility (SID) of crude protein and amino acids (AA) in autoclaved soybean meal (SBM) fed to growing pigs[1,2], Exp. 2.

| Item, g/kg | SBM[3] | Autoclaving temperature | | | | | | | | | Contrast *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 110 °C | | 150 °C | | | | | | | SBM vs. | 110 °C vs. | At 110 °C | | At 150 °C | |
| Duration (min): | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110 °C | 150 °C | Linear | Quadratic | Linear | Quadratic |
| Crude protein | 0.93 | 0.92 | 0.92 | 0.84 | 0.80 | 0.83 | 0.80 | 0.78 | 0.71 | 0.017 | 0.619 | < 0.001 | 0.731 | 0.687 | < 0.001 | 0.594 |
| Indispensable AA | | | | | | | | | | | | | | | | |
| Arg | 0.98 | 0.98 | 0.98 | 0.94 | 0.92 | 0.93 | 0.91 | 0.88 | 0.86 | 0.011 | 0.804 | < 0.001 | 0.913 | 0.760 | < 0.001 | 0.951 |
| His | 0.94 | 0.93 | 0.94 | 0.87 | 0.86 | 0.86 | 0.85 | 0.85 | 0.79 | 0.011 | 0.758 | < 0.001 | 0.854 | 0.768 | < 0.001 | 0.678 |
| Ile | 0.93 | 0.93 | 0.93 | 0.87 | 0.87 | 0.86 | 0.85 | 0.86 | 0.79 | 0.013 | 0.955 | < 0.001 | 0.933 | 0.978 | < 0.001 | 0.935 |
| Leu | 0.93 | 0.93 | 0.93 | 0.87 | 0.88 | 0.88 | 0.87 | 0.88 | 0.82 | 0.012 | 0.916 | < 0.001 | 0.868 | 0.945 | < 0.001 | 0.975 |
| Lys | 0.95 | 0.94 | 0.93 | 0.84 | 0.81 | 0.80 | 0.76 | 0.75 | 0.63 | 0.019 | 0.510 | < 0.001 | 0.431 | 0.991 | < 0.001 | 0.831 |
| Met | 0.94 | 0.93 | 0.93 | 0.88 | 0.88 | 0.88 | 0.86 | 0.86 | 0.80 | 0.010 | 0.306 | < 0.001 | 0.394 | 0.552 | < 0.001 | 0.938 |
| Phe | 0.93 | 0.94 | 0.94 | 0.89 | 0.89 | 0.89 | 0.88 | 0.89 | 0.84 | 0.011 | 0.795 | < 0.001 | 0.703 | 0.903 | < 0.001 | 0.894 |
| Thr | 0.90 | 0.89 | 0.90 | 0.83 | 0.82 | 0.83 | 0.81 | 0.82 | 0.75 | 0.017 | 0.918 | < 0.001 | 0.920 | 0.717 | < 0.001 | 0.907 |
| Trp | 0.89 | 0.88 | 0.90 | 0.84 | 0.83 | 0.83 | 0.81 | 0.82 | 0.77 | 0.015 | 0.932 | < 0.001 | 0.707 | 0.437 | < 0.001 | 0.814 |
| Val | 0.91 | 0.91 | 0.91 | 0.85 | 0.85 | 0.86 | 0.84 | 0.85 | 0.79 | 0.014 | 0.943 | < 0.001 | 0.939 | 0.791 | < 0.001 | 0.943 |
| Dispensable AA | | | | | | | | | | | | | | | | |
| Ala | 0.92 | 0.90 | 0.90 | 0.83 | 0.82 | 0.84 | 0.82 | 0.81 | 0.75 | 0.018 | 0.420 | < 0.001 | 0.485 | 0.670 | < 0.001 | 0.672 |
| Asp | 0.91 | 0.90 | 0.89 | 0.77 | 0.73 | 0.74 | 0.70 | 0.71 | 0.61 | 0.020 | 0.435 | < 0.001 | 0.397 | 0.900 | < 0.001 | 0.014 |
| Cys | 0.87 | 0.85 | 0.86 | 0.73 | 0.70 | 0.69 | 0.63 | 0.64 | 0.47 | 0.032 | 0.567 | < 0.001 | 0.636 | 0.737 | < 0.001 | 0.891 |
| Glu | 0.94 | 0.93 | 0.93 | 0.87 | 0.85 | 0.85 | 0.84 | 0.83 | 0.76 | 0.012 | 0.626 | < 0.001 | 0.702 | 0.750 | < 0.001 | 0.942 |
| Gly | 0.96 | 0.93 | 0.94 | 0.83 | 0.76 | 0.84 | 0.79 | 0.74 | 0.69 | 0.030 | 0.428 | < 0.001 | 0.608 | 0.483 | < 0.001 | 0.310 |
| Pro | 1.31 | 1.28 | 1.20 | 1.12 | 0.77 | 1.11 | 0.97 | 0.62 | 0.79 | 0.128 | 0.552 | < 0.001 | 0.397 | 0.817 | < 0.001 | 0.343 |
| Ser | 0.92 | 0.92 | 0.92 | 0.86 | 0.84 | 0.85 | 0.83 | 0.83 | 0.77 | 0.013 | 0.956 | < 0.001 | 0.884 | 0.894 | < 0.001 | 0.743 |

