Determination of in vitro dry matter, protein, and fiber digestibility and fermentability of novel corn coproducts for swine and ruminants

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ABSTRACT: New processes are being used in some dry-grind ethanol plants in the United States and Brazil to improve ethanol yield and efficiency of production while also providing nutritionally enhanced corn coproducts compared with conventional corn distillers dried grains with solubles (DDGS). The objectives of this study were to determine the chemical composition and in vitro digestibility of 5 conventional corn DDGS sources and 10 emerging novel corn coproducts for swine and ruminants, and compare coproducts produced using similar processes in the United States and Brazil. Chemical composition, on a dry matter (DM) basis, among the 15 coproducts ranged from 18.5% to 54.7% for crude protein (CP), 12.3% to 51.4% for neutral detergent fiber (NDF), 1.6% to 8.6% for acid detergent fiber, 4.7% to 12.3% for ether extract, and 1.6% to 8.6% for ash. For swine, in vitro hydrolysis of DM and CP were greater ($P < 0.01$) for the three U.S. corn DDGS sources compared with the two Brazilian DDGS sources, but in vitro fermentability of DM was comparable ($P > 0.05$) among all sources except one U.S. DDGS source that had less fermentable DM. High-protein and yeast dried distillers grains (Ultramax, UM; StillPro, SP) coproducts also had comparable ($P > 0.05$) DM fermentability for swine, but UM coproducts had greater ($P < 0.01$) DM and CP hydrolysis compared with SP. High-protein distillers dried grains (HP-DDG) from Brazil had greater ($P < 0.01$) DM and CP hydrolysis, but less ($P < 0.01$) DM fermentability for swine than HP-DDG produced in the United States, using the same process. For ruminants, total DM digestibility was greater ($P < 0.01$) in conventional DDGS sources from the United States compared with the two DDGS sources from Brazil. Total protein digestibility for ruminants was comparable and above 81% for all coproducts except for a DDGS source from Brazil, a HP-DDG source from the United States, and a UM sample. Interestingly, the corn fiber + solubles coproduct had not only relatively high digestibility of NDF (67.9%), DM (91.6%), and total CP (81.9%) for ruminants, but it also had relatively high total tract digestibility of DM (86.2%) and CP (69.9%) for swine. These results suggest that nutrient digestibility of conventional DDGS sources produced in the United States appear to be greater than corn Brazilian DDGS sources, but new process technologies being implemented in ethanol and coproduct production in both countries can enhance the nutritional value of corn coproducts for both swine and ruminants.

Key words: corn distillers dried grains with solubles, high-protein dried distillers grains, in vitro digestibility, in vitro fermentability, ruminants, swine

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INTRODUCTION

As the U.S. ethanol industry continues to evolve, new process technologies are continually being developed and tested with the goal of improving operational efficiency of dry-grind ethanol plants and increasing revenue by producing new higher value corn coproducts compared with conventional corn dried distillers grains with solubles (DDGS). During these technology development efforts, new corn coproducts are produced, and their nutrient content and digestibility must be evaluated to determine their nutritional value relative to other commercially available coproducts for various food producing animals. In vitro digestibility procedures for ruminants (Calsamiglia and Stern, 1993) and swine (Boisen and Fernández, 1997; Bindelle et al., 2007) have been developed and used extensively for assessing the relative nutritional value of various feed ingredients because they are less expensive and time consuming than in vivo determinations.

The evolution of diversified corn coproducts produced by dry-grind ethanol facilities began in 2005 with the implementation of technology to separate some of the corn oil from thin stillage to produce distillers corn oil, which is used as a feedstock in biodiesel production and as well as a supplemental energy source in poultry and swine diets (Kerr et al., 2016). As corn oil separation processes became widely implemented throughout the U.S. ethanol industry, it increased the variability in oil and nutrient content of reduced-oil DDGS (Kerr et al., 2013). In addition, the development of various front-end fractionation technologies to convert grain fiber into ethanol and increase the crude protein (CP) content of coproducts has been a major goal of many ethanol and coproduct production technology providers for many years. Early attempts to use front-end fractionation technologies resulted in high-protein distillers dried grains (HP-DDG) with much different nutrient profiles (Widmer et al., 2007; Kim et al., 2009; Jacela et al., 2010; Anderson et al., 2012; NRC, 2012) and greater CP content (~50% on a DM basis; NRC, 2012) than current technologies (U.S. Grain Council, 2018) being used to produce HP-DDG (Rho et al., 2017; Espinosa and Stein, 2018; Yang et al., 2019, 2020) which contain about 36–45% CP (DM basis). In addition, other new process technologies have been implemented to produce high-protein coproducts containing 45–55% CP and about 25–28% spent yeast, which is about 2.5 times greater than the estimated yeast content in conventional DDGS (Shurson, 2018). Although a few studies have been conducted to evaluate nutrient digestibility and feeding value of HP-DDG and other emerging coproducts for swine (Rho et al., 2017; Espinosa and Stein, 2018; Yang et al., 2019, 2020; Cristobal et al., 2020), limited studies have been conducted to determine ruminal degradation characteristics of DM and CP from feeding various HP-DDG coproducts for ruminants (Lee et al., 2016). The limited number of published ruminant studies is presumably because these new coproducts are more applicable and have potentially greater value in more energy and nutrient dense swine, poultry, and aquaculture diets than in ruminant diets.

