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Digestibility of amino acids, but not fiber, fat, or energy, is greater in cold-fermented, low-oil distillers dried grains with solubles (DDGS) compared with conventional DDGS fed to growing pigs

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Abstract

Two experiments were conducted to test the hypothesis that the digestibility of gross energy (**GE**) and nutrients, and concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) in two sources of distillers dried grains with solubles (**DDGS**) are not different despite different concentrations of fat in the two sources. Cold-fermented DDGS (6.82% fat) and a conventional DDGS (9.54% fat) were used. In experiment 1, 12 growing barrows (initial body weight = 55.2 ± 3.6 kg) that had a T-cannula installed in the distal ileum were allotted to one of three diets and two periods. Two diets contained either cold-fermented or conventional DDGS as the sole source of crude protein (**CP**) and amino acids (**AA**). The third diet was an N-free diet that was used to determine the basal endogenous losses of AA from the pigs. Each experimental period lasted 7 d and ileal digesta were collected on days 6 and 7 of each period. Results demonstrated that values for the standardized ileal digestibility (**SID**) of CP and most AA were greater ($P < 0.05$) or tended to be greater ($P < 0.10$) in cold-fermented than in conventional DDGS. In experiment 2, 24 barrows (initial body weight = 17.3 ± 1.3 kg) were randomly allotted to three diets with 8 replicate pigs per diet. A corn-based basal diet and two diets containing corn and either cold-fermented DDGS or conventional DDGS were formulated. Pigs were housed individually in metabolism crates and feces and urine were collected separately for 5 d after 7 d of adaptation. The apparent total tract digestibility (**ATTD**) of neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and acid-hydrolyzed ether extract (**AEE**) was greater ($P < 0.01$) in conventional DDGS than in cold-fermented DDGS, but there was no difference in ATTD of GE between the two sources of DDGS. However, conventional DDGS contained more ($P < 0.001$) DE and ME than cold-fermented DDGS because of greater GE. In conclusion, the SID of AA was greater in cold-fermented DDGS than in the conventional DDGS that was evaluated in this experiment, but the ATTD of NDF, ADF, and AEE, and ME were greater in conventional DDGS than in cold-fermented DDGS.

Key words: amino acids, cold fermentation, digestibility, distillers dried grains with solubles, energy, pigs

Introduction

Corn distillers dried grains with solubles (**DDGS**), a coproduct from the ethanol production, is often included in diets for pigs (Stein and Shurson, 2009). As more corn oil has been extracted from the solubles at the ethanol plants, the oil content in DDGS is reduced. Conventional DDGS usually contains at least 9% crude fat, whereas low-oil DDGS contains 6% to 9% crude fat (NRC, 2012). Addition of fat to diets fed to pigs increases the digestibility

Abbreviations

AA	amino acids
ADF	acid detergent fiber
AEE	acid-hydrolyzed ether extract
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
BPX	Broin Project X
CP	crude protein
DDGS	distillers dried grains with solubles
DE	digestible energy
GE	gross energy
ME	metabolizable energy
NDF	neutral detergent fiber
SID	standardized ileal digestibility

of amino acids (**AA**; Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011) and it is, therefore, possible that AA digestibility is affected by the lower oil concentration in low-oil DDGS, but data to demonstrate this have been inconclusive (Curry et al., 2014). Digestible energy (**DE**) and metabolizable energy (**ME**) in low-oil DDGS are reduced compared with conventional DDGS (NRC, 2012; Kerr et al., 2013; Curry et al., 2016). However, there is limited information about how oil concentration in DDGS influences apparent total tract digestibility (**ATTD**) of neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and acid-hydrolyzed ether extract (**AEE**) by pigs.

POET Nutrition, Inc. (Sioux Falls, SD) uses a heat-free cold fermentation technique to produce a low-oil DDGS. It is possible that this technique positively influences nutrient digestibility by reducing heat damage in the DDGS produced after ethanol is produced. This technique, which is called the Broin Project X (**BPX**) technology, uses a proprietary blend of enzymes for more efficient conversion of starch to ethanol in the fermentation process. This usually results in a final product that contains less AEE than conventional DDGS, but there is limited information about how the BPX technology affects the digestibility of energy and nutrients by growing pigs. Therefore, the objectives of these experiments were to test the hypothesis that the digestibility of crude protein (**CP**) and AA (experiment 1), gross energy (**GE**), NDF, ADF, and AEE, and the DE and ME (experiment 2) are not different despite different concentrations of fat in the two sources.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the two experiments. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN) were used. The same batches of conventional DDGS and cold-fermented DDGS were used in the two experiments (Table 1). Both sources of DDGS were sourced from POET Nutrition, Inc. (Sioux Falls, SD).

