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Non ruminant nutrition

Effect of the pelleting process on diet formulations with varying levels of crystalline amino acids and reducing sugars on digestibility in growing pigs

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Abstract

The objective of this experiment was to determine the effects of pelleting on the standardized ileal digestibility (**SID**) of amino acids (**AA**) and crude protein (**CP**) in diets with or without increased concentrations of free AA and reducing sugars (**RS**). Eight individually housed, ileal cannulated barrows (initially 31.4 kg) were allotted to an 8 × 8 Latin square with eight diets and eight 7-d periods with ileal digesta collected on days 6 and 7. Treatments were arranged in a 2 × 2 × 2 factorial with the main effects of diet form (mash or pellet), crystalline AA (low or high), or RS (low or high), provided by distillers dried grains with solubles and bakery meal. Diets were pelleted to achieve a hot pellet temperature of 85 to 88 °C. Data were analyzed as a Latin square design using the GLIMMIX procedure of SAS 9.4. A feed form × RS interaction ($P < 0.026$) for SID of tryptophan was observed. Feeding pelleted low RS diets increased SID of tryptophan compared with mash high and low RS diets, and pelleted high RS diets. For the main effects of feed form, the SID of total AA, CP, and indispensable AA was greater ($P < 0.042$) in pelleted diets compared with mash diets. For the main effects of crystalline AA, pigs fed high crystalline AA had increased ($P = 0.007$) SID of tryptophan and decreased ($P = 0.050$) SID of histidine compared with those fed low crystalline AA diets. For the main effects of RS, high RS diets had decreased ($P < 0.05$) SID of total AA, CP, and indispensable AA compared with low RS diets. In conclusion, pelleting diets increased AA digestibility, and pelleting diets with increased crystalline AA or RS did not affect the improvement in AA digestibility from pelleting. Diets formulated with high crystalline AA had increased SID of tryptophan. Formulating diets with high RS resulted in decreased AA digestibility compared with corn–soybean meal-based diets.

Lay Summary

The objective of this study was to determine the effects of pelleting on the standardized ileal digestibility (**SID**) of amino acids (**AA**) in diets with or without increased concentrations of free AA and reducing sugars (**RS**). Treatments were arranged in a 2 × 2 × 2 factorial with the main effects of diet form (mash or pellet), crystalline AA (low or high), or RS (low or high), provided by dried distillers grains with solubles and bakery meal. A total of 8 illeal cannulated barrows were fed treatments in an 8 × 8 Latin square design. Results indicated that there was no evidence of interactions between diet types and diet form, indicating that increasing amounts of crystalline AA and RS did not reduce amino acid digestibility when pelleting diets. Additionally, pelleting diets resulted in increased amino acid digestibility compared to mash diets. Diets formulated with 20% dried distillers grains with solubles and 15% bakery resulted in decreased amino acid digestibility compared with the corn–soybean meal-based diets. Crystalline amino acid concentration did not influence amino acid digestibility of indispensable AA, except for SID of tryptophan which was increased in diets with higher concentrations of crystalline AA.

Key words: amino acid digestibility, crystalline amino acids, Maillard reaction, pelleting, pigs, reducing sugars

Abbreviations: AA, amino acids; CP, crude protein; DDGS, dried distillers grains with solubles; DM, dry matter; GE, gross energy; KOH, protein solubility in potassium hydroxide; ME, metabolizable energy; PDI, pellet durability index; RS, reducing sugar; SID, standardized ileal digestibility

Introduction

Pelleting swine diets are commonly used to improve feed efficiency, feed handling characteristics, and bulk density while decreasing feed wastage (Behnke, 1994). Growing concerns for feed safety issues and the importance of pellet quality have led feed manufacturing companies to steam condition swine diets at higher temperatures during the pelleting process. Current trends in diet formulation in the swine industry have focused on increasing the use of crystalline amino acids (**AA**). Another common practice for swine nutritionists is to use byproduct ingredients to reduce feed costs. Common byproduct ingredients include distillers dried grains with solubles (**DDGS**) from the ethanol industry and bakery meal from the food and confectionary industry. Corn and soybean meal are commonly used in finishing pig diets and contain trace amounts of reducing sugars (**RS**). The RS glucose, sucrose,

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maltose, and fructose typically have concentrations of 0.66%, 1.14%, 0.23%, and 0.4% in corn; 5.03%, 4.91%, 2.85%, and 4.71% in bakery meal; and 1.84%, 0.19%, 2.28%, and 0.74% in DDGS (Rojas et al., 2013). Soybeans contain glucose ranging from 0.07% to 0.40% (dry matter [**DM**]-basis). However, glucose is destroyed during soybean crushing to produce soybean meal (Grieshop et al., 2003).

