RESEARCH REPORT
ANIMAL SCIENCE

**Title:** **Strategies to modify fiber structure and increase digestible energy in corn distillers dried grains with solubles – NPB #17-036**

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**Industry summary**

The aims of this project were 1) to develop a new method for quantification of the feeding value of corn DDGS, and 2) to increase extraction of nutrients from corn DDGS (a low energy feedstuffs). We used an *in vitro* digestibility assay that was developed in NPB project #13-014. We introduced modifications to the procedure that allowed us to understand what the characteristics of fiber are that are responsible for low digestible and metabolizable energy (DE and ME) in DDGS. We used 15 sources of DDGS of known DE and ME and that we have preserved at – 20 °C (NPB project #11-136). We measured fermentability of neutral detergent fiber (NDF) after incubating DDGS with fecal inocula for 8, 12, and 72 hours. We observed that a significant portion of NDF (21.6%) was degraded after 8 hours of incubation; while it took another 72 hours to degrade 68% of NDF. In addition, the lower content of undigestible fiber in DDGS (uNDFom8), the greater apparent total tract digestibility (ATTD) of gross energy ($R^2$ = 0.881) and ether extract ($R^2$ = 0.382). These two characteristics of fNDFom8 made it a good predictor of the DE ($R^2$ = 0.93) and ME ($R^2$ = 0.92) content among the 15 sources of DDGS. Subsequently, we used x-ray diffraction to determine the relationship between crystalline configuration of fiber in DDGS and ATTD of NDF. We observed that DDGS with low (44.5%) ATTD of NDF had lower crystallinity index (9.2%) than 12.2% in DDGS with high ATTD of NDF (57.3%). These observations suggest that the differences in digestibility of fiber among sources of DDGS appears to be related to crystalline configuration of fiber. This crystalline structure is more resistant to degradation in the large intestine of pigs. Therefore, for the second objective we used ammonia fiber expansion (AFEX) to degrade the unfermentable portion of fiber in DDGS from two sources (A and B). We measured *in vitro* digestibility of dry matter and energy during fermentation of the non-treated and treated DDGS with and without enzymes. We observed that pretreatment of DDGS increased the digestibility of dry matter and energy, it also increased in vitro DE from 3,579 to 4,502 kcal/kg DM in DDGS A and from 3,699 to 4,225 kcal/kg DM. The effect of adding enzymes was negligible compared with AFEX.

Key observations of this research:

1) Digestibility of fiber differs among sources of corn DDGS and the difference in digestibility of fiber appears to be due to crystalline configuration of fiber.
2) The greater content of undigestible fiber in DDGS, the less digestibility of gross energy, protein, and lipids. Consequently, the greater content of undigestible fiber in DDGS, the less DE and ME in DDGS.
3) Ammonia expansion is an effective technology for increasing the energy value in corn DDGS

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**Key words:** low energy feedstuffs, energy value, energy extraction, feed efficiency

**Scientific abstract**

Volatile fatty acids produced from fermentable fiber contribute to digestible energy (DE) and metabolizable energy (ME) content of high fiber ingredients, while unfermented fiber reduces DE and ME content. Characterizing fiber portions into fermentable and unfermentable fractions may enhance the accuracy of estimating DE and ME content in fiber-rich ingredients. Therefore, the objective of the study was to determine the concentrations of fermented and unfermented neutral detergent fiber (NDF) among sources of corn distillers dried grains with solubles (DDGS) and determine their relative contributions to DE and ME content. A second objective of this study was to increase degradation of fiber in corn DDGS to increase digestibility of energy. For Exp. 1, 15 samples of corn DDGS with known values of apparent total tract digestibility (ATTD) of NDF were selected for their known content of DE and ME. Samples of DDGS (0.5 g) were mixed with fecal inoculum and incubated *in vitro* for 8, 12, and 72 h. Ash corrected NDF (NDFom) content of DDGS residues at each time point were determined. The disappearance coefficients of NDFom were 21.6, 29.0 and 68.6% after inoculated with feces for 8, 12, and 72 h, respectively. The ATTD of gross energy (GE) increased as the unfermented NDF decreased at 8 h (uNDFom8; $R^2 = 0.83$; $P < 0.01$) and 72 h (uNDFom72; $R^2 = 0.83$; $P < 0.01$). Likewise, ME content of DDGS increased with the decreased unNDFom72 ($R^2 = 0.59$; $P < 0.01$). The best fit DE equation was DE (kcal/kg DM) = 2,175 – 3.07 × uNDFom8 (g/kg, DM) – 1.50 × uNDFom72 (g/kg, DM) + 0.55 × GE (kcal/kg DM) [$R^2 = 0.94$, SE = 36.21]. The best fit ME equation was ME (kcal/kg DM) = 1,643 – 2.31 × uNDFom8 (g/kg, DM) – 2.54 × uNDFom72 (g/kg, DM) + 0.65 × GE (kcal/kg DM) – 1.42 × crude protein (g/kg DM) [$R^2 = 0.94$, SE = 39.21]. These results indicate that *in vitro* unfermented fiber is strongly negatively associated with GE and NDF digestibility. Therefore, we studied the structural characteristics of fiber in corn DDGS that are associated with unfermented fiber. We used x-ray diffraction to measure crystallinity index of two sources of DDGS with low (44.5%) and high (57.3%) ATTD of NDF. We observed that crystallinity index of DDGS with low ATTD of NDF (12.2%) was greater than in DDGS with high ATTD of NDF (9.2%). For Exp. 2, we used ammonia fiber expansion (AFEX) to increase degradation of fiber in corn DDGS. We collected DDGS from two ethanol plants with very distinct processes (A and B). The *in vitro* digestibility of dry matter (IVDMD) was less ($P < 0.05$) in DDGS A and in DDGS B and addition of carbohydrase enzymes did no increase IVDMD. Pretreating DDGS with AFEX increased IVDMD of DDGS A to greater extent ($P < 0.05$) than DDGS B. There was an interaction ($P < 0.01$) between DDGS sources and AFEX for energy disappearance during fermentation ($GE_f$) and estimated *in vitro* digestible energy (DE) of DDGS. The pretreatment (AFEX) decreased ($P < 0.05$) $GE_f$ in a greater manner for DDGS A (332 kcal/kg DM feed) compared with DDGS B (154 kcal/kg DM feed). However, pretreatment (AFEX) increased ($P < 0.05$) DE in a greater manner for DDGS A (922 kcal/kg DM feed) compared with DDGS B (526 kcal/kg DM feed). Consequently, the AFEX pretreatment increased the DE of both sources of DDGS but the effect was

greater for source A. Calculated DE of DDGS A after AFEX 4,502 kcal/kg was greater ($P < 0.05$) than DDGS B 4,225 kcal/kg. In conclusion, composition of fiber varies among sources of corn DDGS. This changes in composition of fiber have an impact on the energy value of DDGS; where sources of DDGS with greater content of unfermentable fiber have less energy value because unfermentable fiber appears to decrease digestibility of other dietary components (especially protein and lipids). The reason that some sources of DDGS have less digestible fiber appears to be due to the crystalline configuration of fiber. Pretreating DDGS with ammonia fiber expansion increased digestibility of energy in corn DDGS.

**Introduction**

Utilization of agroindustry coproducts decrease overall feeding cost of pigs. However, most coproducts available in North America (e.g. corn distillers dried grains with solubles, corn germ, corn germ meal, high protein distillers dried grains with solubles) have high content of dietary fiber (Shurson, 2017). This dietary fiber decreases feed efficiency, decreases energy digestibility and decreases overall nutritional efficiency (Kerr and Shurson, 2013; Gutierrez et al., 2016). Improving efficiency of utilization of dietary energy is necessary for maximizing utilization of resources. The content of digestible energy (DE) and metabolizable energy (ME) among feed ingredient with high content of dietary fiber is less than other feed ingredients (Noblet and Le Goff, 2001). This digestibility of energy is less because the extend of degradation of dietary fiber in the gastrointestinal tract of pigs is less than starch, proteins, and lipids (Noblet and Le Goff, 2001; Blok et al., 2015). Likewise, there are large differences in degradability of dietary fiber among high fiber feed ingredients. This was clearly demonstrated in our past NPB funded work (#13-xx) and publications (Huang et al., 2017a). We also demonstrated that using an *in vitro* digestibility system it is possible to accurately measure the digestibility of dietary fiber among feed ingredients (Huang et al., 2017b). However, the digestibility of dietary fiber varies among sources of the same ingredient (Urriola et al., 2010). Therefore, it is necessary to develop new technologies for the quantification of the feeding value of dietary ingredients. Therefore, the first objective of this project is to develop a method for measuring the impact of dietary fiber digestibility on the feeding value of corn DDGS.

Dietary fiber is the least degradable portion in many low-cost feedstuffs. In addition, dietary fiber decreases the ability of pigs to extract other nutrients (e.g. protein, lipids, starch) in low-cost, high fiber feedstuffs. Therefore, it is necessary to increase degradability of dietary fiber in order to increase nutrient extraction from low energy feedstuffs. There are multiple methods for increasing the digestibility of dietary fiber in low energy feedstuffs, these include reduction of particle size (Liu et al., 2012), addition of carbohydrate degrading enzymes (Zeng et al., 2017). Addition of exogenous cabohydrase enzymes (e.g., xylanase, glucanaze, pectinase, and combinations) is a common strategy to increase energy utilization from fibrous/low energy feed ingredients. However, the impact of these enzymes on energy uplift in corn DDGS is rather small and inconsistent. Preliminary results from our pastNPB project (#14-045 - Gut physiology and metabolomic profile of pigs fed diets with carbohydrase enzymes and dried distillers grains with solubles) suggest that the improvement of in vitro ileal digestibility of DM by commercial carbohydrases is associated with a decrease in fermentability of DM using in vitro digestibility procedures to measure hindgut fermentation. This fiber digestion shift from the large intestine to small intestine diminishes the impact of energy uplift in DDGS through feed enzyme addition or similar technologies. It is worth mentioning that this shift in energy and fiber digestibility is a consistent result observed in previous NPB projects (#14-234) and in the literature (Bach Knudsen et al., 2016; Gerrits and Vries, 2016). Therefore, it is critical to understand the reasons of this shift in the digestion of fiber in the intestine of pigs and to understand the conditions under that exogenous enzymes can increase NE in DDGS.

Ammonia fiber expansion (AFEX) is one alkaline pre-treatment technology that disrupts the crystalline structure of cellulose and significantly enhances enzymatic digestibility from fiber rich biomass (Dien et al., 2008). In ruminants, AFEX treated forages were reported to have improved NDF digestibility when evaluated in vitro with rumen inoculum. Actually, the same research group tried to optimize AFEX pre-treatment conditions in corn DDGS, and reported that almost all cellulose in DDGS was removed after 72 h of enzymatic hydrolysis, and released 190 g/glucose dry biomass. Corn DDGS contains 5.8 % cellulose and accounts for about 23.3% of total NSP (Jaworski et al., 2015). If cellulose of DDGS were hydrolyzed before entering the hindgut of a pig, we estimate that it will contribute with approximately 242 kcal/kg DE (Noblet and van Milgen, 2004). More importantly, the portion of arabinoxylans buried in cellulose may be exposed and to degradation from exogenous enzymes, bacteria, organic acids, or their combination. Therefore, we hypothesize that the shift induced by exogenous enzymes may be overcome if cellulose were removed before digesta flows into the hindgut.

