

Long-term steam conditioning is needed to maximize the nutritional value of expander-processed soybean expellers

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Abstract: Two experiments were conducted to determine standardized ileal digestibility (SID) of amino acids (AA) and the concentration of metabolizable energy (ME) in non-heat-treated and heat-treated soybean expellers (L-0, L-12, and L-48). L-0 underwent short-term steam conditioning for 60 s, whereas L-12 and L-48 underwent short-term steam conditioning for 60 s and long-term steam conditioning for 12 or 48 min. All heat-treated soybean expellers were expander processed. In experiment 1, 10 ileal-cannulated barrows (54.22 ± 4.54 kg) were allotted to a replicated 5×4 Youden square design with eight replicate pigs per diet. Each source of soybean expellers was included in one diet, and a nitrogen-free diet was also used. Results indicated that the SID of AA in non-heat-treated soybean expellers was less ($P < 0.01$) than in heat-treated soybean expellers. In experiment 2, 40 barrows (17.52 ± 1.63 kg) housed in metabolism crates were allotted to a corn-based diet or four corn–soybean expellers diets. Feces and urine were collected with 5 d adaptation and 4 d collection periods. The ME in non-heat-treated soybean expellers was less ($P < 0.01$) compared with L-0, L-12, or L-48. In conclusion, the SID of AA and the ME in heat-treated soybean expellers were greater than in non-heat-treated soybean expellers.

Key words: amino acids, digestibility, heat treatment, pigs, soybean expellers.

Résumé : Deux expériences ont été effectuées afin de déterminer la digestibilité iléale standardisée (SID — « standardized ileal digestibility ») des acides aminés (AA) et la concentration d'énergie métabolisable (ME — « metabolizable energy ») dans les presses continues de soja sans ou avec traitement thermique (L-0, L-12, et L-48). L-0 a subi un traitement thermique à la vapeur de courte durée pendant 60 secondes, tandis que L-12 et L-48 ont subi un traitement thermique à la vapeur de courte durée pendant 60 secondes puis un traitement thermique à la vapeur de longue durée pendant 12 ou 48 minutes. Toutes les presses continues ayant eu des traitements thermiques ont été refroidies par extension (« expander »). Dans l'expérience 1, 10 castrats canulés dans l'iléon ($54,22 \pm 4,54$ kg) ont été alloués à un design expérimental répliqué 5×4 carré Youden avec huit porcs répliqués par diète. Chaque source de presse continue de soja a été incluse dans une diète et une diète sans N a aussi été utilisée. Les résultats indiquent que la SID des AA dans les presses continues sans traitement thermique était moindre ($P < 0,01$) par rapport aux presses continues avec traitement thermique. Dans l'expérience 2, 40 castrats ($17,52 \pm 1,63$ kg) logés dans des cages métaboliques ont été alloués à une diète à base de maïs ou quatre diètes maïs-soja obtenues de presse continue. Les fèces et l'urine ont été collectées avec 5 jours d'adaptation et des périodes de collecte de 4 jours. Le ME des presses continues de soja sans traitement thermique était moindre ($P < 0,01$) par rapport à L-0, L-12, ou L-48. En conclusion, la SID des AA et le ME des presses continues de soja avec traitement thermique étaient plus élevés que dans les presses continues sans traitement thermique. [Traduit par la Rédaction]

Mots-clés : acides aminés, digestibilité, traitement thermique, porcs, presse continue de soja.

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Introduction

Globally, soybeans are the most important agricultural protein crop in animal feeding (Lee et al. 2007). Oil can be removed from soybeans using solvent extraction or the extruder-expeller method (Wang and Johnson 2001). If oil is extracted from soybeans via the mechanical expeller method, the resulting co-product is soybean expellers that have greater digestibility of amino acids (AA) than soybean meal, which is the co-product produced after solvent extraction of the oil (Woodworth et al. 2001). In some countries, soybean expellers, unlike solvent-extracted soybean meal, may also be used in organic production of pigs and poultry. However, soybean products contain trypsin inhibitors, which negatively affect nutrient digestibility, feed efficiency, and health status of animals (Palacios et al. 2004; Clarke and Wiseman 2005). Therefore, heat is usually applied to inactivate trypsin inhibitors and other anti-nutritional factors, and thereby improve nutrient digestibility in soy products (Liener 1994). However, excessive heat results in reduced digestibility of AA due to formation of Maillard reaction products (Pahm et al. 2008; González-Vega et al. 2011). Maillard reaction involves the reaction of reducing sugars with the ϵ -amino group of AA, and products of the Maillard reaction can render AA in soybean expellers unavailable (Finot and Magnenat 1981).