Over-processing can potentially lead to a reduction in amino acid availability due to the Maillard reaction. The Maillard reaction is a nonenzymatic browning reaction between an amino group from AA, peptides, or proteins and a carbonyl group of RS such as glucose, fructose, or lactose. The RS in feed ingredients may pose nutritional challenges when pelleting diets at increased temperatures. Variables optimizing the Maillard reaction include high temperatures and moisture levels, which occur during the feed pelleting process (Ajandouz et al., 2008). Thus, pelleting diets containing the combination of crystalline AA and byproducts with RS may increase the risk of reduced amino acid availability (Pahm et al., 2008). Therefore, the hypothesis was that pelleting swine diets containing free AA and RS at high temperatures will reduce the standardized ileal digestibility (**SID**) of crude protein (**CP**) and AA. The objective of this study was to determine the effects of pelleting on the SID of AA in diets with or without increased concentrations of free AA and RS.

Materials and Methods

The University of Illinois Institutional Animal Care and Use Committee approved the animal protocol used in this experiment. The animal part of the experiment was conducted at the University of Illinois, Swine Research Center (Champaign, IL).

Dietary treatments

Dietary treatments were arranged in a 2 × 2 × 2 factorial with the main effects of diet form (mash or pellet), crystalline amino acid level (AA; low or high), and RS (low or high; Table 1). For crystalline AA treatments, diets were considered low or high based on the inclusion of crystalline AA, with high crystalline AA diets having increased concentrations of added lysine, threonine, and tryptophan compared with low crystalline AA diets. Valine and isoleucine were also included as needed in the high crystalline AA diets. RS were naturally occurring in ingredients (corn and soybean meal-based diets; low) or increased by adding DDGS and bakery meal (20% and 15%, respectively; high). All diets contained 0.5% titanium dioxide as an indigestible marker to allow for the calculation of the SID of AA.

Feed processing

Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology and Innovation Center (Manhattan, KS). Whole grain ingredients were ground with a three-high roller mill (RMS Model 924) to an approximate particle size of 600 µm. Feed was mixed in a 1-ton Hayes and Stolz double ribbon mixer. Treatments were pelleted using a 5-ton, 100-horsepower pellet mill (Model PM3016-4, California Pellet Mill) equipped with a 4 × 35-mm die (L:D = 8.75). The conditioning temperature ranged from 79 to 85 °C to achieve a hot pellet temperature of 85 to 88 °C, achieved by adjusting steam addition (Table 4). The pelleted diets were cooled in an experimental counterflow cooler for 15 min. To minimize the effect of pellet quality differences, pellets passed through a sifter to remove fines before transport.

A sample of cool pellets was collected, and the fines were sifted off by using the corresponding sieve stack (ASAE S269.4). Sifted pellets were split using a riffle divider and 100 g was used for analysis. The 100 g sample was placed into the hopper of the Holmen NHP100 for 60 s. The fines were removed as the sample was run. Once completed, the sample was removed from the hopper, sifted, and weighed. The pellet durability index (PDI) was calculated by dividing this final sample weight by the 100 g initial sample weight.

Animals

Eight individually housed growing barrows (initially 31.4 ± 3.4 kg) had a T-cannula installed in the distal ileum and were allotted to an 8 × 8 Latin square design with the eight diets and eight 7-d periods. Thus, each pig was fed each diet in one period and no pig received the same diet more than once. Pigs were limited to 3 times the maintenance requirement for metabolizable energy (**ME**) [i.e., 198 kcal ME per kg body weight$^{0.60}$; NRC 2012]. Throughout the experiment, pigs had free access to water. Pigs were deprived of feed overnight at the end of each experimental period, to be fed a new diet the following morning. Each period lasted 7 d with the initial 5 d being the adaptation period, and ileal digesta was collected for 9 h on days 6 and 7. All collected digesta samples were lyophilized before analysis.

Chemical analysis

Representative samples of corn, bakery meal, DDGS, soybean meal, and treatment diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for DM (AOAC Official Method 934.01, 2006), CP (AOAC Official Method 990.03, 2006), crude fat (AOAC Official Method 920.39 (A)), crude fiber (AOAC Official Method 978.10, 2006), ash (AOAC Official Method 942.05), complete AA profile (AOAC Official Method 982.30 E(a,b,c), Ch. 45.3.05, 2006), available lysine (AOAC Official Method 975.44, Ch. 45.4.03, 2006), protein solubility in potassium hydroxide (**KOH**) (Parsons et al., 1991) and Maillard reaction end products (melanoidins). Melanoidins were measured using ~50 mg of each sample, which was accurately weighed and dispersed in 3 mL ethanol solution (47.5%, v/v) in a 5-mL tube. The tube was then vortexed for 30 s and further shook in a water bath for 30 min at 50 °C and 120 rpm oscillation. After that, the sample was vortexed again for 1 min and then mixed vigorously for another 30 min. Afterward, the sample was centrifuged at 10,000 × *g* for 10 min, and the supernatant was collected for analysis. Each sample was extracted and analyzed in duplicate. The relative amount of melanoidin in the extract was analyzed using a double-beam spectrophotometer (VWR UV-6300PC) at 420 nm (Shen et al., 2018).

