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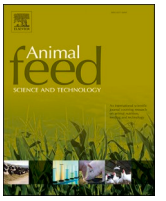

Research Article

Effects of heat damage and crystalline amino acid supplementation on growth performance of weanling pigs fed diets based on soybean meal and enzyme-treated soybean meal

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ABSTRACT

An experiment was conducted to test the hypothesis that growth performance of weanling pigs can be predicted from the concentration of reactive Lys in heat-damaged soybean ingredients and that supplementation with crystalline amino acids (AA) can compensate for reduced reactive Lys. One source of conventional soybean meal (SBM) and one source of enzyme-treated soybean meal (ESBM) were each divided into three batches. One batch of SBM and one batch of ESBM were used without further processing, one batch of SBM and one batch of ESBM were autoclaved at 120 ± 1°C to moderately heat-damage the ingredients and reduce reactive Lys to 85% of total Lys in each ingredient. The last batch of SBM and ESBM were autoclaved at 120 ± 1°C to severely heat-damage the ingredients and reduce reactive Lys to approximately 70% of total Lys. Four diets were formulated for phase 1 (days 0–14 post-weaning) and four additional diets were formulated for phase 2 (days 15–28 post-weaning). The four diets in each phase included a control diet containing the non-autoclaved SBM and ESBM, a diet containing moderately heat-damaged SBM and ESBM, a diet containing severely heat-damaged SBM and ESBM, and a diet containing severely heat-damaged SBM and ESBM supplemented with crystalline AA to match digestible AA levels in the control diet. A total of 160 weanling pigs with an initial body weight (BW) of 5.71 ± 0.55 kg were allotted to the 4 treatments in a randomized complete block design with 10 replicate pens per treatment. Growth performance and feed intake were recorded over 28 days. Results indicated that autoclaving reduced reactive Lys, Lys-to-crude protein ratio, sucrose, stachyose, and raffinose, whereas glucose and fructose increased as autoclaving time increased. Pigs fed severely heat-damaged diets had reduced final BW and average daily gain (ADG) compared with pigs fed the control diet ($P < 0.001$). Supplementation with crystalline AA partially restored growth performance of pigs, but pigs fed the AA supplemented diet had reduced ($P < 0.05$) ADG and final BW compared with pigs fed the control diet. Reactive Lys intake was strongly correlated ($P < 0.001$) with final BW and ADG. In conclusion, heat-damage of soybean ingredients reduces reactive Lys and growth performance of pigs, but supplementation with crystalline Lys, Met, and Thr can partially compensate for the loss of nutritional value.

Abbreviations: AA, Amino acids; ADFI, Average daily feed intake; ADG, Average daily gain; AEE, Acid-hydrolyzed ether extract; BW, Body weight; CP, Crude protein; DM, Dry matter; ESBM, Enzyme-treated soybean meal; G:F, Gain to Feed ratio; SBM, Soybean meal; SID, Standardized ileal digestibility.

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1. Introduction

Soybean meal (SBM) and enzyme-treated soybean meal (ESBM) are excellent sources of amino acids (AA) and may be combined to provide the majority of AA in diets for weanling pigs. However, if ingredients are overheated during processing, concentrations and digestibility of Lys and other AA are reduced due to heat-damage and Maillard reactions (Navarro et al., 2017; Ton et al., 2020). This has been demonstrated in SBM (González-Vega et al., 2011) and other ingredients (Kim et al., 2012; Almeida et al., 2013; 2014). The Maillard reaction causes reducing sugars to bind to Lys, which makes the Lys unavailable for use in protein synthesis. Reactive Lys is the Lys that has not been used in the Maillard reaction, and therefore, can be absorbed from the small intestine and used for protein synthesis (Kim et al., 2012; Kutzli et al., 2021). However, if SBM or ESBM that is heat-damaged are used in diets, growth performance of pigs may be reduced (Almeida et al., 2014c). In the production of soybean meal, the defatted meal has to be toasted to reduce residual hexane and to inactivate trypsin inhibitors, which carries a risk of heat damaging the meals. Likewise, in the production of ESBM, the ingredient is inoculated with enzymes and after inoculation, the product needs to be dried to remove excess moisture, which carries an additional risk of heat damaging the product. There are, however, no data to demonstrate the extent to which heat-damage of soybean ingredients will reduce growth performance, and it is not known if growth performance of pigs can be predicted from the concentration of reactive Lys in a diet. Therefore, the objective of this work was to test the hypotheses that growth performance of weanling pigs can be predicted from the concentration of reactive Lys in soybean ingredients and that the reduced growth performance caused by heat-damage can be corrected by adding synthetic AA to the diets to compensate for the reduced absorption and utilization of AA from heat-damaged ingredients.c

2. Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the

Table 1
Analyzed nutrient composition in soybean meal (SBM) and enzyme-treated soybean meal (ESBM), as-fed basis.[1]

Item	SBM			ESBM		
	No autoclaving	Moderate autoclaving	Severe autoclaving	No autoclaving	Moderate autoclaving	Severe autoclaving
Dry matter, g/kg	890.8	891.3	892.1	929.6	922.8	921.4
Ash, g/kg	61.4	63.9	63.6	70.3	71.2	72.0
Gross energy, kcal/kg	4237	4251	4295	4537	4500	4510
AEE2, g/kg	23.4	24.8	26.0	21.0	12.7	12.9
Glucose, g/kg	< 0.50	0.50	0.90	8.10	1.80	< 0.50
Maltose, g/kg	3.30	2.60	2.10	< 0.50	< 0.50	< 0.50
Sucrose, g/kg	71.0	68.6	53.5	< 0.50	< 0.50	< 0.50
Fructose, g/kg	0.60	1.50	3.20	< 0.50	< 0.50	< 0.50
Stachyose, g/kg	53.0	52.5	42.1	< 0.50	< 0.50	< 0.50
Raffinose, g/kg	11.5	11.5	9.40	< 0.50	< 0.50	< 0.50
Crude protein, g/kg	452.6	456.6	457.9	533.3	526.3	528.9
Lys:CP ratio	6.63	6.07	4.70	6.24	5.62	4.99
Trypsin inhibitor units per mg	4.94	1.69	< 0.45	2.16	0.706	< 0.45
Indispensable amino acids, g/kg						
Arg	34.0	32.1	27.6	39.2	36.9	34.9
His	12.3	12.0	11.6	14.9	14.3	13.8
Ile	22.1	22.0	21.9	26.8	26.5	26.1
Leu	35.8	35.8	35.9	43.8	43.3	42.3
Lys	30.0	27.7	21.5	33.3	29.6	26.4
Reactive Lys	28.4	25.4	15.9	31.1	27.2	23.8
Met	6.30	6.10	6.50	7.80	7.60	7.60
Phe	24.0	24.1	24.5	29.6	29.3	28.3
Thr	17.9	17.9	18.2	22.7	22.4	22.1
Trp	5.60	6.00	5.70	7.00	6.60	6.50
Val	23.2	23.0	23.2	28.6	28.3	28.2
Dispensable amino acids, g/kg						
Ala	20.1	20.0	20.2	24.6	24.2	24.0
Asp	51.9	51.6	51.4	63.2	62.0	61.5
Cys	6.50	6.00	5.60	8.20	7.90	7.50
Glu	85.6	85.7	85.5	101.0	98.7	98.3
Gly	19.8	19.6	19.6	24.3	23.8	23.4
Pro	23.6	23.6	23.2	28.7	28.1	27.9
Ser	19.9	20.0	20.0	25.3	24.7	24.2
Tyr	16.7	16.4	15.8	20.3	19.3	18.6

[1] Analyzed minerals in non-autoclaved SBM and non-autoclaved ESBM were as follows (g/kg): Ca, 2.7 and 3.3; P, 6.8 and 7.6; Mg, 2.4 and 2.3; K, 18.6 and 19.4; Na, 0.1 and 0.1; Cu, 9 and 8 mg/kg; Fe, 110 and 84 mg/kg; Mn, 27 and 25 mg/kg; and Zn, 64 and 42 mg/kg.
[2] AEE, acid-hydrolyzed ether extract.

experiment before animal work was initiated. Pigs that were the offspring of Line 337 males mated to Camborough females (Pig Improvement Company, Henderson, TN) were used in the experiment.

2.1. Ingredient preparation

One source of conventional SBM and one source of ESBM (HP 300, Hamlet Protein, Findlay, OH, USA) were obtained, and each source was divided into three batches. The reactive Lys in both ingredients was analyzed, and it was confirmed that reactive Lys was greater than 95% of total Lys in both ingredients. One batch of each ingredient was used without further processing. The second batch was moderately heat-treated in an autoclave for 30 - 60 min at 120 ± 1 °C to reduce reactive Lys to 85% of total Lys. The last batch was severely heat-treated in an autoclave for 60–120 min at 120 ± 1 °C until reactive Lys was reduced to 70% of total Lys. There were, therefore, six batches in total (three batches of conventional SBM and three batches of ESBM; Table 1). The standardized ileal digestibility (SID) of all AA in ESBM had been determined previously in a separate digestibility experiment, and the SID of AA in each batch of autoclaved ESBM was calculated (Torres-Mendoza et al., 2025).

