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Digestibility by growing pigs of amino acids in heat-damaged sunflower meal and cottonseed meal[1]

F. N. Almeida,* J. K. Htoo,† J. Thomson,‡ and H. H. Stein*[2]

*University of Illinois, Urbana 61801; †Evonik Industries AG,
Rodenbacher Chaussee 4, 63457 Hanau, Germany; and ‡Evonik Degussa Corporation, Kennesaw, GA

ABSTRACT: Two experiments were conducted to determine the effects of heat damage, achieved by autoclaving, on the nutritional composition and on the standardized ileal digestibility (SID) of AA in sunflower meal (SFM) and cottonseed meal (CSM) fed to growing pigs. The second objective was to establish a relationship between the concentration of SID AA in SFM and CSM and the concentration of fiber components, reducing sugars, and AA. In Exp. 1, 10 growing pigs (initial BW: 23.1 ± 1.3 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. A common source of SFM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 20, 40, or 60 min. Four diets (approximately 14.5% CP) that contained each of the 4 batches of SFM were formulated, and SFM was the only source of CP and AA in the diets. A N-free diet that was used to determine the basal endogenous losses of CP and AA from pigs was also formulated. Each period consisted of 5 d of adaptation to the diets followed by 2 d of ileal digesta collection. The SID of Lys in SFM was reduced (linear, $P < 0.05$) from 83.2% in nonautoclaved SFM to 63.5% in SFM autoclaved for 60 min at 130°C. The concentrations of total Lys and reducing sugars in SFM may be used as predictors ($R^2 = 0.85$) of the concentration of SID Lys in SFM. In Exp. 2, 10 growing pigs (initial BW: 35.0 ± 1.5 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. A source of CSM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 15, 35, or 60 min. Four diets (approximately 13.4% CP) containing CSM as the only source of CP and AA were formulated. A N-free diet was also formulated and used as described for Exp. 1. The SID of Lys in nonautoclaved CSM (66.2%) was greater ($P < 0.05$) than in autoclaved (60 min at 130°C) CSM (54.1%). The equation ($R^2 = 0.68$) that best predicted the concentration of SID Lys in CSM included the concentration of ADIN. In conclusion, heat damage reduces the SID of AA in SFM and CSM. For SFM, the concentration of SID Lys may be predicted from the concentrations of total Lys and reducing sugars. The concentration of ADIN may be used to predict the concentration of SID Lys in CSM.

Key words: amino acids, cottonseed meal, digestibility, heat damage, pigs, sunflower meal

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INTRODUCTION

Sunflower meal (**SFM**) and cottonseed meal (**CSM**) are protein sources for swine diets (González-Vega and Stein, 2012). Cottonseed meal contains the antinutritional factor gossypol, which may be deactivated by heat treatment of the meal, but processes involving heat and moisture may cause Maillard reactions (Nursten, 2005), and the application of heat to feed ingredients may decrease the concentration, digestibility, and utilization of Lys and other AA (Van Barneveld et al., 1994; Pahm et al., 2008; Boucher et al., 2009; González-Vega et al., 2011). Amino acids that participate in the Maillard reaction may become unavailable to pigs and Lys is the AA most susceptible to participate in these reactions (Pahm et al., 2008). Conventional AA analysis of heat-damaged feed ingredients is believed to overestimate the concen-

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[2]Corresponding author: hstein@illinois.edu
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Table 1. Ingredient composition of experimental diets (as-fed basis), Exp. 1 and 2

	Sunflower meal (Exp. 1)				Cottonseed meal (Exp. 2)				
		Autoclaved at 130°C				Autoclaved at 130°C			
Ingredient, %	Nonautoclaved	20 min	40 min	60 min	Nonautoclaved	15 min	35 min	60 min	N-free[1]
Sunflower meal	42.00	42.00	42.00	42.00	–	–	–	–	–
Cottonseed meal	–	–	–	–	32.00	32.00	32.00	32.00	–
Cornstarch	42.00	42.00	42.00	42.00	41.97	41.97	41.97	41.97	67.00
Sucrose	10.00	10.00	10.00	10.00	20.00	20.00	20.00	20.00	20.00
Solka floc[2]	–	–	–	–	–	–	–	–	5.00
Soybean oil	3.40	3.40	3.40	3.40	3.50	3.50	3.50	3.50	4.00
Ground limestone	0.85	0.85	0.85	0.85	0.90	0.90	0.90	0.90	0.80
Monocalcium phosphate	0.65	0.65	0.65	0.65	0.50	0.50	0.50	0.50	1.60
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Ferrous sulfate	–	–	–	–	0.03	0.03	0.03	0.03	–
Magnesium oxide	–	–	–	–	–	–	–	–	0.10
Potassium carbonate	–	–	–	–	–	–	–	–	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]A N-free diet was produced separately for Exp. 1 and 2.