Ileal digesta contents were analyzed for DM (duplicate, AOAC Official Method 934.01, 2006), CP (single analysis, AOAC Official Method 990.03, 2006), and complete AA profile (single analyses, AOAC Official Method 982.30 E(a,b,c), Ch. 45.3.05, 2006). Diet and ileal digesta samples were also analyzed in single for titanium dioxide (Myers et al., 2004).

Calculations and statistical analysis

Values for SID (%) were calculated as indicated below using Stein et al. (2007). First, AID of AA was calculated as:

Table 1. Diet composition (as-fed basis)[1]

Crystalline amino acids (AA)	Low	Low	High	High
Reducing sugars	Low	High	Low	High
Ingredient, %				
Corn	75.00	44.11	79.68	52.98
Soybean meal	21.00	15.70	16.19	6.85
Distillers dried grains with solubles	—	20.00	—	20.00
Bakery meal[2]	—	15.00	—	15.00
Soybean oil	1.23	2.70	0.90	2.10
Calcium carbonate	0.70	0.83	0.70	0.85
Monocalcium P, 21%	1.00	0.60	1.10	0.70
Sodium chloride	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.18	0.23	0.33	0.50
DL-Methionine	—	—	0.04	—
L-Threonine	0.09	0.04	0.16	0.16
L-Tryptophan	0.01	0.01	0.04	0.06
L-Valine	—	—	0.07	—
L-Isoleucine	—	—	0.01	0.01
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.15	0.15	0.15	0.15
Titanium dioxide	0.50	0.50	0.50	0.50
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lysine[5]	0.83	0.83	0.83	0.83
Isoleucine:lysine	69	76	60	60
Leucine:lysine	156	187	142	162
Methionine:lysine	29	34	30	29
Methionine and cysteine:lysine	57	65	56	55
Threonine:lysine	70	70	70	70
Tryptophan:lysine	21.0	21.0	21.0	21.0
Valine:lysine	77	90	75	72
Histidine:lysine	47	46	42	36
Total lysine, %	0.95	0.99	0.93	0.97
Metabolizable energy, kcal/kg	3,355	3,393	3,344	3,371
Net energy, kcal/kg	2,599	2,599	2,599	2599
Crude protein, %	16.4	19.1	14.8	16.0
Ca, %	0.59	0.59	0.59	0.59
P, %	0.56	0.55	0.56	0.53
STTD[6] P, %	0.33	0.33	0.33	0.33
Total sugars, %	3.83	5.11	3.42	4.34

[1]Experimental diet was formulated for 22.7- to 38.6-kg pigs.
[2]Quincy Farm Products, Quincy, IL.
[3]Provided per kilogram of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.
[4]Provided per kilogram of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.
[5]To ensure the ability to detect a difference in AA utilization, these diets were formulated to 85% of the recommended SID lysine requirement of pigs (NRC, 2012).
[6]Standardized total tract digestible phosphorus.

$$AID_{AA}\ (\%) = \left[1 - \left(\frac{AA_{diet}}{AA_{digesta}}\right) \times \left(\frac{Ti_{digesta}}{Ti_{diet}}\right)\right] \times 100, \quad (1)$$

where $AA_{digesta}$ and AA_{diet} represent the AA concentrations (g/kg) in digesta and diet DM, respectively, and Ti_{diet} and $Ti_{digesta}$ represent the indigestible marker concentrations (g/kg) in diet and digesta DM, respectively. Then, to calculate SID of AA the following equation was used.

$$SID_{AA}\ (\%) = AID_{AA} + \left[\left(\frac{IAA_{end}}{AA_{diet}}\right) \times 100\right], \quad (2)$$

where AID_{AA} is previously calculated in equation (1), IAA_{end} represents basal endogenous losses where average values were used from digestibility experiments conducted at the University of Illinois, Champaign, IL, and AA_{diet} represents the AA concentrations (g/kg) in diet DM.

Data were analyzed as a Latin square design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC) with pig as the experimental unit. Fixed effects included feed form, crystalline AA, RS inclusion, and all possible interactions. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Results were considered significant at $P \leq 0.05$ and a trend at $P < 0.10$.

