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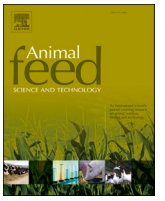

# Standardized ileal digestibility of amino acids and metabolizable energy in three sources of high-protein corn distillers dried grains fed to weanling pigs

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ABSTRACT

Two experiments were conducted to test the null hypothesis that there are no differences in the standardized ileal digestibility (SID) of amino acids (AA) and metabolizable energy (ME) among three sources of high-protein distillers dried grains (HP-DDG) fed to weanling pigs. The three sources included an experimental HP-DDG product and two commercial HP-DDG products (i.e., HP-DDG 40 and HP-DDG 50) that were fed to weanling pigs. In experiment 1, eight barrows (initial body weight: 11.09 kg) were allotted to one of four diets using a replicated 4 × 4 Latin square design with four diets and four periods of seven days in each square. A nitrogen-free diet and three diets containing each source of HP-DDG as the sole source of AA were prepared. Results indicated that values for the SID of Lys in the experimental HP-DDG; HP-DDG 50, and HP-DDG 40 were 0.524, 0.638, and 0.642, respectively. The SID of most AA in the experimental HP-DDG was less ($P < 0.05$) than in the other sources of HP-DDG, but no differences were observed between HP-DDG 40 and HP-DDG 50. In experiment 2, thirty-two weanling pigs (initial body weight: 18.0 kg) were randomly allotted to one of four diets. A corn diet was formulated to contain corn as the sole source of energy and three additional diets contained corn and each source of HP-DDG. Pigs were housed individually in metabolism crates and feces and urine were collected separately for four days after five days of adaptation. Digestible energy (DE) and ME in the three sources of HP-DDG were calculated by difference. Results from experiment 2 indicated that the apparent total tract digestibility (ATTD) of gross energy (GE) in the corn diet was greater ($P < 0.05$) than in the diets containing the experimental HP-DDG or HP-DDG 40, and the ATTD of GE in the diets containing the experimental HP-DDG or HP-DDG 50 was greater ($P < 0.05$) than in the diet containing HP-DDG 40. On a dry matter basis, ME in the experimental HP-DDG, HP-DDG 40, and HP-DDG 50 were 17.05, 16.72, and 18.23 MJ/kg, respectively, with the ME of HP-DDG 50 being greater ($P < 0.05$) than in HP-DDG 40, but not different from the ME in the experimental HP-DDG. In conclusion, the SID of most AA in HP-DDG 40 and HP-DDG 50 was greater than in

*Abbreviations:* AA, amino acids; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; CP, crude protein; DE, digestible energy; DM, dry matter; GE, gross energy; HP-DDG, high-protein distillers dried grains; ME, metabolizable energy; SBM, soybean meal; SEM, standard error of means; SID, standardized ileal digestibility.

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the experimental HP-DDG. The DE and ME in HP-DDG 50 was greater than in HP-DDG 40, but DE and ME in HP-DDG 40 were not different from DE and ME in the experimental HP-DDG.

## 1. Introduction

Soybean meal (SBM) is the main protein source in swine nutrition, but SBM cannot provide all amino acids (AA) in diets for weanling pigs due to the presence of certain anti-nutritional factors, which have negative impacts on growth performance of weanling pigs (Goerke et al., 2012; Rojas and Stein, 2013). Alternative protein sources that may be used instead of SBM include animal proteins, but short supply and high costs have made it difficult to base feeding programs on animal protein sources.

High-protein distillers dried grains (HP-DDG), which is a co-product from the fuel ethanol or the wet milling industries, may be used in diets fed to pigs (Almeida et al., 2011; Rojas et al., 2013), but growth performance of weanling pigs fed diets containing HP-DDG is often less than that of pigs fed diets without HP-DDG (Yang et al., 2018). Most sources of HP-DDG contain around 40% crude protein (CP) and more AA than distillers dried grains with solubles (Widmer et al., 2007; Espinosa and Stein, 2018; Yang et al., 2018), but newer sources of HP-DDG contain up to 50% CP. However, it is not known if the technologies that are used to increase the concentration of CP in HP-DDG from 40% to 50% impact nutrient and energy digestibility. Therefore, the objective of this experiment was to test the null hypothesis that there are no differences in the standardized ileal digestibility (SID) of AA, the apparent total tract digestibility (ATTD) of gross energy (GE), and digestible energy (DE) and metabolizable energy (ME) between HP-DDG that contains 40% CP and HP-DDG that contains 50% CP when fed to weanling pigs.

## 2. Materials and methods

The protocols for two experiments were submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used.