Table 1. Analyzed chemical and physical characteristics of corn and two sources of DDGS[1], experiments 1 and 2

Item	Corn	Cold-fermented DDGS	Conventional DDGS
Dry matter, %	84.90	87.77	82.26
GE, kcal/kg	3,962	4,442	4,831
AEE, %	3.44	6.82	9.54
Ash, %	0.95	5.35	5.92
CP, %	6.93	27.99	26.23
NDF	10.28	29.40	37.59
ADF	3.24	12.47	16.50
Lys:CP, %	—	3.43	3.20
Indispensable AA, %			
Arg	—	1.28	1.23
His	—	0.78	0.71
Ile	—	1.14	1.14
Leu	—	3.31	3.16
Lys	—	0.96	0.84
Met	—	0.53	0.51
Phe	—	1.42	1.42
Thr	—	1.09	1.04
Trp	—	0.17	0.21
Val	—	1.44	1.42
Dispensable AA, %			
Ala	—	2.07	1.89
Asp	—	1.87	1.75
Cys	—	0.53	0.48
Glu	—	4.65	3.70
Gly	—	1.21	1.10
Pro	—	2.31	2.03
Ser	—	1.25	1.20
Tyr	—	1.04	1.01
Carbohydrates, %			
Starch	59.58	6.60	2.82
Glucose	0.55	0.21	0.26
Fructose	0.33	0.25	0.21
Maltose	ND[2]	ND	ND
Sucrose	0.80	ND	ND
Stachyose	ND	ND	ND
Raffinose	0.19	ND	ND
Fructo-oligosaccharides	0.10	0.13	0.10
Physical characteristics			
Objective color[3]			
L*	—	59.67	66.95
a*	—	10.45	9.70
b*	—	18.10	23.70
Bulk density, g/L	623	429	437
Particle size, µm	392	367	410

[1]All values except dry matter and physical characteristics were adjusted to 88% dry matter. AA and objective colors in corn were not analyzed.
[2]ND, not detected.
[3]L* = greater value indicates a lighter color; a* = greater value indicates a more red color; b* = greater value indicates a more yellow color.

Experiment 1: digestibility of CP and AA

Three diets were formulated (Table 2). Two diets contained either cold-fermented or conventional DDGS as the sole source of CP and AA. The third diet was a N-free diet that was used to determine basal endogenous losses of AA from the pigs. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

Twelve growing barrows that had a T-cannula installed in the distal ileum were used (initial body weight = 55.2 ± 3.6 kg). Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted T-bar floors. A feeder and a nipple drinker were installed in each pen. Pigs were randomly allotted to one of three diets based on initial body weight using a quadruplicated 3 × 2

Table 2. Ingredient composition and analyzed composition of experimental diets containing DDGS, experiment 1

Item, %	Cold-fermented DDGS	Conventional DDGS	N-free
Ingredient composition, as-fed basis			
DDGS	50.00	50.00	—
Soybean oil	2.00	2.00	4.00
Ground limestone	0.80	0.80	0.45
Dicalcium phosphate	0.90	0.90	2.15
Sucrose	—	—	20.00
Cornstarch	45.20	45.20	67.80
Solka floc[1]	—	—	4.00
Magnesium oxide	—	—	0.10
Potassium carbonate	—	—	0.40
Sodium chloride	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30
Analyzed composition, 88% dry matter basis			
CP, %	13.36	11.53	0.31
Indispensable AA, %			
Arg	0.64	0.56	ND[3]
His	0.40	0.33	ND
Ile	0.58	0.51	0.01
Leu	1.68	1.46	0.03
Lys	0.50	0.40	0.01
Met	0.26	0.23	ND
Phe	0.72	0.64	0.01
Thr	0.55	0.48	ND
Trp	0.10	0.09	0.02
Val	0.75	0.66	0.01
Dispensable AA, %			
Ala	1.06	0.88	0.01
Asp	0.97	0.84	0.01
Cys	0.26	0.23	ND
Glu	2.49	1.98	0.03
Gly	0.62	0.51	0.01
Pro	1.22	1.00	0.01
Ser	0.62	0.54	0.01
Tyr	0.53	0.46	0.01