The United States is the world’s largest producer of corn-based ethanol and coproducts, and exports about 11 million tonnes of DDGS annually (RFA, 2020). Brazil is the second largest producer of ethanol, and historically has used sugarcane as the primary feedstock. However, during certain times of the year, some of these old ethanol plants also use corn as a feedstock to produce ethanol and corn DDGS. Limited information has been published on nutrient content and digestibility of these traditional corn DDGS sources produced in Brazil for swine (Corassa et al., 2017) and ruminants (Geron et al., 2017). More recently, new corn-based ethanol plants have been constructed and are using fiber and oil separation technologies from U.S. providers to produce HP-DDG and distillers corn oil. Unlike animal nutritionists in the United States, nutritionists in Brazil are less familiar with the nutritional value of corn DDGS and HP-DDG produced in Brazil, and have relied on published data from studies conducted in the United States to guide decisions on feeding value and applications for all food animal species. No studies have been conducted to compare the nutrient content and digestibility of corn coproducts produced using similar processes between the United States and Brazil ethanol plants for swine and ruminants. Therefore, we hypothesized that corn coproducts produced using similar processes in the United States and Brazil would have different nutrient composition and in vitro digestibility for swine and ruminants. To test this hypothesis, the objectives of this study were to: 1) determine and compare the chemical composition of 5 conventional corn DDGS sources and 10 emerging novel corn coproducts, 2) determine in vitro dry matter (DM) hydrolysis and fermentability, along with CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) hydrolysis for swine, 3) determine in vitro DM and NDF digestibility, ruminal undegradable protein (RUP)
and ruminal degradable protein (RDP), intestinally absorbable dietary protein (IADP), and total digestible protein (TDP) for ruminants, and 4) to compare composition and digestibility differences among similar corn coproducts produced in the United States and Brazil.

MATERIALS AND METHODS

Sample Collection

A total of 15 different corn coproduct samples were collected from their respective sources in 2018 (Table 1). Conventional corn DDGS samples were obtained from three U.S. sources representing three different dry-grind engineering and operational designs and included POET (P-DDGS; Lake Crystal, MN), Absolute Energy, LLC (AE-DDGS; St. Ansgar, IA), and Corn Plus (CP-DDGS; Winnebago, MN). In addition, conventional corn DDGS samples were obtained from two sources of old ethanol plants in Brazil and included Libra (BRL-DDGS) and Pantanal (BRP-DDGS). Additional samples of two new corn coproducts (corn fiber + solubles, CF + S; Brazilian HP-DDG, B-HP) produced using fiber separation technology (ICM, Inc., Colwich, KS) in a new facility in Brazil (FS Bioenergia, Lucas do Rio Verde, Brazil) were collected and compared with four U.S. HP-DDG sources from Corn Plus (US-HP; Winnebago, MN) and ICM, Inc. (Colwich, KS; US-HPpellet, US-HPG1.5, US-HP49). Lastly, four samples of high-protein and yeast coproducts from United Wisconsin Grain Processors, LLC (Friesland, Table 1. Summary of corn coproduct samples evaluated

| Coproduct abbreviation | Company/Technology | Country of origin | Brand name/Coproduct type |
|------------------------|--------------------|-------------------|---------------------------|
| P-DDGS*                | POET, POET         | United States     | Conventional DDGS         |
| AE-DDGS†               | Absolute Energy, ICM| United States     | Conventional DDGS         |
| CP-DDGS‡               | Corn Plus, Delta T | United States     | Conventional DDGS         |
| BRL-DDGS'||             | Libra Plant/Flex   | Brazil             | Conventional DDGS         |
| BRP-DDGS$              | Pantanal Plant/Flex| Brazil             | Conventional DDGS         |
| BRCF+S¶               | FS Bioenergia/ICM FST| Brazil          | FS Ouro (Corn fiber + solubles) |
| BR-HP**                | FS Bioenergia/ICM FST| Brazil          | FS Essential (HP-DDG)     |
| US-HP††                | Corn Plus/ICM FST  | United States     | HP-DDG                    |
| US-HPpellet‡‡          | ICM                | United States     | Experimental pelleted HP-DDG |
| US-HPG1.5§§           | ICM                | United States     | Generation 1.5 HP-DDG     |
| US-HP49$$              | ICM                | United States     | Experimental HP-DDG ICM49 |
| SP¶¶                  | UWGP/FluidQuip    | United States     | StillPro/High-protein and yeast DDG |
| UM***                 | ICM                | United States     | Ultramax/High-protein and yeast DDG |
| UMHF¶¶¶               | ICM                | United States     | Experimental high fiber Ultramax |
| UMLF‡‡‡                | ICM                | United States     | Experimental low fiber Ultramax |

*P-DDGS = conventional corn dried distillers grains with solubles (DDGS) produced by POET (Lake Crystal, MN) using POET (Sioux Falls, SD) engineering and process technology.
†AE-DDGS = conventional corn DDGS produced by Absolute Energy (St. Ansgar, IA) using ICM, Inc. (Colwich, KS) engineering and process technology.
‡CP-DDGS = conventional DDGS produced by Corn Plus (Winnebago, MN) using Delta T engineering technology.
§BRL-DDGS = conventional corn DDGS produced by an old generation flex sugarcane/corn Libra ethanol plant in Brazil.
¶BRCF+S = dried corn fiber plus solubles produced using ICM, Inc. fiber separation technology (FST) by FS Bioenergia (Lucas do Rio Verde, Mato Grosso, Brazil) and marketed under the brand name FS Ouro in Brazil.
**BR-HP = high-protein corn distillers dried grains (HP-DDG) produced using ICM, Inc. FST by FS Bioenergia and marketed under the brand name FS Essential in Brazil.
††US-HP = HP-DDG produced using ICM, Inc. FST by Corn Plus (Winnebago, MN).
‡‡US-HPpellet = experimental pelleted HP-DDG produced by ICM, Inc.
§§US-HPGen1.5 = experimental HP-DDG produced using ICM, Inc. Generation 1.5 technology.
$$US-HP49 = experimental HP-DDG produced using ICM, Inc. technology.
¶¶SP = StillPro which is a brand name for a high-protein and yeast corn coproduct produced by United Wisconsin Grain Processors (UWGP; Friesland, WI) using Fluid Quip (Cedar Rapids, IA) Maximized Stillage Co-product technology.
***UM = Ultramax which is a brand name for a high-protein and yeast corn coproduct produced by ICM, Inc. (St. Joseph, MO) Generation 1.5 technology.
¶¶¶UMHF = experimental high fiber UM produced by ICM, Inc. (St. Joseph, MO) Generation 1.5 technology.
‡‡‡UMLF = experimental low fiber UM produced by ICM, Inc. (St. Joseph, MO) Generation 1.5 technology.
WI; StillPro, SP) and ICM, Inc. (Ultramax, UM; Ultramax with 48% CP, UM48, Ultramax high fiber, UMHF; Ultramax low fiber, UMLF) were collected for analysis.