[1]Each least squares mean represents 7 observations except the for SBM that was autoclaved at 110 °C for 15 min and the SBM that was autoclaved at 150 °C for 6 or 9 min, respectively (n = 6).
[2]Values for SID were calculated by correcting the values for apparent ileal digestibility for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of dry matter intake) as crude protein, 19.60; Arg, 0.71; His, 0.16; Ile, 0.27; Leu, 0.46; Lys, 0.29; Met, 0.07; Phe, 0.24; Thr, 0.56; Trp, 0.14; Val, 0.42; Ala, 0.63; Asp, 0.77; Cys, 0.17; Glu, 0.92; Gly, 1.88; Pro, 7.75; and Ser, 0.56.
[3]Conventional SBM that was not autoclaved.

Although there were no linear effects on DE and ME in SBM with increasing duration of autoclaving at 110 °C, there were linear decreases ($P < 0.001$) in DE and ME of SBM as the duration of autoclaving increased at 150 ºC. The DE:GE, ME:DE, and ME:GE ratios in SBM were less ($P < 0.001$) if SBM was autoclaved at 150 ºC than at 110 ºC. In addition, there were linear decreases ($P < 0.001$) for the DE: GE, ME:DE, and ME:GE ratios in SBM as the duration of heat treatment increased at 150 ºC.

### *3.2. Exp. 2. AA digestibility*

The analyzed CP in SBM did not change as autoclaving duration or temperature changed, but concentrations of Arg, Lys, and Cys in SBM that was heat-treated at 110 or at 150 °C decreased as duration of autoclaving increased (Table 1). There were no differences in AID and SID of CP and AA between the control SBM and SBM that was autoclaved at 110 °C (Tables 7 and 8). However, the AID and SID of CP and AA in SBM were less ($P < 0.001$) if SBM was autoclaved at 150 ºC than at 110 ºC. There were no linear reductions in AID and SID of CP and all AA in SBM as the duration of autoclaving increased at 110 °C. In contrast, a linear decrease ($P < 0.001$) for the AID and SID of CP and all AA in SBM was observed with increasing duration of autoclaving at 150 ºC.

Concentrations of standardized ileal digestible CP and of all AA, except Phe were not different between the control SBM and SBM that was autoclaved at 110 °C (Table 9), but the concentration of standardized ileal digestible CP and AA was less ($P < 0.001$) if SBM was autoclaved at 150 ºC than at 110 ºC. There were no linear reduction in concentration of standardized ileal digestible CP and AA if the duration of autoclaving increased at 110 °C, but a linear decrease ($P < 0.001$) in the concentration of standardized ileal digestible CP and all AA was observed with increasing duration of autoclaving at 150 ºC. In addition, the concentration of standardized ileal digestible His, Ile, Lys, Thr, and Cys decreased both linearly and quadratically ($P < 0.05$) if the duration of autoclaving increased at 150 ºC.