After extracting the oil from soybeans, the resulting soybean expellers may undergo steam conditioning and expander treatment (Purushotham et al. 2007). Due to the high pressure and temperature used, these processing technologies can inactivate trypsin inhibitors and may modify the physico-chemical characteristics of soybean expellers (van der Poel et al. 1998). Therefore, different types and degrees of expander treatment and steam conditioning may influence concentrations and digestibility of energy and AA in soybean expellers, but data to demonstrate this are limited. Thus, two experiments were conducted to test the hypothesis that steam conditioning and expander treatment increase the standardized ileal digestibility (SID) of AA, as well as values for digestible energy (DE) and metabolizable energy (ME) in soybean expellers.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for the two experiments before the animal part of the research was initiated. All animal procedures followed "Guide and Care of Laboratory Animals". Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used in both experiments.

One batch of raw soybeans from Romania was procured. The oil was cold extracted from the soybeans using a screw press (type P18.2.1, Florapower

GmbH & Co. KG, Augsburg, Germany), and the resulting soybean expellers were divided into four batches (Table 1). One batch (non-heat-treated) was not further processed, but the remaining three batches were processed using various combinations of short-term steam conditioning, long-term steam conditioning, and expander treatment to manufacture three sources of heat-treated soybean expellers. The short-term conditioner (DLM I), the long-term conditioner (LK 1605-2), and the expander (OEE 15.2) were from Amandus Kahl GmbH & Co. KG, Reinbeck, Germany. The expander had a throughput of 1.5–1.8 t·h⁻¹. The three batches were processed according to the procedure of Hoffmann et al. (2017): L-0 soybean expellers: short-term steam conditioning for 60 s at 90 °C, no long-term steam conditioning, but expander treatment at 110 °C; L-12 soybean expellers: short-term steam conditioning for 60 s at 90 °C, long-term steam conditioning for 12 min at 100 °C, followed by expander treatment at 110 °C; L-48 soybean expellers: short-term steam conditioning for 60 s at 90 °C, long-term steam conditioning for 48 min at 100 °C, followed by expander treatment at 110 °C. All processed soybean expellers were dried in a belt dryer at 85 °C. All diets were fed in mash form.

Experiment 1: Amino acid digestibility

Each source of soybean expellers was included in one diet as the only source of AA, and a nitrogen (N)-free diet was formulated to measure basal endogenous losses of AA; thus, a total of five diets were prepared (Tables 2 and 3). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates [National Research Council (NRC) 2012]. All diets also contained 0.40% chromic oxide as an indigestible marker.

Ten growing pigs (initial body weight: 54.22 ± 4.54 kg) that had a T-cannula installed in the distal ileum were used. Pigs were housed in individual pens (1.2 m × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors. A feeder and a nipple drinker were installed in each pen. Pigs were allotted to a replicated 5 × 4 Youden square design with five diets and four periods for a total of eight replicate pigs per diet. Allotments of pigs to each period in the Youden square followed a spreadsheet application (Kim and Stein 2009). Pigs were fed their respective diets at three times the maintenance requirement for ME (i.e., 197 kcal ME per kg body weight^{0.60}; NRC 2012). Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period to the diets, but ileal digesta were collected on days 6 and 7 for 8 h using standard procedures (Stein et al. 1998). In short, cannulas were opened, and a 225 mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were collected, and bags were removed whenever they were full, or every 30 min, and replaced with a new one. All samples were stored at -20 °C as soon as collected.

Table 1. Analyzed nutrient composition of ingredients, as-fed basis.

Item	Soybean expellers ^a			
	Non-heat-treated	L-0	L-12	L-48
Dry matter (%)	90.39	89.75	89.71	89.65
Gross energy (kcal·kg ⁻¹)	4,651	4,590	4,591	4,596
AEE (%)	10.82	9.27	8.92	8.31
Crude protein (%)	42.49	43.31	43.09	43.10
SDF (%)	1.90	2.20	1.60	2.10
IDF (%)	20.20	17.60	17.70	16.80
TDF (%)	22.10	19.80	19.30	18.90
Ash (%)	6.20	6.13	6.16	6.41
Ca (%)	0.24	0.21	0.22	0.24
Total P (%)	0.79	0.78	0.80	0.81
Phytic acid (%)	1.83	1.80	1.86	1.80
Phytate P ^b (%)	0.52	0.51	0.52	0.51
Nonphytate P ^c (%)	0.27	0.27	0.28	0.30
TIA (mg·g ⁻¹)	34.00	23.10	4.20	2.40
Carbohydrates				
Glucose (%)	0.09	ND	ND	ND
Sucrose (%)	7.63	8.06	7.95	7.78
Maltose (%)	0.39	0.27	0.23	0.23
Fructose (%)	ND	ND	ND	ND
Stachyose (%)	4.37	4.67	4.53	4.42
Raffinose (%)	0.80	0.85	0.92	0.85
Indispensable amino acids (%)				
Arg	3.29	3.35	3.31	3.33
His	1.10	1.13	1.12	1.12
Ile	1.91	1.94	1.93	1.95
Leu	3.18	3.24	3.21	3.24
Lys	2.70	2.75	2.70	2.70
Met	0.56	0.58	0.58	0.58
Phe	2.09	2.13	2.11	2.13
Thr	1.59	1.62	1.60	1.62
Trp	0.57	0.58	0.58	0.58
Val	2.00	2.02	2.02	2.03
Dispensable amino acids (%)				
Ala	1.78	1.80	1.79	1.81
Asp	4.72	4.80	4.74	4.79
Cys	0.62	0.63	0.61	0.61
Glu	7.54	7.67	7.58	7.67
Gly	1.75	1.78	1.77	1.79
Ser	2.06	2.09	2.05	2.08
Lys:CP ratio	6.35	6.35	6.27	6.26