**Chemical Analysis**

All coproduct samples were ground to pass through a 1 mm mesh screen and were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, Table 2). Chemical analyses were performed according to standard AOAC (2006) procedures using the following specific methods for DM (method 930.15), CP (method 990.03), ADF (method 973.18), NDF (method 2002.04), and ether extract (EE; method 920.39 (A)). CP content of DDGS was determined by Kjeldahl method using a Kjeltec 2300 Analyzer Unit (Foss Tectator AB, Höganäs, Sweden). NDF was determined with the ANKOM200 fiber analysis system (Ankom Technology, Macedon, NY) using procedures outlined by Van Soest et al. (1991). Sodium sulfite and α-amylase were used in NDF analysis.

**In Vitro Enzymatic Hydrolysis—Swine**

All 15 corn coproduct samples were ground to pass through a 1-mm mesh screen. The samples underwent a two-step hydrolysis using procedures developed by Boisen and Fernández (1997). About 2 g of each sample (15 total batches, with 2 replicates per batch) were weighed and placed into 500-mL conical flasks. Samples were dried to determine dried weight before hydrolysis. For pepsin hydrolysis, 100 mL of phosphate buffer (0.1 M 7:1 KH₂PO₄:Na₂HPO₄, pH 6.0) and 40 mL of 0.2 M HCl were added to each flask. The pH of each flask was adjusted to 2.0 by either adding 1 M HCl or 1 M NaOH. About 4 mL of 100mg/mL fresh porcine pepsin (P7000, 421 pepsin units/mg solids; Sigma-Aldrich Corp., St. Louis, MO) solution (dissolved in 0.2 M HCl) was added to each flask. Rubber stoppers were placed on the flasks, and replicates were incubated in a water bath at 39°C for 2 h and were gently shaken by hand for 5 s every 15 min. After the incubation period, 40 mL of 0.2 M phosphate buffer (1:1 KH₂PO₄:Na₂HPO₄, pH 6.8) and 20 mL NaOH (0.6M) were added to each flask with the pH being adjusted to 6.8 with 1 M HCl or 1 M NaOH. For pancreatin hydrolysis,
4 mL of 100 mg/mL fresh porcine pancreatic (P1750, 4 times the specifications of United States Pharmacopeia; Sigma-Aldrich Corp.) solution (dissolved in 0.2 M phosphate buffer) was added to each flask. Rubber stoppers were placed on the flasks and replicates were incubated in a water bath at 39°C for 4 h and were gently shaken by hand for 5 s every 15 min.

After enzymatic hydrolysis, the remaining residues were collected via filtration (ANKOM in situ 5 cm × 10 cm nylon concentrate bags, 50 µm porosity), washed with acetone (2 × 20 mL, 99.5%), ethanol (2 × 20 mL, 95%), and distilled water. The residues were then dried for 72 h at 55°C and weighed to determine in vitro DM digestibility. To obtain a sufficient amount of residue for subsequent fermentation, 10 runs of enzymatic hydrolysis were performed with 2 replicates per run (Table 3), and all dried residues within each treatment group were pooled for use in subsequent steps. The in vitro digestibility of CP (N × 6.25), NDF, ADF, and DM (IVDMDh) from small intestinal hydrolysis were calculated as follows:

In vitro digestibility (small intestine) of CP, % = [(CP of sample before hydrolysis – CP of residue)/CP of sample before hydrolysis] × 100

In vitro digestibility (small intestine) of NDF, % = [(NDF of sample before hydrolysis – NDF of residue)/NDF of sample before hydrolysis] × 100.

In vitro digestibility (small intestine) of ADF, % = [(ADF of sample before hydrolysis – ADF of residue)/ADF of sample before hydrolysis] × 100.

IVDMDh = [(dry weight of the sample before hydrolysis – dry weight of residues)/dry weight of the sample before hydrolysis] × 100.

### Table 3. Summary of the number of in vitro batches, replicates per batch, and number of runs of the modified 3-step in vitro fermentation procedure for corn coproducts and blank for swine

| Coproduct | 2-step enzymatic hydrolysis | Fermentation |
|-----------|----------------------------|-------------|
|           | Batch† | Replicates per batch¹ | Run† | Batch | Replicates per batch¹ | Run¹ |
| Coproduct | 15     | 2                    | 10   | 15    | 1                    | 4 |
| Blank     | 1      | 1                    | 4    | 1     | 1                    | 4 |

¹Batch represents the total number of coproducts/samples.

¹Replicates per batch represents the number of flasks per coproduct/sample for each run.

¹Run represents the number of times the entire experiment was performed in order to obtain enough residue for evaluation.

### In Vitro Fermentation—Swine

A cumulative gas production technique developed by Bindelle et al. (2007) was used to assess the rate and amount of in vitro fermentation of the hydrolyzed residues. The residues of the same sample from the enzymatic hydrolysis were pooled for the in vitro fermentation procedure. Blank flasks containing medium and inoculum without substrates were used as controls. Samples were analyzed in four runs with one replicate per batch in each run (Table 3). Approximately 0.2 g of each pooled hydrolyzed sample residue was weighed and placed into 125 mL serum bottles. Fecal inoculum was obtained from growing pigs from Cargill Animal Nutrition Innovation Center (Elk River, MN). The pigs were fed a standard commercial corn–soybean meal diet and were from the same genetic background. After feces were collected, samples were pooled and placed into an airtight sealed Ziploc bag. Sealed bags were kept at 39°C and delivered to the laboratory at the University of Minnesota (St. Paul, MN) within 1 h after collection. To prepare inoculum, feces were blended and diluted in an inoculation solution that contained distilled water (474 mL/L), trace mineral solution (0.12 mL/L containing 132 g/L of CaCl₂, 100 g/L of MnCl₂·4H₂O, 10 g/L of CoCl₂·6H₂O, and 80 g/L of FeCl₃·6H₂O), in vitro buffer solution (237 mL/L containing 4 g/L of NH₄HCO₃ and 35 g/L of NaHCO₃), micromineral solution (237 mL/L containing 5.7 g/L of Na₂HPO₄, 6.2 g/L of KH₂PO₄, 0.583 g/L of MgSO₄·7H₂O, and 2.22 g/L of NaCl), and resazurin (blue dye, 0.1% w/v solution, 1.22 mL/L). The fecal inoculum was filtered through four layers of cheese cloth under vacuum in order to achieve a final inoculum concentration of 0.05 g feces/mL of buffer. About 30 mL of inoculum were added to each serum bottle containing hydrolyzed residue. Reducing solution (containing 47.5 mL/L of distilled water, 2 mL/L of 1 M NaOH, and 335 mg/L of Na₂S₂O₃) and CO₂ was added to the flask until the solution was reduced, as indicated by a change in color of resazurin indicator from purple to colorless, to maintain anaerobiosis in the inoculation solution. Rubber stoppers sealed each flask and each flask was incubated in a water bath at 39°C. Gas production for each flask was measured every 5 min during a 72-h incubation period via ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY). After fermentation, residues were collected via filtration with ANKOM nylon bags.
washed twice with ethanol (95%) and acetone (99.5%), dried at 60°C for 72 h and then weighed. The IVDMD from large intestine fermentation (IVDMDₙ, %) was calculated as follows:

$$\text{IVDMDₙ} = \left( \frac{\text{dry weight of hydrolyzed residues} - \text{dry weight of residues after fermentation}}{\text{dry weight of hydrolyzed residues}} \right) \times 100$$

The IVDMD from total tract digestion (IVDMDₜ, %) was calculated as follows:

$$\text{IVDMDₜ} = \left[ 1 - \left( \frac{100 - \text{IVDMDₙ}}{\text{NDF}} \right) \times \left( \frac{1}{\text{IVDMDₙ}} \right) \right] \times 100.$$  

**Ruminal Fiber Degradation**

An ANKOM Daisy II Incubator was used to determine the in vitro fiber degradability of each corn coproduct. True degradability was determined by grinding samples to 1 mm, and 0.5 g of sample was weighed into acetone rinsed Ankom F57 filter bags. One replicate per sample was placed into one of four rotating fermentation jars within the Ankom incubator. In total, 19 bags including 3 empty bags for correction were placed in each fermentation jar. All 4 jars were operated per run and 3 consecutive runs were conducted resulting in a sample size of 12 samples per coproduct type.

Anaerobic buffer was prepared according to Goering and Van Soest (1970). Rumen fluid was collected from two cannulated lactating dairy cows. The ingredient composition of the diet (% of DM) included 20% corn silage, 9.25% alfalfa hay, 5.35% ground corn, 3.25% cottonseed, 3% corn gluten pellets, 11% milk cow protein, and 2.76% vitamin and mineral supplement. The calculated chemical composition of the diets (% DM) was 16.4% CP, 20.9% NDF, and 20.1% ADF. Rumen fluid was collected and transported to the laboratory in pre-warmed thermoses. After straining through four layers of cheesecloth, rumen contents from each cow were combined and homogenized under constant CO₂ gas. At inoculation, 1,600 mL of pre-warmed buffer, 400 mL of strained rumen fluid and filter bags were added to the fermentation jars. Jars were continuously rotated and maintained at 39.5 ± 0.5°C in the incubator. After 48 h of fermentation, bags were removed from the vessels and washed under cold tap water to cease microbial fermentation. Bags were subsequently exposed to neutral detergent solution for determination of NDF. The ANKOM²⁰⁰ fiber analysis system was used with procedures described by Van Soest et al. (1991). Sodium sulfite and α-amylase were used in the NDF analysis. In vitro total dry matter degradability (IVTDMD), neutral detergent fiber degradability (NDFD), degradable amylase-treated NDF (dNDF), and undegradable amylase-treated NDF (iNDF) were calculated as follows:

$$\% \text{ IVTDMD} = \frac{100 - (C - (A + D))}{(B + DM)} \times 100,$$

$$\% \text{ NDFD} = \left( 1 \left[ \frac{100 - \text{IVTDMD}}{\text{NDF}} \right] \right) \times 100,$$

$$\% \text{ dNDF} = \left[ \text{NDF} - 100\left\{ \frac{\text{g NDF residue at 48h}}{\text{g of DM of in vitro sample}} \right\} \right].$$

$$\% \text{ iNDF} = 100\left\{ \frac{\text{g NDF residue at 48h}}{\text{g of DM of in vitro sample}} \right\}.$$  

where A (W₁) was the bag tare weight, B (W₂) was the sample weight, C (W₃) was the final bag weight after in vitro and ND treatment, D (C₁) was the blank bag correction (final oven dried/original bag weight), and NDF was the NDF content of the original sample.

**Ruminal Protein Degradation**

Ruminal degradability of protein from corn coproduct samples was determined using an in situ technique. Approximately 0.5 g of 2 mm ground coproduct sample was added to pre-weighed ANKOM R510 Dacron polyester bags. Samples were incubated in the rumen for 2, 4, 8, 12, 16, 24, and 48 h after being transported in warmed (38°C) distilled water for 15 min. The incubation time was determined based on previous studies (Kim et al., 2015; Ranathunga et al., 2019), indicating that degradation of in vitro dry matter and organic matter increases up to 48 h of incubation, and extended incubation time did not influence degradability parameters. Each sample was evaluated in duplicate at each time point in separate cannulated lactating cows fed the same diet as previously described. Two blank bags were included at each time point to correct for microbial N contribution to the samples. A pair of bags was incubated only in distilled water 38°C for 15 min to estimate washout at 0 h for each sample. Following incubation, each bag was washed in cold tap water until water ran clear, while limiting mechanical agitation. Bags were dried in a 100°C oven for 24 h. CP (N × 6.25) was determined using Kjeldahl method (AOAC, 2006). The following model, adapted from Mathers and Miller...
In vitro digestibility of corn coproducts

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(1981), was used to calculate rumen degradability of dietary protein:

$$\text{RDP} = a + (1 - a - c) \frac{k_d}{k_p + k_d},$$

where RDP is the rumen degradability of dietary protein, \(a\) is the proportion of N disappearance at 0 h and is assumed soluble and 100% degraded. Undigested CP at 48 h is represented by \(c\) and is used to correct \(a\) by removing the assumption of complete degradability. A rate-constant for degradation \((k_d)\) was calculated by regressing the natural log of remaining N on rumen incubation time. The rate constant for passage of undegraded protein from the rumen \((k_p)\) was held at 0.6, as proposed by Orskov and McDonald (1970) from ad libitum access to soybean diets. Ruminal undegradable protein (RUP) was calculated as 100—RDP.