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

[3]Provided the following per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadionenicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 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 as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 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.

tration of Lys that can be used for protein synthesis (i.e., reactive Lys) because Lys that has reacted with reducing sugars is partially recovered during the acid hydrolysis step although it cannot be used for protein synthesis. Determination of reactive Lys, color of ingredients, and the Lys:CP ratio have been suggested as approaches to estimate the availability (i.e., potential to be metabolically available to the animal) of Lys in heat processed feed ingredients (Moughan and Rutherfurd, 1996; Fontaine et al., 2007; Pahm et al., 2008; Kim et al., 2012).

However, there is limited information about the effects of heat processing on AA digestibility in SFM and CSM. Therefore, the primary objective of these experiments was to determine effects of heat damage induced by autoclaving on the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of AA in SFM and in CSM fed to growing pigs. The second objective was to establish a relationship between concentrations of fiber components, reducing sugars, and AA in SFM and CSM and the concentration of SID AA.

MATERIALS AND METHODS

The protocols for these experiments were reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois (Urbana, IL). Pigs with similar genetic makeup (G-Performer boars × Fertilium 25 females; Genetiporc, Alexandria, MN) were used in both experiments. The duration of autoclaving for SFM or CSM was chosen to produce meals with concentrations of analyzed total Lys that are within reported analyzed values (NRC, 2012).

Experiment 1: AA Digestibility of Sunflower Meal

Animals, Experimental Design, and Diets. Ten growing pigs (initial BW: 23.1 ± 1.3 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were placed in pens (1.2 × 1.5 m) equipped with a nipple drinker and a feeder. Sunflower meal was obtained from a commercial company (Archer Daniels Midland Company, Enderlin, ND). The SFM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 20, 40, or 60 min. Four diets that contained each of the 4 batches of SFM were formulated (Tables 1 and 2). Sunflower meal was the only source of CP and AA in the diets. A N-free diet used to determine the basal endogenous losses of CP and AA in the pigs was also formulated.

Feed allowance was calculated as 3 times the maintenance requirement for energy (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Feed allowance was adjusted according to the BW of pigs at the beginning of each period. Feed was provided once daily at 0800 h and water was available at all times.

Sample Collection. Each period consisted of 7 d. The initial 5 d were considered an adaptation period to

Table 2. Analyzed composition of experimental diets (as-fed basis), Exp. 1 and 2[1]

Item	Sunflower meal (Exp. 1)				Cottonseed meal (Exp. 2)			
		Autoclaved at 130°C				Autoclaved at 130°C		
	Nonautoclaved	20 min	40 min	60 min	Nonautoclaved	15 min	35 min	60 min
DM, %	91.4	90.9	90.9	90.7	92.7	91.9	92.0	92.4
CP, %	13.9	14.6	15.0	14.3	14.0	12.5	13.6	13.6
Indispensable AA, %								
Arg	1.2	1.1	1.2	1.0	1.5	1.3	1.3	1.4
His	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.4
Ile	0.6	0.6	0.6	0.6	0.4	0.4	0.4	0.4
Leu	1.0	0.9	1.0	0.9	0.8	0.7	0.7	0.8
Lys	0.5	0.5	0.5	0.4	0.6	0.5	0.5	0.5
Met	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2
Phe	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7
Thr	0.6	0.5	0.6	0.5	0.4	0.4	0.4	0.4
Trp	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2
Val	0.8	0.7	0.8	0.7	0.6	0.5	0.5	0.6
All indispensable AA	6.3	5.9	6.3	5.5	5.9	5.1	5.2	5.5
Dispensable AA, %								
Ala	0.7	0.6	0.7	0.6	0.5	0.5	0.5	0.5
Asp	1.4	1.3	1.4	1.3	1.2	1.1	1.1	1.2
Cys	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Glu	2.9	2.7	3.0	2.7	2.6	2.3	2.4	2.6
Gly	0.9	0.8	0.9	0.8	0.6	0.5	0.5	0.6
Pro	0.7	0.6	0.7	0.6	0.5	0.5	0.5	0.5
Ser	0.6	0.6	0.7	0.6	0.6	0.5	0.5	0.6
All dispensable AA	7.4	7.0	7.6	6.8	6.2	5.6	5.8	6.2
Total AA	13.6	12.9	13.9	12.3	12.1	10.7	11.0	11.7

[1]The concentrations (%) of DM and CP in the N-free diet were 92.16 and 0.58 in Exp. 1 and 91.94 and 0.24 in Exp. 2, respectively.

the diet. Ileal digesta were collected on d 6 and 7 for 8 h by attaching a plastic bag to the cannula barrel and digesta flowing into the bag were collected. Bags were replaced whenever they were filled with digesta or at least once every 30 min and immediately stored at –20°C to prevent bacterial degradation of AA in the digesta.