Results

Chemical analysis of major ingredients is reported in Table 2. Analyzed CP and lysine corn were numerically greater than the reference values used (10.07 vs. 8.24 for CP and 0.43 vs. 0.25 for lysine). The analyzed lysine content for corn was higher than expected at 0.43% compared to NRC (2012) analyzed nutrient value of 0.25%. Analyzed nutrient values of soybean meal were similar to the reference values used. DDGS had a numerically decreased analyzed crude fat (5.05% vs. 7.50%) and increased total lysine (1.00% vs. 0.79%) compared to the reference values used. Bakery meal had a numerically increased ash content (6.02% vs. 3.22%) and total lysine (0.75% vs. 0.54%) compared to the reference values used. In addition, melanoidins were measured on individual ingredients as evidence of Maillard reaction. All ingredients that undergo processing prior to inclusion in diets (soybean meal, DDGS, and bakery meal) had increased absorbance values for melanoidins as compared to ground corn.

Chemical analysis of experimental diets indicated that analyzed CP ranged from 13.3% to 19.3% with the expected range of 14.8% to 19.1% for both mash and pelleted diets (Table 3). The high crystalline AA × low RS diets contained the lowest CP and the low crystalline AA × high RS diets contained the highest CP. Analyzed available lysine content was 0.85% to 1.02%, where the lowest was high crystalline AA × high RS mash diets and the highest for the low crystalline AA × low RS mash diet. The high RS diets (containing 20% DDGS and 15% bakery meal) had increased absorbance values for melanoidins as compared to the low RS diets (corn and soybean meal-based diets). In addition, when pelleting low RS diets there was little numerical increase in melanoidins absorbance values. However, when pelleting high RS diets there was a greater numerical increase in melanoidins absorbance values. The high RS diets (containing 20% DDGS and 15% bakery meal) had decreased KOH protein solubility compared to low RS diets. In addition, pelleting the high crystalline AA and high RS diets resulted in decreased KOH protein solubility compared to the mash diet. However, pelleting the remaining treatments resulted in increased KOH protein solubility compared to the same diet in mash form.

The experiment was designed to pellet diets at a conditioning temperature of 88 °C. However, at the desired conditioning temperature a consistent pelleting run could not be achieved. Therefore, diets were pelleted to a target hot pellet temperature between 85 and 88 °C, which was achieved by pelleting at a conditioning temperature range of 79 to 85 °C (Table 4). The PDI for diets containing low RS increased by more than 10% compared with those containing high RS. These differences in PDI could possibly be explained by increases in added fat aiding in die lubrication in diets containing high RS. Differences in PDI were alleviated by sifting the pellets postpelleting to remove excessive fines prior to feeding.

There was no feed form × crystalline AA × RS interaction observed for SID of AA. There were also no two-way interactions of feed form × crystalline AA for SID of total AA, indispensable, or dispensable AA. There were no two-way interactions of feed form × RS observed for SID of total AA, CP, or dispensable AA. There were no two-way interactions of feed form × RS observed for SID of indispensable AA, except for tryptophan. Pigs fed pelleted low RS diets resulted in higher SID tryptophan than mash high RS diets, mash low RS diets, and pelleted low RS diets. There were no two-way interactions of crystalline AA × RS observed for SID of total AA, CP, indispensable AA, or dispensable AA. For the main effects of feed form, the SID of total AA, CP, indispensable AA, alanine, aspartic acid, glutamic acid, and serine increased ($P < 0.042$) in the pelleted diets compared with mash diets (Table 5).

For the main effects of crystalline AA, pigs fed low or high crystalline AA had SID of total AA and CP that were not different. Pigs fed high crystalline AA had increased ($P = 0.007$; Table 5) SID of tryptophan compared with those fed the low crystalline AA diet. The SID of lysine tended to increase ($P = 0.076$) in pigs fed high crystalline AA diets compared with those fed low crystalline AA diets. Pigs fed high crystalline AA had decreased ($P = 0.050$) SID of histidine compared with those fed low crystalline AA diets. The SID of arginine and isoleucine tended to decrease ($P < 0.079$) in pigs fed high crystalline AA. In pigs fed high crystalline AA, the SID of serine and glycine decreased ($P < 0.042$) compared with those fed low crystalline AA.

For the main effects of RS diets, pigs fed high RS diets had decreased ($P < 0.05$) SID of total AA, CP, indispensable AA, alanine, aspartic acid, cysteine, glutamic acid, and serine.