Note: AEE, acid-hydrolyzed ether extract; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber; TIA, trypsin inhibitor activity; CP, crude protein; ND, not detected.

^aNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

^bCalculated as 28.2% of phytic acid (Sauvant et al. 2004).

^cCalculated as total P – phytate P.

Table 2. Composition of experimental diets, experiment 1.

Ingredient (%)	Soybean expellers ^a	N-free
Soybean expellers	40.00	—
Soybean oil	3.00	4.00
Solka floc	—	4.00
Dicalcium phosphate	0.85	1.65
Ground limestone	0.30	0.45
Sucrose	10.00	20.00
Cornstarch	44.90	68.45
Magnesium oxide	—	0.10
Potassium carbonate	—	0.40
Chromic oxide	0.40	0.40
Sodium chloride	0.40	0.40
Vitamin–mineral premix ^b	0.15	0.15

Note: N-free, nitrogen-free.

^aFour diets using four different batches of soybean expellers were prepared.

^bThe vitamin–mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11150 IU; vitamin D₃ as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. Samples of all diets and AA-containing ingredients were also collected. Digesta samples were lyophilized, and samples were finely ground using a coffee grinder, which results in particles that can pass through a 1 mm sieve prior to chemical analysis. Samples of diets, ileal digesta, and ingredients were analyzed for N [method 990.03; [Association of Official Analytical Chemists \(AOAC\) Int. 2007](#)] on an FP628 N Determinator (Leco Corporation, St. Joseph, MI, USA), and crude protein (CP) was calculated as N × 6.25. Dry matter (DM; method 930.15; [AOAC Int. 2007](#)) was also analyzed in diets, ingredients, and ileal digesta samples. These samples were also analyzed for AA using ion-exchange chromatography with post-column derivatization with ninhydrin as the internal standard ([Llames and Fontaine 1994](#)). Diets and ileal digesta samples were analyzed for chromium (method 990.08; [AOAC Int. 2007](#)).

The apparent ileal digestibility (AID) and the SID of CP and AA were calculated for the four diets containing soybean expellers ([Stein et al. 2007](#)). Values calculated for these diets also represent the values for each ingredient. The basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet ([Stein et al. 2007](#)).

Data were analyzed using the PROC MIXED procedure (SAS Institute Inc., Cary, NC, USA). Homogeneity of the variances was confirmed using the UNIVARIATE procedure in PROC MIXED. The model included diet as the fixed effect, whereas pig and period were considered random effects. Mean values were calculated using the LSMeans statement, and pig was the experimental unit. If significant, means were separated using the PDIFF option in SAS. Results were considered significant at $P \leq 0.05$.

Experiment 2: Energy measurements

A corn-based diet and four diets containing corn and each source of soybean expellers were formulated; thus, a total of five diets were prepared ([Table 4](#)). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates ([NRC 2012](#)). Forty barrows (initial body weight: 17.52 ± 1.63 kg) were randomly allotted to the five diets in a completely randomized design with eight replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials. Pigs were fed their respective diets at three times the maintenance requirement for ME (i.e., 197 kcal ME per kg body weight^{0.60}; [NRC 2012](#)), each of which was provided each day in two equal meals at 0800 and 1600. Throughout the study, pigs had free access to water. Feed consumption was recorded daily. The initial 5 d was considered the adaptation period to the diet. Urine and fecal materials were collected during the following 4 d according to standard procedures using the marker-to-marker approach ([Adeola 2001](#)). Urine was collected in urine buckets over a preservative of 50 mL of hydrochloric acid. Fecal samples and 20% of the collected urine were stored at -20 °C immediately after collection.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was lyophilized before analysis. Fecal samples were thawed and mixed within pig and diet, and then dried in a 50 °C forced air-drying oven prior to analysis. Diets and fecal samples were analyzed for DM as explained for experiment 1. Ingredients, diets, fecal, and urine samples were analyzed for gross energy (GE) using bomb calorimetry (model 6400; Parr Instruments, Moline, IL, USA). The four sources of soybean expellers were analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 ([AOAC Int. 2007](#)) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary

Table 3. Analyzed composition of experimental diets, experiment 1.