2.2. Experimental diets

A two-phase feeding program was used, with days 1–14 as phase 1 and days 15–28 as phase 2. Four diets were formulated with the control diet containing non-autoclaved SBM and ESBM and providing 90% of the NRC (2012) requirement for digestible Lys for pigs weighing 5–7 kg and 7–11 kg, respectively (Tables 2 and 3). The moderately and severely autoclaved diets were identical to the diets with non-autoclaved SBM and ESBM with the exception that these diets contained SBM and ESBM that were moderately or severely autoclaved, and therefore, were expected to have reactive Lys that was 85% and 70%, respectively, of the total Lys. The last treatment also contained severely autoclaved SBM and ESBM, but synthetic Lys, Met, and Thr were added to phase 1 and phase 2 diets to bring the concentration of digestible AA and reactive Lys to the same level as in the non-autoclaved diets. All diets were analyzed for Lys and reactive Lys before the animal part of the experiment was initiated to confirm correct diet mixing.

2.3. Growth performance and fecal scoring

A total of 160 weaned pigs with an initial body weight (BW) of 5.71 ± 0.55 kg were allotted to the four dietary treatments. The experiment was conducted using a randomized complete block design with room as the blocking factor. Two blocks were used, each representing a separate room and containing 80 pigs. Within each room (block), pigs were randomly allotted to 20 pens, with five replicate pens per dietary treatment and four pigs per pen (two barrows and two gilts). Therefore, a total of 40 pens were used,

Table 2
Ingredient composition of experimental diets for phases 1 and 2 containing soybean meal (SBM) and enzyme-treated soybean meal (ESBM).[1]

	Phase 1				Phase 2			
Ingredient, g/kg	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe + AA[2]	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe + AA
Corn	440.5	440.5	440.5	433.4	455.6	455.6	455.6	447.7
SBM	210.0	210.0	210.0	210.0	250.0	250.0	250.0	250.0
ESBM	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Lactose	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Whey, dried	40.0	40.0	40.0	40.0	15.0	15.0	15.0	15.0
Blood Plasma	30.0	30.0	30.0	30.0	-	-	-	-
Soybean oil	24.0	24.0	24.0	24.0	27.0	27.0	27.0	27.0
L-Lys-HCl	1.80	1.80	1.80	7.10	1.20	1.20	1.20	7.20
DL-Met	1.30	1.30	1.30	2.20	1.10	1.10	1.10	2.10
L-Thr	0.10	0.10	0.10	1.00	-	-	-	0.90
Dicalcium phosphate	14.3	14.3	14.3	14.3	12.7	12.7	12.7	12.7
Limestone	9.00	9.00	9.00	9.00	8.40	8.40	8.40	8.40
Sodium chloride	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.0
Vitamin mineral premix[3]	5.00	5.00	5.00	500	5.00	5.00	5.00	5.00

[1] Calculated concentrations of standardized ileal digestible AA other than Lys were as follows in Phase 1 and Phase 2 diets containing non-autoclaved SBM and ESBM (g/kg): Arg, 14.1 and 14.2; His, 5.7 and 5.5; Ile, 9.0 and 9.0; Leu, 17.4 and 16.6; Met, 4.3 and 4.1; Met + Cys, 7.5 and 6.8; Phe, 10.3 and 10.0; Thr, 7.9 and 7.3; Trp, 2.6 and 2.4; and Val, 10.2 and 9.7.

[2] AA, amino acids.

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 3
Analyzed nutrient composition in experimental diets, as fed basis.