Three sources of HP-DDG were used and included an experimental HP-DDG product containing approximately 50% CP and two commercial HP-DDG products containing 40 or 50% CP (Table 1). The experimental HP-DDG product was produced using Fluid Equip Technology (Green Plains Energy, Omaha, NE, USA). This technology involves separation via centrifugation of the whole stillage that

**Table 1**
Analyzed nutrients and energy in high-protein distillers dried grains (HP-DDG) and corn, as-fed basis.

| Item, g/kg | Experimental HP-DDG | HP-DDG 40 | HP-DDG 50 | Corn |
|---|---|---|---|---|
| Dry matter | 946.1 | 874.5 | 937.6 | 867.0 |
| Gross energy, MJ/kg | 21.1 | 20.1 | 22.1 | 16.1 |
| Ash | 27.4 | 18.0 | 14.4 | 10.4 |
| Acid-hydrolyzed ether extract | 31.4 | 84.6 | 99.0 | 38.5 |
| Total dietary fiber | 299 | 376 | 320 | 98 |
| Soluble dietary fiber | 3 | 22 | 28 | 5 |
| Insoluble dietary fiber | 296 | 354 | 292 | 93 |
| Crude protein | 501.5 | 390.5 | 517.9 | - |
| Lys to crude protein | 34.1 | 34.3 | 34.6 | - |
| Indispensable amino acids | | | | - |
| Arg | 22.2 | 16.5 | 23.6 | - |
| His | 13.8 | 10.6 | 14.8 | - |
| Ile | 20.8 | 15.9 | 20.6 | - |
| Leu | 59.5 | 50.6 | 64.5 | - |
| Lys | 17.1 | 13.2 | 17.6 | - |
| Met | 11.8 | 8.5 | 12.8 | - |
| Phe | 26.2 | 21.4 | 27.4 | - |
| Thr | 20.2 | 15.3 | 19.9 | - |
| Trp | 4.0 | 2.8 | 4.2 | - |
| Val | 26.7 | 19.6 | 26.4 | - |
| Total indispensable amino acids | 222.3 | 174.4 | 231.8 | - |
| Dispensable amino acids | | | | - |
| Ala | 36.4 | 28.6 | 38.2 | - |
| Asp | 36.1 | 26.8 | 34.7 | - |
| Cys | 9.9 | 7.9 | 10.8 | - |
| Glu | 82.6 | 66.4 | 89.4 | - |
| Gly | 20.8 | 14.6 | 20.5 | - |
| Pro | 40.0 | 33.6 | 42.8 | - |
| Ser | 24.4 | 17.9 | 24.1 | - |
| Tyr | 20.3 | 15.5 | 21.4 | - |
| Total dispensable amino acids | 270.5 | 211.3 | 281.9 | - |
| Total amino acids | 495.7 | 387.8 | 515.9 | - |

is produced after fermentation and distillation and results in production of a protein cake and thin stillage. Suspended solids that were fermented with yeast were then combined with the protein cake and dried to produce the experimental HP-DDG product.

The two commercial HP-DDG products were procured from the Andersons Inc. (Maumee, OH, USA). The two products are sold under the brand names ANDVantage 40 and ANDVantage 50 and contain 40 and 50% CP, respectively, and both were produced using technology from ICM Inc. (Colwich, KS, USA). These two products were produced using a front-end separation technology, which involves an initial removal of the corn pericarp and additional fiber is removed after liquefaction and prior to fermentation using the FST Fiber Separation Technology™. The remaining material is fermented, distilled, and centrifuged to separate corn protein from thin stillage. Thin stillage is then processed using the TS4 Thin Stillage Solids Separation System™ to remove suspended solids (predominantly spent yeast), which is added back to the corn protein and dried to produce the HP-DDG 40. By using the FOT Feed Optimization Technology™, residual fiber from the initial post-fermentation protein cake is removed, which increases protein concentration and results in production of HP-DDG 50.

### 2.1. Diets and feeding

In experiment 1, four diets were formulated (Tables 2 and 3). Three diets contained each HP-DDG source as the sole source of AA and a nitrogen-free diet was used to determine the basal endogenous losses of AA. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements of weanling pigs (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

In experiment 2, four diets were formulated (Table 4). The corn diet contained corn as the sole source of energy. Three additional diets were formulated to include corn and each source of HP-DDG. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements of weanling pigs (NRC, 2012).

In both experiments, all diets were fed in meal form. Pigs were limit fed at three times the energy requirement for maintenance (i.e., 824 kJ/kg body weight$^{0.60}$; NRC, 2012). Daily allowance of feed was provided at 0700 h each day in experiment 1, whereas the daily allowance of feed was provided each day in two equal meals at 0800 and 1700 h in experiment 2. Throughout both experiments, pigs had free access to water.

### 2.2. Animal and housing

In experiment 1, eight barrows (initial body weight: 11.09 ± 0.46 kg) had a T-cannula installed in the distal ileum (Stein et al., 1998). Pigs were allotted to one of the four diets using a replicated 4 × 4 Latin square design with four diets and four periods of seven days in each square (Kim and Stein, 2009). Therefore, there were eight replicate pigs per diet. On the completion of one experimental period, animals were deprived of feed overnight. The following morning, a new experimental diet was offered. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors and a feeder and a nipple drinker were installed in each pen.