Ruminant Intestinal Protein Degradation

A three-step procedure described by Calsamiglia and Stern (1993) was used to determine the intestinal degradability of corn coproduct samples. Similar procedures were used as described to ruminally incubate the coproduct samples. Briefly, approximately 1.5 g of sample ground to 2 mm was weighed into nylon bags (5 × 10 cm, 50 μm pore size; Ankom R510, Ankom Technology, NY). Bags were prepared in triplicate and incubated alongside those used for the determination of ruminal degradable protein in two ruminally cannulated lactating Holstein cows consuming an alfalfa/corn silage mixed diet, such that six bags per sample were incubated. A soybean meal standard was included to determine efficacy of ruminal degradation. After 16 h of incubation, bags were washed in cold tap water and dried for 48 h in a 55°C oven. All dried residues from each treatment were composited and mortared to ensure homogeneity. A subsample was used to determine N content of the sample residue using the Kjeldahl method. About 15 mg of N from the sample residue was weighed into 50 mL polypropylene centrifuge tubes in duplicate. Incubated soybean meal, unincubated soybean meal, and two blank tubes were also included. Pre-warmed (38°C) HCl-pepsin solution was prepared and added to centrifuge tubes. Tubes were incubated for 1.25 h in a 38°C shaking water bath. After incubation, 0.5 mL of 1 N NaOH and 13.5 mL of phosphate-pancreatin buffer were added to the centrifuge and incubated for 24 h in the same water bath while swirling every 8 h. About 3 mL of a TCA solution was added to the tubes and samples were allowed to stand for at least 15 min. Samples were subsequently centrifuged at 10,000 × g for 15 min. The N content of 5 mL of supernatant was determined by the Kjeldahl method. Intestinal degradable protein (IDP) was calculated as TCA soluble N divided by amount of N in the initial sample. Intestinally absorbable dietary protein (IADP) was calculated as RUP × IDP. Total degradable dietary protein (TDP) was calculated as RDP + IADP.

Statistical Analysis

All swine hydrolysis data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC) with individual bottles considered the experimental unit. The model included treatment as the fixed effect and batches of samples as the random effect. The least square means of individual treatments were separated by Tukey adjustment. Ruminal protein, intestinal protein, and ruminal fiber degradation statistics were analyzed using GLM procedure of SAS 9.4. Differences between coproducts were determined by least square means and adjusted by Tukey–Kramer option. Fiber degradation was analyzed as a completely randomized block design with sample and fermentation jar as fixed effects and run as a random effect. Ruminal protein and intestinal protein degradation were analyzed as a completely randomized block design with sample as a fixed effect and batch as a random effect. Least squares means comparisons with values of \(P < 0.05\) were considered significant.

RESULTS AND DISCUSSION

Nutrient Composition

Only some nutrient composition values of coproducts evaluated in this study could be compared with published data. The chemical composition of the 15 corn coproducts (DM basis) varied substantially (Table 2), with a range in CP from 18.5% to 54.7%, NDF from 12.3% to 51.4%, ADF from 3.0% to 30.0%, EE from 4.7% to 12.3%, crude fiber from 3.1% to 13.4%, and ash from 1.6% to 8.6%. These wide ranges in composition of various nutrients among conventional and emerging corn coproducts emphasizes the need for developing and using clear definitions of these ingredients to minimize confusion in composition and feeding value for all animal species because they are produced by
using different processes (AAFCO, 2017). However, the extent to which various emerging technologies are adopted in the U.S. ethanol industry to produce high-protein corn coproducts is uncertain. In a previous study, Anderson et al. (2012) evaluated the chemical composition associated with predicting the digestible (DE) and metabolizable energy (ME) content of 20 existing and emerging corn coproducts from wet-mill and dry-grind ethanol plants. Many of the coproducts and technologies used to produce them are no longer used today, but nutritional evaluations of diverse corn coproducts can be extremely valuable because robust datasets (Anderson et al., 2012) representing a significant number of diverse corn coproduct samples were necessary for developing accurate DE and ME prediction equations for DDGS that subsequently became the foundation of the final validated prediction equations for swine (Urriola et al., 2014; Wu et al., 2016). Huang et al. (2018) suggested that increasing the number of samples and their variability in chemical composition may improve the accuracy of DE and ME prediction equations for corn DDGS sources derived from in vitro determinations compared to in vivo determined digestibility values because in vitro digestible DM and EE, but not digestible NDF, were selected for use in some prediction equations.

Beginning with the conventional DDGS sources evaluated in the current study, the concentrations (DM basis) of CP, NDF, ADF, EE, crude fiber, and ash of the 3 U.S. DDGS sources (P-DDGS, AE-DDGS, CP-DDGS) were comparable to the range in composition of 15 corn DDGS sources evaluated by Kerr et al. (2013), where CP ranged from 28.97% to 31.19%, NDF ranged from 28.79% to 43.97%, EE ranged from 4.88% to 13.23%, and ash ranged from 4.32% to 6.14%. Similarly, Huang et al. (2017a) determined in vitro hydrolysis and fermentation of 16 corn DDGS sources which contained 28.8–44.0% NDF and 8.6–15.0% ADF content. However, the NDF content of P-DDGS (27.7%) and AE-DDGS (29.2%) in the present study were substantially at the low end of these ranges in composition and less than 34.1% reported in NRC (2012) for corn DDGS containing more than 6% but less than 9% oil. Furthermore, the ash content of the 3 U.S. DDGS sources (5.1–6.0%) was greater than 4.52% reported for DDGS with >6 and <9% oil in NRC (2012). Unfortunately, no nutrient composition data for Brazilian DDGS and other corn coproducts produced by the Brazilian ethanol industry were published in the Brazilian tables of feedstuff composition for swine and poultry (Rostagno et al., 2011). However, the Brazilian DDGS sources evaluated in the current study had slightly greater CP (35.5–35.7%), much greater NDF (50.4–51.4%), similar EE content (4.7–5.5%), but much lower ash content (1.6–2.3%) compared with U.S. DDGS sources. Corassa et al. (2017) determined the nutrient composition as well as DE and ME content of a corn DDGS source produced in Brazil and reported that it contained (DM basis) slightly less CP (31.4%), and greater NDF (55.0%), EE (7.35%), and ash (5.14%) content that the Brazilian DDGS sources in the current study, and had DE and ME content similar to minimum values reported by Anderson et al. (2012). The specific reasons for these apparent differences in nutritional composition between U.S. and Brazilian DDGS sources evaluated in this study are unclear, but Liu (2011) indicated multiple causes for varying DDGS composition among sources including differences in composition of feedstock sources, process methods and parameters, amount of condensed solubles added to distillers wet grains before drying, the effect of fermentation yeast, and use of different analytical methods.