Discussion

The present experiment was designed to determine if pelleting various swine diets influenced the digestibility of AA. Diets were formulated with low or high crystalline AA, low or high RS (from DDGS and bakery meal) and fed in either mash or pelleted form to increase the likelihood of binding lysine via the Maillard reaction. Components of the pelleting process, such as steam conditioning and feed retention time in the conditioner and die, expose feed to various degrees of heat, moisture, pressure, and sheer altering the feeds' physical and chemical characteristics. When ingredients that make up a diet formulation are exposed to the steam conditioning process and extrusion through the die, the heat and moisture plasticize the soluble fractions of the diet and increase the agglomeration of dietary components (Lundblad et al., 2009). Pelleting swine diets are commonly used to improve nutrient utilization, feed efficiency, feed handling characteristics, and bulk density (Behnke, 1994). In addition, previous research observed that feeding pelleted diets increased DM, N, and GE digestibility compared to feeding meal diets when fed to pigs (Wondra et al., 1995). More recently, Rojas et al. (2016) observed pelleting a corn–soybean meal diet increased digestibility of DM, N, and GE by 5% to 8% compared when feeding the same diet in a mash form. Observed improvements in protein and AA digestibility could be a result of pelleting due

Table 2. Chemical composition of individual major ingredients (as-fed basis)[1,2]

Item, %	Corn	Soybean meal	DDGS[3]	Bakery meal
Dry matter	88.10	88.94	90.39	87.28
Crude protein	10.07	45.44	29.77	17.32
Fat	2.25	0.54	5.05	4.03
Crude fiber	1.81	3.7	7.64	8.00
Ash	1.57	6.09	5.00	6.02
Melanoidin,[4] abs	0.105	0.206	0.215	0.306
KOH protein solubility[5]	49.95	76.10	26.67	49.71
Available lysine	0.42	2.87	0.92	0.68
Total AA	9.67	45.09	27.95	16.29
Lysine:CP	4.27	6.49	3.36	4.32
Indispensable amino acids (AA)				
Arginine	0.51	3.27	1.36	1.04
Histidine	0.29	1.22	0.81	0.43
Isoleucine	0.39	2.23	1.21	0.79
Leucine	1.03	3.52	3.40	1.42
Lysine	0.43	2.95	1.00	0.75
Methionine	0.19	0.63	0.54	0.25
Phenylalanine	0.47	2.36	1.49	0.94
Threonine	0.36	1.77	1.10	0.67
Tryptophan	0.08	0.66	0.21	0.15
Valine	0.48	2.27	1.50	1.04
Dispensable AA				
Alanine	0.64	1.97	2.04	0.95
Aspartic acid	0.76	5.08	1.94	1.33
Cysteine	0.22	0.71	0.60	0.28
Glutamic acid	1.80	8.28	4.45	2.50
Serine	0.44	2.01	1.29	0.71
Tyrosine	0.22	1.66	1.06	1.10
Glycine	0.42	1.91	1.15	0.84
Proline	0.73	2.25	2.35	0.86

[1]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[2]Values in parentheses indicate values used in diet formulation. These values were acquired from the NRC (2012) and bakery meal values were provided by supplier. The soybean meal net energy value assigned was 88% of corn.
[3]Dried distillers grains with solubles.
[4]Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.
[5]Protein solubility in potassium hydroxide (KOH).

to protein denaturation under processing conditions (Lancheros et al., 2020). The results of this experiment agree with previous research in that pigs fed pelleted diets had increased SID of total AA, CP, indispensable AA, alanine, aspartic acid, glutamic acid, and serine compared with pigs fed mash diets.

Evidence of the Maillard reaction can be determined from a reactive cyclic intermediate compound containing amino groups that polymerize to a dark-colored, insoluble material-containing nitrogen called melanoidin, which varies in color, molecular weight, nitrogen content, and solubility (Fennema et al., 2008). These end products from this “browning reaction” are commonly observed in food science processes such as baking bread. Melanoidin research in feed ingredients and complete diets is limited with most research evaluating early reaction productions (Amadori compounds). However, Amadori compounds can interfere with amino acid analysis giving an inaccurate measure of lysine concentrations by overestimating lysine content in the sample (Stein et al., 2005). On the human food side, melanoidins are measured for indication of favorable products for flavor and antioxidant potential. Shen et al. (2018) demonstrated that melanoidins increased during bread making with longer baking time, higher baking temperature, or higher sugar inclusion. In this study, ingredients that undergo processing prior to diet formulation (i.e., soybean meal, DDGS, and bakery meal) had increased melanoidin absorbance values compared to ground corn. Therefore, diets containing DDGS and bakery meal and reduced concentrations of ground corn had increased melanoidin absorbance values. Pelleting the corn and soybean meal-based diets (low RS) only resulted in minor numerical increases in melanoidin absorbance values. There tended to be a greater numerical increase in melanoidin absorbance values when high RS diets were pelleted compared to the mash high RS diets. Additionally, diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with corn–soybean meal-based diets. However, further research is needed to determine the relationship between melanoidins and nutrient digestibility in swine.