**Objectives as stated in the project proposal**

The overall objective of this project is to increase energy uplift from low energy feedstuffs, by characterizing DDGS according to digestibility of fiber, studying the structure of fiber, and developing degradation procedures. Specifically, we will enhance:

1. Understanding the relationship between crystallinity of DDGS and *in vivo* fiber and energy digestibility.
2. Understanding structural changes on fiber from DDGS before and after ammonia fiber expansion (AFEX) pre-treatment, as well as *in vitro* and *in vivo* digestion.
3. Energy uplift in corn DDGS by combining exogenous carbohydrases with fiber pre-treatment to improve digestibility in the small and large intestine as well as digestibility of nutrients (protein, starch, fat and cellulose, non-cellulose polysaccharides) using *in vitro* and *in vivo* methods.

A component of the second and third objectives was to measure energy uplift after pretreatment of DDGS with AFEX and carbohydrase enzymes. This were described in the original grant. However, funding for *in vivo* digestibility measurements was deferred after analyzing results from objectives 1 and 2

**Materials and methods**

The project is divided into two major experiments. Experiment 1 for the first objective and experiment 2 for objectives 2 and 3.

**Experiment 1**

***Sample collection***

Fifteen sources of corn DDGS were obtained from different ethanol plants that represented diverse geographical locations of U.S. corn production and different co-product processing technologies, to capture variability in chemical composition among sources in the U.S. market. These 15 corn DDGS samples were used in our previous experiment to determine chemical composition *in vivo* apparent total tract digestibility (ATTD) of nutrients, as well as DE and ME content for growing pigs (Kerr et al., 2013). The digestible nutrient content of DDGS was calculated by multiplying total concentration by the corresponding ATTD values, and the indigestible portion was subsequently calculated by the difference between total and digestible nutrient content.

***Crystallinity index (CrI)***

Crystallin configuration of structural carbohydrates in grains have been associated with accessibility of degrading enzymes and digestibility of fiber. Our hypothesis is that greater crystallinity index will be a good measurement for the apparent total tract digestibility of fiber in corn DDGS. We selected two sources of DDGS with different ATTD of NDF (44.5 vs. 57.3%) that collected from Kerr et al. (2013). We scanned the samples by x-ray diffraction (XRD) to determine the degree of crystallinity of the structural fiber in these samples. Briefly, samples were front-loaded into shallow (ca. 0.5 mm) wells machined into zero background holders manufactured from off-axis single crystal quartz. The XRD measurements were performed on a Bruker D8 Advance System (Billerica, MA). The diffracted intensity of Cu K$\alpha$ radiation ($\lambda$ = 0.1542 nm; 40 kV and 40 mA) was measured in a 2$\theta$ range between 10$^o$ and 60$^o$. Crystallinity index (CrI) was calculated from the area ratio between the intensity of the crystalline peak and total intensity after subtraction of the background signal.

***In vitro fermentation***

Feces were obtained from 3 finishing pigs (90 kg BW) from Cargill Animal Nutrition (Elk River, MN). The pigs were fed a corn-wheat-soybean meal diet with no antibiotics. Fecal samples were collected directly from the rectum, immediately placed in zipper plastic bags without air, and kept in a water bath at 39 °C until used as inoculum for incubation. The time from fecal collection until incubation was less than 1 h. Briefly, 500 mg of DDGS samples were added to 125 mL serum bottles with rubber-stoppers containing 40 mL buffer solution containing macro- and micro-minerals (Rymer et al., 2005). The inoculum was prepared by diluting blended feces in an inoculation solution composed of distilled water (474 mL/L), trace mineral solution (0.12 mL/L containing $CaCl_2$ 132 g/L, $MnCl_3 \cdot 4H_2O$ 100 g/L, $CoCl_2 \bullet 6H_2O$ 10 g/L, and $FeCl_3 \bullet 6H_2O$ 80 g/L), *in vitro* buffer solution (237 mL/L containing $NH_4HCO_3$ 4.0 g/L and $NaHCO_3$ 35 g/L), macro-mineral solution (237 mL/L composed of $Na_2HPO_4$ 5.7 g/L, $KH_2PO_4$ 6.2 g/L, $MgSO4 \cdot 7H_2O$ 0.583 g/L, and NaCl 2.22 g/L), and resazurin (blue dye, 0.1% w/v solution; 1.22 mL/L), and was filtered through four layers of cheesecloth. The final inoculum concentration was adjusted to 0.094 g feces per mL of buffer, which represented the same feces to substrate ratio used in previous studies (Huang et al., 2017b; Huang et al., 2017a). Forty mL of inoculum was transferred into bottles containing DDGS samples, and bottles were sealed with rubber stoppers before placing in a 39$^o$C water bath for incubation. Anaerobiosis was maintained in the inoculation solution by the addition of a reducing solution (distilled water 47.5 mL/L, 1 *M* NaOH 2 mL/L, $Na_2S$ 335 mg/L) and $CO_2$. Bottles were sealed with a rubber stopper and placed in the water-bath for incubation. The fermentation was terminated after 8, 12, or 72 h of incubation by placing bottles in ice, and gas production was recorded at 2, 5, 8 12, 16, 20, 24, 30, 36, 48, and 72 h.

After the termination of fermentation, the inoculum (40 mL) was directly mixed with NDF washing detergents (60 mL) and loaded on the reflux apparatus for NDF analyses as described by Mertens (2002). After reflux solubilization, residues were filtered with the use of a glass microfiber filter (934-AH™ by Whatman®, Whatman Limited-GE Healthcare, Maidstone, UK) with porosity of 1.5 µm in Pyrex® Gooch crucibles (40 to 60 µm; Corning, Inc., Corning, NY). Subsequently, residues were ashed at 550°C to determine ash free NDF (NDFom). Blanks were created by inoculating the bottles with buffer, fecal inoculum, and were subjected to the same process as the test feed ingredients to adjust for any particles introduced into the *in vitro* fermentation system. The NDF content of DDGS samples was analyzed in duplicate, and all unfermented NDF residues after 8, 12 and 72 h fecal incubation (uNDFom8, uNDFom12, and uNDFom72, respectively) were analyzed in triplicate. The *in vitro* fermented NDFom (fNDFom8, 12, and 72) was calculated by subtracting total NDFom and uNDFom after 8, 12 and 72 h fecal incubation. The digestibility coefficients of NDFom (DigNDFom8, 12, and 72) were calculated by the ratio of digestible NDFom and total NDFom content.

Gas accumulation curves were recorded during the 72 h of fermentation and were modified according to (France et al., 2005):

$G\ (mL\ g/DM) = 0$, if $0 < t < L$

$G\ (mL\ g/DM) = G_f(1\text{-}\exp\ (-[b(t-L) + c\ (\sqrt{t} - \sqrt{L})]))$, if $t \geq L$

where G denotes the gas accumulation at a specific time (t), $G_f$ (ml $g^{-1}$ DM) was the maximum gas volume for $t = \infty$, and L (h) represents the lag time before the fermentation began. Gas accumulation rapidly reached one-fourth of the maximum accumulation in 2 h, and the parameter L was very close to 0, which resulted in the model failing to converge. Therefore, L(h) data were removed from the final model. The constants b ($h^{-1}$) and c ($h^{-1/2}$) were used to determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$\mu = b + c/(2\sqrt{t})$, t=T/2, representing the time to half-asymptote when $G = G_f/2$.

**Experiment 2**

***Sample collection and ammonia expansion pretreatment (AFEX)***

Two sources of DDGS were obtained (A and B) and sent to Michigan Biotechnology Institute (Lansing, MI) for pretreatment with the ammonia expansion process (AFEX). Briefly, samples were premixed with water to the desired moisture (60%) and then were loaded in a 19 L reactor. Air was removed from the reactor using a vacuum. Anhydrous ammonia was preheated to the desired pressure (around 300 psi) in the ammonia loading vessel, and the biomass was preheated to a target temperature (100 °C). The reactor was maintained at 100 ±5 °C, and 300 ±20 psi for 30 min after all the preheated ammonia was loaded with 1 g/g of dry DDGS. In the end, the pressure was gradually released, and then the treated biomass was removed from the reactor.

***Fiber fermentability***

The fermentability of fiber in corn DDGS (with or without AFEX) was determined by using modified *in vitro* fermentation. Briefly, about 0.5 g (± 0.01) DDGS samples were directly weighted into pre-weighed filter bags (F57, Ankom Technology, Macedon, NY), which was sealed and put into 310 mL bottles with 75 mL buffer solution containing macro- and micro-minerals along with fecal inoculum (0.05g/mL), and incubated at 39 °C. A blank bag was inocubated and served as corrected blank control. Feces were obtained from 3 finishing pigs (90 kg BW) from Cargill Animal Nutrition (Elk River, MN). The pigs were fed a corn-wheat-soybean meal diet with no antibiotics. Fecal samples were collected directly from the rectum, immediately placed in zipper plastic bags without air, and kept in a water bath at 39 °C until used as inoculum for incubation. The time from fecal collection until incubation was less than 1 h. Anaerobiosis was maintained in the inoculation solution by the addition of a reducing solution (distilled water 47.5 mL/L, 1 M NaOH 2 mL/L, Na2S 335 mg/L) and $CO_2$. The fermentation was terminated after 8, 12, or 72 h of incubation by placing bottles in ice (n = 3 for each time point). The bags were put into oven and dried for 48 h at 60 ◦C before neutral detergent fiber (NDF) analysis.

***Three step in vitro digestion and fermentation***

Samples of DDGS were ground to pass 1 mm-mesh screen before undergoing in vitro pepsin and pancreatin hydrolysis according to the first step of the method (Boisen and Fernández, 1997). Briefly, about 2 g (± 0.01) samples were weighed into 500 mL conical flasks. A phosphate buffer solution (100 mL, 0.1 M, pH 6.0) and a HCl solution (40 mL, 0.2 M) were added into each flask. Solution pH was adjusted to 2.0 with 1 M HCl or 1 M NaOH. In addition, 2 mL of chloramphenicol (Sigma C-0378, Sheboygan Falls, WI) solution (0.5 g 100 mL/L ethanol) were added to inhibit microbial activity. One milliliter of carbohydrases solution (Archer Daniels Midland Animal Nutrition, Decatur, IL) was prepared to provide 10 g enzyme/kg of DDGS and pipetted into the 500-mL conical flask. The carbohydrases cocktail was composed of 1500

U/g xylanase, 1100 U/g β-glucanase, 110 U/g mannanase, and 35 U/g galactosidase. Prepared phytase solution (1 mL, 20 FTU, AB Vista, Marborugth, UK) was transferred to each flask across all treatments. Fresh porcine pepsin solution (4 mL, 25 g /L, P-7000, Sigma Aldrich, St. Louis, MO) was added, and flasks were closed with a rubber stopper and placed in a water-bath at 39 ± 0.5 ◦C for 2 h under gentle agitation. Subsequently, 40 mL phosphate buffer (0.2 M, pH 6.8) and 20 mL of 0.6 M NaOH were added to the solution. The pH was adjusted to 6.8 with 1 M HCl or 1 M NaOH. Fresh pancreatin solution (2 mL, 100 g/L pancreatin, P-1750, Sigma) was added, and hydrolysis was continued for 4 h using the same water-bath conditions as the ones for pepsin.