Chemical Analyses. All samples were analyzed in duplicates. At the conclusion of the experiment, ileal digesta samples were thawed and mixed within animal and diet, and a subsample was lyophilized, finely ground, and analyzed. A sample of each diet and of each batch of SFM was collected at the time of diet mixing. Diets, ingredients, and ileal samples were analyzed for AA by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with sodium metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 *N* HCl for 24 h at 110°C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. The concentration of Pro was quantified after post-column derivatization using ninhydrin at a wavelengths of 440 nm and Trp was determined by HPLC with fluorescence detection (extinction, 280 nm, and emission, 356 nm) after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000). Diets, ingredients, and ileal samples were also analyzed for DM (method 935.29; AOAC International, 2007) and for CP following the Dumas procedure (method 968.06; AOAC International, 2007), and diets and ileal digesta samples were analyzed for Cr (method 990.08; AOAC International, 2007). Each batch of SFM was also analyzed for ash (method 942.05; AOAC International, 2007), ADF (method 973.18; AOAC International, 2007), NDF (Holst, 1973), lignin (method 973.18 [A–D]; AOAC International, 2007), ADIN (method 990.03; AOAC International, 2007), and total reducing sugars (Dubois et al., 1956), for Ca and P by inductively coupled plasma spectroscopy (method 985.01; AOAC International, 2007), and for total fat by acid hydrolysis using 3 *N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (method 2003.06; AOAC International, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). The Minolta L* value for each batch of SFM was also determined (8 mm aperture, D65 light source, and 0° observer; Minolta Camera Company, Osaka, Japan).

Calculations and Statistical Analysis. Values for AID and SID of CP and AA in each batch of SFM were calculated (Stein et al., 2007), and the Lys:CP in each batch was calculated by expressing the concentration of Lys in

Table 3. Chemical composition of sunflower meal and effects of heat damage on nutritional composition of sunflower meal[1]

	Sunflower meal					*P*-value[2]	
Item	0	20	40	60	SEM	Linear	Quadratic
DM, %	91.3	89.4	90.1	89.2	0.5	0.04	0.08
Ash, %	8.1	8.3	8.0	7.9	0.2	0.41	0.60
CP, %	37.0	36.9	36.7	36.4	0.6	0.48	0.99
ADF, %	21.8	20.3	21.1	23.3	0.8	0.16	0.80
NDF, %	29.5	30.9	31.7	35.9	2.4	0.10	0.72
Lignin, %	6.6	5.7	6.4	8.7	0.7	0.06	0.99
ADIN, %	0.2	0.2	0.3	0.5	0.1	0.01	0.76
Reducing sugars, %	3.5	3.5	3.3	3.2	0.5	0.66	0.83
Lys:CP[3]	3.6	3.4	3.2	2.8	0.2	<0.01	0.53
AEE,[4] %	1.9	0.9	1.8	1.6	nd[5]	nd	nd
Ca, %	0.4	0.3	0.4	0.3	nd	nd	nd
P, %	1.3	1.3	1.3	1.1	nd	nd	nd
L*	53.8	51.7	49.7	51.8	nd	nd	nd
Indispensable AA, %							
Arg	2.8	2.8	2.8	2.6	0.1	0.23	0.71
His	0.9	0.9	0.9	0.8	0.0	0.33	0.31
Ile	1.4	1.5	1.5	1.4	0.1	0.85	0.86
Leu	2.2	2.3	2.3	2.3	0.1	0.65	0.97
Lys	1.3	1.2	1.2	1.0	0.1	0.01	0.54
Met	0.8	0.8	0.8	0.8	0.0	0.82	0.79
Phe	1.6	1.6	1.7	1.6	0.1	0.48	0.93
Thr	1.3	1.3	1.3	1.3	0.0	0.64	0.78
Trp	0.5	0.5	0.5	0.4	0.0	<0.01	0.51
Val	1.7	1.8	1.8	1.7	0.1	0.74	0.99
Dispensable AA, %							
Ala	1.5	1.5	1.6	1.6	0.1	0.58	0.88
Asp	3.1	3.2	3.2	3.2	0.1	0.62	0.87
Cys	0.6	0.6	0.6	0.5	0.0	0.48	0.73
Glu	6.4	6.6	6.6	6.7	0.2	0.38	0.95
Gly	2.0	2.0	2.1	2.0	0.1	0.65	0.80
Pro	1.4	1.5	1.5	1.5	0.1	0.18	0.47
Ser	1.3	1.3	1.4	1.4	0.0	0.46	0.27

[1]Data are means of 3 observations, except for AEE, Ca, P, and L*.

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

[3]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

[4]AEE = acid hydrolyzed ether extract.

[5]nd = not determined.