Item	Phase 1				Phase 2			
	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA[1]	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA
Dry matter, g/kg	897.8	897.8	899.2	900.8	896.5	896.5	898.6	899.6
Ash, %	57.7	56.2	57.2	602	55.1	53.5	54.9	55.9
Gross energy, kcal/kg	4065	4100	4088	4101	4088	4087	4111	4133
Glucose, g/kg	2.50	1.80	1.70	1.80	2.20	1.60	1.60	1.40
Maltose, g/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
Sucrose, g/kg	20.8	22.2	16.9	19.4	25.2	25.3	19.5	20.4
Fructose, g/kg	1.50	1.60	1.80	1.40	1.60	1.80	1.60	1.80
Stachyose, g/kg	12.7	12.3	8.40	10.7	14.2	13.9	10.3	11.0
Raffinose, g/kg	3.20	3.20	2.30	2.90	3.60	3.50	2.80	3.10
Crude protein, g/kg	227.4	223.5	230.1	234.1	222.6	221.4	218.0	225.0
Indispensable AA, g/kg								
Arg	14.5	13.9	12.2	13.3	14.7	14.2	12.8	12.6
His	6.10	5.90	5.60	5.90	5.90	5.80	5.60	5.60
Ile	10.0	9.70	9.30	10.1	10.3	10.1	10.2	9.90
Leu	19.7	19.3	19.2	19.6	19.0	18.8	19.0	18.5
Lys	15.1	14.2	12.0	17.3	13.9	13.2	10.9	15.6
Reactive Lys	14.3	12.7	10.3	15.6	12.6	11.9	9.30	13.9
Met	4.50	4.10	3.80	4.80	4.30	3.80	3.60	4.50
Phe	11.7	11.4	11.4	11.6	11.5	11.4	11.5	11.3
Thr	9.50	9.30	9.70	10.3	8.40	8.40	8.40	9.40
Trp	2.80	2.70	2.50	2.70	2.60	2.40	2.20	2.30
Val	11.7	11.2	11.1	11.9	11.2	11.3	11.1	11.0
Dispensable AA, g/kg								
Ala	11.1	11.0	10.9	11.1	10.8	10.8	10.9	10.8
Asp	23.4	22.8	22.8	23.4	23.1	23.1	22.8	23.1
Cys	4.00	3.90	3.60	3.80	3.40	3.40	3.10	3.10
Glu	40.9	40.1	39.7	40.8	41.3	41.3	40.9	40.7
Gly	9.30	9.10	8.90	9.10	9.30	9.20	9.20	9.20
Pro	12.8	12.7	12.6	12.8	12.6	12.5	12.6	12.4
Ser	10.3	10.3	10.3	9.90	9.60	9.60	9.40	9.40
Tyr	8.00	7.80	7.50	7.60	7.50	7.40	7.30	7.00

[1] AA, amino acid.

resulting in 10 replicate pens per treatment. Pigs were weighed at the beginning of the experiment, on day 14, and on day 28, and feed allotments were recorded daily. Quantities of feed left in the feeders were also recorded on the days when pig weights were recorded. Data were summarized to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) for each treatment group. If a pig died or was removed from the experiment, the weight of that pig was recorded at the time of removal, and the weight of the feed in the feeder of the pen where the pig was housed was recorded to determine feed intake up to the time of removal (Laskoski et al., 2021). Feed intake was corrected based on the number of pigs that remained in the pen. Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea).

2.4. Chemical analyses

At the end of the experiment, diets and feed ingredients were analyzed for dry matter (DM; method 930.15). Ash in ingredients and diets was also analyzed (method 942.05; AOAC Int., 2019). Diets and ingredients were analyzed for gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Ingredients were analyzed for acid-hydrolyzed ether extract (AEE) using 3 *N* HCl (AnkomHCl, Ankom Technology, Macedon, NY, USA), followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY, USA). Glucose, maltose, sucrose, fructose, stachyose, and raffinose in ingredients and diets were analyzed by High-Performance Liquid Chromatography using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA, USA; Navarro et al., 2018). These samples were also analyzed for nitrogen by combustion using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA; method 990.03). Crude protein (CP) was calculated as nitrogen × 6.25. Trypsin inhibitor concentrations were analyzed in ingredients (method Ba 12–75; AOCS, 2006). Amino acids in feed ingredients and diets were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N*

hydrochloric acid for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E (b)]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(b); AOAC Int., 2019]. Reactive Lys was analyzed using Ultra High-Performance Liquid Chromatography, Model QSight LX50 (PerkinElmer, Waltham, MA, USA). Samples were incubated with O-methylisourea to initiate the guanidination reaction (Moughan and Rutherfurd, 1996; Pahm et al., 2008). After incubation, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019].

Calcium, P, K, Mg, Na, Cu, Fe, Mn, and Zn in the non-autoclaved sources of SBM and ESBM were analyzed (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600°C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. U.S. U.S. Environmental Protection Agency, 2000).