After hydrolysis, the residues (n = 4 per treatment) were collected by filtration through a nylon bag (R510, 50 μm porosity, Ankom Technology, Macedon, NY), washed with ethanol (2 × 25 ml 95% ethanol) and acetone (2 × 25 ml 99.5% acetone), dried for 48 h at 60 ˚C, and weighed. The enzymatic hydrolysis was repeated 12 times under the conditions described previously, to obtain sufficient residue for multiple analyses. Hydrolyzed residues from the different replicates and batches of same treatments (n = 6) were pooled for subsequent in vitro fermentation. The remaining 6 replicates were pooled for GE determination using an isoperibol bomb calorimeter (Parr 6400, Parr Instrument Company, Moline, IL).

The rate of fermentation of the hydrolyzed substrates was assessed *in vitro*, using a cumulative gas-production technique (Bindelle et al., 2007). Briefly, 500 mg of pooled enzymatic hydrolytic residues for each treatment was added to 125 mL-glass bottles with 75 mL buffer solution containing macro- and micro-minerals (Menke and Steingass, 1988), along with fecal inoculum (0.05g/mL), and incubated at 39 °C. Feces were obtained from 3 finishing pigs (90 kg BW) from Cargill Animal Nutrition (Elk River, MN). The inoculum was diluted to 0.05 g of wet feces per mL of the buffer solution. The prepared inoculum was filtered through a 250 μm screen and transferred into the bottle with fermentation substrates. Bottles were sealed with a rubber stopper and placed in the water-bath for incubation. An anaerobic environment was maintained throughout the experiment, from the time of inoculum preparation until the incubation step, by adding CO2 gas to bags and bottles. The gas generated from fermentation was manually recorded at 0, 2, 5, 8, 12, 18, 24, 36, 48 and 72 h by using inverted burette assembly for gas measurement. The bottles were vented to release gas that accumulated after every measurement. Fermentation was terminated at 72 h of incubation by quenching the bottles in ice cold water. At the end of the fermentation period, the supernatant from each bottle was collected and frozen until analysis for short chain fatty acids (SCFA; e. g. acetic, propionic, butyric, and branched chain fatty acids). The unfermented residues were collected by filtration (R510, 50 μm porosity, Ankom Technology), washed, dried and weighed following the same procedures described for the hydrolyzed residues. Samples of the fecal inoculum prior to fermentation were also analyzed for SCFA concentrations.

***Chemical analyses***

All samples and feed ingredients were ground with a laboratory mill to pass through 1 mm mesh screen. Chemical analyses were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007)(AOAC, 2007) with specific methods as follows: DM (135 °C for 2 h, AOAC 930.15), nitrogen (AOAC 968.06; using an elemental analyzer LECO FP528, St Joseph MI, USA; crude protein = nitrogen × 6.25), acid-hydrolyzed fat using Soxhlet apparatus and petroleum ether (AOAC 920.39), and ash (AOAC 942.05). The GE was determined by bomb calorimeter (Parr 6400; Parr Instrument Company, Moline, IL) and benzoic acid was used as standard. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined using fiber bags and Fiber Analyzer (Ankom Technology, Macedon, NY, USA) following an adaptation of the procedure described by (Van Soest et al., 1991). The total and free AA profile were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). The non-protein nitrogen was calculated by differences of total nitrogen and total AA. The SCFA were determined via gas chromatography (Agilent 6890 System,

Böblingen, Germany) after extraction with diethyl ether. Briefly, 2 mL of the samples were transferred into the centrifuge tube, and 0.5 mL of sulfuric acid (1/1), 0.4 g sodium chloride, 0.4 mL internal standard, and 2 mL of diethyl ether were added (Urriola and Stein, 2010). The samples were mixed for 2 min and centrifuged at 3000 × g for 3 min. Subsequently, the ether layer was transferred to vials and loaded on the gas chromatography analyzer (Agilent 6890 System).

***Calculations and statistical analyses***

The fermentability (%) of DM (NDF) at 8, 12, and 72 h were calculated as equations: disappeared DM (NDF) after 8, 12, and 72 h fermentation/total DM (NDF) content

The fermented or unfermented NDF (g/kg DM) at 8, 12, and 72 h were calculated as equation: total NDF content × fermentability or (1 – fermentability)

*In vitro* digestibility of GE (IVDGE) and IVDDM during the pepsin and pancreatin hydrolysis were calculated as follows:

IVDDM (GE) = (dry weight (GE) of the sample before hydrolysis − dry weight (GE) of the residue) /dry weight (GE) of the sample before hydrolysis

*In vitro* fermentability of DM (IVFDM) during feces fermentation was calculated as follows:

IVFDM = (dry weight of the hydrolyzed residue − dry weight of the residue after fermentation) / dry weight of the hydrolyzed residue

Gas accumulation curves recorded during the 72 h of fermentation were modeled according to France et al. (1993):

$G\ (mL\ g/DM) = 0$, if $0 < t < L$

$G\ (mL\ g/DM) = Gf\ (1-\exp\ (-[b(t-L) + c\ (\sqrt{t}-\sqrt{L})]))$, if $t \geq L$

where G denotes the gas accumulation at a specific time (t), Gf (ml g−1 DM) was the maximum gas volume for $t = \infty$, and L (h) represents the lag time before the fermentation began. The constants b (h-1) and c (h−1/2) determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$\mu = b + c/(2\sqrt{t})$, if $t \geq L$

Kinetics parameters (Gf, t = T/2, and μ at T/2) were compared in the statistical analysis, with T/2 representing the time to half-asymptote when G = Gf/2.

The SCFA (acetic [A], propionic [P], butyric [B], and valeric [V] acids) production per kg of enzymatic digestion residues DM, was estimated by the following equation: [(A+ P + B+ V)]72h – (A+ P + B+ V)]0h/g of residue DM. The equivalent energy for each SCFA was assumed to be 0.209, 0.365, 0.522, and 0.678 Mcal/mol for acetic, propionic, butyric, and valeric acids, respectively (Anguita et al., 2006). The DE of DDGS (DM basis) was then calculated according to the following equations:

$GE_f$ = GE of SCFA × (1-IVDDM)

$GE_i$ = GE × IVDGE

$DE = GE_i + GE_f$

where $GE_f$ denotes the energy (kcal/kg, DM basis) derived from SCFA per kg of feed DM, GE of SCFA was energy of SCFA production per kg of enzymatic digestion residues DM. GEi (kcal/kg, DM basis) denotes energy disappeared during two-step *in vitro* enzymatic digestion, which is equivalent to digested GE at ileum.

***Statistical analyses***

The PROC CORR of SAS (Version 9.3; SAS Inst. Inc., Cary, NC) was used to determine if there was an association among DE, ME, fNDF and uNDF fractions, and the chemical composition of corn DDGS samples. Correlations with a value of $P < 0.05$ were considered significant. The PROC REG STEPWISE of SAS was used to select input variables for the equations to predict DE and ME content from chemical

composition, and *in vitro* fNDFom and uNDFom of the corn DDGS samples. Variance Inflation Factor (VIF) was used to determine multicollinearity, variables with VIF > 10 were considered as multicollinear and were removed from the prediction equations. The *P* value, $R^2$, and root of the mean square error (SEM) were used as parameters to determine the accuracy of the prediction equations.

All data were analyzed by using the GLM procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC), with individual bottles considered the experimental unit. For NDF fermentability data, the model included DDGS sources, AFEX, and their interaction. For 3-step digestion and fermentation data, the model included DDGS sources, AFEX, carbohydrases addition and their interactions. The equality of variances was tested by Levene test in GLM procedure of SAS 9.4. If variances were not equal, then heterogeneous variance model in MIXED procedure was used. If interaction effects tended to be significant ($P < 0.10$), treatment differences were separated using PDIFF and adjusted by the Tukey option. Results were considered significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

**Results and discussion**

**Experiment 1**

***Crystallinity in Distillers Dried Grains with Solubles***

The x-ray diffraction pattern of the sample of DDGS with low ATTD of NDF (44.5%) was similar and overlapping to the pattern of the sample with high ATTD of NDF (57.3%). Despite that patterns between both sources of DDGS overlapped, there was sufficient information in the data to calculate crystallinity index for both samples. Consequently, the CrI of the sample with low ATTD of NDF (12.2%) was greater ($P < 0.01$) than that of the sample with high ATTD of NDF (9.2%), suggesting that crystallinity index is a potential measure of ATTD of NDF.

In spite these encouraging results, we observe challenges that will need to be resolved for developing a more robust assay that measures ATTD of NDF. The reason is that DDGS is not a purified material but contains other amorphous components such as lignin and hemicellulose (Jaworski et al., 2015). The content of other amorphous components affect XRD patterns (Xu et al., 2009). Consequently, XRD is unable to distinguish between the crystallinity index of the cellulose inside the biomass and the crystallinity index of other components (protein, starch) in the biomass in DDGS. Therefore, more samples of DDGS with known ATTD of NDF are needed to verify the relationship between fiber structure and fiber digestibility.

***Variability of energy and fiber related composition***

Based on variability of chemical composition (e.g., DE, ME, NDF), the sources of DDGS collected for this project represent the variability observed in previous publications (Urriola et al., 2014). The differences between maximum and minimum energy values were 387, 396, and 430 kcal/kg for gross energy (GE), DE, and ME, respectively (Table 1.1). Dietary fiber (as measured by ADF, NDF, and TDF). This suggests that measuring the content of fiber would improve prediction of DE and ME among sources of DDGS (Kerr et al., 2013). However, our hypothesis is that additional information regarding fiber composition may improve our ability to predict the impact of dietary fiber on nutrient digestibility and ultimately DE or ME content in corn DDGS.

Among all 15 sources of DDGS, the disappearance of NDFom after 8 h fecal incubation (fNDFom8) was 21.6% (range 11.0 to 30.4%). Interestingly, this value is close to the apparent ileal digestibility (AID, 21.5 %) of total dietary fiber (TDF) reported by Urriola and Stein (2010) for 10 sources of corn DDGS. This observation suggest that DigNDFom8 may represent the portion of NDF in corn DDGS that is readily degradable in the small intestine of pigs. About 68.6 % of NDFom disappeared after 72 h fecal incubation

(fNDFom72). This value is greater than the 32.7% *in vitro* total tract digestibility of non-starch polysaccharides (Jaworski et al., 2015). Likewise, this value is also greater than the ATTD of NDF (59.3%) in corn DDGS reported by Urriola et al. (2010). The exact reason for the difference is unknown, but we speculate that this difference may have been a result of using the 72-h *in vitro* incubation time compared with a typical 30 to 51 h *in vivo* digesta transit time (Le Goff et al., 2002). If the use of in vitro digestible fiber is of use for predicting energy value of corn DDGS or other high fiber feed ingredients, it would be necessary to adjust *in vitro* incubation time to a time that represents normal physiological conditions in the gastrointestinal tract of pigs.

In addition, the difference between *in vitro* and *in vivo* assays may be due to the fact that excretion of many nutrients *in vivo* is composed of the undigestible portion of the diet and 'endogenous' nutrients lost during the digestion process (Sauer and Ozimek, 1986). In the *in vitro* assay, the digestion residue is only composed on undigestible portion of the diet and it is a representation of sometimes called 'real' digestibility (de Lange et al., 1990). This phenomenon has been primarily demonstrated for amino acids, phosphorus and lipids. However, it has been recently reported that analysis of TDF (and possibly NDF) in gastrointestinal content and feces may be biased by non-dietary materials (e.g., mucin, microbial biomass). This non-dietary material is excreted in gastrointestinal content and feces and may decrease calculated AID and ATTD of NDF and TDF (Montoya et al., 2016). In fact, negative values for AID of NDF was observed in diets with very low content of NDF; these AID values increased linearly with increasing intake of NDF (a suggestion that non-dietary material may be excreted in digesta).