Table 4. Apparent ileal digestibility of CP and AA in sunflower meal subjected to increasing duration of autoclaving by growing pigs (Exp. 1)[1]

	Sunflower meal						
		Autoclaved at 130°C				*P*-value[2]	
Item	Nonautoclaved	20 min	40 min	60 min	SEM	Linear	Quadratic
CP, %	69.5	70.1	64.7	57.9	2.1	<0.01	0.41
Indispensable AA, %							
Arg	88.0	87.2	84.6	81.0	1.0	<0.01	0.88
His	81.5	80.1	78.5	71.6	1.2	<0.01	0.23
Ile	81.6	81.4	80.0	75.0	1.1	<0.01	0.56
Leu	82.1	81.7	80.3	75.3	1.1	<0.01	0.53
Lys	73.9	71.3	64.9	51.4	2.1	<0.01	0.66
Met	89.2	88.3	87.9	83.4	0.7	<0.01	0.08
Phe	84.3	84.1	83.6	79.6	1.0	<0.01	0.34
Thr	74.3	73.3	70.7	63.3	1.6	<0.01	0.58
Trp	77.0	78.6	74.4	69.3	1.5	<0.01	0.37
Val	80.6	80.3	78.7	73.3	1.2	<0.01	0.56
Average	82.0	81.1	79.3	73.3	1.1	<0.01	0.43
Dispensable AA, %							
Ala	73.5	73.2	69.2	60.5	2.1	<0.01	0.89
Asp	79.1	77.4	74.1	65.1	1.3	<0.01	0.34
Cys	75.9	74.8	70.4	62.2	2.0	<0.01	0.95
Glu	87.7	87.2	85.6	81.8	1.0	<0.01	0.71
Gly	54.6	57.1	44.6	36.0	4.8	<0.01	0.28
Ser	74.2	72.9	71.5	64.2	1.2	<0.01	0.26
Average	74.2	73.8	69.7	62.2	1.7	<0.01	0.94

[1]Data are means of 10 observations.

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

the sample as a percentage of the CP ratio in the sample (Kim et al., 2012). Data were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). Normality of the data and the presence of outliers were evaluated using the UNIVARIATE procedure of SAS. The model included diet as a fixed effect and pig and period as random effects. Linear and quadratic effects of increasing duration of heat treatment on the AID and SID of AA were analyzed by orthogonal polynomial contrasts. Regression equations to estimate the concentration of SID AA were developed using the REG procedure in SAS. The forward selection method was used to choose the equations that best fit the data (i.e., equations with the least root mean square error and greatest R^2). The pig was the experimental unit and significance among means was assessed with an α level of 0.05. If *P*-values were greater than 0.05 and less than 0.10, those were considered a tendency.

Experiment 2: AA Digestibility of Cottonseed Meal

Animals, Experimental Design, and Diets. Ten growing pigs (initial BW: 35.0 ± 1.5 kg) were used as described for Exp. 1. Cottonseed meal was procured from a commercial company (Delta Oil Mill, Jonestown, MS). The CSM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 15, 35, or 60 min. Four diets that contained each of the 4 batches of CSM were formulated, and CSM was the only source of CP and AA in the diets (Tables 1 and 2). A N-free diet that was used to determine the basal endogenous losses of CP and AA in the pigs was also formulated. Feed allowance and feeding schedule followed the same pattern as for Exp. 1.

Ileal digesta samples were collected from pigs as described for Exp. 1 and ileal samples, diets, and ingredients were processed and chemically analyzed as described for Exp. 1. Each batch of CSM was also ana-

Table 5. Standardized ileal digestibility of CP and AA in sunflower meal subjected to increasing duration of autoclaving by growing pigs (Exp. 1)[1]

	Sunflower meal						
		Autoclaved at 130°C				*P*-value[2]	
Item	Nonautoclaved	20 min	40 min	60 min	SEM	Linear	Quadratic
CP, %	82.7	82.6	76.8	70.6	2.1	<0.01	0.35
Indispensable AA, %							
Arg	92.5	92.1	89.3	86.5	1.0	<0.01	0.55
His	86.9	86.0	83.9	77.8	1.2	<0.01	0.48
Ile	87.6	87.6	85.8	81.4	1.1	<0.01	0.83
Leu	88.2	88.1	86.2	81.9	1.1	<0.01	0.85
Lys	83.2	81.2	74.8	63.5	2.1	<0.01	0.96
Met	92.8	92.3	91.6	87.8	0.7	<0.01	0.25
Phe	89.8	89.9	89.0	85.6	1.0	<0.01	0.68
Thr	84.6	84.2	80.8	74.4	1.6	<0.01	0.96
Trp	85.4	86.5	82.8	78.6	1.5	<0.01	0.40
Val	87.2	87.1	85.0	80.3	1.2	<0.01	0.88
Average	88.5	88.0	85.7	80.7	1.1	<0.01	0.79
Dispensable AA, %							
Ala	84.9	85.1	80.2	72.8	2.1	<0.01	0.69
Asp	85.4	84.0	80.2	71.9	1.3	<0.01	0.62
Cys	84.6	84.1	79.3	72.4	2.0	<0.01	0.78
Glu	91.6	91.3	89.4	86.0	1.0	<0.01	0.99
Gly	73.8	77.1	63.4	52.9	3.9	<0.01	0.17
Ser	83.2	82.3	80.1	73.9	1.2	<0.01	0.56
Average	83.9	84.0	79.1	71.9	1.7	<0.01	0.59

[1]Data are means of 10 observations; Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI) determined by feeding pigs a N-free diet: CP, 20.08; Arg, 0.61; His, 0.22; Ile, 0.40; Leu, 0.66; Lys, 0.55; Met, 0.13; Phe, 0.42; Thr, 0.63; Trp, 0.18; Val, 0.54; Ala, 0.84; Asp, 0.94, Cys, 0.24; Glu, 1.24; Gly, 1.85; and Ser, 0.63.