The dried corn fiber and solubles (BR-CF + S) coproduct that was produced using fiber separation technology in Brazil is unique compared with the other coproducts evaluated in this study because of its relatively low CP content (18.5%), but relatively high NDF (42.0%) and EE (8.8%) content. Anderson et al. (2012) evaluated a similar corn bran with solubles coproduct, which contained 15.1% CP, 25.21% NDF, 5.35% ADF, and 9.68% EE, and determined that it contained 3,031 kcal/kg DM of ME (swine), which was only 80% of the ME content of corn (3,805 kcal/kg DM). Further studies are needed to determine the in vivo DE and ME content of the Brazilian corn fiber and solubles coproduct for swine.

There was significant variation in nutrient composition among the five HP-DDG coproducts evaluated in this study with range in concentrations of CP (25.9–45.1%), NDF (30.0–49.3%), ADF (10.3–30.0%), EE (7.0–12.5%), crude fiber (5.1–11.0%), and ash (2.4–8.6%; Table 2). The nutrient composition (converted to DM basis) for HP-DDG from NRC (2012) indicates greater CP content (49.73%), comparable NDF (36.88%), ADF (22.62%), and crude fiber (8.00%) content, but substantially less EE (3.88%) and ash (2.62%) content than the HP-DDG sources evaluated in the current study. As indicated by Yang et al. (2019), the swine NRC (2012) nutrient composition values for HP-DDG should not be used in diet formulation because they were derived from processing technology that is no longer being used in
the ethanol industry, and the energy and nutrient composition of HP-DDG is substantially different than the HP-DDG coproducts currently being produced. Furthermore, the 3 HP-DDG sources evaluated by Anderson et al. (2012) were more variable in CP (39.98–57.45%), NDF (32.00–51.09%), ADF (12.61–25.42%), crude fiber (7.87–9.42%), EE (2.86–6.97%), and ash (1.10–2.09%) content (DM basis) than the HP-DDG samples evaluated in the current study.

No studies have been conducted to compare the nutrient composition and digestibility among high-protein, high yeast corn coproducts (SP, UM, UMHF, UMLF), but there is an official AAFCO (2017) definition for them (grain distillers dried yeast, AAFCO 96.5). These coproducts generally contained the greatest amount of CP (44.1–54.7%), but more variable concentrations of NDF (12.3–35.3%), ADF (3.0–16.7%), and EE (5.3–9.8%) compared with other general categories of coproducts evaluated in this study. Using mannan content of yeast cell walls as a proxy for estimating residual yeast content in corn coproducts, the yeast content of SP was estimated to be 29% compared with the estimate of 10% yeast in conventional DDGS sources (Shurson, 2018). As a result, the relatively high residual yeast content in these high-protein and yeast coproducts contributes to their relatively high CP and moderate to low fiber content. Therefore, because of the relatively high yeast content with favorable nutrient content and digestibility, it was expected that digestibility of these coproducts would be substantially greater than conventional DDGS and HP-DDG coproducts evaluated in this study for both swine and ruminants.

**In Vitro Digestibility—Swine**

Overall, DM digestibility from hydrolysis (IVDMD<sub>h</sub>) varied among corn coproducts and was greatest (P < 0.001) for UMLF (87.13%), followed by UM (81.68%), and UMHF (73.44%, Table 4). The lowest (P < 0.001) IVDMD<sub>h</sub> was observed and similar for US-HP49 (29.34%) and US-HPG1.5 (30.94%). Lower (P < 0.001) IVDMD<sub>h</sub> was also observed for US-HP (34.53%), which was similar for BRL-DDGS (37.23%) and BRP-DDGS (34.98%), but less than the 3 U.S. DDGS sources (P-DDGS = 65.02%, AE-DDGS = 58.92%, and CP-DDGS = 60.15%) and BRCF+S (55.06%). Anderson et al. (2012) determined organic matter (OM) digestibility of 20 diverse corn coproducts including 7 DDGS sources, 3 HP-DDG sources, and a sample of corn bran with solubles using a modified enzymatic assay (Boisen and Fernandez, 1997), which was comparable to the procedures used in the current study for determining IVDMD<sub>h</sub>. The OM digestibility values obtained in the Anderson et al. (2012) study ranged from 57.14% in a reduced oil DDGS source (3.15% EE) to 74.22% in a high oil DDGS source (11.45% EE), and were comparable to IVDMD<sub>f</sub> for 3 U.S. DDGS sources in the current study (58.92–65.02%), but substantially greater than IVDMD<sub>h</sub> for Brazilian DDGS samples (34.98–37.23%). The IVDMD<sub>h</sub> values for the U.S. DDGS sources evaluated in the current study were similar to the maximum values of IVDMD<sub>h</sub> (45.3–63.2%) reported by Huang et al. (2017a) when evaluating 16 U.S. corn DDGS sources with variable NDF content (28.8–44.0%). Interestingly, the OM digestibility of the corn bran with solubles (73.32%) in the Anderson et al. (2012) study was substantially greater than the IVDMD<sub>h</sub> value of 55.06% for BRCF+S in the current study. Likewise, most of the HP-DDG samples in the current study, except US-HPpel, had much lower IVDMD<sub>h</sub> (29.34–45.02%) compared with OM digestibility of HP-DDG sources (54.36–71.54%) from the Anderson et al. (2012) study. The explanation for these differences in digestibility (hydrolysis) estimates among similar categories of coproducts between the current study and those reported by Anderson et al. (2012) is unclear.