The DigNDFom8 had greater CV (> 20%) than DigNDFom12 or DigNDFom72. This observation suggests that the ethanol production process, and differences among technologies, may modify dietary fiber among sources of DDGS. Interestingly, the fact that the most variable portion is DigNDFom8 may suggest that ethanol production technologies have greater impact of the portion of dietary fiber (NDF) in corn DDGS that is readily degradable (possibly arabinoxylans). The use of different types and amounts of enzymes (e.g., xylanases) in the bioethanol process among different ethanol plants may contribute to high variance of readily degradable fiber in corn DDGS samples evaluated in this study (Luangthongkam et al., 2015).

***Unfermented fiber and apparent total tract digestibility***

An interesting observation of the studies reported in this NPB project, is that different portions of NDF, specially undigestible portions (e.g., uNDFom8, uNDFom12, and uNDFom72) have strong relationship to digestibility of other components of DDGS (e.g., protein, lipids). The ATTD of GE, DM, and carbon decreased linearly ($P < 0.01$) in response to increased uNDFom8 and uNDFom72 (Fig. 1). The ATTD of ether extract (EE) was negatively ($P < 0.01$) associated with uNDFom8 and uNDFom12, but not affected by uNDFom72. The ATTD of nitrogen (N) tended to decrease linearly ($P = 0.067$) with increasing uNDFom72. It has been well documented that there is a linear decrease in apparent ileal and total tract digestibility of GE, DM, and N with increased levels of dietary fiber (Yin et al., 2001; Gutierrez et al., 2016). In a meta-analysis review, Zeng et al., (2018) also reported that standardized ileal digestibility of N and amino acids decreased linearly with increasing NDF or ADF content of different sources of DDGS. The greater content of uNDFom8 in DDGS, the lesser ATTD of EE ($R^2 = 0.38$; $P < 0.01$). However, the ATTD of EE was not correlated to uNDFom8, uNDFom12, or uNDFom72 (Figures 1.1, 1.2, 1.3, and 1.4). These two observations suggest that readily degradable fiber, but not the recalcitrant portions degraded at 12 and 72 hours of incubation, are responsible for encapsulating and decreasing ATTD of EE. This observation is also in agreement with previous reports that ATTD of EE is greater in extracted corn oil compared with the intact oil from corn germ meal (Kil et al., 2010). It is important to note that this report contains values for ATTD of EE and not AID of EE or fatty acids. Therefore, it is possible that another cause for the relationship between readily degradable fiber (NDFom8) and ATTD of EE is that the former

may stimulate hindgut fermentation and modification of the excretion of EE and fatty acids (Kim et al., 2013).

***Correlation among energy components, gas production, and chemical composition***

The content of DE among sources of DDGS was correlated negatively with uNDFom8 (r = -0.86, $P < 0.01$), uNDFom12 (r = -0.86, $P < 0.01$), uNDFom72 (r = -0.86, $P < 0.01$), and NDFom (r = -0.84, $P < 0.01$). While it was positively correlated with digestible DM (r = 0.73, $P < 0.01$, Figure 1.4). Similarly, ME was negatively ($P < 0.05$) associated with uNDFom8 (r = -0.86, $P < 0.01$), uNDFom12 (r = -0.86, $P < 0.01$), uNDFom72 (r = -0.86, $P < 0.01$), and NDFom (r = -0.84, $P < 0.01$).

Gas production at 8, 12, and 72 h was positively ($P < 0.05$) associated with fermented NDFom at the corresponding time points (Table 1.2). The disappearance of NDFom at 8 and 12 h was negatively associated ($P < 0.05$) with time of achieving half maximal gas production (T/2). These results are consistent with previous data reported by our research group, where an increase in ATTD of TDF was observed as the maximal gas production increased among DDGS, wheat straw, and soybean hulls (Huang et al. 2017a). *In vitro* gas accumulation measurements can be used to estimate substrate degradation and yield valuable information about feed ingredient fermentation kinetics of feed ingredients (France et al. 1993). However, the disappearance of NDF may not precisely match with gas accumulation from fermentation because gas is generated from fermenting a wide range of substrates, including both soluble and insoluble fiber components (Williams et al., 2001).

***DE and ME prediction equations***

Stepwise regression analysis of fiber-related measurements was used to generate a series of prediction equations for DE (Table 1.3). The initial regression equation (Eq. [1]) included uNDFom8 as the most important component to predict DE followed by Eq. [2], which included both uNDFom8 and GE, and ultimately resulting in the best-fit equation (Eq. [3]), which included uNDFom8, uNDFom72, and GE. A series of prediction equations were also developed for ME content of corn DDGS (Table 4). The initial regression (Eq. [4]) included uNDFom72 as the most important component to predict ME followed by Eq. [5], which included both uNDFom72 and GE. Equation 6 included uNDFom8, uNDFom72, and GE. Lastly, the inclusion of CP improved prediction of ME for the best fit equation (Eq. [7]); which included uNDFom8, uNDFom72, GE, and CP.

The *in vitro* uNDFom8 and uNDFom72 are initial variables in the DE and ME regression model, indicating that unfermented fiber is an important factor that affects DE and ME value of corn DDGS for growing pigs. The uNDFom8 contains slow fermented fiber (uNDFom8 – uNDFom72) that appears to affect digestibility of other nutrients mainly protein, starch, and fat. It has been reported that cell walls can encapsulate nutrients in grains, nuts, and other vegetable ingredients making them inaccessible to digestive enzymes and decreasing utilization of the nutrient (Grundy et al., 2016).

In conclusion, the use of the *in vitro* fermentation assay appears to be an effective method to estimate the content of fermentable and unfermentable fiber in corn DDGS. The portion of unfermentable fiber (uNDFom8 and uNDF72) are good predictors of the DE and ME content among the 15 sources of DDGS because uNDFom8 and uNDFom72 were correlated to ATTD of GE, EE, and N. Further investigations are encouraged to develop prediction equation of energy based on *in vitro* digestible nutrients (CP, fat, starch, and carbohydrates) and unfermented residues (ash and fiber).

**Experiment 2**

***Chemical composition of DDGS after pretreatment***

The sample of DDGS from source A contained less acid hydrolyzed fat, crude protein, than source B (Table 2.1). The sample of DDGS from source A had greater content of neutral detergent fiber, acid detergent fiber, and lignin than source B. There were no differences in the amino acid composition between the two sources of DDGS. Pretreatment of DDGS with AFEX decreased the content of acid

hydrolyzed fat in both sources of DDGS by 12 - 22 g/kg DM. Likewise, pretreatment of DDGS with AFEX increased the content of non-protein nitrogen by 131 g/kg DM and subsequently resulted in an increased CP by around 120 g/kg DM. The NDF content decreased by 168 and 128 g/kg DM for DDGS from sources A and B after AFEX pretreatment. However, AFEX only resulted in a decrease in ADF (49 g/kg DM) and lignin (17 g/kg DM) for DDGS from source A, whereas ADF and lignin of DDGS from source B were not affected by AFEX pretreatment of DDGS. The content of free AA and the corresponding profile of either source of DDGS was also not modified after AFEX pretreatment (Table 2.2).

***Fiber fermentability after AFEX***

The pretreatment (AFEX) increased ($P < 0.01$) fermentability of DM after *in vitro* fermentation for 8, 12, and 72 h. The fermentability of NDF and fermented NDF at 12 and 72 h were improved ($P < 0.01$) by AFEX pretreatment. There were interactions between DDGS sources and AFEX for unfermented NDF after *in vitro* fermentation of 8, 12, and 72 h. The DDGS sample from source B, unfermented NDF (8, 12, and 72 h) were decreased in a greater extent after AFEX pretreatment for DDGS source A.

***Three step in vitro digestion and fermentation***

There was a significant interaction ($P = 0.02$) among the effect of DDGS sources, carbohydrases, and AFEX; where the IVDMD of DDGS from source A was less than source B and adding carbohydrases increased the IVDMD of both sources of DDGS (Figure 2.1). Likewise, AFEX increase the IVDMD of DDGS from source A, but it was relatively less than AFEX and carbohydase. These results for IVDMD were similar for in IVDGE (Figures 2.1 and 2.2). Carbohydrases supplementation only increased ($P < 0.05$) IVDDM and IVDGE in DDGS from source B with AFEX pretreatment. There was also an interaction ($P < 0.01$) among DDGS sources, AFEX and carbohydrases for IVFDM (Figure 2.3). For DDGS without AFEX pretreatment, DDGS source B had a greater ($P < 0.05$) IVFDM in the presence of carbohydrases compared to DDGS obtained from source A. However, carbohydrases supplementation decreased ($P < 0.05$) IVFDM in DDGS from source B with AFEX pretreatment. For both DDGS sources, AFEX increased ($P < 0.05$) the IVFDM in the situation without carbohydrases supplementation. The DDGS from source B had a greater ($P < 0.05$) $G_f$ compared with DDGS from source A (Table 4). The pretreated (AFEX) DDGS had an increased ($P < 0.05$) $G_f$ and $\mu$ and decreased T/2 compared with the untreated DDGS. Compared with DDGS obtained from source B, DDGS from source A had an increased ($P < 0.05$) $\mu$ only in the presence of AFEX pretreatment, but not in the untreated situation. The pretreatment (AFEX) increased ($P < 0.05$) the production of acetic acid and total SCFA (Mmol/g DM hydrolyzed residue) in the both DDGS sources.

We observe that the effect of carbohydrases supplementation and AFEX were different depending on the source of DDGS. Treating both sources of DDGS with AFEX increased ($P < 0.05$) energy disappearing in the small intestinal portion of the *in vitro* assay ($G_i$) compared with DDGS without pretreatment, whereas carbohydrases supplementation only increased ($P < 0.05$) $G_i$ in the DDGS source B with AFEX pretreatment. There was an interaction ($P < 0.01$) between DDGS sources and AFEX for energy disappearance during fermentation ($GE_f$) and estimated *in vitro* digestible energy (DE) of DDGS. The pretreatment (AFEX) decreased ($P < 0.05$) $GE_f$ in a greater manner for DDGS source A (332 kcal/kg DM feed) compared with DDGS from source B (154 kcal/kg DM feed). However, pretreatment (AFEX) increased ($P < 0.05$) DE in a greater manner for DDGS A (922 kcal/kg DM feed) compared with DDGS B (526 kcal/kg DM feed). Consequently, the AFEX pretreatment increased the DE of both sources of DDGS.

In conclusion, the composition of fiber varies among sources of corn DDGS. This changes in composition of fiber have an impact on the energy value of DDGS; where sources of DDGS with greater content of unfermentable fiber have less energy value because unfermentable fiber appears to decrease digestibility of other dietary components (especially protein and lipids). The reason that some sources of DDGS have less

digestible fiber appears to be due to the crystalline configuration of fiber. Pretreating DDGS with ammonia fiber expansion increased digestibility of energy in corn DDGS.