## 4. Discussion

Amino acid composition of the control SBM is in close agreement with published book values (NRC, 2012; Rostagno et al., 2017), and the SID of AA in the control SBM is also within the range of reported values (González-Vega et al., 2011; NRC, 2012; Sotak-Peper et al., 2017). However, the DE and ME for the control SBM is slightly greater than some data published previously (NRC, 2012; Rostagno et al., 2017), but in agreement with Sotak-Peper et al. (2015) who demonstrated that the ME of SBM generally is greater than values published by NRC (2012).

In commercial production of SBM, heat is applied during de-solventizing and during the drying-cooling process. During de-

**Table 9**
Concentrations of standardized ileal digestible crude protein (CP) and amino acids (AA) in autoclaved soybean meal (SBM) fed to growing pigs[1], Exp. 2.

| Item, g/kg | SBM[3] | Autoclaving temperature | | | | | | | | Contrast *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 110 °C | | 150 °C | | | | | | | SBM vs. | 110 °C vs. | At 110 °C | | At 150 °C | |
| Duration (min): | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110 °C | 150 °C | Linear | Quadratic | Linear | Quadratic |
| Crude protein | 441.2 | 438.2 | 443.2 | 401.8 | 381.1 | 395.4 | 374.5 | 374.8 | 341.4 | 7.467 | 0.950 | < 0.001 | 0.823 | 0.627 | < 0.001 | 0.267 |
| Indispensable AA | | | | | | | | | | | | | | | | |
| Arg | 33.6 | 34.3 | 33.9 | 30.1 | 27.7 | 26.9 | 25.4 | 22.5 | 20.2 | 0.278 | 0.175 | < 0.001 | 0.540 | 0.102 | < 0.001 | 0.299 |
| His | 11.2 | 11.4 | 11.3 | 10.3 | 10.8 | 10.9 | 10.8 | 10.8 | 9.8 | 0.124 | 0.483 | < 0.001 | 0.674 | 0.500 | < 0.001 | 0.006 |
| Ile | 18.9 | 19.2 | 19.2 | 18.3 | 18.3 | 18.6 | 18.2 | 18.5 | 16.9 | 0.259 | 0.358 | < 0.001 | 0.487 | 0.519 | < 0.001 | 0.039 |
| Leu | 32.6 | 33.4 | 33.4 | 31.4 | 31.5 | 32.0 | 31.6 | 32.2 | 29.7 | 0.413 | 0.060 | < 0.001 | 0.098 | 0.336 | 0.001 | 0.067 |
| Lys | 27.1 | 27.1 | 26.6 | 21.9 | 19.5 | 18.2 | 16.5 | 15.1 | 11.9 | 0.392 | 0.488 | < 0.001 | 0.198 | 0.438 | < 0.001 | < 0.001 |
| Met | 5.6 | 5.7 | 5.7 | 5.3 | 5.2 | 5.3 | 5.3 | 5.2 | 4.8 | 0.059 | 0.656 | < 0.001 | 0.635 | 0.932 | < 0.001 | 0.085 |
| Phe | 21.9 | 22.7 | 22.8 | 21.3 | 20.9 | 21.4 | 20.9 | 21.3 | 19.9 | 0.245 | 0.003 | < 0.001 | 0.005 | 0.203 | < 0.001 | 0.242 |
| Thr | 15.6 | 16.1 | 16.0 | 14.7 | 14.8 | 15.2 | 14.8 | 14.9 | 13.4 | 0.265 | 0.159 | < 0.001 | 0.239 | 0.417 | < 0.001 | 0.029 |
| Trp | 5.6 | 5.7 | 5.7 | 5.3 | 5.1 | 5.2 | 5.0 | 5.1 | 4.7 | 0.089 | 0.208 | < 0.001 | 0.239 | 0.604 | < 0.001 | 0.897 |
| Val | 19.6 | 19.9 | 19.9 | 18.6 | 18.7 | 18.9 | 18.6 | 19.0 | 17.3 | 0.294 | 0.358 | < 0.004 | 0.407 | 0.671 | < 0.001 | 0.204 |
| Dispensable AA | | | | | | | | | | | | | | | | |
| Ala | 18.6 | 18.2 | 18.2 | 16.4 | 16.6 | 17.5 | 16.9 | 16.9 | 15.5 | 0.338 | 0.610 | < 0.001 | 0.635 | 0.831 | 0.001 | 0.314 |
| Asp | 47.4 | 47.9 | 47.3 | 39.6 | 38.3 | 39.5 | 37.6 | 37.8 | 31.8 | 0.995 | 0.857 | < 0.001 | 0.881 | 0.553 | < 0.001 | 0.148 |
| Cys | 5.8 | 5.7 | 5.5 | 3.9 | 3.2 | 2.9 | 2.6 | 2.4 | 1.6 | 0.127 | 0.094 | < 0.001 | 0.051 | 0.974 | < 0.001 | < 0.001 |
| Glu | 77.4 | 79.2 | 79.1 | 72.3 | 71.2 | 73.2 | 71.9 | 71.8 | 65.3 | 0.963 | 0.082 | < 0.001 | 0.128 | 0.365 | < 0.001 | 0.166 |
| Gly | 18.6 | 18.4 | 18.6 | 15.9 | 15.0 | 17.0 | 15.9 | 14.9 | 13.8 | 0.567 | 0.757 | < 0.001 | 0.935 | 0.641 | < 0.001 | 0.725 |
| Pro | 28.0 | 27.5 | 26.5 | 23.7 | 17.4 | 25.5 | 23.5 | 14.8 | 18.8 | 2.791 | 0.711 | 0.001 | 0.622 | 0.931 | 0.001 | 0.636 |
| Ser | 21.3 | 21.8 | 21.8 | 19.7 | 19.6 | 20.0 | 19.7 | 19.4 | 17.7 | 0.273 | 0.086 | < 0.001 | 0.134 | 0.369 | < 0.001 | 0.180 |