[1]Fiber Sales and Development Corp., Urbana, OH.
[2]Provide the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.
[3]ND, not detected.

incomplete Latin square design with three diets and two periods (Kim and Stein, 2009). Therefore, there were 8 replicate pigs per treatment in the two periods. Feed was provided in the amount of three times the maintenance energy requirement (i.e., 197 kcal ME/kg body weight$^{0.60}$; NRC, 2012). Water was available at all times. The daily feed allotment was provided at 0700 hours. Each experimental period lasted 7 d with the initial 5 d being the adaptation period and ileal digesta were collected on days 6 and 7 for 8 h using standard procedures (Stein et al., 1998).

At the conclusion of the experiment, ileal digesta samples collected from each pig were thawed and mixed, and one subsample for chemical analysis was collected for each pig and period. Each sample of feed ingredient, diet, and ileal digesta was analyzed in duplicate. Digesta samples were lyophilized in a freeze dryer (Gamma 1-16 LSCplus, IMA Life, Tanowanda, NY) and samples were finely ground before analysis. Samples of diets, each source of DDGS, and ileal digesta were analyzed for dry matter (method 930.15; AOAC Int., 2007) and CP (method 990.03; AOAC Int., 2007), and AA were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110 °C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C (method 982.30 E(c); AOAC Int., 2007). Diets and ileal digesta samples were also analyzed for Cr using Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2007) after digestion using nitric acid-perchloric acid (method 968.08D(b); AOAC Int., 2007).

The Lys:CP ratio (%) was calculated for each source of DDGS (Stein et al., 2009). The apparent ileal digestibility (**AID**) of CP and AA was calculated in the two diets containing cold-fermented or conventional DDGS (Stein et al., 2007). The basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet (Stein et al., 2007) and these values were used to correct AID values for basal endogenous losses to calculate standardized ileal digestibility (**SID**) of CP and AA (Stein et al., 2007).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). Homogeneity and normality of the variances were confirmed using the UNIVARIATE procedure of SAS. The model included diet as fixed effect and pig and period as random effects. Mean values were calculated using the LSMeans statement, and if significant differences were detected, means were separated using the PDIFF option. Pig was the experimental unit. Results were considered significant at $P < 0.05$ and considered a trend at $P < 0.10$.

Experiment 2: digestibility of NDF, ADF, AEE, and GE

A corn-based basal diet and two diets containing either cold-fermented DDGS or conventional DDGS were formulated (Table 3). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Twenty-four barrows (initial body weight = 17.3 ± 1.3 kg) were randomly allotted to the three diets with 8 replicate pigs per diet in a completely randomized design. Pigs were housed individually in metabolism crates that were equipped with a feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials during the collection period. Pigs were fed at three times the energy requirement for maintenance based on individual body weight of pigs (i.e., 197 kcal ME/kg body weight$^{0.60}$; NRC, 2012). Diets were provided each day at 0800 and 1600 hours in two equal meals. The amount of feed left in the feeders was recorded daily and feed intake of pigs was calculated based on total feed provided and feed refusals. Pigs had ad libitum access to water throughout the 14-d experiment. The initial 7 d were considered the adaptation period to the diet, whereas urine and fecal

Table 3. Ingredient composition and analyzed composition of experimental diets containing DDGS, experiment 2

Item, %	Basal	Cold-fermented DDGS	Conventional DDGS
Ingredient composition, as-fed basis			
Ground corn	97.0	47.4	47.4
DDGS	—	50.0	50.0
Ground limestone	0.8	1.3	1.3
Dicalcium phosphate	1.5	0.6	0.6
Sodium chloride	0.4	0.4	0.4
Vitamin-mineral premix[1]	0.3	0.3	0.3
Analyzed composition, 88% dry matter basis			
GE, kcal/kg	3,833	4,094	4,200
CP, %	7.27	18.42	17.07
NDF, %	10.09	19.86	23.42
ADF, %	4.83	7.39	9.00
AEE, %	3.34	5.32	7.22
Calculated value[2]			
ME, kcal/kg	3,293	3,307	3,307