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

lyzed for free gossypol (method Ba 8a-99; AOCS, 1998). Data were analyzed as described for Exp. 1.

RESULTS

Experiment 1: Sunflower Meal

The concentrations of most nutrients in SFM were not affected by duration of autoclaving (Table 3). A linear decrease ($P < 0.05$) was observed for the concentration of DM with increasing duration of autoclaving. The concentration of lignin tended ($P = 0.06$) to increase and the concentration of ADIN increased linearly ($P < 0.05$) with increased duration of autoclaving. There was a linear reduction ($P < 0.05$) in the Lys:CP and in the concentrations of Lys and Trp as the duration of autoclaving increased.

The AID of CP decreased (linear, $P < 0.01$) as the duration of autoclaving increased (Table 4). Likewise, increasing the duration of autoclaving decreased (linear, $P < 0.01$) the AID of all AA. The SID of CP was also reduced (linear, $P < 0.01$) by increasing the duration of autoclaving (Table 5). For all AA, increasing the duration of autoclaving reduced (linear, $P < 0.01$) the SID of AA. The concentration of SID Lys in SFM may be predicted ($P < 0.01$) from the concentration (%) of analyzed Lys in combination with the concentration (%) of reducing sugars using the following equation: SID Lys (%) = –1.00 + 0.54 × Lys + 0.30 × reducing sugars ($R^2 = 0.85$; Table 6).

Experiment 2: Cottonseed Meal

Increasing the duration of autoclaving linearly decreased ($P < 0.05$) the concentration of DM and reducing sugars (Table 7). The concentrations of CP and NDF, however, increased linearly ($P < 0.05$) as duration of autoclaving increased from 0 to 60 min. Acid detergent fiber and ADIN tended ($P < 0.10$) to increase whereas the Lys:CP tended ($P < 0.10$) to decrease as duration of autoclaving increased.

Table 6. Linear regression to predict the concentration (%) of standardized ileal digestible (SID) AA from the concentrations (%) of AA, NDF, lignin, analyzed Lys as percentage of CP, and reducing sugars as independent variables in sunflower meal fed to pigs[1]

Dependent	Intercept			Independent variables[2]									
variable	Estimate	SE	*P*-value	Variable 1	Estimate	SE	*P*-value	Variable 2	Estimate	SE	*P*-value	RMSE[2]	Adjusted R^2
SID Arg	–0.99	0.30	<0.01	Arg	0.89	0.15	<0.01	Lys:CP	0.32	0.09	<0.01	0.08	0.77
SID His	0.45	0.17	<0.05	His	0.60	0.19	<0.01	NDF	–0.07	0.00	<0.01	0.03	0.67
SID Ile	0.64	0.29	<0.05	Ile	0.59	0.29	<0.01	NDF	–0.07	0.00	<0.01	0.04	0.43
SID Leu	0.99	0.44	<0.05	Leu	0.61	0.20	<0.01	NDF	–0.01	0.00	<0.01	0.07	0.49
SID Lys	–1.00	0.12	<0.01	Lys	0.54	0.31	0.08	RS[3]	0.30	0.08	<0.01	0.06	0.85
SID Met	0.20	0.08	<0.05	Met	0.81	0.10	<0.01	NDF	–0.003	0.001	<0.01	0.01	0.81
SID Phe	0.46	0.26	0.08	Phe	0.73	0.16	<0.01	NDF	–0.01	0.00	<0.01	0.04	0.50
SID Thr	1.45	0.08	<0.01	NDF	–0.01	0.00	<0.01	–	–	–	–	0.06	0.48
SID Trp	–0.23	0.05	<0.01	Trp	0.53	0.23	<0.05	Lys:CP	0.12	0.04	<0.01	0.02	0.81
SID Val	1.59	0.11	<0.01	NDF	–0.03	0.01	<0.01	Lignin	0.13	0.05	<0.05	0.06	0.46

[1]n = 40 observations; for all models, $P < 0.01$.

[2]RMSE = root mean square error.

[3]RS = reducing sugars.

Table 7. Chemical composition of cottonseed meal and effects of heat damage on the nutritional composition of cottonseed meal[1]