[1] The concentrations of standardized ileal digestible CP and AA for each ingredient was calculated multiplying the standardized ileal digestibility of CP and each AA by the concentration of CP or the corresponding AA (as-fed basis) each ingredient.
[3] Conventional SBM that was not autoclaved.

solventizing, the meal is heated via steam and meal temperature will usually be between 100 and 110 °C for 25–30 min (Kemper, 2020). Because of the steam applied, moisture in the SBM increases to 170–220 g/kg. Following the de-solventizer, the SBM enters the dryer-cooler where hot air that may be up to 150 °C is applied to the SBM to reduce moisture concentration to less than 125 g/kg (Kemper, 2020). Thus, the temperatures applied to the SBM used in this research mimic the temperatures reached during commercial production in the SBM in the de-solventizer and the air temperature in the dryer-cooler.

The observed decreases in the AID and SID of AA in SBM resulting from increasing duration of autoclaving indicates that autoclaving at 150 °C results in heat damage. Similar results have been reported for autoclaved distillers dried grains with solubles, soybean meal, sunflower meal, cottonseed meal, and canola meal (Fontaine et al., 2007; González-Vega et al., 2011; Almeida et al., 2013, 2014a, 2014b). The negative effects of autoclaving on AA digestibility is likely a result of Maillard reactions, which form insoluble complexes that cannot be digested by pigs. Heating may also result in crosslinking of protein with reduced access for digestive enzymes as a result (Almeida et al., 2014a).

Lysine is the AA that is most susceptible to Maillard reactions due to the presence of an ε-amino group in the sidechain that can react directly with reducing sugars under moist and heat conditions (Eklund et al., 2015). The Maillard reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars and leads to a decrease in the availability of the AA involved (Singh et al., 2007). The decrease in the concentration of sucrose and increase in the concentration of glucose and fructose as the SBM was autoclaved at 150 °C indicates that sucrose was hydrolyzed to glucose and fructose, which are reducing sugars that can then participate in the Maillard reaction (Camire et al., 1990; Rufián-Henares et al., 2006; Singh et al., 2007). As a result of this reaction, the concentration of Lys and the SID of Lys and all other AA will be reduced. The observation that the Lys:CP ratio was reduced as autoclaving time increased clearly demonstrates that the Mailard reaction had reduced the amount of Lys as heat treatment increased. In SBM that has not been heat damaged, the Lys:CP ratio is greater than 60 g/kg (Gonzalez-Vega et al., 2011), which was also observed for the control SBM used in this experiment. This demonstrates that the starting material was from a non-heat damaged source of SBM. However, as the severity and time of autoclaving increased, the Lys:CP ratio was reduced and this ratio was less than 40 g/kg for the most heat damaged SBM, which clearly demonstrates that the meals were heat damaged because a Lys:CP ratio less than 60 g/kg demonstrates heat damage of SBM.