[1]Provide the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethyl primidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.
[2]ME in all diets was calculated from previous data (NRC, 2012).

materials were collected during the following 5 d according to the marker to marker approach (Adeola, 2001). Fecal collections were initiated when the start marker, which was included in the morning meal on day 8 first appeared in the feces, and ceased when the stop marker, which was included in the morning meal on day 13 appeared. Urine was collected in buckets that contained a preservative of 50 mL of 6 N HCl. Fecal samples and 20% of the collected urine were stored at −20 °C immediately after collection.

At the conclusion of the experiment, fecal samples were thawed and pooled for each pig and diet, and then dried in a 50 °C forced-air drying oven. Fecal samples were ground through a 1-mm screen using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ). Urine samples were also thawed and mixed for each pig and diet, and a subsample was dripped onto cotton balls that were placed in a plastic bag and lyophilized before analysis (Kim et al., 2009). One subsample of each feed ingredient, diet, feces, and urine was analyzed in duplicate. Ingredients (corn and the two sources of DDGS), diets, ground fecal samples, and lyophilized urine samples were analyzed for GE using a bomb calorimeter (Model 6400; Parr Instruments, Moline, IL). Ingredients, diets, and fecal samples were also analyzed for dry matter and CP as indicated for experiment 1, and ADF and NDF were also analyzed in these samples (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Diets, ingredients, and fecal samples were analyzed for AEE using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Bulk density was analyzed in corn and the two sources of DDGS (Cromwell et al., 2000), and color was measured using a Minolta CR-400 apparatus (Minolta, Camera Company Osaka, Japan). Particle size of corn and the two DDGS sources was also measured (ASABE, 2008). Corn and each source of DDGS were analyzed for starch using the glucoamylase procedure (Method 979.10; AOAC Int., 2007). Ingredients were also analyzed for sugars and oligosaccharides including glucose, fructose, maltose, sucrose, stachyose, and raffinose (Method 977.2; AOAC Int., 2007), and fructo-oligosaccharides were analyzed using refractive index high-performance liquid chromatography (Campbell et al., 1997).

Following chemical analysis, the ATTD of GE, DM, ADF, NDF, and AEE was calculated for each diet and the DE and ME in each diet were calculated as well (NRC, 2012). The DE and ME of corn were then calculated by dividing the DE and ME of the corn diet by the inclusion rate of corn in that diet (i.e., 97.0%). The contribution of DE and ME from corn to the DE and ME in the diets containing each source of DDGS was then subtracted from the DE and ME of these diets and the DE and ME of cold-fermented and conventional DDGS were calculated by difference (Adeola, 2001). The ATTD of ADF, NDF, and AEE in the two sources of DDGS was also calculated from the proportional contribution of each source to the diets.

Homogeneity of the variances and normality were confirmed and data were analyzed using the PROC MIXED in SAS. Diet or ingredient was the fixed effect and pig was the random effect. Mean values were calculated using the LSMeans statement and pig was the experimental unit. A contrast statement was used to compare the diets (i.e., basal diet vs. DDGS diets; cold-fermented vs. conventional DDGS) and ingredients (i.e., corn vs. the two DDGS sources; cold-fermented vs. conventional DDGS). Results were considered significant at $P < 0.05$ and considered a trend at $P < 0.10$.

Results

The chemical composition of the ingredients used in this experiment was adjusted to 88% dry matter. The CP concentrations in cold-fermented and conventional DDGS were 27.99% and 26.23%, and Lys concentrations were 0.96% and 0.84%, respectively. Consequently, the Lys:CP ratio was 3.43 in cold-fermented DDGS and 3.20 in conventional DDGS. Cold-fermented DDGS had lower AEE concentrations compared with conventional DDGS, and GE was almost 400 kcal/kg greater in conventional DDGS compared with cold-fermented DDGS. Cold-fermented DDGS had lower concentrations of fiber compared with conventional DDGS. Conventional DDGS had a lighter (greater L*) and more yellow (greater b*), but less red (less a*), color than cold-fermented DDGS, whereas cold-fermented DDGS had smaller particle size and slightly less bulk density than conventional DDGS.