In experiment 2, thirty-two weanling pigs (initial body weight: 18.02 ± 1.21 kg) were randomly allotted to one of the four diets using a completely randomized design. Therefore, there were eight replicate pigs per diet. Pigs were placed in individual metabolism crates that were equipped with a self-feeder, a nipple waterer, and slatted floors to allow for the total, but separate, collection of urine

**Table 2**
Ingredient composition of experimental diets, as-fed basis (experiment 1).

| Item, g/kg | Experimental high-protein distillers dried grains (HP-DDG) | HP-DDG 40 | HP-DDG 50 | N-free |
|---|---|---|---|---|
| Cornstarch | 606.0 | 517.0 | 606.0 | 676.5 |
| Experimental HP-DDG | 360.0 | - | - | - |
| HP-DDG 40 | - | 450.0 | - | - |
| HP-DDG 50 | - | - | 360.0 | - |
| Sucrose | - | - | - | 200.0 |
| Soybean oil | - | - | - | 40.0 |
| Solka floc[a] | - | - | - | 40.0 |
| Dicalcium phosphate | 10.0 | 7.0 | 10.0 | 21.0 |
| Ground limestone | 11.0 | 13.0 | 11.0 | 4.5 |
| Sodium chloride | 4.0 | 4.0 | 4.0 | 4.0 |
| Potassium carbonate | - | - | - | 4.0 |
| Magnesium oxide | - | - | - | 1.0 |
| Vitamin-mineral premix[b] | 5.0 | 5.0 | 5.0 | 5.0 |
| Chromic oxide | 4.0 | 4.0 | 4.0 | 4.0 |

[a]Fiber Sales and Development Corp., Urbana, OH, USA.
[b]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 of experimental diets, as-fed basis (experiment 1).

| Item, g/kg | Experimental high-protein distillers dried grains (HP-DDG) | HP-DDG 40 | HP-DDG 50 | Nitrogen-free |
|---|---|---|---|---|
| Dry matter | 917.1 | 902.0 | 906.1 | 918.7 |
| Crude protein | 182.8 | 180.7 | 186.4 | 1.0 |
| Indispensable amino acids | | | | |
| Arg | 8.0 | 7.4 | 8.5 | 0.1 |
| His | 5.0 | 4.8 | 5.3 | - |
| Ile | 7.5 | 7.2 | 7.4 | 0.2 |
| Leu | 21.4 | 22.8 | 23.2 | 0.2 |
| Lys | 6.2 | 5.9 | 6.3 | 0.1 |
| Met | 4.2 | 3.8 | 4.6 | - |
| Phe | 9.4 | 9.6 | 9.9 | 0.1 |
| Thr | 7.3 | 6.9 | 7.2 | 0.1 |
| Trp | 1.4 | 1.3 | 1.5 | 0.2 |
| Val | 9.6 | 8.8 | 9.5 | 0.1 |
| Total indispensable amino acids | 80.0 | 78.5 | 83.4 | 0.9 |
| Dispensable amino acids | | | | |
| Ala | 13.1 | 12.9 | 13.8 | 0.1 |
| Asp | 13.0 | 12.1 | 12.5 | 0.2 |
| Cys | 3.6 | 3.6 | 3.9 | - |
| Glu | 29.7 | 29.9 | 32.2 | 0.2 |
| Gly | 7.5 | 6.6 | 7.4 | 0.1 |
| Pro | 14.4 | 15.1 | 15.4 | 0.2 |
| Ser | 8.8 | 8.1 | 8.7 | 0.1 |
| Tyr | 7.3 | 7.0 | 7.7 | 0.1 |
| Total dispensable amino acids | 97.4 | 95.1 | 101.5 | 1.0 |
| Total amino acids | 178.5 | 174.5 | 185.7 | 3.3 |

**Table 4**
Ingredient composition and analyzed nutrient composition of experimental diets, as-fed basis (experiment 2).

| Item, g/kg | Corn | Experimental high-protein distillers dried grains (HP-DDG) | HP-DDG 40 | HP-DDG 50 |
|---|---|---|---|---|
| Ingredient | | | | |
| Ground corn | 968 | 750 | 671 | 770 |
| Experimental HP-DDG | - | 220 | - | - |
| HP-DDG 40 | - | - | 300 | - |
| HP-DDG 50 | - | - | - | 200 |
| Dicalcium phosphate | 16 | 10 | 8 | 10 |
| Ground limestone | 7 | 11 | 12 | 11 |
| Sodium chloride | 4 | 4 | 4 | 4 |
| Vitamin-mineral premix[a] | 5 | 5 | 5 | 5 |
| Analyzed nutrient composition | | | | |
| Dry matter | 866.0 | 886.4 | 873.8 | 881.9 |
| Gross energy, MJ/kg | 13.7 | 13.9 | 13.9 | 13.9 |
| Ash | 34.6 | 34.5 | 33.6 | 33.3 |
| Acid-hydrolyzed ether extract | 32.6 | 40.2 | 47.7 | 47.2 |
| Total dietary fiber | 115 | 174 | 198 | 181 |
| Soluble dietary fiber | 16 | 25 | 16 | 40 |
| Insoluble dietary fiber | 99 | 149 | 182 | 141 |

[a]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.

and fecal materials.

### *2.3. Sample collection*

In experiment 1, the initial five days of each period were considered an adaptation period to the diets, and ileal digesta were collected on days 6 and 7 for nine hours per day. For ileal digesta collection, cannulas were opened and a 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 min and replaced with a new one. All samples were stored at −20 °C as soon as collected. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized, finely ground using a coffee grinder, and analyzed.