2.5. Calculations

Total reactive Lys intake (kg) was calculated by multiplying the analyzed concentration of reactive Lys in the diet (%) by the total feed intake (kg) in each period. Reactive Lys intake (g/day) was then determined by dividing the total reactive Lys intake by the number of days in the feeding period. These calculations allowed for quantifying the daily supply of reactive Lys provided from each diet.

2.6. Statistical analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Institute Inc.., 2018). Outliers were identified as values with internally studentized residuals outside the range of −3–3 (Tukey, 1977). The pen was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS. The statistical model included diet as fixed effect, and block, and replicate within block as random effects. Mean values were calculated using the LSMeans statement. Contrast coefficients were used to compare the control diet versus the diet containing crystalline AA. Statistical significance was considered at $P \leq 0.05$, and tendencies were considered if $0.05 < P \leq 0.10$.

Pearson correlation coefficients were calculated using the CORR procedure of SAS to evaluate associations between total reactive Lys intake (kg) and final BW, and between reactive Lys intake (g/d) and ADG. Prediction equations for final BW and ADG were developed using the REG procedure of SAS. In the initial models, final BW and ADG were regressed on total reactive Lys intake (kg) or daily intake (g/d), respectively. Separate models were constructed for each experimental phase and for the overall period. Final prediction equations were generated using least squares regression estimates, and model fit was evaluated based on the coefficient of determination (R^2).

3. Results

During phase 1, two pigs fed the diet containing non-autoclaved SBM and ESBM died for unknown reasons. For phase 2, three pigs fed the severely heat-treated diet, and one pig fed the diet supplemented with crystalline AA were removed due to poor health. All other pigs completed the experiment.

3.1. Ingredients and diet composition

Glucose and fructose were < 0.50 and 0.60; 0.50 and 1.50; and 0.90 and 3.20 g/kg in non-autoclaved, moderately autoclaved, and severely autoclaved SBM, whereas sucrose was 71.0, 68.6, and 53.6 g/kg; stachyose was 53.0, 52.5, and 42.1 g/kg; and raffinose was 11.5, 11.5, and 9.40 g/kg in non-autoclaved, moderately autoclaved, and severely autoclaved SBM, respectively (Table 1). In ESBM, stachyose and raffinose were less than 0.50 g/kg regardless of heat treatment. The Lys:CP ratio was 6.63, 6.07, and 4.70 g per 100 g in the non-autoclaved, moderately autoclaved, and severely autoclaved SBM and 6.24, 5.62, and 4.99 g per 100 g in non-autoclaved, moderately autoclaved, and severely autoclaved ESBM. Lysine and reactive Lys were 30.0 and 28.4; 27.7 and 25.4, and 21.5 and 15.9 g/kg in the non-autoclaved, moderately autoclaved, and severely autoclaved SBM, but Lys and reactive Lys were 33.3 and 31.1; 29.6 and 27.2, and 26.4 and 23.8 g/kg in non-autoclaved, moderately autoclaved, and severely autoclaved ESBM. Other indispensable AA did not change with heat-treatment. Trypsin inhibitors were 4.94 units per mg in non-autoclaved SBM and < 0.45 units per mg in the severely autoclaved product. In ESBM there were 2.16, 0.71 and < 0.45 trypsin inhibitor units per mg in non-autoclaved, moderately autoclaved, and severely autoclaved samples. Reactive Lys was 14.3 g/kg, 12.7 g/kg, and 10.3 g/kg in phase 1 diets with non-autoclaved, moderately autoclaved, and severely autoclaved SBM and ESBM, and 15.6 g/kg in the diet supplemented with AA. In phase 2 diets, reactive Lys was 12.6 g/kg in the diet with non-autoclaved SBM and ESBM, but reactive Lys was 11.9 g/kg and 9.3 g/kg in the moderately and severely autoclaved diets and 13.9 g/kg in the diet with AA supplementation.

3.2. Growth performance

Initial BW did not differ among treatments. However, on day 28, pigs fed the severely autoclaved diet had reduced final BW compared with pigs fed the diet with non-autoclaved SBM and ESBM ($P < 0.05$; Table 4). From days 1–14, ADG tended ($P < 0.10$) to be less by pigs fed the severely autoclaved diet compared with pigs fed the other diets. From days 15–28 and overall, pigs fed the diet with

Table 4
Growth performance and fecal score of pigs fed experimental diets.