	Cottonseed meal					*P*-value[2]	
Item	0	15	35	60	SEM	Linear	Quadratic
DM, %	90.9	90.4	87.1	87.9	0.5	<0.01	0.06
Ash, %	8.7	8.8	8.6	9.2	0.3	0.30	0.44
CP, %	41.8	41.9	43.7	43.8	0.7	0.04	0.67
ADF, %	18.8	18.5	19.9	21.9	1.3	0.08	0.54
NDF, %	25.2	23.7	29.4	29.8	1.5	0.02	0.98
Lignin, %	6.4	8.8	8.4	9.2	1.2	0.21	0.52
ADIN, %	0.3	0.3	0.5	0.5	0.1	0.07	0.41
Reducing sugars, %	3.5	3.4	2.6	2.8	0.3	0.03	0.29
Lys:CP ratio[3]	4.1	4.0	3.9	3.8	0.1	0.08	0.54
AEE,[4] %	1.9	0.9	1.8	1.6	nd[5]	nd	nd
Ca, %	0.4	0.3	0.4	0.3	nd	nd	nd
P, %	1.3	1.3	1.3	1.1	nd	nd	nd
L*	53.8	51.7	49.7	51.8	nd	nd	nd
Free gossypol, %	<0.02	<0.02	<0.02	<0.02	nd	nd	nd
Total gossypol, %	0.7	0.6	0.5	0.4	nd	nd	nd
Indispensable AA, %							
Arg	4.4	4.3	4.3	4.2	0.1	0.25	0.63
His	1.1	1.1	1.2	1.2	0.0	0.14	0.97
Ile	1.3	1.3	1.4	1.4	0.0	0.03	0.92
Leu	2.4	2.3	2.5	2.5	0.1	0.05	0.89
Lys	1.7	1.7	1.7	1.7	0.1	0.56	0.74
Met	0.6	0.6	0.7	0.7	0.0	0.26	0.90
Phe	2.1	2.1	2.3	2.3	0.1	0.02	0.74
Thr	1.3	1.3	1.3	1.4	0.0	0.04	0.98
Trp	0.4	0.4	0.4	0.4	0.0	0.98	0.87
Val	1.8	1.7	1.9	1.9	0.1	0.11	0.92
Dispensable AA, %							
Ala	1.6	1.6	1.7	1.7	0.0	0.09	0.96
Asp	3.6	3.6	3.7	3.6	0.1	0.59	0.94
Cys	0.6	0.6	0.6	0.6	0.0	0.52	0.94
Glu	7.7	7.6	7.9	7.8	0.2	0.42	0.87
Gly	1.7	1.7	1.8	1.8	0.0	0.10	0.91
Pro	1.5	1.4	1.5	1.5	0.1	0.39	0.62
Ser	1.5	1.6	1.7	1.6	0.1	0.34	0.56

[1]Data are means of 3 observations, except for AEE, Ca, P, and L*, and the data for gossypol.

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

[3]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

[4]AEE = acid hydrolyzed ether extract.

[5]nd = not determined.

The AID of all indispensable AA in CSM decreased quadratically ($P < 0.01$) as duration of autoclaving increased from 0 to 60 min (Table 8). Likewise, the SID of all AA in CSM was reduced (quadratic, $P < 0.01$) with increasing duration of autoclaving (Table 9). The SID of Lys (54.42, 49.75, and 54.10%) in CSM autoclaved for 15, 35, and 60 min, respectively, was less ($P < 0.05$) than the SID of Lys in nonautoclaved CSM (66.21%).

The SID AA (%) for all indispensable AA, except Arg and Trp, in CSM may be predicted from the concentration (%) of ADIN and from the concentration of other nutritional components, either alone or in combination with other predictor variables (Table 10). The concentration (%) of SID Lys may be predicted using the following equation: SID Lys = 1.81 – 3.67 × ADIN ($R^2 = 0.68$).

DISCUSSION

Pigs maintained good health status throughout the experiments and pigs used in Exp. 1 consumed their diets well. At the end of period 3 in Exp. 2, some pigs refused to consume all of their daily allotments. It has been shown that free gossypol, which is an antinutritional factor in CSM, is toxic to animals and if present

Table 8. Apparent ileal digestibility of CP and AA in cottonseed meal subjected to increasing duration of autoclaving by growing pigs (Exp. 2)[1]

	Cottonseed meal						
		Autoclaved at 130°C				*P*-value[2]	
Item	Nonautoclaved	15 min	35 min	60 min	SEM	Linear	Quadratic
CP, %	61.3	52.6	51.2	53.7	2.4	<0.01	<0.01
Indispensable AA, %							
Arg	82.2	77.3	74.6	77.8	1.3	<0.01	<0.01
His	75.5	67.9	64.4	68.1	1.2	<0.01	<0.01
Ile	64.0	54.5	51.9	57.9	1.6	0.04	<0.01
Leu	66.8	60.2	57.8	63.2	1.5	0.15	<0.01
Lys	59.1	45.8	41.1	45.6	1.8	<0.01	<0.01
Met	67.6	61.5	58.9	63.9	1.4	0.10	<0.01
Phe	77.4	72.1	69.9	74.1	1.2	0.11	<0.01
Thr	58.4	51.0	47.4	53.0	1.8	0.06	<0.01
Trp	67.9	55.3	57.1	59.1	1.6	0.01	<0.01
Val	67.4	58.6	56.4	62.0	1.5	0.05	<0.01
Mean	71.2	63.9	61.1	65.5	1.2	<0.01	<0.01
Dispensable AA, %							
Ala	52.7	44.2	40.7	48.8	2.6	0.12	<0.01
Asp	71.0	63.0	56.0	60.0	1.4	<0.01	<0.01
Cys	70.6	64.7	60.7	64.4	1.6	0.01	<0.01
Glu	81.0	76.3	73.3	76.4	1.1	<0.01	<0.01
Gly	25.6	16.0	9.3	22.7	6.7	0.42	<0.01
Ser	66.5	62.4	58.5	63.2	1.3	0.07	<0.01
Mean	61.2	54.4	49.7	55.8	1.9	<0.01	<0.01

[1]Data are means of 10 observations.