The fermentability of DM (IVDMD<sub>f</sub>) was greater (P < 0.001) and similar among all three Ultramax coproducts (80.20–89.00%), SP (76.80%), and US-HP (72.80%) compared with other coproducts. The lowest (P < 0.001) fermentability of DM was observed for US-HP49 (48.40%), while IVDMD<sub>f</sub> was similar among U.S. and Brazilian DDGS sources (61.00–64.40%) except CP-DDGS, which had lower (P < 0.001) IVDMD<sub>f</sub> (58.00%). The IVDMD<sub>f</sub> values for U.S. and Brazilian DDGS and HP-DDG sources, except for US-HP49, were comparable to the maximum values in the range in IVDMD<sub>f</sub> values for 16 corn DDGS sources evaluated by Huang et al. (2017a). These in vitro fermentation values can be used to accurately predict apparent total tract digestibility of total dietary fiber (TDF) among sources of corn DDGS (Huang et al., 2017b).

Total tract DM digestibility values were calculated from pooled samples and were greater than 75% for all coproducts except US-HP49 (63.54%). These results are consistent with those reported by Huang et al. (2017a, 2017b) and Jang et al. (2019). For example, Huang et al. (2017a) reported that IVDMD<sub>f</sub> of 16 corn DDGS sources ranged from 76.0% to 83.5%. The greatest total tract digestibility was for SP.
Table 4. Swine in vitro CP, NDF, and ADF digestibility (hydrolysis) and in vitro dry matter digestibility (IVDMD) of corn coproducts

| Source* | CP† | NDF‡ | ADF|| | Hydrolysis$ | Fermentation¶ | Total tract** |
|---------|-----|-----|-----|-----|-------------|--------------|--------------|
| P-DDGS | 78.67d | 12.16f | 21.10d | 65.02d | 62.80e | 86.99 |
| AE-DDGS | 68.20a | 22.23a | 29.03abc | 58.92a | 64.40b | 85.38 |
| CP-DDGS | 68.13a | 33.17f | 37.19d | 60.15a | 58.00a | 83.26 |
| BRL-DDGS | 54.39a | 13.86g | 31.65ab | 37.23b | 61.00c | 75.52 |
| BRP-DDGS | 43.38a | 13.35a | 28.78a | 34.98b | 62.80c | 75.81 |
| BRCF+S | 46.48b | 39.36ab | 29.53a | 45.02b | 65.40c | 80.98 |
| BR-HP | 55.98b | 22.68f | 36.49d | 34.53b | 72.80c | 82.19 |
| US-HP | 90.77ab | 43.16d | 67.22a | 78.79b | 63.00c | 92.15 |
| US-HPG1.5 | 38.98c | 17.29g | 20.98a | 30.94c | 64.20c | 75.27 |
| US-HP49 | 32.32d | 14.20e | 15.89d | 29.34hi | 72.80a | 92.08 |
| SP | 71.07a | 60.51b | 51.22c | 65.85d | 76.80b | 92.08 |
| UM | 88.13b | 53.71c | 79.30a | 81.68d | 89.00c | 93.98 |
| UMHF | 84.71c | 71.77a | 23.64d | 73.44a | 80.20c | 94.74 |
| UMLF | 91.18c | 62.49b | 37.78a | 87.13b | 85.60c | 98.14 |
| SEM | 0.8 | 0.9 | 1.5 | 1.4 | 3.9 | - |
| P-value | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | - |

In vitro digestibility (small intestine) CP = [(CP of sample before hydrolysis − CP of residue)/CP of sample before hydrolysis] × 100.

In vitro digestibility (small intestine) NDF = [(NDF of sample before hydrolysis − NDF of residue)/NDF of sample before hydrolysis] × 100.

In vitro digestibility (small intestine) ADF = [(ADF of sample before hydrolysis − ADF of residue)/ADF of sample before hydrolysis] × 100.

IVDMD hydrolysis [incubation: pepsin (2h) + pancreatin (4h)] = [(dry weight of sample before hydrolysis − dry weight of residues)/dry weight of the sample before hydrolysis] × 100.

IVDMD fermentation [incubation: fecal inoculum (72h)] = [(dry weight of hydrolyzed residues − dry weight of residues after fermentation)/dry weight of hydrolyzed residues] × 100.

IVDMD total tract = [1 − (1 − IVDMD hydrolysis/100) * (1 − IVDMD fermentation/100)] × 100, values are calculated from pooled samples, not actual observed values.

*Conventional corn dried distillers grains with solubles (DDGS) from U.S. POET (P-DDGS), U.S. Absolute Energy (AE-DDGS), U.S. Corn Plus (CP-DDGS); conventional corn DDGS from Brazil Libra (BRL-DDGS), Brazil Pantanal (BR-P); dried corn fiber + solubles produced using fiber separation technology by FS Bioenergy in Brazil (BR-CF+S); high-protein distillers dried grains produced using fiber separation technology by FS Bioenergy in Brazil (BR-HP); high-protein distillers dried grains produced using fiber separation technology by Corn Plus in the U.S. (US-HP); experimental high-protein distillers dried grains produced using ICM technologies and pelleted (US-HPpellet), and ICM Generation 1.5 grain fiber to cellulosic technologies (US-HP G1.5 and US-HP49); a branded high-protein and yeast coproduct called StillPro (SP) produced using FluidQuip Maximized Stillage Co-product technology; a branded high-protein and yeast coproduct called Ultramax (UM) produced using ICM, Inc. processes and two experimental UM coproducts containing high fiber (UMHF) and low fiber (UMLF).

†In vitro digestibility (small intestine) CP = [(CP of sample before hydrolysis − CP of residue)/CP of sample before hydrolysis] × 100.

