Use of in vitro dry matter digestibility and gas production to predict apparent total tract digestibility of total dietary fiber for growing pigs¹

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ABSTRACT: In vitro DM disappearance (IVDMD) and gas production methods have been developed and used to measure in vivo nutrient digestibility of feed ingredients, but further validation is needed for ingredients containing high concentrations of insoluble fiber such as corn distiller's dried grains with solubles (DDGS). A 3-step in vitro procedure and resulting gas production were used to predict in vivo apparent total tract digestibility (ATTD) of total dietary fiber (TDF) among 3 sources each of wheat straw (WS), soybean hulls (SBH), and DDGS. A total of 34 barrows and 2 gilts (84 ± 7 kg BW) were used in a changeover design to determine the ATTD of 9 dietary treatments. The WS, SBH, or DDGS sources were the only ingredients containing fiber in each diet, and all diets were formulated to contain the same TDF concentration (22.3%). The in vivo experiment was conducted in 2 consecutive 13-d periods, each including a 10-d adaptation and a 3-d collection period to provide 8 replications/dietary treatment, and 0.5% TiO₂ was added to each diet as an indigestible marker. Pigs had ad libitum access to water and were fed an amount of feed equivalent to 2.5% of initial BW in each period. The in vitro experiment was used to determine IVDMD and gas production of the 9 ingredients (5 to 8 replicates/ingredient) fed during the in vivo experiment. Gas production kinetics were

fitted using a nonlinear model and analyzed using a mixed model, and predictions were evaluated using correlations and regression models. There were differences ($P < 0.01$) in ATTD of TDF among WS (26.7%), SBH (78.9%), and DDGS (43.0%) and among sources of DDGS (36.0 to 49.8%). Differences ($P < 0.05$) in IVDMD from simulated gastric and small intestinal hydrolysis were observed among WS (13.3%), SBH (18.9%), and DDGS (53.7%) and among sources of WS (12.8 to 13.8%), SBH (17.0 to 20.5%), and DDGS (52.0 to 56.9%). Differences ($P < 0.05$) in IVDMD from simulated large intestine fermentation (IVDMDf) were also observed among WS (23.3%), SBH (84.6%), and DDGS (69.6%) and among sources of WS (18.7 vs. 26.8%). In vitro DM disappearance from simulated total tract digestion of SBH (88.9%) and DDGS (86.1%) were greater ($P < 0.01$) than that of WS (33.5%). Differences ($P < 0.01$) in asymptotic gas production (A; mL/g DM substrate) were observed among WS (121), SBH (412), and DDGS (317), and ATTD of TDF was highly correlated with IVDMDf and A. In conclusion, low variability in ATTD of TDF and IVDMD among sources of WS and SBH evaluated in the current study may not justify the use of in vitro measurements, but in vitro fermentation accurately predicts ATTD of TDF among sources of corn DDGS.

Key words: apparent total tract digestibility, corn distiller's dried grains with solubles, growing pigs, in vitro digestibility, soybean hulls, wheat straw

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INTRODUCTION

Fiber fermentation in the large intestine can contribute a significant amount of energy to pigs (Yen et al., 1991). When developing ME prediction equations for growing pigs fed distiller's dried grains with solubles (DDGS), inclusion of NDF or total dietary fiber (TDF) improved the accuracy of prediction compared with not including it (Kerr et al.,

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2013). However, improved prediction accuracy may be possible by using digestible and fermentable NDF or TDF in these equations. Therefore, using estimates of digestible nutrients and fermentable carbohydrates may improve prediction accuracy of NE equations for high-fiber ingredients (Kil et al., 2013), especially given that apparent total tract digestibility (ATTD) of TDF of DDGS has been shown to vary from 29.3 to 57.0% (Urriola et al., 2010). The low fiber digestibility of corn DDGS is mainly due to its high content of insoluble dietary fiber (IDF; 95 to 100%; Urriola et al., 2010) and less-digestible fiber components (Kim et al., 2008). Improving the accuracy of prediction equations may be possible by using in vitro fiber digestibility values.

A modified 3-step in vitro procedure has been used to measure IVDMD and total gas production of swine feed ingredients, which includes pepsin hydrolysis, pancreatin hydrolysis, and fecal fermentation (Boisen and Fernández, 1997; Bindelle et al., 2007). The IVDMD method has been used to predict in vivo ATTD of GE and DM for swine among sources of barley (Regmi et al., 2008) and wheat (Regmi et al., 2009) but not for DDGS, soybean hulls (SBH), or wheat straw (WS). The procedure has been used to predict ATTD of NDF (Chen et al., 2014), but TDF is a better measure of the physiologically relevant content of fiber than NDF (Mertens, 2003). To date, no predictions of in vivo ATTD of TDF have been evaluated using the modified 3-step procedure among sources of DDGS. Therefore, the hypothesis of this study was that the modified 3-step procedure can accurately predict in vivo ATTD of TDF by IVDMD and gas production kinetics among and within samples of WS, SBH, and DDGS.