Experiment 1: digestibility of CP and AA

Greater ($P < 0.05$) AID and SID of CP and Lys were observed in pigs fed cold-fermented DDGS compared with pigs fed conventional DDGS (Table 4). The AID and SID of most other AA were also greater ($P < 0.05$) or tended to be greater ($P < 0.10$) in cold-fermented than in conventional DDGS with the exception that there was no difference between the two sources of DDGS for SID of Trp, Cys, Pro, and Ser.

Table 4. AID and SID of CP and AA in DDGS[1,2], experiment 1

	AID				SID			
Item, %	Cold-fermented DDGS	Conventional DDGS	SEM	*P*-value	Cold-fermented DDGS	Conventional DDGS	SEM	*P*-value
CP	67.0	54.6	2.54	0.021	84.6	74.8	2.54	0.031
Indispensable AA								
Arg	85.9	78.8	1.16	0.010	93.3	87.2	1.16	0.013
His	82.2	76.8	1.07	0.030	86.6	82.1	1.07	0.043
Ile	78.4	72.6	1.36	0.033	84.7	79.8	1.36	0.051
Leu	86.8	82.7	1.18	0.061	90.5	86.9	1.18	0.075
Lys	47.4	27.6	4.60	0.003	68.1	53.0	4.60	0.007
Met	85.9	81.5	0.88	0.004	89.3	85.4	0.88	0.007
Phe	82.9	78.1	1.10	0.008	88.4	84.2	1.10	0.017
Thr	70.2	65.3	1.15	0.009	80.8	77.4	1.15	0.052
Trp	72.3	69.1	2.66	0.489	79.1	76.7	2.66	0.582
Val	74.8	68.1	1.48	0.015	83.2	77.6	1.48	0.026
Dispensable AA								
Ala	80.3	72.0	1.86	0.003	87.2	80.2	1.86	0.004
Asp	72.7	67.4	1.14	0.006	81.6	77.6	1.14	0.027
Cys	73.9	71.0	1.37	0.147	81.8	79.9	1.37	0.301
Glu	83.8	78.1	1.45	0.015	88.5	84.0	1.45	0.045
Gly	57.9	36.8	5.61	0.018	88.5	74.2	5.61	0.051
Pro	66.7	33.7	10.10	0.034	118.3	95.9	10.10	0.106
Ser	78.6	74.4	0.99	0.057	86.5	83.4	0.99	0.110
Tyr	85.4	82.4	0.75	0.012	90.8	88.5	0.75	0.048

[1]Each least squares mean represents eight observations.
[2]SID of CP and AA was calculated by correcting values for AID for basal endogenous losses. Basal endogenous losses (g/kg dry matter intake) were determined from pigs fed the N-free diet: CP, 25.7; Arg, 0.52; His, 0.19; Ile, 0.40; Leu, 0.69; Lys, 1.13; Met, 0.10; Phe, 0.43; Thr, 0.64; Trp, 0.08; Val, 0.69; Ala, 0.80; Asp, 0.95; Cys, 0.23; Glu, 1.28; Gly, 2.10; Pro, 6.90; Ser, 0.54; Tyr, 0.31.

Experiment 2: digestibility of NDF, ADF, AEE, and GE

Feed intake of pigs fed the corn diet tended to be greater ($P < 0.10$) than that of pigs fed DDGS diets (Table 5), but daily GE intake did not differ between corn and DDGS diets. The reason feed intake tended to be greater for pigs fed the basal diet compared with the two diets containing DDGS was that there was more feed refusal from pigs fed the two diets containing DDGS. However, the intake of NDF, ADF, and AEE was greater ($P < 0.001$) for pigs fed DDGS diets than for pigs fed the corn diet reflecting the greater concentrations of these nutrients in diets containing DDGS than in the diet containing corn. Pigs fed the corn diet had less ($P < 0.01$) excretion of GE, NDF, ADF, and AEE in feces and less ($P < 0.01$) urinary excretion of GE than pigs fed the two DDGS diets. The ATTD of dry matter, GE, NDF, ADF, and AEE for the corn diet was greater ($P < 0.05$) than for the two DDGS diets. The DE and ME in the corn diet was also greater ($P < 0.001$) than in the two DDGS diets.