Item	Diets				SEM	*P* -value	P-value No autoclaving vs. severely autoclaved + AA
	No autoclaving	Moderately autoclaved	Severely autoclaved	Severely autoclaved + AA[1]			
Body weight, kg							
Initial body weight	5.75	5.72	5.73	5.70	0.463	0.433	0.116
d 14	6.62	6.51	6.12	6.33	0.738	0.111	0.183
d 28	11.10[a]	10.44[ab]	8.13[c]	9.89[b]	1.268	< 0.001	0.006
ADG, g							
d 1–14	62	57	28	45	20.750	0.099	0.236
d 15–28	320[a]	280[ab]	143[c]	254[b]	39.440	< 0.001	0.006
d 1–28	191[a]	169[ab]	86[c]	149[b]	29.140	< 0.001	0.004
ADFI, g							
d 1–14	122	117	103	114	25.331	0.494	0.550
d 15–28	432[a]	440[a]	326[b]	397[ab]	56.821	0.001	0.200
d 1–28	268[a]	278[a]	205[b]	253[ab]	44.720	0.009	0.490
G:F							
d 1–14	0.51[a]	0.49[a]	0.27[b]	0.39[ab]	0.085	0.006	0.022
d 15–28	0.74[a]	0.64[ab]	0.44[b]	0.64[ab]	0.046	< 0.001	0.042
d 1–28	0.71[a]	0.61[ab]	0.43[b]	0.59[ab]	0.050	< 0.001	0.011
Fecal score							
d 1–14	1.55[ab]	1.58[a]	1.37[b]	1.43[ab]	0.058	0.039	0.123
d 15–28	1.55	1.61	1.45	1.36	0.078	0.084	0.070
d 1–28	1.53[ab]	1.58[ab]	1.39[b]	1.38[b]	0.050	0.009	0.029

[1] AA, amino acid; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.
[ab] Means within a row without a common superscript letter are different ($P < 0.05$).

non-autoclaved SBM and ESBM had greater ADG than pigs fed the moderately or severely autoclaved diets ($P < 0.01$). Average daily feed intake was also reduced ($P < 0.01$) by pigs fed the severely autoclaved diet compared with the diet containing non-autoclaved SBM and ESBM from days 15–28 and overall. Gain to feed was greater ($P < 0.001$) in pigs fed the diet with non-autoclaved SBM and ESBM during phase 1 and overall compared with pigs fed a severely autoclaved diet and tended ($P < 0.10$) to be greater in phase 2. Supplementation with crystalline AA improved ($P < 0.05$) final BW on day 28 and ADG in phase 2 and overall. However, pigs fed the diets with severely autoclaved SBM and ESBM and added AA had a final BW on day 28, and ADG in phase 2 and overall, that were less ($P < 0.05$) than for pigs fed the diets with non-autoclaved SBM and ESBM. Fecal scores were generally low for all treatments, although pigs on the two treatments with severely heat damaged SBM and ESBM had firmer feces overall compared with pigs fed the diet with moderately heat damaged SBM and ESBM ($P < 0.05$).

Reactive Lys intake was positively correlated ($P < 0.05$) with final BW at the end of both phases of the experiment (Table 5). Similarly, a strong positive association ($P < 0.001$) was observed between reactive Lys intake and ADG in phase 1, phase 2, and for the overall experimental period.

4. Discussion

4.1. Ingredients

Soybean meal and ESBM are widely used protein sources in diets for weanling pigs. Because of antinutritional factors in SBM, and the inability of young pigs to ferment oligosaccharides, it is not common to use diets for early weaned pigs that contain SBM as the only AA containing ingredient, and there is, therefore, usually a maximum inclusion of SBM in starter diets for pigs of around 200 g/kg in phase 1 and 250 g/kg in phase 2. As a consequence, there is a need to add additional sources of AA and one of the commonly used sources is ESBM, which does not contain oligosaccharides and has a reduced concentration of antigenic proteins compared with SBM (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011). Because SBM is less expensive than ESBM, practically all diets that contain ESBM also contain SBM, which is the reason the two ingredients were mixed in the present experiment. However, because both ingredients need to be heat processed during toasting and drying, there is a risk for overheating, which can result in heat damage in the ingredients (Stein et al., 2008). Heat damage of soybean ingredients usually results in reduced digestibility of Lys and other AA and the digestible energy may be reduced as well due to destruction of AA and lipids (González-Vega et al., 2011; Oliveira et al., 2020b; Espinosa et al., 2021). The concentrations of CP and most indispensable AA in the non-autoclaved ESBM and conventional SBM used in this experiment were consistent with reported values (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012). The differences in nutrient composition between ESBM and SBM reflect the processing steps involved in the production of ESBM, in which sucrose and oligosaccharides are fermented, which results in increased concentrations of CP and other nutrients.