Item (%)	Soybean expeller diets ^a				
	Non-heat-treated	L-0	L-12	L-48	N-free
Dry matter	91.61	91.19	91.30	91.33	91.27
Crude protein	16.88	17.75	16.82	16.93	0.39
Indispensable amino acids					
Arg	1.22	1.38	1.26	1.32	0.01
His	0.44	0.46	0.43	0.45	0.01
Ile	0.80	0.82	0.75	0.79	0.01
Leu	1.26	1.36	1.25	1.31	0.02
Lys	1.09	1.14	1.05	1.08	0.02
Met	0.21	0.24	0.22	0.23	0.01
Phe	0.86	0.89	0.82	0.86	0.01
Thr	0.62	0.68	0.62	0.65	0.01
Trp	0.18	0.24	0.22	0.22	0.02
Val	0.82	0.85	0.78	0.82	0.02
Dispensable amino acids					
Ala	0.71	0.77	0.70	0.73	0.02
Asp	1.85	2.03	1.86	1.93	0.02
Cys	0.24	0.26	0.24	0.24	0.01
Glu	3.02	3.22	2.96	3.09	0.04
Gly	0.70	0.75	0.69	0.72	0.01
Ser	0.71	0.88	0.81	0.84	0.01

Note: N-free, nitrogen-free.

^aNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. The four sources of soybean expellers were analyzed for acid-hydrolyzed ether extract (AEE) by acid hydrolysis using 3 mol·L⁻¹ HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Soybean expellers were analyzed for ash (method 942.05; AOAC Int. 2007), for phytic acid (Ellis et al. 1977), and for calcium and phosphorus by inductively coupled plasma optical emission spectrometry using an internally validated method (method 985.01 A, B, and C; AOAC Int. 2007) after wet ash sample preparation [method 975.03 B(b); AOAC Int. 2007]. Samples were analyzed for sugars including glucose, fructose, maltose, sucrose, stachyose, and raffinose using high-performance liquid chromatography (Dionex App Notes 21 and 92). The trypsin inhibitor activity in soybean expellers was also determined [method 71-10; American Association of Cereal Chemists (AACC) 1995].

Following analysis, the apparent total tract digestibility (ATTD) of GE and DM was calculated for each diet, and the DE and ME in each diet were calculated as well. The DE and ME of corn were then calculated by dividing the

DE and ME of the corn diet by the inclusion rate of corn in that diet. The contribution of DE and ME from corn to the DE and ME in the diets containing the four sources of soybean expellers was subtracted from the DE and ME of each diet, and the DE and ME of each source of soybean expellers were calculated by difference (Adeola 2001). The ATTD of GE in each source of soybean expellers was calculated using the same procedure.

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC, USA) with pig as the experimental unit. Homogeneity of the variances was confirmed as explained for experiment 1. Diet or ingredient was the fixed effect, and replicate was considered the random effect. Treatment means were calculated and separated using the LSMEANS statement and the PDIF option of PROC MIXED, respectively. Results were considered significant at $P \leq 0.05$.

Results

Nutrient composition of soybean expellers

The DM in all sources of soybean expellers was greater than 89%, and the GE in the four sources of soybean expellers ranged from 4590 to 4651 kcal·kg⁻¹. The AEE concentration in non-heat-treated soybean expellers

Table 4. Composition of experimental diets, experiment 2.

Item (%)	Corn	Soybean expeller diets ^a			
		Non-heat-treated	L-0	L-12	L-48
Ground corn	97.45	58.40	58.40	58.40	58.40
Soybean expellers	—	40.00	40.00	40.00	40.00
Ground limestone	0.60	0.30	0.30	0.30	0.30
Dicalcium phosphate	1.40	0.75	0.75	0.75	0.75
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix ^b	0.15	0.15	0.15	0.15	0.15
Analyzed composition					
Dry matter (%)	87.80	88.30	88.30	88.10	88.20
Gross energy (kcal·kg ⁻¹)	3728	4062	4067	4041	4075

^aNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

^bThe vitamin–mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11 150 IU; vitamin D₃ as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

was 10.82%, which is greater compared with heat-treated soybean expellers (i.e., 8.31%–9.27%). The concentration of total dietary fiber in all sources of soybean expellers ranged from 18.90% to 22.10%, and the concentration of insoluble dietary fiber in heat-treated soybean expellers was less than in non-heat-treated soybean expellers. The analyzed CP in non-heat-treated, L-0, L-12, and L-48 soybean expellers was 42.49%, 43.31%, 43.09%, and 43.10%, respectively. The Lys in non-heat-treated, L-0, L-12, and L-48 soybean expellers was 2.70%, 2.75%, 2.70%, and 2.70%, respectively, which resulted in Lys being 6.35%, 6.35%, 6.27%, and 6.26% of CP in non-heat-treated, L-0, L-12, and L-48 soybean expellers, respectively. The analyzed concentration of oligosaccharides (stachyose plus raffinose) in all sources of soybean expellers was greater than 5%. The analyzed trypsin inhibitor activity in non-heat-treated, L-0, L-12, and L-48 soybean expellers was 34.00, 23.10, 4.20, and 2.40 mg·g⁻¹, respectively.

Experiment 1: Amino acid digestibility

Diet analyses indicated that the intended concentrations of CP and AA were present in all diets, and pigs readily consumed their assigned diets and remained healthy throughout the experiment. Short-term steam conditioning and expander treatment increased ($P < 0.01$) the AID and SID for CP in soybean expellers from 27.9% to 44.5% and from 45.0% to 60.7%, respectively

(Tables 5 and 6). Likewise, the AID and SID of all AA increased ($P < 0.01$) by at least 10% units if short-term steam conditioning and expander treatment were applied to soybean expellers. The AID and SID of all AA in L-12 or L-48 soybean expellers were greater ($P < 0.01$) compared with that of L-0 soybean expellers. Therefore, if the treatments for L-12 or L-48 soybean expellers were applied, the AID and SID of CP and all AA in soybean expellers increased ($P < 0.01$) by at least 45% units compared with the non-heat-treated expellers.

Experiment 2: Energy measurements

Pigs fed the corn diet had reduced ($P < 0.01$) GE intake, fecal output, fecal GE loss, and urine GE loss compared with pigs fed the soybean expeller diets (Table 7). The GE intake, fecal output, fecal GE loss, and urine GE loss of pigs fed the L-0 soybean expeller diets were not different from that of pigs fed the L-12 or L-48 soybean expeller diets. The observed reduction in fecal output resulted in greater ($P < 0.01$) ATTD of DM in the corn diet compared with the soybean expeller diets. The ATTD of GE in the corn diet was also greater ($P < 0.01$) than in the non-heat-treated or L-0 soybean expeller diets. However, due to greater GE concentration in soybean expeller diets, the DE concentration in the corn diet was less ($P < 0.01$) than in the soybean expeller diets. The ME concentration in the corn diet was also less ($P < 0.01$) than in the L-0,

Table 5. Apparent ileal digestibility of crude protein and amino acids in four sources of soybean expellers, experiment 1.^a

Item (%)	Soybean expellers ^b				SEM	P value
	Non-heat-treated	L-0	L-12	L-48		
CP	27.9c	44.5b	79.5a	80.3a	2.98	<0.001
Indispensable AA						
Arg	42.3c	59.3b	90.2a	91.7a	2.39	<0.001
His	43.1c	55.4b	87.1a	88.9a	2.32	<0.001
Ile	33.9c	47.2b	82.3a	85.4a	2.45	<0.001
Leu	30.3c	46.9b	83.0a	86.2a	2.47	<0.001
Lys	55.4c	66.7b	91.2a	92.1a	1.80	<0.001
Met	37.9c	53.5b	86.6a	88.7a	2.42	<0.001
Phe	34.9c	48.5b	84.3a	87.5a	2.48	<0.001
Thr	29.0c	46.6b	76.2a	79.0a	2.55	<0.001
Trp	9.42c	42.9b	80.7a	83.0a	3.04	<0.001
Val	35.9c	48.8b	81.4a	84.7a	2.37	<0.001
Total	37.8c	52.8b	85.0a	87.4a	2.31	<0.001
Dispensable AA						
Ala	33.8c	48.5b	78.9a	81.7a	2.44	<0.001
Asp	34.1c	51.3b	84.0a	85.3a	2.47	<0.001
Cys	14.8c	33.6b	75.9a	78.2a	3.54	<0.001
Glu	40.6c	55.1b	86.1a	87.7a	2.38	<0.001
Gly	11.8c	32.9b	67.8a	68.8a	4.14	<0.001
Ser	15.8c	45.2b	80.8a	83.1a	2.91	<0.001
Total	23.6c	42.9b	78.2a	79.4a	3.32	<0.001
All AA	30.5c	47.6b	81.4a	83.2a	2.76	<0.001

Note: Means within a row lacking a common lowercase letter are different ($P < 0.05$). CP, crude protein; AA, amino acids; SEM, standard error of the mean.