In experiment 2, feed provisions were recorded daily, and diets were fed for 12 days. The initial five days were considered the adaptation period to the diet, and urine and fecal materials were collected from the feed provided during the following four days using the marker to marker procedure (Adeola, 2001). Urine was collected in urine buckets over 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, urine samples were thawed and mixed within animal and diet, and a sub-sample was lyophilized before analysis (Kim et al., 2009). Fecal samples were thawed, and then dried in a 50 °C forced air drying oven prior to grinding, mixing, sub-sampling, and analysis.

### 2.4. Chemical analysis

Ingredient, diet, ileal digesta, and fecal samples were analyzed for dry matter (DM; method 930.15; AOAC Int.., 2019) and diet, ingredient, fecal, and urine samples were analyzed for GE using an isoperibol bomb calorimeter (Model 6400; Parr Instruments, Moline, IL, USA). Ingredient and diet samples were also analyzed for ash (method 942.05; AOAC Int.., 2019), and acid-hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA; method 2003.06; AOAC Int.., 2019). Diet and ingredient samples were analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int.., 2019) using AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Crude protein in feed ingredient, diet, and ileal digesta samples was calculated as nitrogen × 6.25 and nitrogen was measured by the combustion procedure (method 990.03; AOAC Int.., 2019) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA). Ingredient, diet, and ileal digesta samples were also analyzed for AA [method 982.30 E(a,*b*,c); AOAC Int.., 2019] using a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA). Diets and ileal digesta samples were also analyzed for chromium (method 990.08; AOAC Int.., 2019) after sample preparation with nitric acid-perchloric acid [method 968.08 D(b); AOAC Int.., 2019].

### 2.5. Calculations

In experiment 1, the basal endogenous losses of AA were calculated using the analyzed AA and chromium concentrations in ileal digesta samples from pigs fed the nitrogen-free diet (Stein et al., 2007). Apparent ileal digestibility (AID) was calculated using analyzed AA and chromium concentrations in diets and ileal digesta samples, and SID values were calculated by correcting AID values for basal endogenous losses of AA (Stein et al., 2007).

In experiment 2, the ATTD of GE and DM was calculated for each diet, and DE and ME in each diet were calculated as well (NRC, 2012). The DE and ME in the corn diet were divided by the inclusion rate of corn in the diet (i.e., 968 g/kg) to calculate the DE and ME

**Table 5**
Apparent ileal digestibility of dry matter, crude protein, and amino acids (AA) in three sources of high-protein distillers dried grains (HP-DDG; experiment 1).[a]

| Item | Experimental HP-DDG | HP-DDG 40 | HP-DDG 50 | SEM | P-value |
|---|---|---|---|---|---|
| Dry matter | 0.726 $^{y}$ | 0.680$^{z}$ | 0.752$^{x}$ | 0.006 | < 0.001 |
| Crude protein | 0.586 $^{y}$ | 0.656$^{x}$ | 0.690$^{x}$ | 0.023 | 0.001 |
| Indispensable AA | | | | | |
| Arg | 0.719 $^{y}$ | 0.766$^{xy}$ | 0.799$^{x}$ | 0.021 | 0.009 |
| His | 0.664 $^{y}$ | 0.720$^{xy}$ | 0.759$^{x}$ | 0.016 | 0.002 |
| Ile | 0.605 $^{y}$ | 0.704$^{x}$ | 0.706$^{x}$ | 0.013 | < 0.001 |
| Leu | 0.748 $^{y}$ | 0.832$^{x}$ | 0.835$^{x}$ | 0.011 | < 0.001 |
| Lys | 0.435 $^{y}$ | 0.552$^{x}$ | 0.552$^{x}$ | 0.023 | 0.003 |
| Met | 0.766 $^{y}$ | 0.823$^{x}$ | 0.839$^{x}$ | 0.011 | < 0.001 |
| Phe | 0.691 $^{y}$ | 0.777$^{x}$ | 0.785$^{x}$ | 0.012 | < 0.001 |
| Thr | 0.563 $^{y}$ | 0.641$^{x}$ | 0.652$^{x}$ | 0.017 | 0.003 |
| Trp | 0.450 $^{y}$ | 0.569$^{x}$ | 0.619$^{x}$ | 0.032 | 0.003 |
| Val | 0.601 $^{y}$ | 0.683$^{x}$ | 0.699$^{x}$ | 0.013 | < 0.001 |
| Total | 0.657 $^{y}$ | 0.741$^{x}$ | 0.753$^{x}$ | 0.013 | < 0.001 |
| Dispensable AA | | | | | |
| Ala | 0.667 $^{y}$ | 0.743$^{x}$ | 0.761$^{x}$ | 0.014 | < 0.001 |
| Asp | 0.575 $^{y}$ | 0.682$^{x}$ | 0.654$^{x}$ | 0.015 | < 0.001 |
| Cys | 0.610 $^{y}$ | 0.652$^{xy}$ | 0.699$^{x}$ | 0.019 | 0.012 |
| Glu | 0.717 $^{y}$ | 0.773$^{xy}$ | 0.800$^{x}$ | 0.018 | 0.012 |
| Gly | 0.441$^{xy}$ | 0.402 $^{y}$ | 0.511$^{x}$ | 0.062 | 0.045 |
| Pro | 0.606 | 0.571 | 0.663 | 0.111 | 0.302 |
| Ser | 0.704 $^{y}$ | 0.764$^{x}$ | 0.772$^{x}$ | 0.012 | 0.002 |
| Tyr | 0.754 $^{y}$ | 0.810$^{x}$ | 0.825$^{x}$ | 0.011 | 0.001 |
| Total | 0.651 $^{y}$ | 0.697$^{xy}$ | 0.730$^{x}$ | 0.029 | 0.008 |
| Total AA | 0.649 $^{y}$ | 0.712$^{x}$ | 0.736$^{x}$ | 0.019 | 0.001 |