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**Table1.1.** Chemical composition, fermentation characteristics, and parameters of cumulative gas production among 15 sources of corn distillers dried grains with solubles, DM basis[1]

| Item | Mean | Min | Max | Interval | SD | CV |
|---|---|---|---|---|---|---|
| Chemical composition | | | | | | |
| GE, kcal/kg | 4,996 | 4,780 | 5,167 | 387 | 111 | 2.2 |
| DE, kcal/kg | 3,650 | 3,474 | 3,870 | 396 | 130 | 3.6 |
| ME, kcal/kg | 3,435 | 3,266 | 3,696 | 430 | 140 | 4.1 |
| TDF, g/kg | 342 | 308 | 378 | 69 | 19 | 5.6 |
| NDF, g/kg | 354 | 288 | 440 | 152 | 40 | 11.3 |
| ADF, g/kg | 117 | 90 | 140 | 50 | 18 | 15.3 |
| Fiber fractions characterized by *in vitro* fecal incubation | | | | | | |
| NDFom, g/kg | 388 | 335 | 457 | 122 | 28 | 7.2 |
| uNDFom8, g/kg | 304 | 247 | 350 | 103 | 30 | 9.8 |
| uNDFom12, g/kg | 276 | 234 | 329 | 95 | 28 | 10.3 |
| uNDFom72, g/kg | 123 | 84 | 165 | 81 | 28 | 22.6 |
| fNDFom8, g/kg | 84 | 43 | 121 | 78 | 19 | 23.2 |
| fNDFom12, g/kg | 112 | 73 | 146 | 73 | 18 | 16.4 |
| fNDFom72, g/kg | 265 | 232 | 297 | 65 | 21 | 7.7 |
| DigNDFom8, % | 21.6 | 11.0 | 30.4 | 19.4 | 4.8 | 22.4 |
| DigNDFom12, % | 29.0 | 18.7 | 36.6 | 17.9 | 4.6 | 15.8 |
| DigNDFom72, % | 68.6 | 59.1 | 75.9 | 16.8 | 5.7 | 8.4 |
| Gas accumulation kinetics | | | | | | |
| Gas8 | 97 | 81 | 117 | 36 | 8.5 | 8.8 |
| Gas12 | 120 | 105 | 139 | 33 | 9.2 | 7.7 |
| Gas72 | 199 | 174 | 214 | 39 | 13 | 6.5 |
| Gf, mL/g | 240 | 211 | 271 | 60 | 22.4 | 9.3 |
| T/2, hour | 17.2 | 10.9 | 27.1 | 16.2 | 4.8 | 27.6 |
| $\mu_{T/2}$ | 0.036 | 0.024 | 0.048 | 0.024 | 0.007 | 18.8 |

[1]Samples of corn distillers dried grains with solubles from previous experiment (Kerr et al., 2013). SD: standard deviation; CV: coefficient of variation; GE: gross energy; DE: digestible energy; ME: metabolizable energy; TDF: total dietary fiber; ADF: acid detergent fiber; NDFom: ash free NDF; DigNDFom: percentage of fermented NDFom after 8, 12 or 72 h fecal incubation; fNDFom: fermented NDF after 8, 12 or 72 h fecal incubation; uNDFom: unfermented NDF after 8, 12 or 72 h fecal incubation; Gas: cumulative gas production after 8, 12 or 72 h fecal incubation; T/2: half-time to asymptote (hour); $\mu_{T/2}$: fractional rate of degradation ($h^{-1}$) at t = T/2; Gf: maximal gas production.

**Table 1.2.** Correlation coefficients (r) between energy values, total dietary fiber and fermented neutral detergent fiber of 15 corn distillers dried grains with solubles samples[1]

| Item | NDFom | uNDFom8 | uNDFom12 | uNDFom72 | fNDFom8 | fNDFom12 | fNDFom72 | DigNDFom8 | DigNDFom12 | DigNDFom72 | Gas8 | Gas12 | Gas72 | Gf | T/2 | $\mu_{T/2}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NDFom | 1.00 | | | | | | | | | | | | | | | |
| uNDFom8 | 0.78** | 1.00 | | | | | | | | | | | | | | |
| uNDFom12 | 0.79** | 0.94** | 1.00 | | | | | | | | | | | | | |
| uNDFom72 | 0.73** | 0.81** | 0.88** | 1.00 | | | | | | | | | | | | |
| fNDFom8 | 0.24 | -0.43 | -0.31 | -0.21 | 1.00 | | | | | | | | | | | |
| fNDFom12 | 0.30 | -0.27 | -0.35 | -0.25 | 0.84** | 1.00 | | | | | | | | | | |
| fNDFom72 | 0.38 | -0.04 | -0.12 | -0.36 | 0.60* | 0.75** | 1.00 | | | | | | | | | |
| DigNDFom8 | -0.11 | -0.71** | -0.60* | -0.47 | 0.94** | 0.76** | 0.49 | 1.00 | | | | | | | | |
| DigNDFom12 | -0.14 | -0.63* | -0.72** | -0.59* | 0.76** | 0.90** | 0.61* | 0.83** | 1.00 | | | | | | | |
| DigNDFom72 | -0.52* | -0.72** | -0.79** | -0.96** | 0.36 | 0.43 | 0.60* | 0.56* | 0.68** | 1.00 | | | | | | |
| Gas8 | -0.15 | -0.51 | -0.58* | -0.45 | 0.57* | 0.67** | 0.40 | 0.62* | 0.75** | 0.48 | 1.00 | | | | | |
| Gas12 | -0.07 | -0.41 | -0.53* | -0.38 | 0.54* | 0.72** | 0.43 | 0.57* | 0.77** | 0.44 | 0.77** | 1.00 | | | | |
| Gas72 | -0.06 | -0.42 | -0.43 | -0.64* | 0.56* | 0.58* | 0.78** | 0.59* | 0.62* | 0.78** | 0.62* | 0.59* | 1.00 | | | |
| Gf, mL/g | 0.06 | -0.02 | -0.07 | -0.46 | 0.12 | 0.20 | 0.70** | 0.11 | 0.18 | 0.60* | 0.13 | 0.18 | 0.73** | 1.00 | | |
| T/2, hour | 0.10 | 0.42 | 0.41 | 0.12 | -0.51 | -0.49 | -0.03 | -0.54* | -0.55* | -0.09 | -0.58* | -0.48 | -0.15 | 0.54* | 1.00 | |
| $\mu_{T/2}$ | -0.44 | -0.40 | -0.44 | -0.12 | -0.03 | 0.01 | -0.44 | 0.13 | 0.22 | -0.04 | 0.21 | 0.25 | -0.30 | -0.70** | -0.66** | 1.00 |

[1]GE: gross energy; DE: digestible energy; ME: metabolizable energy; NDFom: ash free neutral detergent fiber; DigNDFom: percentage of fermented NDF after 8, 12 or 72 h fecal incubation; fNDFom: fermented NDFom after 8, 12 or 72 h fecal incubation; uNDFom: unfermented NDFom after 8, 12 or 72 h fecal incubation. Gas: accumulative gas production after 8, 12 or 72 h fecal incubation; T/2: half-time to asymptote (hour); $\mu_{T/2}$: fractional rate of degradation ($h^{-1}$) at t = T/2; Gf: maximal gas production.

*Means $P < 0.05$,** means $P < 0.01$.

**Table 1.3.** Stepwise regression equation to predict digestible energy (DE) content among 15 sources of corn distillers dried grains with solubles

| Item | Regression coeficient[1] | | | | Statistics[2] | | |
|---|---|---|---|---|---|---|---|
| | Intercept | uNDFom8 | uNDFom72 | GE | SE | $R^2$ | Adjust $R^2$ |
| Equation[1] | 4,783 | -37.28 | | | 69.96 | 0.73 | 0.71 |
| SE[3] | 191 | 6.24 | | | | | |
| *P*-value[3] | <0.01 | <0.01 | | | | | |
| Equation2 | 2,388 | -4.19 | | 0.51 | 42.89 | 0.91 | 0.89 |
| SE[3] | 517 | 0.395 | | 0.11 | | | |
| *P*-value[3] | <0.01 | <0.01 | | <0.01 | | | |
| Equation3 | 2,175 | -1.5 | -23.59 | 0.55 | 35.41 | 0.94 | 0.93 |
| SE[3] | 435 | 0.54 | 0.58 | 0.09 | | | |
| *P*-value[3] | <0.01 | <0.01 | 0.026 | <0.01 | | | |

[1]Equations based on analyzed nutrient content expressed on a DM basis. GE: gross energy. Units are kcal/kg DM for GE and DE and g/kg DM for unfermented NDFom after 8 and 72 h fecal incubation (uNDFom8 and uNDFom72).

[2]SE = SE of the regression estimate defined as the root of the mean square error.

[3]SE and P-values of the corresponding regression coefficient.

**Table 1.4.** Stepwise regression equation for prediction of metabolizable energy (ME) among 15 sources of corn distillers dried grains with solubles

| Item | Regression coeedicient[1] | | | | | Statistics[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Intercept | uNDFom8 | uNDFom72 | GE | CP | SE | $R^2$ | Adjust $R^2$ |
| Equation4 | 3,911 | | -38.78 | | | 93.53 | 0.59 | 0.55 |
| SE[3] | 113 | | 9.02 | | | | | |
| *P*-value[3] | <0.01 | | <0.01 | | | | | |
| Equation5 | 648.7 | | -4.49 | 0.67 | | 58.49 | 0.85 | 0.83 |
| SE[3] | 711 | | 0.58 | 0.14 | | | | |
| *P*-value[3] | 0.380 | | <0.01 | <0.01 | | | | |
| Equation6 | 899 | -2.23 | -2.57 | 0.71 | | 42.64 | 0.93 | 0.91 |
| SE[3] | 524 | 0.65 | 0.71 | 0.11 | | | | |
| *P*-value[3] | 0.114 | <0.01 | <0.01 | <0.01 | | | | |
| Equation7 | 1,643 | -2.31 | -2.54 | 0.65 | -1.42 | 39.21 | 0.94 | 0.92 |
| SE[3] | 645 | 0.60 | 0.65 | 0.10 | 0.82 | | | |
| *P*-value[3] | 0.029 | <0.01 | <0.01 | <0.01 | 0.013 | | | |

[1]Equations based on analyzed nutrient content expressed on a DM basis. CP: crude protein; GE: gross energy. Units are kcal/kg DM for GE and ME and g/kg DM for unfermented NDFom after 8 and 72 h fecal incubation (uNDFom8 and uNDFom72).

[2]SE = SE of the regression estimate defined as the root of the mean square error.

[3]SE and P-values of the corresponding regression coefficient.