Feed intake and intake of GE, NDF, and ADF did not differ between pigs fed the two DDGS diets, but AEE intake was greater ($P < 0.05$) for pigs fed the diet with conventional DDGS compared with pigs fed the diet containing cold-fermented DDGS. Fecal and urine excretion and ATTD of dry matter and GE did not differ between the two DDGS diets. However, the ATTD of NDF, ADF, and AEE was greater ($P < 0.01$) for the diet containing conventional DDGS than for the diet containing cold-fermented DDGS. There was no difference in DE and ME concentrations between the two diets containing cold-fermented DDGS and conventional DDGS.

The ATTD of GE, NDF, ADF, and AEE and concentrations of DE and ME were greater ($P < 0.05$) in corn than in the two sources of DDGS (Table 6). The ATTD of NDF, ADF, and AEE and concentrations of DE and ME were greater ($P < 0.01$) in conventional DDGS than in cold-fermented DDGS, but there was no difference in the ATTD of GE between the two sources of DDGS.

Discussion

Because Lys has the epsilon amino group in the side chain, Lys is the AA that is most susceptible to heat damage, and heat damage of a feed ingredient will, therefore, reduce the concentration of analyzed Lys, whereas CP usually is not reduced (González-Vega et al., 2011; Almeida et al., 2013, 2014). As a consequence, the Lys:CP ratio will be reduced if a feed ingredient is heat-damaged and this ratio may, therefore, be used as an indicator of heat damage (Stein et al., 2009; González-Vega et al., 2011). The Lys:CP ratio in corn grain is approximately 3.1% (NRC, 2012) and a Lys:CP ratio in corn DDGS that is greater than this value, therefore, indicates that there was minimal damage from processing. The Lys:CP ratios in the two DDGS sources used in the present experiments were greater than in some sources of DDGS used in previous experiments (Stein and Shurson, 2009; Kim et al., 2012), which indicates that both sources of DDGS were less heat-damaged compared with DDGS used previously. This observation confirms that the Lys:CP ratio of DDGS produced in recent years is greater than previously reported, which indicates that ethanol plants are causing less heat damage during drying than they used to (Espinosa and Stein, 2018; Espinosa et al., 2019). This results in greater Lys concentration in current sources of DDGS compared with DDGS produced 10 to 20 years ago. It was expected that cold-fermented DDGS would have less heat damage than the conventional DDGS because of the cold-processing technique used during fermentation, but the

Table 5. ATTD of nutrients and energy and concentrations of DE and ME in experimental diets[1] (as-fed basis), experiment 2

	Diet				Contrast *P*-value	
Item	Basal	Cold-fermented DDGS	Conventional DDGS	SEM	Basal vs. DDGS	DDGS source
Intake						
Feed intake, kg/d	0.70	0.63	0.57	0.05	0.079	0.332
GE, kcal/d	2,584	2,530	2,301	206	0.426	0.349
NDF, g/d	69	123	128	9	<0.001	0.606
ADF, g/d	33	46	49	4	<0.001	0.391
AEE, g/d	23	33	40	3	<0.001	0.043
Fecal excretion						
Dry feces output, kg/d	0.06	0.12	0.11	0.01	<0.001	0.169
Fecal GE, kcal/d	278	569	524	41	<0.001	0.383
NDF, g/d	26	58	51	5	<0.001	0.174
ADF, g/d	9	20	17	2	<0.001	0.097
AEE, g/d	7	15	13	1	0.001	0.179
Urine excretion						
Urine output, kg/d	2	3	2	0.3	0.036	0.541
Urinary GE, kcal/d	56	113	97	8	<0.001	0.193
ATTD						
Dry matter, %	91.5	80.3	80.8	0.4	<0.001	0.410
GE, %	89.2	77.6	77.2	0.5	<0.001	0.623
NDF, %	61.7	52.7	60.8	1.9	0.046	0.007
ADF, %	73.1	57.5	66.6	1.6	<0.001	<0.001
AEE, %	69.6	53.6	68.5	2.9	0.026	0.002
Energy in diets, kcal/kg						
DE	3,303	3,119	3,130	19	<0.001	0.671
ME	3,221	2,940	2,958	24	<0.001	0.548

[1]Each least squares mean represents eight observations.