SEM = standard error of the means.
$^{x\text{-}z}$ Within a row, means without a common superscript letter differ (P < 0.05).
[a] Each least squares mean represents 8 observations.

in corn. The contributions of DE and ME from corn to DE and ME in the three test diets containing corn and the three sources of HP-DDG were subtracted from the DE and ME of each diet, and the DE and ME in each HP-DDG were calculated by difference (Adeola, 2001).

### 2.6. Statistical analyses

Homogeneity of the variances and normality were confirmed and data were analyzed using the PROC MIXED in SAS (2018). Outliers were identified using Internally Studentized Residuals (Tukey, 1977). In experiment 1, the statistical model included diet as the fixed effect and square, pig within square, and period within square as the random effects. In experiment 2, the statistical model included diet or ingredient as the fixed effect. Pig was the experimental unit in both experiments. Least squares means were calculated, and means were separated using the PDIFF option with Tukey's adjustment if the model was significant. Results were considered significant at $P \leq 0.05$.

## 3. Results

### 3.1. Experiment 1 (SID of AA)

Pigs remained healthy during the experiment and minimal feed refusals were observed (average feed remaining in feeders ≤ 15% of total feed provided). The AID of DM was the greatest ($P < 0.05$) in the diet containing HP-DDG 50, followed by diets containing the experimental HP-DDG and HP-DDG 40 (Table 5). The AID of CP in HP-DDG 40 and HP-DDG 50 was greater ($P < 0.05$) than in the experimental HP-DDG. The AID of all AA except Gly and Pro in HP-DDG 50 was greater ($P < 0.05$) than in the experimental HP-DDG. The AID of Gly was greater in HP-DDG 50 ($P < 0.05$) compared with HP-DDG 40, but for all other AA, no difference between HP-DDG 50 and HP-DDG 40 was observed.

The SID of CP in the experimental HP-DDG was less ($P < 0.05$) than in HP-DDG 40 and HP-DDG 50 (Table 6) and the SID of all AA except Gly and Pro in the experimental HP-DDG was less ($P < 0.05$) than in HP-DDG 50. The SID of all indispensable AA except Arg and His and the SID of Ala, Asp, Ser, and Tyr were also greater ($P < 0.05$) in HP-DDG 40 compared with the experimental HP-DDG. No difference in SID of AA was observed between HP-DDG 40 and HP-DDG 50.

**Table 6**
Standardized ileal digestibility of crude protein and amino acids (AA) in three sources of high-protein distillers dried grains (HP-DDG; experiment 1).[a, b]

| Item | Experimental HP-DDG | HP-DDG 40 | HP-DDG 50 | SEM | P-value |
|---|---|---|---|---|---|
| Crude protein | 0.695 $^{y}$ | 0.765$^{x}$ | 0.796$^{x}$ | 0.023 | 0.002 |
| Indispensable AA | | | | | |
| Arg | 0.805 $^{y}$ | 0.857$^{xy}$ | 0.879$^{x}$ | 0.021 | 0.013 |
| His | 0.709 $^{y}$ | 0.766$^{xy}$ | 0.801$^{x}$ | 0.016 | 0.002 |
| Ile | 0.665 $^{y}$ | 0.766$^{x}$ | 0.766$^{x}$ | 0.013 | < 0.001 |
| Leu | 0.780 $^{y}$ | 0.862$^{x}$ | 0.865$^{x}$ | 0.011 | < 0.001 |
| Lys | 0.524 $^{y}$ | 0.642$^{x}$ | 0.638$^{x}$ | 0.023 | 0.003 |
| Met | 0.792 $^{y}$ | 0.851$^{x}$ | 0.862$^{x}$ | 0.011 | < 0.001 |
| Phe | 0.736 $^{y}$ | 0.820$^{x}$ | 0.827$^{x}$ | 0.012 | < 0.001 |
| Thr | 0.659 $^{y}$ | 0.742$^{x}$ | 0.749$^{x}$ | 0.017 | 0.002 |
| Trp | 0.589 $^{y}$ | 0.726$^{x}$ | 0.751$^{x}$ | 0.032 | 0.003 |
| Val | 0.664 $^{y}$ | 0.751$^{x}$ | 0.763$^{x}$ | 0.013 | < 0.001 |
| Total | 0.715 $^{y}$ | 0.799$^{x}$ | 0.808$^{x}$ | 0.013 | < 0.001 |
| Dispensable AA | | | | | |
| Ala | 0.728 $^{y}$ | 0.803$^{x}$ | 0.817$^{x}$ | 0.014 | < 0.001 |
| Asp | 0.641 $^{y}$ | 0.752$^{x}$ | 0.722$^{x}$ | 0.015 | < 0.001 |
| Cys | 0.677 $^{y}$ | 0.718$^{xy}$ | 0.760$^{x}$ | 0.019 | 0.019 |
| Glu | 0.756 $^{y}$ | 0.812$^{xy}$ | 0.835$^{x}$ | 0.018 | 0.015 |
| Gly | 0.689 | 0.680 | 0.760 | 0.062 | 0.124 |
| Pro | 0.979 | 0.921 | 1.008 | 0.111 | 0.331 |
| Ser | 0.765 $^{y}$ | 0.829$^{x}$ | 0.833$^{x}$ | 0.012 | 0.001 |
| Tyr | 0.801 $^{y}$ | 0.859$^{x}$ | 0.870$^{x}$ | 0.011 | 0.001 |
| Total | 0.766 $^{y}$ | 0.813$^{xy}$ | 0.839$^{x}$ | 0.029 | 0.012 |
| Total AA | 0.740 $^{y}$ | 0.803$^{x}$ | 0.822$^{x}$ | 0.019 | 0.001 |