**Table 2.1.** Chemical composition of corn distillers dried grains with solubles (DDGS) from two sources before and after of ammonia fiber expansion (AFEX)

| Item | Untreated | | AFEX | |
|---|---|---|---|---|
| | A | B | A | B |
| Gross energy, kcal/kg DM | 5,141 | 4,902 | 5,197 | 4,939 |
| Nutrients, g/kg DM | | | | |
| Dry matter | 884 | 890 | 922 | 933 |
| Ash | 53 | 59 | 53 | 57 |
| Acid hydrolyzed fat | 106 | 139 | 94 | 117 |
| Neutral detergent fiber | 363 | 346 | 199 | 218 |
| Acid detergent fiber | 150 | 121 | 100 | 118 |
| Lignin | 29 | 26 | 12 | 24 |
| Crude protein | 308 | 312 | 426 | 432 |
| Non-protein nitrogen | 32 | 27 | 163 | 158 |
| Essential amino acids, g/kg DM | | | | |
| Arginine | 14 | 13 | 12 | 13 |
| Histidine | 8.5 | 8.8 | 8.1 | 8.5 |
| Isoleucine | 12 | 13 | 12 | 13 |
| Leucine | 35 | 35 | 34 | 35 |
| Lysine | 8.6 | 9.4 | 7.9 | 8.8 |
| Methionine | 5.9 | 5.7 | 5.6 | 5.7 |
| Phenylalanine | 15 | 15 | 15 | 15 |
| Threonine | 11 | 12 | 11 | 11 |
| Tryptophan | 2.1 | 1.9 | 1.3 | 1.3 |
| Valine | 16 | 16 | 16 | 16 |
| Nonessential amino acids, g/kg DM | | | | |
| Alanine | 21 | 21 | 21 | 21 |
| Aspartic acid | 20 | 20 | 19 | 20 |
| Cysteine | 6.8 | 7.1 | 2.0 | 2.6 |
| Glutamic acid | 43 | 45 | 40 | 44 |
| Glycine | 12 | 12 | 12 | 12 |
| Proline | 22 | 25 | 22 | 24 |
| Serine | 13 | 13 | 12 | 12 |
| Tyrosine | 11 | 12 | 12 | 12 |
| Total amino acids | 276 | 285 | 263 | 274 |

**Table 2.2.** Free amino acid content of corn distillers dried grains with solubles (DDGS) from two sources before and after of ammonia fiber expansion (AFEX)

| Item, g/kg DM basis | Untreated | | AFEX | |
|---|---|---|---|---|
| | A | B | A | B |
| Essential amino acids | | | | |
| Arginine | - | 0.22 | - | 0.21 |
| Histidine | - | 0.11 | - | - |
| Isoleucine | - | 0.22 | - | 0.75 |
| Leucine | 0.11 | 0.56 | 0.11 | 0.43 |
| Lysine | 0.11 | 0.79 | - | 0.64 |
| Methionine | - | - | - | - |
| Phenylalanine | 0.11 | 0.45 | 0.11 | 0.43 |
| Threonine | 0.11 | 0.22 | 0.33 | 0.43 |
| Tryptophan | - | - | - | - |
| Valine | - | 0.22 | 0.65 | 0.75 |
| Nonessential amino acids | | | | |
| Alanine | 0.68 | 1.24 | 0.65 | 1.18 |
| Aspartic acid | 0.11 | 0.67 | 0.33 | 0.86 |
| Cysteine | - | - | - | - |
| Glutamic acid | 0.23 | 0.45 | 0.33 | 0.54 |
| Glycine | 0.11 | 0.34 | 0.11 | 0.21 |
| Proline | - | - | - | - |
| Serine | 0.11 | 0.34 | 0.22 | 0.32 |
| Tyrosine | 0.23 | 0.56 | 0.22 | 0.43 |
| Total free amino acids | 1.92 | 6.18 | 3.04 | 6.97 |

**Table 2.3.** The effects of ammonia fiber expansion (AFEX) on fermentability (Fer) of dry matter and neutral detergent fiber in corn distillers dried grains with solubles (DDGS) from two sources

| Item | Untreated | | AFEX | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | | Source | AFEX | Interaction[1] |
| Fermentation 8 h | | | | | | | | |
| Fer of DM, % | 24.7 | 27.4 | 36.0 | 35.3 | 2.6 | 0.707 | <0.01 | 0.521 |
| Fer of NDF, % | -5.0 | -2.7 | 2.0 | 0.0 | 3.4 | 0.959 | 0.191 | 0.543 |
| Fer NDF, g/kg | -18.0 | -9.0 | 4.0 | 0.0 | 7.1 | 0.752 | 0.055 | 0.378 |
| Unfer NDF, g/kg | 381.0[a] | 355.0[a] | 195.0[b] | 218.0[b] | 7.3 | 0.792 | <0.01 | 0.010 |
| Fer 12 h | | | | | | | | |
| Fer of DM, % | 32.2 | 33.6 | 46.4 | 47.1 | 1.4 | 0.457 | <0.01 | 0.826 |
| Fer of NDF, % | 7.4 | 9.3 | 20.5 | 19.3 | 1.3 | 0.813 | <0.01 | 0.265 |
| Fer NDF, g/kg | 27.0 | 32.0 | 41.0 | 42.0 | 3.0 | 0.367 | <0.01 | 0.486 |
| Unfer NDF, g/kg | 336.0[a] | 314.0[b] | 158.0[d] | 176.0[c] | 2.8 | 0.391 | <0.01 | <0.01 |
| Fer 72 h | | | | | | | | |
| Fer of DM, % | 46.9 | 52.6 | 62.8 | 64.4 | 1.3 | 0.012 | <0.01 | 0.128 |
| Fer of NDF, % | 47.2 | 52.8 | 66.9 | 66.4 | 1.8 | 0.176 | <0.01 | 0.114 |
| Fer NDF, g/kg | 171.0 | 183.0 | 133.0 | 145.0 | 4.5 | 0.018 | <0.01 | 0.985 |
| Unf NDF, g/kg | 192.0[a] | 163.0[b] | 66.0[c] | 73.0[c] | 4.5 | 0.025 | <0.01 | 0.001 |

[1]Interaction between AFEX and DDGS sources

**Table 2.4.** The effects of ammonia fiber expansion (AFEX) and exogenous carbohydrases supplementation on gas accumulation kinetics parameters, short-chain fatty acid profile (SCFA) and fermented energy in corn distillers dried grains with solubles (DDGS).

| Item[1] | No AFEX | | AFEX | | Enzyme | | SEM | *P* value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | N | Y | | AFEX | DDGS | Enz | A×D[2] |
| Kinetics of gas production[1] | | | | | | | | | | | |
| Gf, mL/g DM | 236[b] | 281[a] | 217[b] | 226[b] | 240 | 238 | 10.91 | <0.01 | <0.01 | 0.46 | 0.03 |
| T/2, h | 29.2 | 31.3 | 13.4 | 15.5 | 21.4 | 22.7 | 2.33 | <0.01 | 0.20 | 0.78 | 0.91 |
| $\mu$, $h^{-1}$ | 0.03[c] | 0.03[c] | 0.10[a] | 0.07[b] | 0.06 | 0.05 | 0.01 | <0.01 | 0.01 | 0.46 | 0.02 |
| Lag time, h | 2.86 | 3.23 | 3.11 | 1.56 | 2.86 | 2.50 | 0.99 | 0.301 | 0.45 | 0.56 | 0.18 |
| SCFA profile, Mmol/g DM | | | | | | | | | | | |
| Acetic | 3.05 | 3.28 | 4.55 | 4.52 | 3.75 | 3.89 | 0.56 | <0.01 | 0.79 | 0.67 | 0.80 |
| Propionic | 1.01 | 1.01 | 1.03 | 0.99 | 1.02 | 1.00 | 0.07 | 0.98 | 0.61 | 0.68 | 0.69 |
| Butyric | 0.59 | 0.57 | 0.56 | 0.46 | 0.55 | 0.54 | 0.06 | 0.13 | 0.23 | 0.73 | 0.34 |
| Valeric | 0.09 | 0.10 | 0.10 | 0.07 | 0.09 | 0.09 | 0.02 | 0.30 | 0.73 | 0.54 | 0.18 |
| Total SCFA | 4.74 | 4.95 | 6.23 | 6.04 | 5.41 | 5.52 | 0.53 | <0.01 | 0.94 | 0.73 | 0.65 |
| Energy, kcal/kg DM of feed[3] | | | | | | | | | | | |
| $GE_i$[4] | 2,861 | 3,122 | 4,120 | 3,834 | 3,466 | 3,481 | 37.72 | <0.01 | 0.19 | 0.54 | <0.01 |
| $GE_f$ | 713[a] | 577[b] | 381[c] | 423[c] | 527 | 532 | 39.23 | <0.01 | 0.09 | 0.91 | <0.01 |
| DE | 3,579[d] | 3,699[c] | 4,502[a] | 4,225[b] | 3,978 | 3,995 | 39.21 | <0.01 | 0.02 | 0.53 | <0.01 |

[1]Gf, maximum gas volume (ml per g DM incubated); T/2, half-time to asymptote (h); μ, fractional rate of degradation (h−1) at t = T/2; L, lag time.

[2]Interaction between AFEX and DDGS sources.

[3]$GE_i$, GE × IVDGE estimated *in vitro* dissapearance of energy in the small intestine; $GE_f$, energy estimated from stoichiometry of SCFA during *in vitro* fermentation; DE, $GE_i + GE_f$

[4]*P*-values (AFEX × DDGS × enzyme) = 0.074, *P*-value (DDGS B with AFEX vs. DDGS B with AFEX + enzyme) < 0.05.

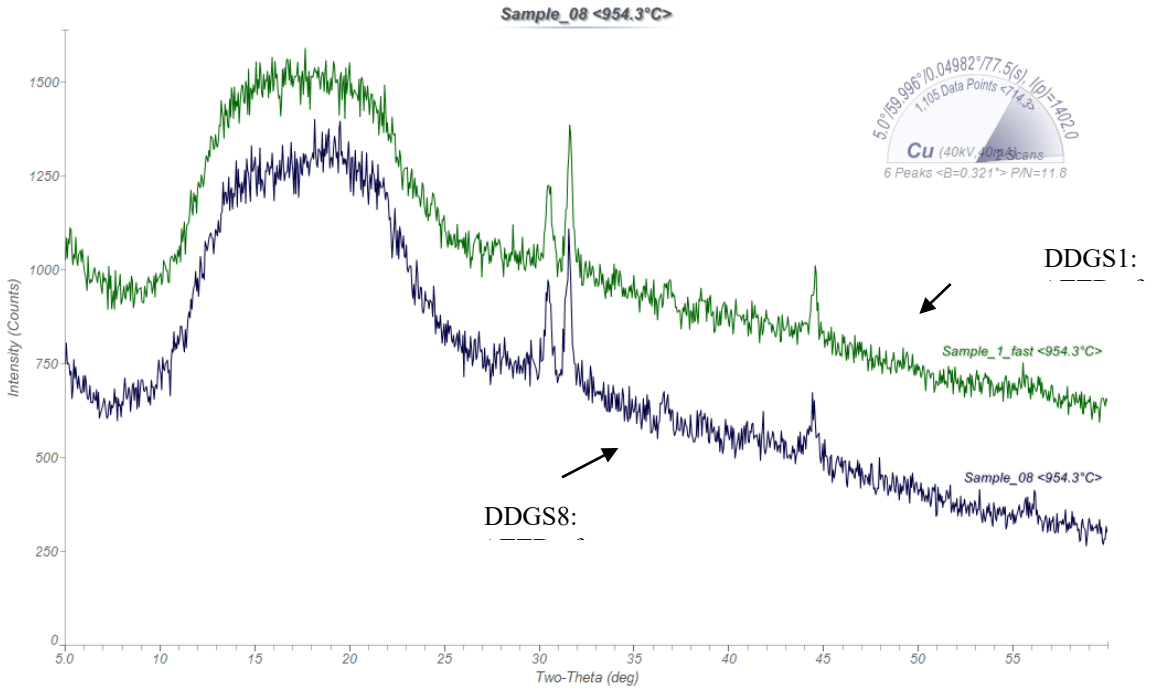


**Figure 1.1**. Measured X-ray diffraction patterns of DDGS samples, scaled to the same maximum intensity and offset for clarity.