Table 3. Chemical composition of experimental diets (as-fed basis)[1,2]

	Mash				Pellets			
Crystalline AA	**Low**	**Low**	**High**	**High**	**Low**	**Low**	**High**	**High**
Reducing sugars	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Item, %								
Dry matter	87.37	87.81	87.43	87.88	87.88	87.91	87.83	88.65
Crude protein	15.78	18.71	13.31	15.73	15.61	19.29	14.42	16.99
Fat	1.20	3.69	0.95	2.89	2.13	4.53	2.30	4.40
Fiber	1.60	3.26	1.51	3.04	1.87	3.68	1.78	3.08
Ash	4.19	5.28	4.09	4.84	4.47	5.54	4.31	5.11
Melanoidin, abs[3]	0.081	0.119	0.081	0.123	0.088	0.146	0.087	0.143
KOH protein solubility[4]	63.45	48.62	62.07	54.66	81.76	57.33	71.73	46.15
Available lysine	1.02	0.96	0.93	0.85	0.98	0.94	0.90	0.98
Total AA	15.04	18.16	13.90	14.88	16.38	19.36	13.95	16.29
Lysine:CP	5.90	6.59	5.83	6.15	6.40	7.04	5.83	6.78
Indispensable AA								
Arginine	0.93	1.05	0.81	0.79	1.02	1.15	0.82	0.88
Histidine	0.41	0.49	0.37	0.39	0.44	0.53	0.37	0.43
Isoleucine	0.66	0.80	0.60	0.60	0.72	0.86	0.60	0.67
Leucine	1.36	1.77	1.27	1.51	1.47	1.87	1.28	1.63
Lysine	0.93	1.04	0.92	0.97	1.01	1.11	0.92	1.07
Methionine	0.26	0.32	0.27	0.28	0.28	0.34	0.27	0.29
Phenylalanine	0.75	0.94	0.67	0.74	0.82	1.00	0.68	0.81
Threonine	0.62	0.72	0.63	0.68	0.67	0.78	0.62	0.74
Tryptophan	0.18	0.20	0.20	0.20	0.22	0.22	0.21	0.23
Valine	0.74	0.94	0.72	0.73	0.80	1.01	0.71	0.81
Dispensable AA								
Alanine	0.81	1.08	0.75	0.94	0.87	1.14	0.77	1.02
Aspartic acid	1.43	1.60	1.24	1.14	1.57	1.72	1.24	1.26
Cysteine	0.28	0.34	0.26	0.29	0.31	0.37	0.25	0.31
Glutamic acid	2.70	3.12	2.43	2.44	2.93	3.30	2.44	2.68
Serine	0.65	0.77	0.58	0.61	0.71	0.84	0.59	0.70
Tyrosine	0.53	0.71	0.49	0.61	0.57	0.75	0.48	0.66
Glycine	0.63	0.79	0.56	0.62	0.69	0.84	0.57	0.70

[1]Dietary treatments were arranged in a 2 × 2 × 2 factorial with the main effects of crystalline amino acids (AA) (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet).
[2]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[3]Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.
[4]Protein solubility in potassium hydroxide (KOH).

Table 4. Feed processing and pellet quality of pelleted diets[1,2]

Crystalline amino acids	**Low**	**Low**	**High**	**High**
Reducing sugars	**Low**	**High**	**Low**	**High**
Item				
Production rate, kg/h	3810	3628	3719	3447
Conditioning temperature, °C	85.1	83.6	83.0	79.7
Hot pellet temperature, °C	89.3	87.4	87.6	85.0
Pellet durability index (PDI), %[3]	71.5	53.7	72.15	59.6

[1]Treatments were pelleted using a 5-ton 100-hp pellet mill (Model PM3016-4, California Pellet Mill) equipped with a 4 × 35-mm die (L:D = 8.75).
[2]Pellets were sifted to remove fines to ensure no effect of pellet quality on pig performance.
[3]Holmen NHP100 for 60 s.