‡In vitro digestibility (small intestine) NDF = [(NDF of sample before hydrolysis − NDF of residue)/NDF of sample before hydrolysis] × 100.

||In vitro digestibility (small intestine) ADF = [(ADF of sample before hydrolysis − ADF of residue)/ADF of sample before hydrolysis] × 100.

$IVDMD hydrolysis [incubation: pepsin (2h) + pancreatin (4h)] = [(dry weight of sample before hydrolysis − dry weight of residues)/dry weight of the sample before hydrolysis] × 100.

¶IVDMD fermentation [incubation: fecal inoculum (72h)] = [(dry weight of hydrolyzed residues − dry weight of residues after fermentation)/dry weight of hydrolyzed residues] × 100.

**IVDMD total tract = [1 − (1 − IVDMD hydrolysis/100) * (1 − IVDMD fermentation/100)] × 100, values are calculated from pooled samples, not actual observed values.

*Least square means with different superscripts within the columns are different (P < 0.01).
In vitro digestibility of corn coproducts has been studied to determine dry matter (DM), neutral detergent fiber (NDF), and protein degradability for the new high-protein coproducts evaluated in the current study. Overall, UM (93.21%) and UMLF (92.27%) had the greatest digestibility compared to other coproducts. The lowest digestibility was observed in BRP-DDGS (69.26%) and US-HP49 (72.17%).

The NDF degradability was greater in AE-DDGS (78.65%), but negative NDF degradability values were observed in UM (−8.12%) and UMHF (−69.83%). The average range of NDF degradability of the conventional U.S. corn DDGS observed in the current experiment are similar to those reported by Miron et al. (2001). The negative degradability observed for UM and UMHF was likely due to small particle size of these coproducts, resulting in loss of product through the filter bags during incubation (Nocek et al., 1988). We speculated that the use of smaller pore size bags (less than 50 ± 10 μm porosity) or modifying the rinsing method may reduce the problem of particulate matter loss when analyzing degradability of these corn coproducts with small particle size.

The DM degradability values of the conventional corn dried distillers grains with solubles (DDGS) from U.S. POET (P-DDGS), U.S. Absolute Energy (AE-DDGS), U.S. Corn Plus (CP-DDGS); conventional corn DDGS from Brazil Libra (BRPL-DDGS), Brazil Pantanal (BR-P); dried corn fiber + solubles produced using fiber separation technology by FS Bioenergia in Brazil (BR-CF+S); high-protein distillers dried grains produced using fiber separation technology by FS Bioenergia in Brazil (BR-HP); high-protein distillers dried grains produced using fiber separation technology by Cora Plus in the U.S. (US-HP); experimental high-protein distillers dried grains produced using ICM technologies and pelleted (US-HP Pellet), and ICM Generation 1.5 grain fiber to cellulosic technologies (US-HP G1.5 and US-HP49); a branded high-protein and yeast coproduct called StillPro (SP) produced using FluidQuip Maximized Stillage Co-product technology; a branded high-protein and yeast coproduct called Ultramax (UM) produced using ICM, Inc. processes and two experimental UM coproducts containing high fiber (UMHF) and low fiber (UMLF).
Palowski et al. 2010). Mertens (2016) indicated that there is a strong negative correlation between undigested NDF (i-NDF) and IVTDMD, which suggests that i-NDF can be an ideal analytical measure instead of using NDFD to more accurately explain differences in DM degradability among ingredients. Although the current study did not determine the correlation between i-NDF and IVTDMD, DDGS sources that contained greater i-NDF content (e.g., BRP-DDGS = 30.74% and US-HP49 = 27.83%) had lower IVTDMD.

Intestinally degradable protein (IDP) from the conventional U.S. DDGS sources varied from 68.28% to 77.30%, which are consistent with results obtained from previous studies using the same 3-step procedure (59.2%–76.8% in Kleinschmit et al., 2007; 62.3%–66.5% in Cao et al., 2009; 67.6% in Krogsdalen et al., 2020). However, other studies (Boucher et al., 2009; Mjoun et al., 2010) using the modified 3-step procedure (Gargallo et al., 2006) reported greater IDP in corn DDGS sources than observed in the current study. In these reports, IDP in DDGS ranged from 84.0% to 92.8%, which is around 16% greater than observed in the current study. It is likely that the different analytical procedures used to estimate intestinal degradability may have resulted in the lower estimated IDP value.

All conventional DDGS coproducts in the current study had similar TDP degradability ranging 81.87%–87.25%, except for BRL-DDGS (66.67%, P < 0.001). Among the high-protein and high yeast coproducts, UMHF (90.64%), and UMLF (88.46%) had similar but greater (P < 0.001) TDP degradability compared with UM (74.93%, P < 0.001). Lee et al. (2016) reported that DM fraction of corn DDGS had 79.9% TDP compared with a lower percentage (68.9%) in HP-DDGS due to the greater proportion of undegradable C fraction, which can originate from heat damage during the drying process to produce HP-DDGS. The high-protein and high-protein and yeast corn coproducts produced using new technologies had greater or similar TDP degradability compared with conventional DDGS, indicating that there is minimal heat damage in these coproduct sources.

When comparing digestibility of the categories of coproducts between swine and ruminants, TDP digestibility of conventional DDGS sources, except for BR1, appeared to be greater for ruminants than CP digestibility of these coproducts for swine. Total tract DM digestibility of conventional DDGS samples was similar between ruminants and swine, while NDF digestibility was greater in ruminants. Within the HP-DDG sources, total tract DM digestibility was similar between swine and ruminants, while N digestibility was less in swine than ruminants except for US-HP Pellet. For NDF digestibility of HP-DDG sources, US-HP and US-HP Pellet were similar between swine and ruminants, but overall, ruminant digestibility values were greater than those for swine.

In conclusion, the results from this study suggest that while all of these coproducts appear suitable feed ingredients for use in swine and ruminants diets, Ultramax and StillPro have greater nutritional value than conventional DDGS and HP-DDG sources for swine due to greater DM, CP, and NDF digestibility. Although the high-protein and high-protein and yeast corn coproducts are highly degradable CP sources for use in ruminant diets, dietary inclusion rates will likely be limited to avoid supplying excess protein relative to the requirements, especially if these coproducts are used as a primary dietary energy source.

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