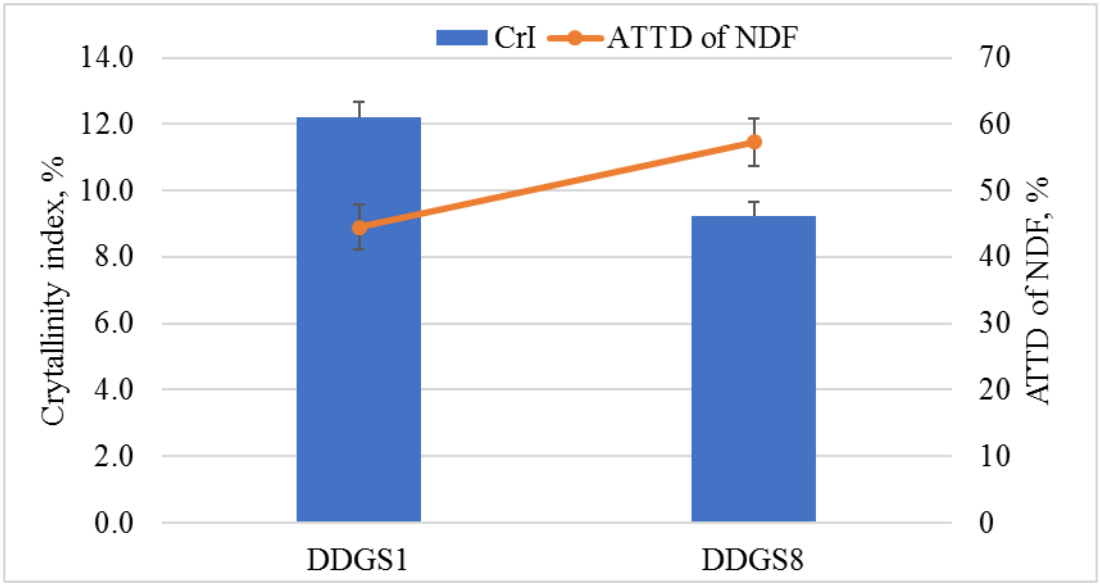


**Figure 1.2.** The crystallinity index and apparent total tract digestibility (ATTD) of NDF for DDGS samples adopted from Kerr et al., (2013).

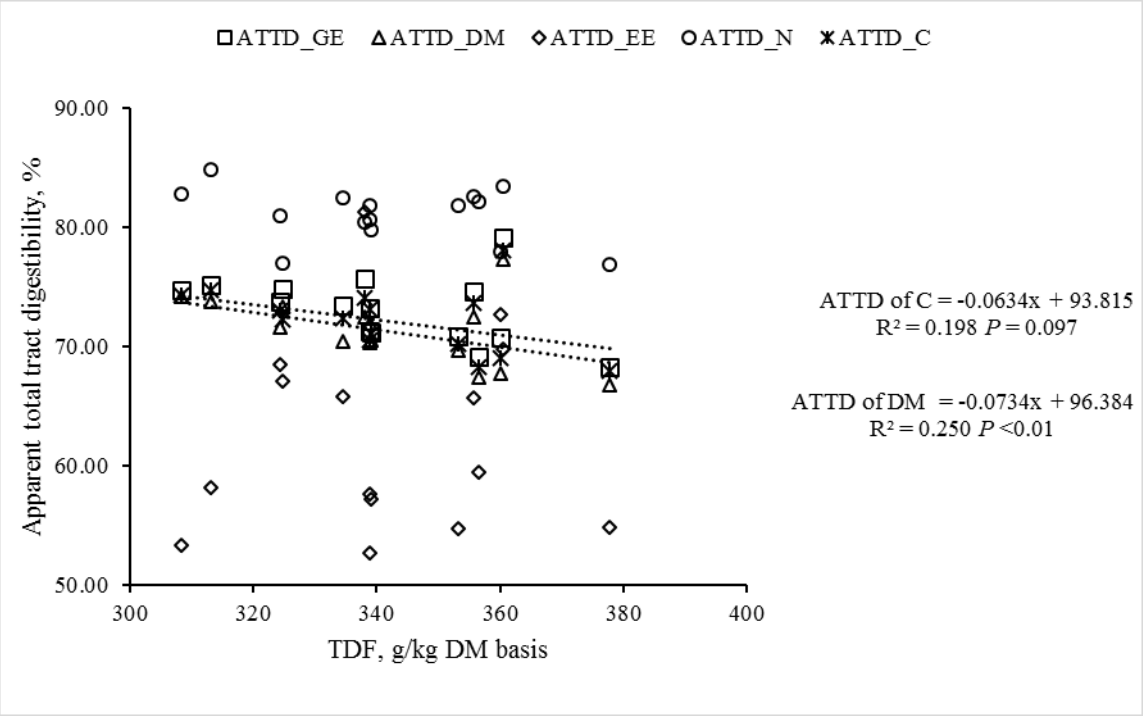


**Figure 1.1.** Prediction of apparent total tract digestibility of carbon (C) and dry matter (DM) from the concentration of total dietary fiber (TDF) among 15 sources of corn distillers dried grains with solubles

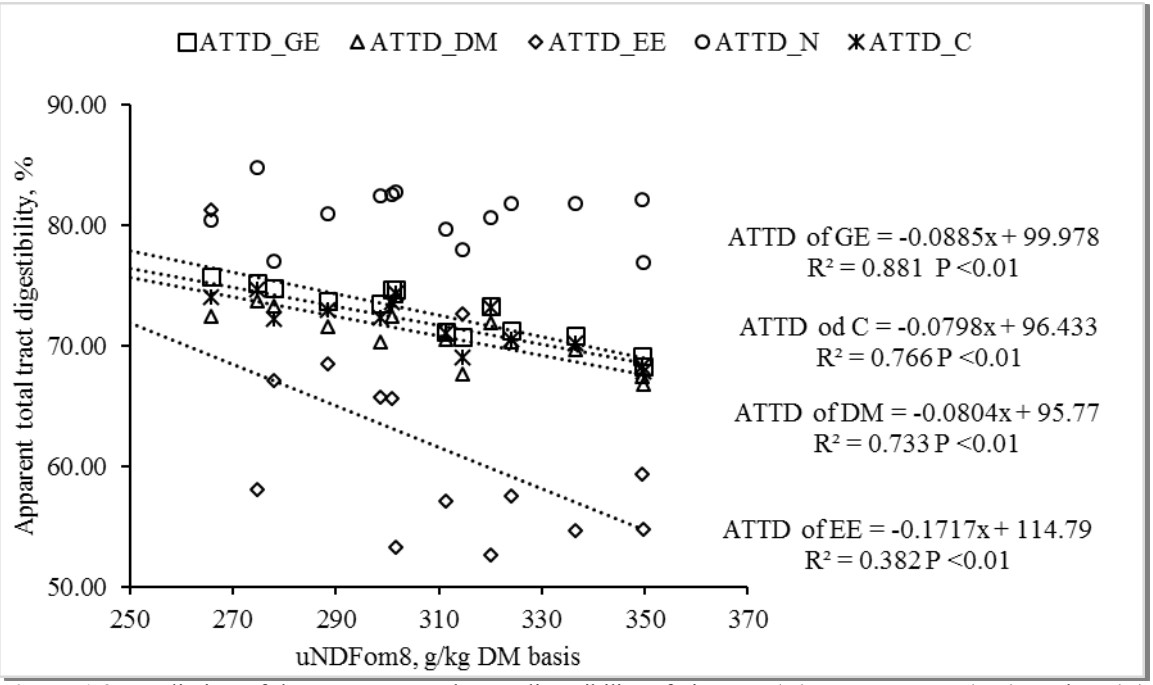


**Figure 1.2.** Prediction of the apparent total tract digestibility of nitrogen (N), gross energy (GE), carbon (C), and dry matter (DM) from the concentration of unfermentable neutral detergent fiber after 8-hour incubation (uNDF)

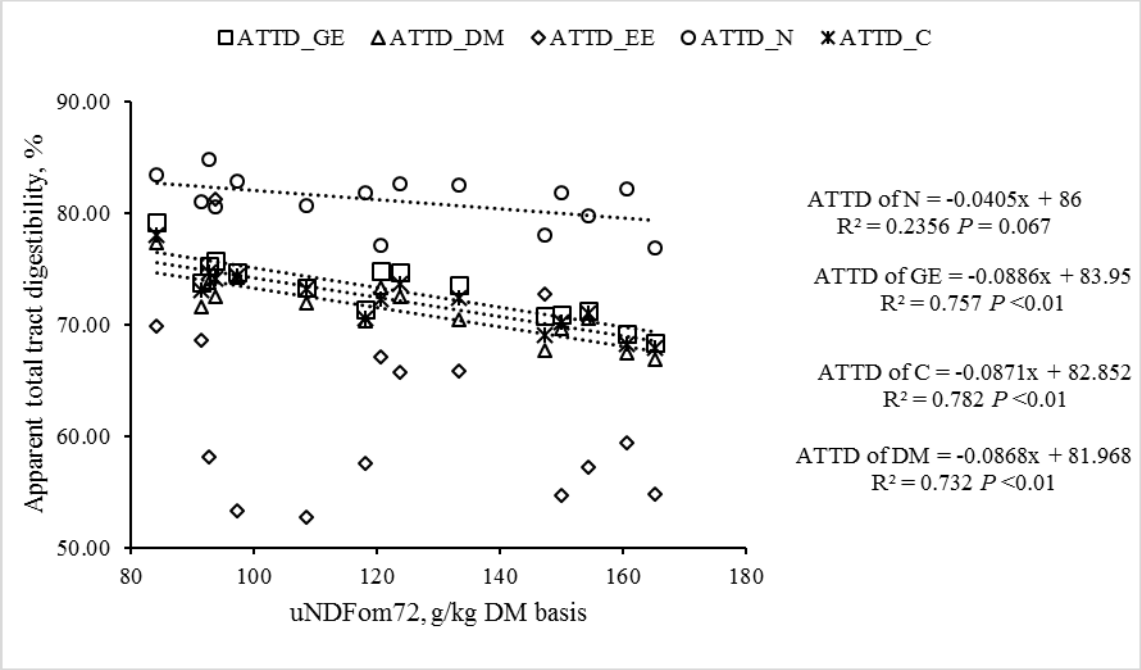


**Figure 1.3.** Prediction of apparent total tract digestibility of nitrogen (N), gross energy (GE), carbon (C), and dry matter (DM) from the concentration of unfermentable neutral detergent fiber after 72-hour incubation (uNDF)