^aData are least-squares means of seven to eight observations per treatment.

^bNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

L-12, or L-48 soybean expeller diets. Pigs fed the non-heat-treated soybean expeller diet had greater ($P < 0.01$) fecal output and fecal GE loss compared with the L-0, L-12, or L-48 soybean expeller diets. This resulted in a reduced ($P < 0.01$) ATTD of GE, as well as reduced ($P < 0.01$) concentrations of DE and ME in the non-heat-treated soybean expeller diet compared with the L-0, L-12, or L-48 soybean expeller diets.

Concentrations of DE and ME (as-fed and DM basis) in corn and non-heat-treated soybean expellers were less ($P < 0.01$) than in L-0, L-12, or L-48 soybean expellers (Table 8). The DE to GE ratio, as well as the ME to GE ratio in non-heat-treated soybean expellers were less ($P < 0.01$) than in corn, L-0, L-12, and L-48 soybean expellers. The ME to DE ratio in corn was greater ($P < 0.01$) than in non-heat-treated or L-0 soybean expellers, but there was no difference in ME to DE ratio between corn and the L-12 and L-48 soybean expellers. The ATTD of GE and concentrations of DE and ME between heat-treated soybean expellers were not different regardless of

whether or not long-term steam conditioning was applied. However, if steam conditioning and expander treatment were applied, the concentrations of DE and ME in soybean expellers were increased by at least 325 kcal·kg⁻¹ compared with non-heat-treated expellers.

Discussion

The analyzed concentrations of GE, CP, ash, and AA in soybean expellers are in agreement with published data (NRC 2012; Rodriguez et al. 2020). However, the analyzed AEE in all soybean expellers was greater compared with the AEE reported by Rodriguez et al. (2020). The soybean expellers used by Rodriguez et al. (2020) were produced using a high shear dry extrusion-pressing procedure, and this technology is likely more efficient in extracting oil from soybeans than the procedure used in this experiment. The observed reduction in insoluble dietary fiber and total dietary fiber in heat-treated soybean expellers as steam-conditioning time increased is likely due to modifications in the physico-chemical characteristics of

Table 6. Standardized ileal digestibility of crude protein and amino acids in four sources of soybean expellers, experiment 1.^{a,b}

Item (%)	Soybean expellers ^c				SEM	P value
	Non-heat-treated	L-0	L-12	L-48		
CP	45.0c	60.7b	96.5a	97.3a	2.98	<0.001
Indispensable AA						
Arg	50.2c	66.1b	97.8a	98.8a	2.39	<0.001
His	48.3c	60.3b	92.4a	94.0a	2.32	<0.001
Ile	41.5c	54.5b	90.3a	93.0a	2.45	<0.001
Leu	36.7c	52.7b	89.3a	92.2a	2.47	<0.001
Lys	58.2c	69.4b	94.1a	94.9a	1.80	<0.001
Met	42.4c	57.5b	91.0a	92.9a	2.42	<0.001
Phe	39.5c	52.9b	89.0a	92.0a	2.48	<0.001
Thr	40.8c	57.3b	87.9a	90.2a	2.55	<0.001
Trp	20.7c	51.4b	89.8a	91.9a	3.04	<0.001
Val	42.6c	55.3b	88.4a	91.3a	2.37	<0.001
Total	44.3c	58.8b	91.5a	93.6a	2.31	<0.001
Dispensable AA						
Ala	45.3c	59.1b	90.4a	92.9a	2.44	<0.001
Asp	39.8c	56.5b	89.6a	90.7a	2.47	<0.001
Cys	26.6c	44.5b	87.7a	89.7a	3.54	<0.001
Glu	44.8c	59.0b	90.4a	91.7a	2.38	<0.001
Gly	49.5c	67.9b	105.7a	105.3a	4.14	<0.001
Ser	27.4c	54.5b	90.9a	92.9a	2.91	<0.001
Total	38.9c	56.5b	92.4a	94.8a	3.32	<0.001
All AA	41.7c	57.9b	92.0a	93.7a	2.76	<0.001

Note: Means within a row lacking a common lowercase letter are different ($P < 0.05$). CP, crude protein; AA, amino acids; SEM, standard error of the mean.

^aData are least-squares means of seven to eight observations per treatment.