The greater reduction in the SID of Lys, Arg, and sulfur AA compared with other AA that was observed in the present study confirms that these AA are most affected by heat treatment. Absorption of AA may also be reduced because of decreased transport in the intestinal lumen and proteolysis may also be inhibited if AA participated in Maillard reactions (Rérat et al., 2002). Similar observations were reported with heat treated soybean meal and canola meal (Fontaine et al., 2007; Messerschmidt et al., 2012; Almeida et al., 2014b).

The observation that autoclaving at 150 °C rather that at 110 °C reduced the concentrations of standardized ileal digestible AA, clearly demonstrates the negative effect of heating at 150 °C. The digestibility of most AA appears to be more negatively affected if autoclaved at 150 °C for 18 min, and this is likely a result of the Maillard reaction continuing to more advanced stages as the time of autoclaving increased with an increased conversion of Amadori compounds to advanced Maillard reaction products such as pre-melanoidins and melanoidins that can also react with other AA. This may result in many AA becoming unavailable (González-Vega et al., 2011; Almeida et al., 2014b). Results also indicated that the extent of the reduction in the concentration of digestible AA varies among AA and is particularly severe for Lys and Cys.

The decrease in insoluble dietary fiber and increase in soluble dietary fiber that was observed if SBM was autoclaved at 110 °C or 150 °C may indicate that autoclaving solubilized some insoluble dietary fiber. Heat damage of feed ingredients is associated with an increase in analyzed values for ADF and NDF and a decrease in AA digestibility (Almeida et al., 2013, 2014b). However, to our knowledge, this is the first time it has been demonstrated that heat treatment may also affect the analyzed concentration of insoluble and soluble dietary fiber in soybean meal.

Results from this study also demonstrated that overheating reduce DE and ME of SBM. The magnitude of the reduction in DE and ME indicates that the economic consequences of the reduction in DE and ME are at least as big as the consequences of reduced SID of AA. To our knowledge, this is the first time it has been demonstrated that overheating may negatively affect the DE and ME of SBM when fed to pigs. The decrease in sucrose concentration in SBM as the duration of autoclaving increased at 150 °C, likely contributed to the reduced DE and ME because sugars are bound to Lys and other AA during the Maillard reaction, and therefore, these sugars are not available for absorption. The activity of amylase and amyloglucosidase may also be reduced if Maillard reaction products enters the small intestine (Chung et al., 2012), which likely also contributed to the reduced DE and ME in the SBM that was autoclaved at 150 °C. The increase in the concentration of soluble dietary fiber in SBM that occurred as autoclaving time increased was expected to increase ME because soluble dietary fiber is much more fermentable than insoluble dietary fiber (Urriola et al., 2010). However, the observation that ME was instead reduced with increased autoclaving time indicates that the combined negative effects of the Maillard reaction were greater than the positive effects of solubilizing the fiber.

## 5. Conclusions

Amino acid digestibility and digestible energy and metabolizable energy of soybean meal are not reduced by autoclaving at 110 °C for 15 or 30 min. However, digestibility of amino acid, and concentrations of digestible and metabolizable energy, are reduced if soybean meal is autoclaved at 150 °C and the longer the duration of autoclaving is the more the standardized ileal digestibility of amino acids and digestibility of energy are reduced. These results indicate that if soybean crushing plants can avoid heating soybean meal to more than 110 °C during processing, the risk of overheating is greatly reduced.

## Author statement

MKW and HHS conceptualized the experiment. MKW prepared the SBM and recommended on autoclaving parameters. WBK conducted the animal part of the experiment. WBK and MSFO analyzed the data, and wrote the first draft of the manuscript. HHS and SAL contributed with data interpretation and proofreading of manuscript. HHS also supervised the project. All authors edited and approved the final version of the manuscript.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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