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

in diets in amounts greater than 100 mg/kg may cause depressed appetite (Tanksley and Knabe, 1981; Akande et al., 2010). However, CSM used in this experiment contained concentrations of free gossypol below detection levels and even at relatively high inclusion levels in the diets, the concentration of free gossypol in the CSM diets was below 100 mg/kg. Diets used in the CSM experiment were also supplemented with ferrous sulfate, which has been reported to mitigate gossypol toxicity (Moreira et al., 2006). Nevertheless, after period 3, all pigs were fed regular commercial diets for 10 d and, when given the experimental diets for the subsequent experimental periods, no issues with feed consumption were observed.

Effects of Autoclaving on Nutrient Composition

The nutrient composition of nonautoclaved SFM and CSM are in agreement with the values reported for these ingredients (Rostagno et al., 2011; NRC, 2012). Some variation in the nutritional composition among different sources of feed ingredients exists, and these variations may be caused by heat processing during production of SFM and CSM. As an example, heat damage increases the analyzed concentrations of ADF and lignin in hay because of the formation of Maillard products that are analyzed as lignin (Miao et al., 1994). The concentration of ADIN in orchardgrass and alfalfa also increases as length of exposure to heat increases although the increase in ADIN concentration in orchardgrass was of a greater proportion than that observed for alfalfa (Goering et al., 1973). Heat processing of sunflower expellers at 150°C for different time periods also resulted in increased analyzed concentrations of ADIN (Schroeder et al., 1996). Our results for the concentrations of ADIN in SFM support the above observations. Heat damage of feed ingredients does not usually affect the concentration of CP although the concentration of Lys is reduced (González-Vega et al., 2011; Kim et al., 2012). As a consequence, the concentration of Lys expressed as a percentage of the concentration of CP can be used as an indicator of heat damage in feed ingredients. Therefore, it is expected that the greater the degree of heat damage, the lower the Lys:CP ratio will be (Stein et al., 2009; Cozannet et al., 2010; Skiba et al., 2011). Results observed in these experiments for both SFM and CSM support this assumption.

Effects of Autoclaving on AA Digestibility

Values for the SID of CP and for the SID of AA determined for nonautoclaved SFM are in close agreement with values reported by NRC (2012). Likewise, the SID of CP and SID of AA determined for nonautoclaved CSM concur with the SID values presented by NRC (2012). The SID of Lys in SFM has been reported in a range from 75.8 to 80.0%, which is narrower than the range observed in this experiment although it encompasses some of the values we observed (Jondreville et al., 2000; González-Vega and Stein, 2012). Values for the SID of Lys in CSM reported by NRC (2012) ranged from 52.15 to 73.85% and the SID of Lys determined for CSM in this experiment ranged from 49.75 to 66.21%. These differences may be results of differences in the composition of the oilseed meals. However, although autoclaving is not used in commercial production of SFM and CSM, the present data indicate that some of the variation in the SID of Lys in commercial sources of SFM and CSM may be a result of differences in heat processing of the meals.

The observed decreases in the SID of AA in both SFM and CSM resulting from increasing duration of autoclaving was expected, and this also has been observed in distillers' dried grains with solubles and soybean meal (Fontaine et al., 2007; González-Vega et al., 2011). Heat processing reduces the digestibility of AA because AA and protein undergoes Maillard reactions to form insoluble complexes and cross-linking proteins (Nursten, 2005). Therefore, these reactions may yield AA and protein containing products that are less accessible to digestive enzymes. Consequently, the overall digestibility of CP and AA is reduced.

Table 9. Standardized ileal digestibility of CP and AA in cottonseed meal subjected to increasing levels of heat treatment by growing pigs (Exp. 2)[1]