^bValues for SID were calculated by correcting the values for AID for basal ileal endogenous losses. Basal ileal endogenous losses were determined ($\text{g}\cdot\text{kg}^{-1}$ of dry matter intake) as CP, 30.89; Arg, 1.02; His, 0.24; Ile, 0.64; Leu, 0.86; Lys, 0.33; Met, 0.10; Phe, 0.42; Thr, 0.78; Trp, 0.22; Val, 0.59; Ala, 0.87; Asp, 1.13; Cys, 0.30; Glu, 1.35; Gly, 2.82; and Ser, 0.88.

^cNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

soybean expellers during the expander treatment process (van der Poel et al. 1998). Heat can reduce oil viscosity, which subsequently increases the efficiency of oil removal in oilseeds (Subroto et al. 2015), and this is likely the reason heat-treated soybean expellers had slightly less AEE than non-heat-treated soybean expellers because they may have lost a little bit of oil during processing. The observation that the concentration of oligosaccharides was not different among soybean expellers indicates that heat treatment does not eliminate oligosaccharides in soybeans, and that other types of processing (e.g., alcohol extraction, fermentation, or enzyme treatment) is needed to reduce oligosaccharide concentrations (Cervantes-Pahm and Stein 2010). The analyzed trypsin inhibitor activity ($34 \text{ mg}\cdot\text{g}^{-1}$) in non-heat-treated soybean expellers is in close agreement

with data reported by Goebel and Stein (2011) for raw full-fat soybeans. This indicates that the initial processing of soybeans did not destroy trypsin inhibitors, which is likely a consequence of the cold-extraction procedure that was used to expel oil from the soybeans. The observed reduction in trypsin inhibitor activity in L-0, L-12, and L-48 soybean expellers indicates that the longer time heat was applied, the greater was the inactivation of trypsin inhibitors. However, results indicated that only using short-term steam conditioning and expander treatment was not sufficient to reduce trypsin inhibitors to an acceptable level, and only if long-term steam conditioning was used, was it possible to reduce the level of trypsin inhibitor activity sufficiently. Heat treatment, although important to reduce trypsin inhibitor activity, may result in formation of Maillard reaction products

Table 7. Apparent total tract digestibility of dry matter and gross energy, digestible energy, and metabolizable energy in experimental diets fed to pigs, experiment 2.^a

Item	Corn	Soybean expeller diets ^b				SEM	P value
		Non-heat-treated	L-0	L-12	L-48		
Intake							
Feed (kg·d ⁻¹)	0.82b	0.89a	0.91a	0.89a	0.92a	0.03	<0.001
GE (kcal·d ⁻¹)	3072b	3611a	3697a	3598a	3733a	111.95	<0.001
Fecal excretion							
Dry feces output (kg·d ⁻¹)	0.07c	0.12a	0.10b	0.09b	0.10b	0.01	<0.001
GE (kcal·d ⁻¹)	336c	592a	490b	435b	464b	28.12	<0.001
ATTD (%)							
DM	91.2a	86.4c	88.6b	89.5b	89.0b	0.58	<0.001
GE	89.0a	83.7c	86.8b	88.0ab	87.6ab	0.68	<0.001
DE in diet (kcal·kg ⁻¹)	3316c	3401b	3531a	3555a	3571a	27.48	<0.001
Urine output (kg·d ⁻¹)	1.75b	4.40a	3.16a	3.93a	3.34a	0.53	0.005
Urinary GE output (kcal·d ⁻¹)	55b	125a	114a	95a	91a	15.03	0.004
ME in diet (kcal·kg ⁻¹)	3251b	3261b	3379a	3443a	3473a	28.71	<0.001

Note: Means within a row lacking a common lowercase letter are different ($P < 0.05$). GE, gross energy; ATTD, apparent total tract digestibility; DM, dry matter; DE, digestible energy; ME, metabolized energy; SEM, standard error of the mean.

^aData are least-squares means of seven to eight observations per treatment.

^bNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

Table 8. Energy values in corn and in four sources of soybean expellers fed to pigs, experiment 2.^a

Item	Corn	Soybean expellers ^b				SEM	P value
		Non-heat-treated	L-0	L-12	L-48		
Energy values							
DE (as-fed basis)	3403b	3533b	3858a	3920a	3959a	65.33	<0.001
DE (dry matter basis)	3897b	3909b	4298a	4369a	4416a	72.64	<0.001
ME (as-fed basis)	3336b	3283b	3641a	3746a	3811a	67.40	<0.001
ME (dry matter basis)	3821b	3632b	4057a	4176a	4251a	74.97	<0.001
Energy digestibility and metabolizability (%)							
DE to GE ratio	89.0a	76.0c	84.1b	85.4ab	86.1ab	1.42	<0.001
ME to DE ratio	98.0a	93.0c	94.4bc	95.6ab	96.3ab	0.97	0.002
ME to GE ratio	87.4a	70.6c	79.3b	81.6b	82.9b	1.47	<0.001

Note: Means within a row lacking a common lowercase letter are different ($P < 0.05$). DE, digestible energy; ME, metabolizable energy; GE, gross energy; SEM, standard error of the mean.