MATERIALS AND METHODS

All experimental procedures involving animals were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Sample Collection and Diet Formulation

Three sources each of 3 ingredients containing high concentrations of IDF were obtained to provide a range in IVDMD and chemical composition: WS (98 to 99% IDF; Panthapulakkal et al., 2006; Alemдар and Sain, 2008), SBH (83 to 94% IDF; Cole et al., 1999), and corn DDGS (95 to 100% IDF; Urriola et al., 2010). The chemical composition of each ingredient and source is shown in Table 1.

Nine experimental diets were formulated and mixed for the in vivo experiment (Table 2), and WS,

SBH, or corn DDGS sources served as the only source of fiber in each diet. Diets were formulated to meet the nutrient requirements of 80- to 90-kg growing pigs (NRC, 2012) and to contain similar concentrations (22.3%) of TDF. Titanium dioxide (TiO₂) was added at 0.5% to all diets as an indigestible marker.

Determination of In Vivo Apparent Total Tract Digestibility of Total Dietary Fiber

Thirty-four growing barrows and 2 gilts (84 ± 7 kg BW; Large White × Danish Landrace) were individually housed in metabolism cages (198 by 84 by 71 cm) at the Southern Research and Outreach Center in Waseca, MN. The experiment used a changeover design, where 36 pigs were allotted to 4 blocks based on initial BW to provide 9 pigs in each block, and all 4 blocks had the same average BW. The experiment was conducted over a 30-d time period, which included 4 d for pigs to adapt to the metabolism cages when they were fed a commercial diet. In the subsequent 2 consecutive 13-d periods, experimental diets were fed and feces and urine were collected on the final 3 d of each period. In each period, the 9 pigs within each block were randomly allotted and fed 1 of 9 experimental diets. At the end of period 1, each diet was consumed by 4 pigs, and at the end of period 2, each diet was consumed by an additional 4 pigs, resulting in a total of 8 replicates per dietary treatment.

During the adaptation period, the commercial corn-soybean diet was gradually replaced by experimental diets. Depending on the type of experimental diet, 1 to 2 d were required for pigs to consume all of their daily amount of the corn DDGS diets, 1 to 4 d were required for pigs to consume all of their SBH diets, and 3 to 10 d were required for pigs to consume all of the daily amount of the WS diets. Due to the greater volume and dry texture of WS diets, the WS diets were mixed with increasing amounts of water before they were fed to pigs until they consumed all of it. Pigs were provided an amount of their respective experimental diets equivalent to 2.5% of their initial BW divided into 2 equal meals and fed twice daily (0800 and 1600 h). Water was available ad libitum from nipple drinkers. Pigs were weighed at the beginning and the end of each period, before the morning meal. Feces of each pig were collected twice daily at 0800 and 1600 h using a fine wire mesh screen placed under each cage for 3 d during each collection period. About 200 g of feces per day were collected in sealed plastic bags and frozen (−20°C) until further processing. At the conclusion of the 2 collection periods, fecal samples were weighed and oven-dried at 60°C for 4 d, ground through a 1-mm screen, and subsampled for further analysis.

Table 1. Analyzed composition of wheat straw (WS), soybean hulls (SBH), and corn distiller's dried grains with solubles (DDGS), DM basis

Item	WS			SBH			Corn DDGS		
	1	2	3	4	5	6	7	8	9
GE, kcal/kg	4,050	4,160	4,032	4,070	4,065	3,998	4,879	4,842	4,776
CP, %	4.1	6.2	3.8	11.5	12.6	11.3	29.9	31.6	30.7
EE, ¹ %	2.2	2.5	2.8	2.4	2.8	2.8	9.8	9.0	8.2
ADF, %	53.0	53.0	52.7	51.0	48.2	50.2	13.8	17.4	14.5
NDF, %	79.1	80.0	78.2	68.4	63.2	66.4	32.7	35.1	33.8
TDF, ² %	77.2	82.6	80.0	80.2	75.9	75.9	38.2	37.5	37.8
NDF:TDF, %	102.5	96.9	97.8	85.3	83.3	87.5	85.6	93.6	89.4
Lignin, %	6.6	7.6	7.5	3.5	3.9	4.9	1.8	4.6	2.6
Hemicellulose, ³ %	26.0	27.0	25.5	17.4	15.1	16.2	18.8	17.7	19.2
Cellulose, ⁴ %	46.4	45.4	45.2	47.5	44.3	45.2	12.0	12.8	11.9
Bulk density, g/cm ³	0.18	0.19	0.16	0.39	0.42	0.41	0.48	0.50	0.54
Particle size, μ m	NA ⁵	NA	NA	720	600	715	793	804	644

¹EE = ether extract with acid hydrolysis.²TDF = total dietary fiber.³Calculated as NDF – ADF.⁴Calculated as ADF – lignin.⁵NA = not applicable. The particle size of WS could not be determined because of the long and rigid shape of ground particles.