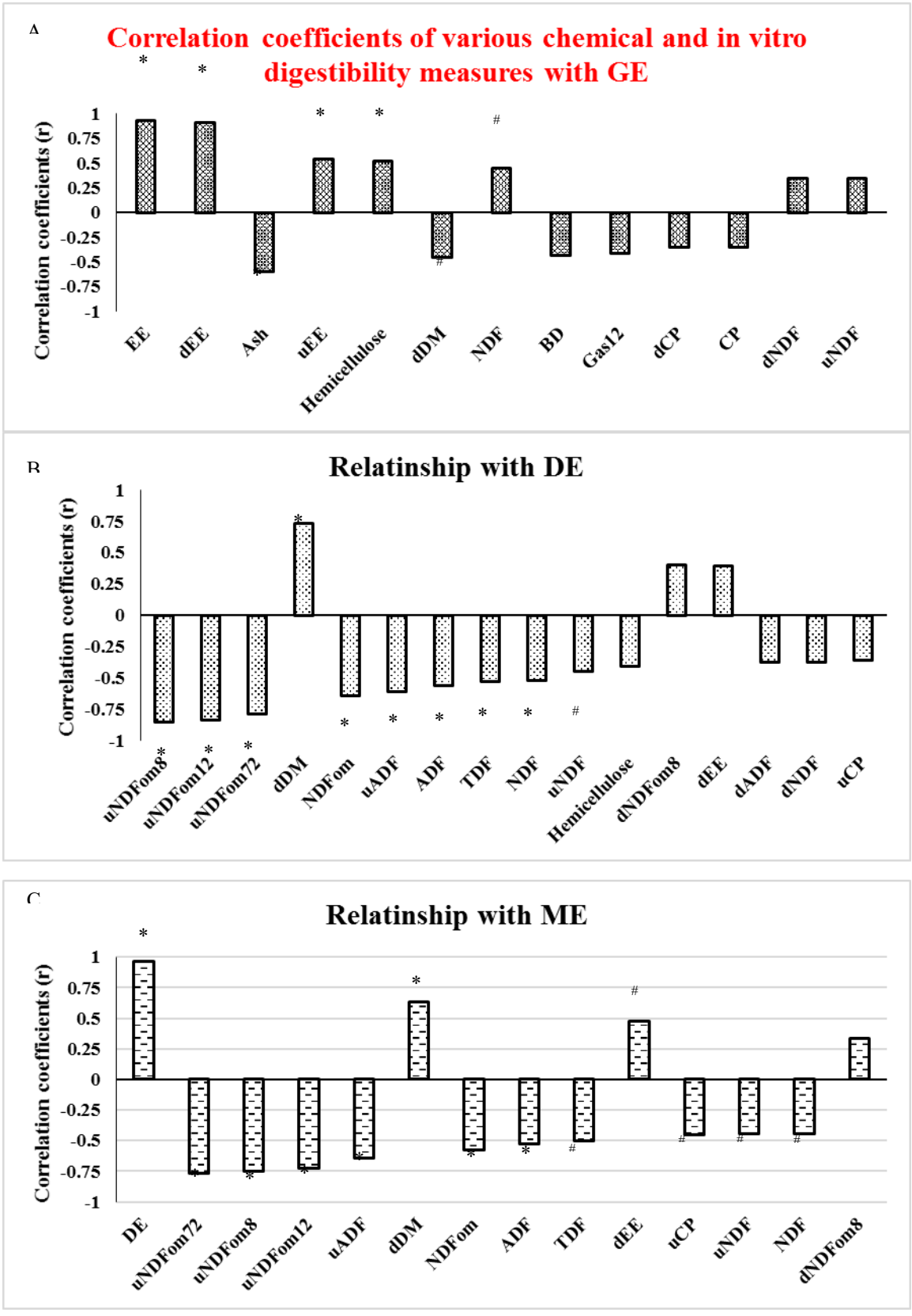
A
Correlation coefficients of various chemical and in vitro digestibility measures with GE
Correlation coefficients (r)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
EE
dEE
Ash
uEE
Hemicellulose
dDM
NDF
BD
Gas12
dCP
CP
dNDF
uNDF
B
Relatinship with DE
Correlation coefficients (r)
uNDFom8
uNDFom12
uNDFom72
dDM
NDFom
uADF
ADF
TDF
NDF
uNDF
Hemicellulose
dNDFom8
dEE
dADF
dNDF
uCP
C
Relatinship with ME
Correlation coefficients (r)
DE
uNDFom72
uNDFom8
uNDFom12
uADF
dDM
NDFom
ADF
TDF
dEE
uCP
uNDF
NDF
dNDFom8

**Figure 1.4.** Correlation coefficients (r) between chemical composition (digestible and unfermented) and GE (A), DE (B), and ME (C) of 15 corn distillers dried grains with solubles samples. ADF: acid detergent fiber; DE: digestible energy; DM: dry matters; EE: ether extract; GE: gross energy; NDF: neutral detergent fiber; NDFom: ash free NDF; ME: metabolizable energy; TDF: total dietary fiber; dDM, dEE, dADF, and dNDF: digestible DM, EE, ADF, and NDF (multiplying by total content and apparent total tract digestibility); dNDFom: fermented NDFom after 8, 12 or 72 h fecal incubation; Gas12: gas production after 12 h fecal incubation; uNDF and uCP: undigested NDF and CP (total – digestible); uNDFom: unfermented NDFom after 8, 12 or 72 h fecal incubation; NDFom: ash free NDF; TDF: total dietary fiber. $^{\#}$Means $P < 0.10$, * means $P < 0.05$.

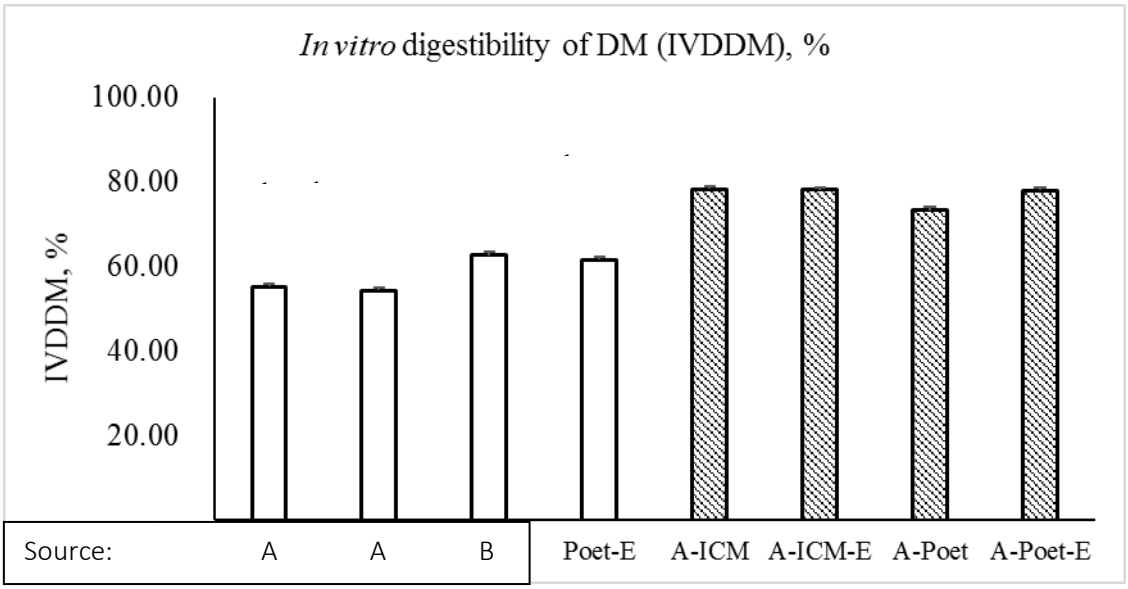


**Figure 2.1.** The effects of ammonia fiber expansion (AFEX) and carbohydrases addition on *in vitro* digestibility of dry matter in corn distillers dried grains with solubles (DDGS). The interaction among DDGS sources, carbohydrases, and AFEX was significant ($P$ = 0.02).

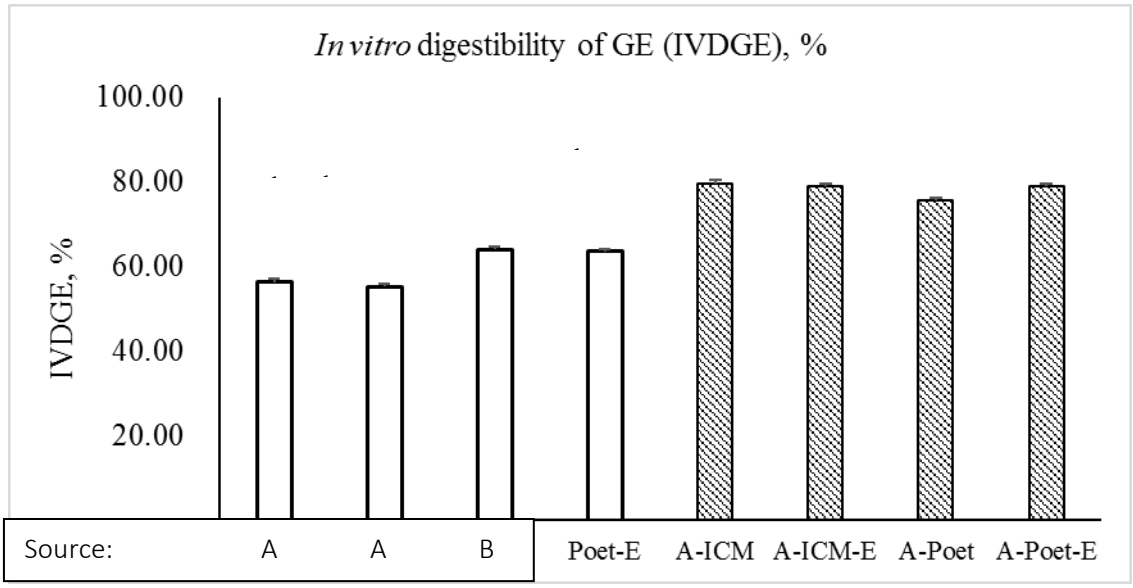


**Figure 2.2.** The effects of ammonia fiber expansion and carbohydrases addition on *in vitro* digestibility of gross energy (GE) in corn distillers dried grains with solubles (DDGS). There was a trend ($P = 0.07$) for an interaction among DDGS sources, carbohydrases, and AFEX.

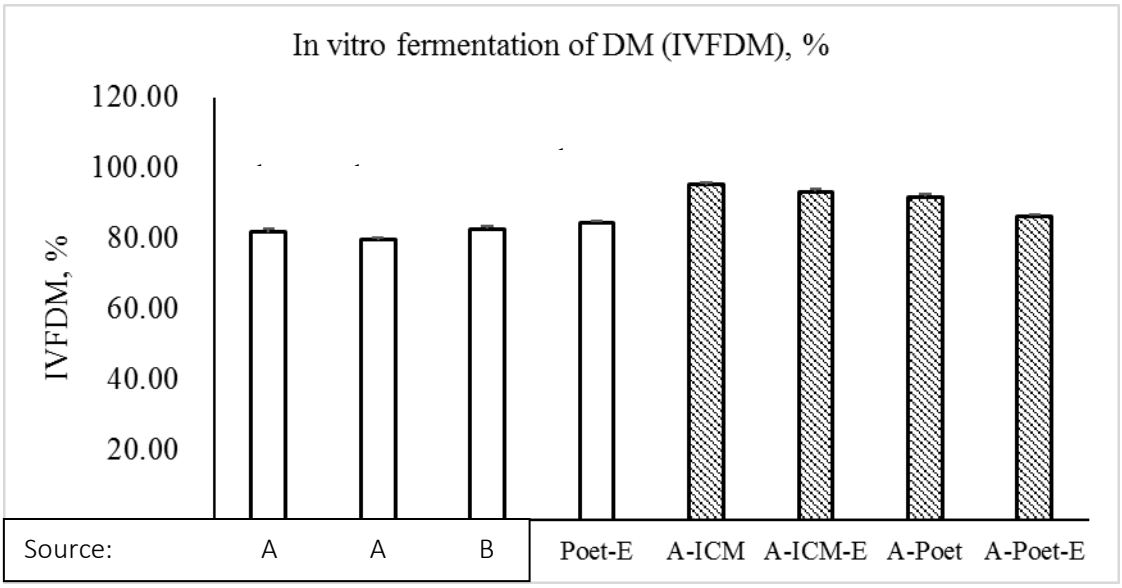


**Figure 2.3.** The effects of ammonia fiber expansion and carbohydrases addition on *in vitro* fermentation of dry matter in corn distillers dried grains with solubles (DDGS). The interaction among DDGS sources, carbohydrases, and AFEX was significant ($P < 0.01$).