	Cottonseed meal						
		Autoclaved at 130°C				P-value[2]	
Item	Nonautoclaved	15 min	35 min	60 min	SEM	Linear	Quadratic
CP, %	76.0	69.1	66.3	68.8	2.4	<0.01	<0.01
Indispensable AA, %							
Arg	88.4	84.4	81.7	84.6	1.3	<0.01	<0.01
His	80.9	74.2	70.6	73.9	1.2	<0.01	<0.01
Ile	70.7	62.2	59.5	64.9	1.6	0.04	<0.01
Leu	73.1	67.2	64.6	69.5	1.5	0.13	<0.01
Lys	66.2	54.4	49.8	54.1	1.8	<0.01	<0.01
Met	71.9	66.2	63.7	68.4	1.4	0.12	<0.01
Phe	81.8	77.1	74.7	78.6	1.2	0.10	<0.01
Thr	70.5	64.3	60.3	65.1	1.8	0.05	<0.01
Trp	75.5	65.0	65.6	67.6	1.6	0.02	<0.01
Val	74.3	66.6	64.1	69.2	1.5	0.05	<0.01
Mean	75.3	68.2	65.5	69.6	1.4	0.01	<0.01
Dispensable AA, %							
Ala	66.4	59.2	55.4	62.4	2.6	0.09	<0.01
Asp	77.4	70.1	62.9	66.6	1.4	<0.01	<0.01
Cys	78.4	73.6	69.6	73.0	1.6	0.02	<0.01
Glu	84.8	80.5	77.3	80.2	1.1	<0.01	<0.01
Gly	63.0	57.6	49.3	60.0	6.7	0.27	<0.01
Ser	76.4	73.0	69.0	73.0	1.3	0.05	<0.01
Mean	74.4	69.0	63.9	69.1	1.9	<0.01	<0.01

[1]Data are means of 10 observations; Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI) determined by feeding pigs a N-free diet: CP, 22.29; Arg, 1.00; His, 0.23; Ile, 0.32; Leu, 0.55; Lys, 0.44; Met, 0.10; Phe, 0.35; Thr, 0.57; Trp, 0.15; Val, 0.45; Ala, 0.80; Asp, 0.84, Cys, 0.19; Glu, 1.06; Gly, 2.30; and Ser, 0.61.

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

Regression Equations

From the regression equations developed to predict the concentration of SID AA in SFM, there was a clear pattern, indicating that the concentrations of NDF in combination with the concentrations of AA were relatively accurate predictors. This observation, however, must be interpreted with caution as the concentration of NDF in different sources of SFM may differ because of factors other than heat damage, such as the proportion of hulls remaining before oil extraction (Chiba, 2001). Therefore, the equations developed from this experiment may be used to predict the concentration of SID AA within a source of SFM if it is known that the only

Table 10. Linear regression to predict the concentration (%) of standardized ileal digestible (SID) AA from the concentrations (%) of AA, NDF, ADF, lignin, acid detergent insoluble N, analyzed Lys as percentage of CP, and reducing sugars as independent variables in cottonseed meal fed to pigs[1]

Dependent	Intercept			Independent variables									
variable	Estimate	SE	P-value	Variable 1	Estimate	SE	P-value	Variable 2	Estimate	SE	P-value	RMSE[2]	Adjusted R^2
SID Arg	–4.48	1.27	<0.01	Arg	1.87	0.29	<0.01	–	–	–	–	0.16	0.53
SID His	1.06	0.10	<0.01	Lignin	0.06	0.03	0.04	ADIN	–2.18	0.39	<0.01	0.04	0.62
SID Ile	5.49	1.49	<0.01	Lys:CP	–0.92	0.32	<0.01	ADIN	–4.95	1.30	<0.01	0.07	0.40
SID Leu	0.78	0.44	0.08	NDF	0.06	0.02	<0.01	ADIN	–3.22	0.77	<0.01	0.11	0.30
SID Lys	1.81	0.11	<0.01	ADIN	–3.67	0.42	<0.01	–	–	–	–	0.09	0.68
SID Met	0.21	0.11	0.06	NDF	0.01	0.005	<0.01	ADIN	–0.78	0.19	<0.01	0.03	0.28
SID Phe	1.01	0.33	<0.01	NDF	0.05	0.02	<0.01	ADIN	–2.38	0.57	<0.01	0.08	0.30
SID Thr	0.56	0.29	0.06	NDF	0.03	0.01	0.05	ADIN	–1.75	0.50	<0.01	0.07	0.23
SID Trp	–0.08	0.11	0.13	ADF	0.02	0.01	<0.05	RS	0.004	0.005	<0.05	0.02	0.28
SID Val	8.27	1.87	<0.01	Lys:CP	–1.40	0.39	<0.01	ADIN	–7.19	1.62	<0.01	0.08	0.43

[1]n = 40 observations; for all models, $P < 0.01$.

[2]RMSE = root mean square error.

source of variation in the nutrient composition of SFM is due to heat processing. Nevertheless, the present results indicate that the concentration of NDF and AA and the Lys:CP ratio in SFM may serve as indicators of heat damage.

Regression equations developed to predict the concentration of SID AA in CSM indicate that the concentration of ADIN alone or combined with other nutrients may be used although a relatively low R^2 was calculated for most equations. Nevertheless, the SID Lys in CSM may be predicted from the concentration of ADIN although a validation of the equation is required using other data sets.

In conclusion, the concentrations and digestibility of AA are reduced as the degree of heat damage increases and Lys is the AA most affected by heat damage, but the AID and SID of all other AA may also be reduced by severe heat damage. Therefore, heat processing of SFM and CSM should be optimized to prevent reducing the digestibility of AA. Regression equations for the prediction of SID AA that use the concentrations of NDF, ADIN, and AA may be used to identify the nutritional quality of heat-damaged SFM and CSM, but the practical use of the regression equations developed in the current work need to be validated.

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