The reduction of CP, carbohydrates, Lys, and reactive Lys in the autoclaved ingredients is due to the combined effects of thermal degradation, Maillard reactions, and formation of indigestible complexes. Prolonged time in the autoclave may result in loss of volatiles and structural changes in the sample (Oliveira et al., 2020b; Espinosa et al., 2021). The reduction in CP and Lys is primarily attributed to heat-damage, especially the Maillard reaction, in which the ε-amino group of Lys binds with reducing sugars to form

Table 5
Pearson correlation coefficients (r), coefficients of determination (R^2), and prediction equations for final body weight (BW) and average daily gain (ADG) based on reactive Lys intake (as-is basis) in weanling pigs.

Item	Pearson r	P-value	R^2	Prediction Equation
Final BW				
Phase 1	0.841	< 0.001	0.71	Y = 4.33 + (26.50 × total reactive Lys intake, kg)
Phase 2	0.881	< 0.001	0.78	Y = 4.92 + (19.97 × total reactive Lys intake, kg)
ADG				
Phase 1	0.910	< 0.001	0.83	Y = - 45.43 + (67.33 × reactive Lys intake, g/d)
Phase 2	0.790	< 0.001	0.62	Y = 15.54 + (51.64 × reactive Lys intake, g/d)
Overall	0.880	< 0.001	0.77	Y = - 1.58 + (51.37 × reactive Lys intake, g/d)

Amadori products and melanoidins, making Lys unavailable for protein synthesis. However, non-enzymatic protein denaturation and crosslinking may also take place during heating (González-Vega et al., 2011; Almeida et al., 2014c; Oliveira et al., 2020b). The reduction in carbohydrates observed in autoclaved SBM is likely due to the consumption of reducing sugars during Maillard reactions, which results in formation of glycation end products (Fontaine et al., 2007; Oliveira et al., 2020a) and the reduction in stachyose and raffinose indicates degradation of oligosaccharides and partial hydrolysis of complex carbohydrates (Navarro et al., 2018). The increased concentrations of glucose and fructose after autoclaving are likely a result of hydrolysis of sucrose and other complex sugars, and release of these simple sugars provides additional substrates that promote Maillard reactions during heat processing (Fontaine et al., 2007; Murai et al., 2024).

In undamaged soybean protein, Lys:CP is typically greater than 6 g per 100 g, which is indicative of minimal heat-damage, whereas a Lys:CP < 6 g per 100 g indicates heat-damage (González-Vega et al., 2011; Almeida et al., 2014c; Pedersen et al., 2016; Oliveira et al., 2021). Therefore, the non-autoclaved SBM and ESBM that were used in this experiment likely were not heat-damaged, whereas most of the autoclaved ingredients had Lys:CP below 6 g per 100 g indicating they were indeed heat-damaged.

4.2. Diets

The negative impact on digestibility of AA and energy of overheating SBM has been reported (Gonzalez-Vega et al., 2011; Oliveira et al., 2020b), but no data have been published for the combination of SBM and ESBM being heat damaged. However, because these ingredients are never used as the sole source of AA in diets for weanling pigs, mixing the two ingredients to determine the combined effect of heat damage in diets containing heat damaged SBM and ESBM provides a more practical scenario for effects of heat damage. By autoclaving the ingredients, the heat damage that may happen in the toasting or drying of SBM and ESBM in a crushing plant was simulated. This method has previously been demonstrated to infer heat damage that is similar to practical conditions (Gonzalez-Vega et al., 2011).

The diets containing autoclaved SBM and ESBM without crystalline AA supplementation were formulated using the analyzed concentrations of AA from non-autoclaved SBM and ESBM, simulating a scenario where overestimation of AA concentration and digestibility occurred in the moderately and severely heat-damaged diets. The reduced concentrations of indispensable AA in these diets indicate that autoclaving reduces the concentration of not only Lys, but also Arg, His, and Cys. The metabolizable energy may also be reduced in heat damaged ingredients, but no corrections were made for potential reductions in energy digestibility because we did not have data to demonstrate how much the metabolizable energy potentially was reduced. However, it is acknowledged that the concentration of digestible and metabolizable energy in 00-rapeseed meal, SBM, and soybean expellers have been reduced in diets containing over-heated ingredients (Oliveira et al., 2020a; 2020b; Espinosa et al., 2021). It is, therefore, possible that digestible energy and metabolizable energy also was reduced in diets containing heat treated feed ingredients in the present experiment, which may have contributed to the reduced growth performance observed in pigs fed these diets.