SEM = standard error of the means.
$^{x\text{-}y}$ Within a row, means without a common superscript letter differ ($P < 0.05$).
[a] Each least squares mean represents 8 observations.
[b] Standardized ileal digestibility was calculated by correcting the apparent ileal digestibility for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of dry matter intake) as crude protein, 21.73; Arg, 0.75; His, 0.25; Ile, 0.49; Leu, 0.76; Lys, 0.60; Met, 0.12; Phe, 0.46; Thr, 0.77; Trp, 0.22; Val, 0.66; Ala, 0.86; Asp, 0.94; Cys, 0.26; Glu, 1.27; Gly, 2.03; Pro, 5.86; Ser, 0.58; and Tyr, 0.38.

### 3.2. Experiment 2 (ATTD of GE and DE and ME)

One pig fed the diet containing the experimental HP-DDG was removed from the experiment due to a leg injury. During statistical analysis, 1 pig fed the diet containing HP-DDG 40 was identified as an outlier and this pig was removed from data analysis. All other data were included in the analysis.

Feed intake was not different among diets, but GE intake of pigs fed the diet containing HP-DDG 40 was greater ($P < 0.05$) compared with that of pigs fed the corn diet or the diet containing the experimental HP-DDG (Table 7). The weight of dry feces and fecal GE excretion were greater ($P < 0.05$) by pigs fed the diet containing HP-DDG 40 compared with pigs fed the other three diets. There was no difference in urine weight among pigs fed the four diets, but urine GE excretion was greater ($P < 0.05$) from pigs fed the diet containing HP-DDG 50 than from pigs fed the corn diet or the diet containing the experimental HP-DDG. The ATTD of DM in the corn diet was greater ($P < 0.05$) than in the other diets and the ATTD of DM in the diets containing the experimental HP-DDG or HP-DDG 50 was greater ($P < 0.05$) than in the diet containing HP-DDG 40. The ATTD of GE in the corn diet was greater ($P < 0.05$) than in the other diets, with the exception that no difference was observed in ATTD of GE between the diet containing HP-DDG 50 and the corn diet. The DE was greater ($P < 0.05$) in diets containing the experimental HP-DDG or HP-DDG 50 than in the corn diet and DE in the diet containing HP-DDG 50 was also greater ($P < 0.05$) than in the diet containing HP-DDG 40. The ME in the diet containing HP-DDG 50 was greater ($P < 0.05$) than in the corn diet and the diet containing HP-DDG 40, but there was no difference in the ME between diets containing the experimental HP-DDG and HP-DDG 50, and there was also no difference in the ME between the corn diet and the diet containing HP-DDG 40.

On an as-fed basis, DE in HP-DDG 50 was greater ($P < 0.05$) than in the other ingredients (Table 8). The ME in the experimental HP-DDG and HP-DDG 50 was greater ($P < 0.05$) than in corn and HP-DDG 40, but ME did not differ between the experimental HP-DDG and HP-DDG 50 or between corn and HP-DDG 40. On a DM basis, DE in HP-DDG 50 was greater ($P < 0.05$) than in the other ingredients, but ME in HP-DDG 50 was not different from the ME in the experimental HP-DDG. The DE and ME on a DM basis were not different between the experimental HP-DDG and HP-DDG 40, but DE in corn (DM basis) was less than in the three sources of HP-DDG and ME in corn was less than in HP-DDG 50. The DE:GE, ME:DE, and ME:GE in corn were greater ($P < 0.05$) than in the three sources of HP-DDG except for the DE:GE. The DE:GE was not different between corn and HP-DDG 50, but the DE:GE in HP-DDG 50 was greater ($P < 0.05$) than in HP-DDG 40. No differences in ME:DE or ME:GE among the three sources of HP-DDG were observed.

## 4. Discussion

The three sources of HP-DDG that were used in this experiment were developed using technologies that have been introduced to the dry-grind ethanol industry. The pre-fermentation separation of fiber is one of these new technologies, which reduces energy usage in production and increases the concentration of protein in the resulting co-product. The end-products also contain yeast protein, which may influence the nutritional value (Yang et al., 2018).