Table 5. Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) main effects of feed form, crystalline AA, and reducing sugar (RS)[1,2]

	Form		Crystalline AA		RS			*P* value[3]		
	Mash	Pellet	Low	High	Low	High	SEM	Form	AA	RS
SID, %										
Total AA	80.8	84.6	83.0	82.3	85.2	80.2	0.74	0.001	0.500	0.001
Crude protein	76.9	82.2	80.2	78.9	82.0	77.1	1.08	0.001	0.340	0.002
Indispensable AA										
Arginine	91.6	96.1	94.5	93.2	96.1	91.5	0.54	0.001	0.079	0.001
Histidine	80.7	83.7	83.1	81.3	86.0	78.4	0.64	0.002	0.050	0.001
Isoleucine	79.6	84.3	83.0	81.0	85.1	78.8	0.76	0.001	0.059	0.001
Leucine	80.6	85.3	83.3	82.7	85.8	80.8	0.74	0.001	0.577	0.001
Lysine	85.8	87.8	85.9	87.7	89.8	83.7	0.69	0.041	0.076	0.001
Methionine	86.4	90.9	88.9	88.4	92.1	85.2	0.76	0.001	0.698	0.001
Phenylalanine	80.6	85.1	83.4	82.3	84.8	80.8	0.73	0.001	0.269	0.001
Threonine	75.7	78.7	76.2	78.2	80.4	74.0	0.89	0.017	0.113	0.001
Tryptophan[4]	87.7	90.0	87.3	90.4	90.0	87.7	0.80	0.042	0.007	0.046
Valine	75.9	80.6	79.0	77.4	81.8	74.7	0.88	0.001	0.200	0.001
Dispensable AA										
Alanine	78.8	84.3	81.7	81.4	83.6	79.5	0.86	0.001	0.776	0.002
Aspartic acid	75.7	78.9	78.0	76.6	81.4	73.1	0.69	0.002	0.160	0.001
Cysteine	68.3	70.7	70.5	68.4	73.3	65.6	0.88	0.058	0.090	0.001
Glutamic acid	84.1	84.9	83.2	83.9	85.6	81.5	0.64	0.004	0.442	0.001
Serine	80.0	85.4	83.9	81.4	86.3	79.1	0.85	0.001	0.042	0.001
Tyrosine	85.0	89.1	87.3	86.7	87.1	87.0	0.57	0.001	0.436	0.897
Glycine	62.3	65.4	66.2	61.5	65.3	62.4	1.59	0.157	0.039	0.194
Proline	101.4	105.8	105.2	101.9	102.2	104.9	3.57	0.380	0.517	0.587

[1]A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 8 Latin square design with the eight diets and eight 7-d periods.
[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with the main effects of crystalline AA (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet).
[3]Probability, $P <$ for the main effects of form, crystalline AA, or reducing sugar.
[4]A feed form × RS interaction ($P < 0.026$) for SID of tryptophan was observed. Feeding pelleted low RS diets increased SID of tryptophan compared with mash high and low RS diets, and pelleted high RS diets.

Additional analytical techniques to measure degree of processing for feed ingredients or complete diets included Lysine:CP, KOH protein solubility, and available lysine. The relationship of Lysine:CP gives an indication of the extent of conversion of lysine to melanoidins, and the heat damage that has occurred, where lower than expected values can indicate heat damage of processed ingredients (Stein et al., 2005). Expected Lysine:CP values for corn, DDGS, and bakery meal are about 3.0%, 3.1%, and 3.2%, respectively, where the expected soybean meal value is about 6.2% (NRC, 2012). In this experiment, the Lysine:CP ratio did not decrease in the pelleted diets compared to the mash diets. Therefore, there was no evidence of the Maillard reaction based on Lysine:CP.

The KOH solubility (%) indicates the nitrogen (%) that is solubilized in a potassium hydroxide solution. Previous research has determined that KOH solubility is well correlated to the digestibility of protein in the animal (Araba and Dale, 1990). In individual ingredients, KOH solubility was greatest in soybean meal (75.1%) and least in DDGS (26.7%), with corn (50.0%) and bakery meal (49.7%) being intermediate. For complete diets, high RS diets (containing 20% DDGS and 15% bakery meal) had decreased KOH protein solubility compared to low RS diets. This is largely driven by DDGS and bakery meal having the lowest KOH solubility as well as high RS diets having lower inclusions of soybean meal. Although KOH solubility has previously been correlated with soybean meal lysine digestibility, Stein et al. (2005) suggest that the KOH solubility was not accurate at predicting AA digestibility of DDGS. In this experiment, pelleting low RS and low crystalline AA, low RS and high crystalline AA, and high RS and low crystalline AA diet did not reduce KOH solubility compared to the respective mash diets. However, pelleting the high crystalline AA and high RS diets numerically decreased KOH protein solubility compared to the mash diet, which could be explained by the possible initiation of the Maillard reaction. However, this did not correspond with the AA digestibility results. Thus, analyzing KOH solubility in complete diets warrants further research to understand the association of KOH solubility with digestibility. Pelleting the remaining treatments resulted in increased KOH protein solubility compared to the same diet in mash form. Higher KOH solubility would indicate a greater nitrogen digestibility.