Table 6. Concentrations of DE and ME and ATTD of nutrients in corn and two sources of DDGS[1,2], experiment 2

	Ingredient				Contrast *P*-value	
Item	Corn	Cold-fermented DDGS	Conventional DDGS	SEM	Corn vs. DDGS	DDGS source
ATTD, %						
GE	89.2	67.9	67.2	0.8	<0.001	0.505
NDF	61.7	49.9	60.6	2.2	0.025	0.002
ADF	73.1	56.0	66.1	1.7	<0.001	<0.001
AEE	69.6	51.9	68.4	3.1	0.023	0.001
Energy, kcal/kg						
GE	3,962	4,442	4,831	—	—	—
DE	3,529	3,017	3,244	36	<0.001	<0.001
ME	3,446	2,743	2,965	50	<0.001	0.001

[1]Each least squares mean represents eight observations.
[2]All values for GE and DE and ME were adjusted to 88% dry matter.

high Lys:CP ratio in conventional DDGS, although less than in cold-fermented DDGS, indicates that this source of DDGS was also of high quality. This observation was confirmed by the color measurements, which did not indicate that the conventional DDGS was heat-damaged. Color measurements are usually used as an indication of sample quality. Among color measurements, lightness (L) and yellowness (b) are more correlated with heat damage of DDGS than the "a" score for redness (Cromwell et al., 1993). In overprocessed sources of DDGS, the lightness score (L) is less than 32 and the yellowness score (b) is less than 13 (Cromwell et al., 1993). Thus, the color values obtained for the two sources of DDGS used in this experiment were greater than in heat-damaged DDGS (Cromwell et al., 1993; Almeida et al., 2013), which is also in agreement with the data for Lys:CP ratio.

However, whereas both the Lys:CP ratio and the color measurements indicate that the two sources of DDGS were not heat-damaged, the SID value for Lys obtained in the conventional DDGS was very low and corresponds to the SID of Lys in a heat-damaged DDGS (Almeida et al., 2013). Likewise, the SID of Lys in both sources of DDGS was less than that of all other indispensable AA including Thr, which clearly indicates some heat damage in these sources of DDGS because the SID of Thr is expected to always be the lowest among the indispensable AA due to the high concentration of Thr in endogenous protein. It is possible that this discrepancy in indicators of heat damage is a result of a moderate heat damage that resulted in Lys being converted to early Amadori compounds, which reduces SID of Lys, but not severe enough to convert Lys to more advanced Maillard reaction products, which results in reduced analyzed Lys. Some

of the early Amadori compounds may convert back to Lys during the acid hydrolysis that precedes AA analysis, and although having a low digestibility, these compounds will be analyzed as normal Lys, and thus, contribute to a high calculated value for Lys:CP. Likewise, early Amadori compounds do not contribute to the browning reactions that often are associated with the Maillard reaction, which explains why no change in color was observed. It therefore appears that neither the Lys:CP ratio, nor color measurements are completely accurate indicators of heat damage in DDGS if the heat damage only results in conversion of Lys to early Amadori compounds.

The SID values for AA other than Lys in conventional DDGS that were calculated in this experiment are in agreement with values for SID of AA in conventional DDGS (Kim et al., 2009; NRC, 2012), but the values for cold-fermented DDGS were greater than average values for low-oil DDGS (Stein et al., 2016). The basal endogenous losses of AA were also close to expected values (Park et al., 2013).

Even though cold-fermented DDGS contains less fat, the digestibility of AA was greater than in the conventional DDGS. Reduced AID and SID of AA were reported for low-fat DDGS compared with conventional DDGS (Curry et al., 2014), likely because dietary fat promotes AA digestibility (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). It is possible that the fact that cold-fermented DDGS was produced using the BPX technology is the reason for the increased SID of AA (Robinson et al., 2008; Gutierrez et al., 2014). In the BPX process, heat is not applied during the production of ethanol as is the case in conventional fermentation and the enzymes used in the cold fermentation process are also different from those used in traditional fermentation. It is possible that these differences also contribute to the increase in SID of AA that was observed.