Concentrations of most AA and the SID of AA in the experimental HP-DDG were in agreement with data obtained for HP-DDG in a previous experiment (Acosta et al., 2021), but HP-DDG 50 contained more AA and had greater SID of AA and HP-DDG 40 contained less AA compared with the experimental HP-DDG. Concentrations of AA in HP-DDG 50 were not different from concentrations in high-protein distillers dried grains with solubles, but the SID of AA was less in HP-DDG 50 than in high-protein distillers dried grains with solubles (Cristobal et al., 2020), which may be because different production technologies were used to produce HP-DDG 50 compared with the product used by Cristobal et al. (2020). However, concentrations of AA and the SID of AA in HP-DDG 40 were in agreement with values for a different source of high-protein distillers dried grains with solubles (Son et al., 2019).

The Maillard reaction is a chemical reaction between the $NH_2$ group of an AA and a reducing sugar that occurs in the presence of heat and moisture. This reaction produces Amadori compounds and melanoidins, which are the main reasons for changes in color and organoleptic properties of heated feed ingredients. Because the Amadori compounds and melanoidins are not bioavailable for pigs, the AA and energy digestibility in heat-damaged feed ingredients is reduced (González-Vega et al., 2011; Kim et al., 2012; Oliveira et al., 2020). Among indispensable AA, Lys is usually most susceptible to the Maillard reaction due to the amino group in the side chain of Lys (i.e., the epsilon amino group) and it is possible that heat damage occurs during production of HP-DDG. In undamaged protein, it is expected that Thr has the lowest SID among indispensable AA because of the high concentration of Thr in endogenous protein. Although SID values are corrected for the basal endogenous losses, they are not corrected for specific endogenous losses of AA. As an example, dietary fiber increases the specific endogenous loss of Thr, and pigs fed high-fiber diets, therefore, have an increased Thr requirement (Mathai et al., 2016; Wellington et al., 2019). The observation that the SID of Lys in all three sources of HP-DDG was lower than the SID of all other indispensable AA including Thr, therefore, indicates that all three sources of HP-DDG likely were heat damaged during fermentation or drying as has been demonstrated for other sources of distillers co-products (Kim et al., 2012).

The observation that the SID of most indispensable AA in HP-DDG 40 and HP-DDG 50 were greater than in the experimental HP-DDG may be a result of the different production processes including drying procedures that were used. Addition of yeast during the final production step of the experimental HP-DDG may also have reduced SID of AA because yeast protein sometimes has low SID of AA. It is also likely that the lower acid-hydrolyzed ether extract in the experimental HP-DDG than in the other two sources contributed to the reduced SID of AA because acid-hydrolyzed ether extract slows gastric emptying and passage rate during the intestinal system, which gives more time for proteases to digest the protein and for transporters to absorb AA (Cervantes-Pahm and Stein, 2008).

The DE and ME in the experimental HP-DDG, HP-DDG 40, and HP-DDG 50 were greater than in HP-DDG used in a previous experiment (Acosta et al., 2021). However, DE and ME in the experimental HP-DDG were less than DE and ME reported for corn gluten meal, but greater than values reported for corn, hominy feed, distillers dried grains with solubles, corn gluten feed, and corn germ meal

**Table 7**
Apparent total tract digestibility (ATTD) of gross energy (GE) and digestible energy and metabolizable energy in experimental diets (experiment 2).[a]

| | Diet | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| Item | Corn | Experimental HP-DDG[b] | HP-DDG 40 | HP-DDG 50 | | |
| Intake | | | | | | |
| Feed, kg/day | 0.79 | 0.73 | 0.87 | 0.81 | 0.04 | 0.078 |
| GE, MJ/day | 12.32 $^{y}$ | 12.23 $^{y}$ | 14.80$^{x}$ | 13.77$^{xy}$ | 0.59 | 0.014 |
| Fecal excretion | | | | | | |
| Dry feces output, kg/day | 0.07 $^{y}$ | 0.08 $^{y}$ | 0.12$^{x}$ | 0.09 $^{y}$ | 0.01 | < 0.001 |
| GE, MJ/day | 1.46 $^{y}$ | 1.74 $^{y}$ | 2.47$^{x}$ | 1.85 $^{y}$ | 0.12 | < 0.001 |
| Urine excretion | | | | | | |
| Urine output, kg/day | 2.43 | 3.01 | 3.07 | 2.98 | 0.61 | 0.864 |
| GE, MJ/day | 0.24$^{z}$ | 0.33$^{yz}$ | 0.40$^{xy}$ | 0.43$^{x}$ | 0.02 | < 0.001 |
| ATTD of dry matter | 0.899$^{x}$ | 0.878 $^{y}$ | 0.853$^{z}$ | 0.882 $^{y}$ | 0.004 | < 0.001 |
| ATTD of GE | 0.882$^{x}$ | 0.858 $^{y}$ | 0.834$^{z}$ | 0.866$^{xy}$ | 0.005 | < 0.001 |
| Energy in diets, MJ/kg (as-is) | | | | | | |
| Digestible energy | 13.76$^{z}$ | 14.41$^{xy}$ | 14.17 $^{y}$ | 14.64$^{x}$ | 0.08 | < 0.001 |
| Metabolizable energy | 13.44$^{z}$ | 13.96$^{xy}$ | 13.70$^{yz}$ | 14.11$^{x}$ | 0.08 | < 0.001 |

SEM = standard error of the means.
$^{x\text{-}z}$Within a row, means without a common superscript letter differ (P < 0.05).
[a]Each least squares mean is the mean of 8 observations, except for the two diets containing of the experimental HP-DDG or HP-DDG 40 ($n$ = 7).
[b]HP-DDG = high-protein distillers dried grains.