^aData are least-squares means of seven to eight observations per treatment.

^bNon-heat-treated, absence of steam conditioning and expander treatment; L-0, short conditioned for 60 s at 90 °C followed by expander treatment at 110 °C; L-12, short conditioned for 60 s at 90 °C, followed by 12 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C; L-48, short conditioned for 60 s at 90 °C, followed by 48 min of long-term steam conditioning at 100 °C, followed by expander treatment at 110 °C.

(Pahm et al. 2008; González-Vega et al. 2011). However, the observation that the Lys to CP ratio in all four sources of soybean expellers ranged from 6.26% to 6.35% indicates that the soybean expellers were not heat damaged (González-Vega et al. 2011).

The observation that the AID and SID for CP and all AA in non-heat-treated soybean expellers were less than in

heat-treated soybean expellers demonstrates that heat treatment was necessary to increase the digestibility of AA in soybean expellers. The observation that L-0 soybean expellers had reduced digestibility of AA when compared with L-12 or L-48 soybean expellers further indicates that the processing conditions applied to L-0 soybean expellers were not sufficient to completely

ameliorate the negative effect of trypsin inhibitors on AA digestibility. Soy products that have trypsin inhibitor activity greater than 5 mg·g⁻¹ should not be fed to pigs or poultry to prevent reduction in growth performance (Batterham et al. 1993; Clarke and Wiseman 2005). Therefore, the observed increase in the digestibility of AA in L-12 and L-48 soybean expellers compared with L-0 expellers is likely a result of the inactivation of trypsin inhibitors upon application of long-term steam conditioning of the expellers. This observation is in agreement with data indicating that heat treatment reduced trypsin inhibitor concentrations with a subsequent improvement in the digestibility of AA in soy products (Herkelman et al. 1992; Qin et al. 1996; Goebel and Stein 2011).

The difference procedure was used to determine concentrations of DE and ME in the four sources of soybean expellers, and a corn-based diet was used for the energy balance experiment. Values for the DE and ME obtained for corn in the present experiment are in close agreement with previous data (Sauvant et al. 2004; NRC 2012; Espinosa and Stein 2018), which gives confidence that calculated values for DE and ME in soybean expellers are also accurate. Values for DE and ME in soybean expellers are in agreement with reported data (Rodhouse et al. 1992; Woodworth et al. 2001; NRC 2012; Rodriguez et al. 2020). To the best of our knowledge, no data demonstrating the effects of heat treatment on concentrations of DE and ME in soybean expellers have been reported. Despite the greater AEE concentration in non-heat-treated soybean expellers, the observed reduction in concentrations of DE and ME in non-heat-treated soybean expellers is most likely a result of the presence of heat-labile anti-nutritional factors in soybean expellers. Trypsin inhibitors reduce the availability of digestible AA in soybean expellers, which subsequently resulted in a reduction in DE and ME concentrations in non-heat-treated soybean expellers. The observed reduction in energy values may also be a result of greater concentration of insoluble dietary fiber in non-heat-treated soybean expellers than in other sources of soybean expellers. Increased insoluble dietary fiber likely resulted in increased fecal output from pigs fed non-heat-treated soybean expellers, resulting in pigs fed non-heat-treated soybean expellers being able to absorb and utilize less energy than pigs fed the heat-treated soybean expellers.

Conclusions

The DE and ME and the SID of AA in non-heat-treated soybean expellers were less than in heat-treated soybean expellers, which demonstrates that heat must be applied to increase nutrient availability in soybean expellers. The SID of AA in L-0 soybean expellers was also less than in L-12 or L-48 soybean expellers; thus, the application of short-term steam conditioning (60 s) at 90 °C followed by expander treatment at 110 °C is not enough to inactivate

trypsin inhibitors in soybean expellers. Therefore, long-term steam conditioning must be applied to completely inactivate trypsin inhibitors and subsequently maximize digestibility of AA in soybean expellers.

However, the DE and ME in L-0 soybean expellers were not different from that of L-12 or L-48 soybean expellers indicating that the long-term conditioning is not needed to maximize energy digestibility. The SID of AA and concentrations of DE and ME were not different between L-12 and L-48 soybean expellers, which indicates that 12 min of long-term steam conditioning at 100 °C followed by expander treatment at 110 °C is sufficient to inactivate trypsin inhibitors to optimize the nutritional value of soybean expellers.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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