The control diets were formulated to provide 90 g per 100 g of the requirement for Lys to make sure Lys was limiting in these diets. Any reduction in Lys availability due to heat damage was, therefore, expected to result in reduced growth performance of the pigs.

4.3. Growth performance

The reduction in ADG and final BW observed for pigs fed the severely autoclaved diet is in agreement with reported data and reflects the detrimental effects of heat-damage on AA and energy digestibility (Almeida et al., 2014c). This reduction is likely due to the decreased concentration of reactive Lys caused by Maillard reactions, and possibly also the reduction in the concentration of other indispensable AA caused by non-enzymatic heat damage during autoclaving (Fontaine et al., 2007; González-Vega et al., 2011; Almeida et al., 2014c). The reduction in reactive Lys and other AA compromises the ability of pigs to synthesize proteins efficiently, which likely is the reason for the impaired growth for pigs fed diets with autoclaved SBM and ESBM (Pahm et al., 2008; Lund and Ray, 2017). It is possible that some of the advanced Amadori compounds and melanoidins that were generated during heat treatment reduced diet palatability, and therefore, contributed to the reduced ADFI that was observed for pigs fed the diets with heat treated SBM and ESBM. However, the observation that inclusion of additional Lys, Met, and Thr in the diets with severely autoclaved ingredients partially restored ADFI indicates that the reduction in ADFI primarily was a result of AA imbalances. The reduced ADFI of pigs fed the diets with severely autoclaved SBM and ESBM contributed to the reduced ADG by limiting overall nutrient and energy intake (Pahm et al., 2008; Almeida et al., 2014c; Oliveira et al., 2020a; 2020b). These results confirm that excessive heat treatment negatively affects

not only AA digestibility, but also overall growth performance and fecal score.

Supplementation with crystalline AA to the diet containing severely autoclaved SBM and ESBM was done in an attempt to compensate for AA deficiencies in the heat-damaged ingredients. However, the fact that growth performance was not fully restored by adding synthetic AA to the diets indicates that heat damage may affect more than AA concentration and digestibility. Other factors such as reduced energy digestibility, altered palatability, or impaired availability of digested AA may also contribute to the negative effects of heat damage (Almeida et al., 2014c; Oliveira et al., 2021). These observations align with results of experiments that indicated that nutrient availability was reduced if heat-damaged ingredients were used, which ultimately had negative effects on growth performance (Almeida et al., 2014c).

The data presented for the prediction models assume that only Lys was limiting in the diets, but because Lys was included in the control diet at only 90 g per 100 g of the requirement and other AA were added at or above the requirement, we attempted to meet this requirement. It is also assumed that for nutrients other than Lys, no differences among diets were induced, which we also attempted to achieve in diet formulations.

The positive correlation between reactive Lys intake and final BW and ADG demonstrates the importance of reactive Lys as a predictor of growth performance in weanling pigs, which is in agreement with results of research demonstrating that bioavailable Lys is important for protein accretion and growth because reactive Lys is a marker for available Lys in heat-processed ingredients (Pahm et al., 2008; Almeida et al., 2014c). Increasing dietary Lys may improve growth performance of pigs fed diets containing heat-damaged ingredients (Aymerich et al., 2020). Therefore, if reactive Lys in SBM and ESBM can be determined, it will be possible to determine if ingredients are heat damaged or not and if they are heat damaged, corrective measures such as adding extra synthetic AA to the diets can be taken.

5. Conclusion

Heat treatment of soybean meal and enzyme treated soybean meal at 120 ± 1 °C reduced concentrations of lysine, reactive lysine, and other amino acids. Growth performance and feed efficiency declined when heat-damaged ingredients were used without adjusting for amino acid losses during heating, but addition of crystalline amino acids to diets containing heat damaged soybean meal and enzyme treated soybean meal partially mitigated the negative effects of heat damage. The strong positive correlation between reactive lysine intake and average daily gain of pigs indicates that reactive lysine can be used as a predictor for growth of weanling g pigs.

CRediT authorship contribution statement

Stein Hans H: Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Leidy J. Torres-Mendoza:** Writing – original draft, Formal analysis, Data curation.

Declaration of Competing Interest

The authors have no conflicts of interest.

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Data availability

All data from this research are included in the manuscript.

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