There are several factors that influence the rate of Maillard product formation, including temperature, pH, water activity, pressure, and reactant concentration (Rutherford and Moughan, 2007). Pelleting provides the potential for ideal conditions for the Maillard reaction. Ajandouz et al. (2008) utilized an aqueous model system via spectrophotometer absorbance of the intermediate and final stages of the reaction indicating that increasing temperature increases the Maillard reaction. In contrast to reducing AA digestibility, Lundblad et al. (2012) found that hydrothermal treatment increased AID for total indispensable AA, arginine, isoleucine, lysine, and threonine when compared to pigs fed a mash control diet. Additionally, AID of lysine was increased by hydrothermal treatment compared to mash with further improvement in pigs fed an extruded diet compared to a mash diet. These improvements in AID of AA could be due to processing partly denaturing dietary proteins, increasing digestion by proteases in the small intestinal (Svihus and Zimonja, 2011; Rojas et al., 2016).

Unlike protein-bound AA, crystalline AA are in the free, unbound form, and thus have more available amino groups to bind RS. The structure of lysine in particular makes it very susceptible to the Maillard reaction as it has two free amino groups. Maillard reactions with lysine result in a reduction of both lysine concentration and lysine digestibility (González-Vega et al., 2011). It has been established that crystalline AA are 100% digested and rapidly absorbed (Chung and Baker, 1992). Recent trends in swine diet formulation are to increase the levels of crystalline AA and byproduct ingredients to reduce diet cost and reduce environmental impact. In this study, no differences were observed for SID of total AA, indispensable AA or CP pigs fed low or high crystalline AA. However, the SID of tryptophan increased in diets containing higher concentrations of crystalline AA. The SID of lysine tended to increase in pigs fed high crystalline AA diets compared with those fed low crystalline AA diets.

It has been demonstrated that the Maillard reaction can negatively impact AA digestibility (Pahm, 2008). González-Vega et al. (2011) determined that SID of all AA in autoclaved SBM was reduced as the autoclaving time increased from 0 to 30 min. Therefore, as soybean meal was exposed to moisture, pressure, high temperature (125 °C), and increasing time in the autoclave, the Maillard reaction occurred in the soybean meal. However, as soybeans were exposed to an oven-drying thermal process, at the same time and temperature (125 °C for 30 min) there were minimal effects on the SID of AA in SBM (González-Vega et al., 2011). This suggests more severe processing conditions with added moisture and pressure increase the formation of Maillard reaction products and destruction of AA. Further, González-Vega et al. (2011) suggest that with increased pressure AAs become less stable (Qain et al., 1993) and an increase in humidity will increase the reaction rate between the amino group of the AA and glucose (Schwartz and Lea, 1952). Rojas et al. (2016) demonstrated that CP and AA digestibility increased in pelleted diets compared to mash diets containing corn, soybean meal, and DDGS. In this experiment, there was no evidence for interaction between diets, demonstrating that diet type did not accelerate the Maillard reaction to a point to reduce AA digestibility during the pelleting process. Pigs fed pelleted diets did demonstrate increased SID of AA, in agreement with Rojas et al. (2016).

In this study, both DDGS and bakery meal byproducts were utilized and accounted for 35% of total dietary inclusion. Diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with the corn–soybean meal-based diets. Amino acid digestibility of byproduct ingredients has been evaluated throughout literature which can explain the main effect differences observed for RS diets. Almeida et al. (2011) observed that bakery meal is a poor source of digestible AA when compared with corn and corn coproducts, likely due to bakery meal variability. Additionally, the SID of lysine and CP for bakery meal was not different from SID values found for DDGS. For all other indispensable AA except tryptophan, SID values in bakery meal were less than in pigs fed corn, with no differences found between corn and DDGS (Almeida et al., 2011). This did not translate into differences in AA digestibility due to diet type and the pelleting process.

Amino acid digestibility of swine diets containing free AA and RS pelleted at high temperatures was evaluated. Therefore, the data rejected the null hypothesis that pelleting swine diets containing free AA and RS at the reported temperatures will reduce SID of CP and AA. There was no evidence of interactions between diet types and diet form, indicating that increasing amounts of crystalline AA and RS did not reduce AA digestibility when pelleting diets following the described processing conditions. Additionally, pelleting diets resulted in increased AA digestibility compared to mash diets. Diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with the corn–soybean meal-based diets. Crystalline AA concentration did not influence AA digestibility of indispensable AA, except for SID of tryptophan which was increased in diets with higher concentrations of crystalline AA. Further research should focus on the pelleting temperature breakpoint (conditioning and/or hot pellet temperature) where digestibility decreases due to the Maillard reaction.

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