The reason for the increased GE in conventional DDGS compared with cold-fermented DDGS is most likely that the concentration of AEE was reduced in cold-fermented DDGS. The DE and ME in corn that were obtained in this experiment were close to expected values (NRC, 2012), but DE and ME in conventional DDGS that were determined in this experiment were less than values previously reported for conventional DDGS (>6% and <9% oil DDGS; Stein et al., 2006; NRC, 2012). However, the values for DE and ME in conventional DDGS obtained in this experiment are in agreement with the average DE and ME recently reported for 20 sources of DDGS that contained less than 10% oil (Curry et al., 2016). Values from this experiment are also in agreement with the DE and ME recently reported for eight sources of low-oil DDGS (Espinosa et al., 2018). Thus, despite a low digestibility of fat in DDGS (Kim et al., 2013), removal of oil from the solubles, which is now a common procedure in the industry, results in production of low-oil DDGS that contains less DE and ME than DDGS with greater concentrations of fat. The implication of this observation is that diets containing low-oil DDGS will contain less ME than if diets contain conventional DDGS with more than 10% fat, which was produced before oil removal from the solubles became a standard in the industry.

The observation that DE and ME concentrations in cold-fermented DDGS were lower compared with conventional DDGS is in agreement with data for low-oil DDGS compared with conventional DDGS (Anderson et al., 2012; Gutierrez et al., 2014). It is likely that the reason for this difference is that the concentration and ATTD of AEE was less in cold-fermented DDGS than in conventional DDGS although fat concentration is not always related with DE and ME in DDGS (Kerr et al., 2013). The ATTD of AEE in the two sources of DDGS was within the range of reported values (Stein et al., 2009; Kim et al., 2013). The reason for the lower ATTD of AEE in the cold-fermented DDGS than in the conventional DDGS may be that the concentration of AEE was lower in the cold-fermented DDGS. The ATTD of AEE usually increases as fat concentration in the diet increases due to reduced contributions of endogenous fat in the output of AEE in the feces (Kil et al., 2010; Kim et al., 2013). The lower concentration of fat in the DDGS used in this experiment is likely also the reason for the lower ATTD of AEE obtained in this experiment compared with the values reported by Stein et al. (2009) who used DDGS that contained 10% to 12% ether extract.

The ATTD of ADF and NDF obtained in this experiment for conventional DDGS is in agreement with previous data (Stein and Shurson, 2009; Urriola et al., 2010). The observation that the ATTD of ADF and NDF was less in cold-fermented DDGS than in conventional DDGS is likely because of the reduced concentration of ADF and NDF in cold-fermented DDGS. The BPX technology that is used in the production of cold-fermented DDGS results in some of the fibers being fermented in the ethanol plant and it is likely that it is the most fermentable fiber that are fermented in the ethanol plant resulting in a lower ATTD of the remaining fiber in cold-fermented DDGS. Likewise, the reason for the lower ATTD of AEE in cold-fermented DDGS compared with conventional DDGS is likely that it is the fat in the solubles that are removed from low-oil DDGS, whereas the intrinsic fat in the grain remains in the DDGS. The ATTD of intrinsic fat in corn grain is less than in extracted fat (Kil et al., 2010), and with a greater proportion of the fat in cold-fermented being intrinsic fat compared with conventional DDGS, it is expected that the ATTD is reduced. Nevertheless, the ATTD of AEE of both sources of DDGS that were determined in this experiment is within the range of values previously reported for DDGS (Kim et al., 2013).

The reason the ATTD of GE was not reduced in cold-fermented DDGS compared with conventional DDGS despite the lower ATTD of AEE, ADF, and NDF is likely that the SID of AA was greater in cold-fermented DDGS and cold-fermented DDGS also contained more starch. However, because the GE was greater in conventional DDGS than in cold-fermented DDGS, the DE and ME were also greater despite the lack of a difference in the ATTD of GE.

Conclusion

Values for SID of most AA were greater in cold-fermented DDGS compared with conventional DDGS evaluated in this experiment. However, the ATTD of NDF, ADF, and AEE was less in cold-fermented DDGS than in conventional DDGS when fed to growing pigs. Although the ATTD of GE was not different between the two sources of DDGS, the greater GE in conventional DDGS resulted in DE and ME also being greater in conventional DDGS than in cold-fermented DDGS.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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