**Table 8**
Digestible energy (DE) and metabolizable energy (ME) in corn and three sources of high-protein distillers dried grains (HP-DDG) (experiment 2).[a]

| Item | Corn | Experimental HP-DDG | HP-DDG 40 | HP-DDG 50 | SEM | P-value |
|---|---|---|---|---|---|---|
| As-is basis, MJ/kg | | | | | | |
| DE | 14.21$^{z}$ | 17.07$^{x}$ | 15.43 $^{y}$ | 18.50$^{w}$ | 0.28 | < 0.001 |
| ME | 13.88$^{x}$ | 16.13$^{w}$ | 14.63$^{x}$ | 17.09$^{w}$ | 0.29 | < 0.001 |
| Dry matter basis, MJ/kg | | | | | | |
| DE | 16.41 $^{y}$ | 18.04$^{x}$ | 17.65$^{x}$ | 19.73$^{w}$ | 0.31 | < 0.001 |
| ME | 16.03$^{x}$ | 17.05$^{wx}$ | 16.72$^{x}$ | 18.23$^{w}$ | 0.32 | < 0.001 |
| Digestibility and metabolizability | | | | | | |
| DE:GE | 0.885$^{w}$ | 0.811$^{xy}$ | 0.766 $^{y}$ | 0.837$^{wx}$ | 0.014 | < 0.001 |
| ME:DE | 0.977$^{w}$ | 0.945$^{x}$ | 0.947$^{x}$ | 0.924$^{x}$ | 0.007 | < 0.001 |
| ME:GE | 0.865$^{w}$ | 0.766$^{x}$ | 0.726$^{x}$ | 0.773$^{x}$ | 0.014 | < 0.001 |

GE = gross energy; SEM = standard error of the means.
$^{w\text{-}z}$ Within a row, means without a common superscript letter differ (P < 0.05).
[a] Each least squares mean is the mean of 8 observations, except for the two diets containing the experimental HP-DDG or HP-DDG 40 ($n$ = 7).

(Widmer et al., 2007; Rojas et al., 2013; Espinosa and Stein, 2018). The greater DE and ME in HP-DDG 50 compared with corn is likely a result of greater concentrations of GE, protein, and acid-hydrolyzed ether extract in HP-DDG 50 than in corn. In contrast, the concentration of acid-hydrolyzed ether extract was not different between HP-DDG 40 and HP-DDG 50, but protein was greater and total dietary fiber was less in HP-DDG 50 than in HP-DDG 40, which is the reason for the greater DE and ME in HP-DDG 50. The reason, there was no difference in ME (DM basis) between the experimental HP-DDG and HP-DDG 40, despite the greater concentration of acid-hydrolyzed ether extract in HP-DDG 40, is that the experimental HP-DDG contained less total dietary fiber and more protein than HP-DDG 40. The observation that the ratio between DE and GE in the three sources of HP-DDG was much less than in corn is a result of the increased GE in feces from pigs fed diets containing HP-DDG compared with pigs fed the corn diet, which is likely a result of the much greater concentration of dietary fiber in these ingredients. Because the majority of GE in urine is nitrogen, it is likely that the reason for the increased GE in urine from pigs fed HP-DDG instead of corn was the increased CP in the HP-DDG ingredients compared with corn. Thus, the differences in ME and in the ME:GE ratio among ingredients are largely a result of differences in the chemical composition of the ingredients although it is also possible that differences in production processes may have contributed to these differences. To our knowledge, this it is the first time the nutritional value of HP-DDG ingredients differing in acid-hydrolyzed ether extract, CP, and total dietary fiber have been compared and we also believe it is the first time an HP-DDG ingredient produced using the Fluid Equip technology has been compared with HP-DDG produced using the ICM technology. Because results of the experiments could largely be explained by differences in the nutritional composition of the ingredients, it is likely that the processing procedures per se may have had limited impact on the nutritional quality of the end-products.

## 5. Conclusion

Results from the experiments rejected the null hypothesis that there were no differences in standardized ileal digestibility of amino acids, in the apparent total tract digestibility of gross energy, or in digestible energy and metabolizable energy among the three sources

of high-protein distillers dried grains that were used. The standardized ileal digestibility of crude protein and most amino acids in the experimental high-protein distillers dried grains was less than in high-protein distillers dried grains 50 and high-protein distillers dried grains 40. The reduced standardized ileal digestibility of Lys in the three sources of high-protein distillers dried grains compared with the standardized ileal digestibility of Thr indicated that these ingredients may have been heat damaged. On a dry matter basis, the experimental high-protein distillers dried grains contained less digestible energy than high-protein distillers dried grains 50, but metabolizable energy was not different between these two ingredients. Likewise, digestible energy and metabolizable energy in the experimental high-protein distillers dried grains were not different from corn or high-protein distillers dried grains 40.

## Declaration of Competing Interest

The authors have no conflicts of interest.

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