Determination of *In Vitro* DM Digestibility and Gas Production

All 9 sources of ingredient samples were ground to pass through a 1-mm screen for determining *in vitro* DM disappearance and gas production. A modified 3-step enzymatic and microbial fermentation procedure was used (Boisen and Fernández, 1997; Bindelle et al., 2007). Briefly, 2 g of each sample was subjected to hydrolysis through the first 2 steps of this procedure with pepsin (100 mg/mL 0.2 M HCl; P7000, 421 units/mg solids; Sigma-Aldrich Corp., St. Louis, MO) for 2 h and pancreatin (100 mg/mL 0.2 M phosphate buffer; P1750, 4 × United States Pharmacopeia unit specifications; Sigma-Aldrich Corp.) for 4 h. After enzymatic digestion, the residues were collected by filtration through a nylon bag (5 by 10 cm; 50- μ m pore size; Ankom Technology Corp., Macedon, NY); washed with distilled water, ethanol (2 × 20 mL, 95%), and acetone (2 × 20 mL, 99.5%); dried for 72 h at 55°C; and weighed for determination of IVDMD. The residues of 5 to 8 digestion replicates were pooled for each sample, and 200 mg was used for the third step of this procedure involving microbial fermentation. Residues were incubated at 39°C in a glass bottle with 30 mL buffer solution, including macro- and microminerals (Menke and Steingass, 1988), along with swine fecal inoculum (Bindelle et al., 2007). The fecal inocula were prepared by pooling feces obtained from 9 pigs representing each dietary treatment in the *in vivo* experiment. The inoculum was prepared by diluting blended feces with the macro- and micromineral buffer solution and filtered through folded cheesecloth. The final inoculum

concentration was 0.05 g feces/mL of buffer. Each of the 30 mL inocula were transferred into bottles containing the digested residues, and the bottles were sealed with a rubber stopper and placed in 39°C water bath for incubation. Throughout the entire process (inoculum preparation until the incubation step), an anaerobic environment was maintained by adding reducing agents (1 M NaOH 0.2% and Na₂S, nonahydrate, 0.335 g/L) into the buffer solution along with CO₂ gas.

The amount of gas produced during fermentation was measured at 2, 5, 8, 12, 16, 20, 24, 30, 36, 48, and 72 h using an inverted 25-mL burette, with its stopcock end attached to a vacuum line and its open end submerged in a 39°C water bath. Before assembling the burette apparatus, the headspace volume of the burette was determined. To measure the gas volume produced, the inverted burette was filled with water to remove the air, and then a 20-gauge needle was quickly inserted through stopper of the fermentation bottle and attached to the burette apparatus. The burette valve was opened to release the gas into the burette, and the volume displaced by the gas was immediately recorded. After *in vitro* fermentation, the residues were collected, filtered, and washed as previously described for the residues and then subsequently dried for 72 h at 55°C and weighed for determination of IVDMD.

Physicochemical Analysis

All samples were analyzed at a commercial lab (Midwest Laboratories Inc., Omaha, NE). The analysis methods for ingredients, diets, and feces were as follows:

Table 2. Ingredient composition and nutrient content of wheat straw (WS), soybean hulls (SBH), and corn distiller's dried grains with solubles (DDGS) diets, DM basis

Item	WS			SBH			Corn DDGS		
	1	2	3	4	5	6	7	8	9
Ingredient composition, %									
WS	23.00	23.00	23.00	0.00	0.00	0.00	0.00	0.00	0.00
SBH	0.00	0.00	0.00	30.00	30.00	30.00	0.00	0.00	0.00
Corn DDGS	0.00	0.00	0.00	0.00	0.00	0.00	55.00	55.00	55.00
Spray-dried plasma	4.73	4.73	4.73	4.73	4.73	4.73	4.73	4.73	4.73
Cornstarch	61.10	61.10	61.10	56.66	56.66	56.66	34.73	34.73	34.73
Casein	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Fish meal, menhaden	6.74	6.74	6.74	3.77	3.77	3.77	0.00	0.00	0.00
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate (18.5%)	0.00	0.00	0.00	0.30	0.30	0.30	0.00	0.00	0.00
Limestone	0.32	0.32	0.32	0.44	0.44	0.44	1.44	1.44	1.44
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin–trace mineral premix ¹	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	99.99	99.99	99.99	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed nutrient composition, DM basis									
GE, kcal/kg	4,160	4,169	4,174	4,106	4,105	4,099	4,517	4,480	4,429
CP, %	13.00	13.60	12.40	13.20	13.70	13.10	22.20	24.00	23.00
EE, ² %	2.90	2.60	3.10	2.40	2.50	2.30	6.30	6.30	6.00
ADF, %	12.40	12.50	13.00	15.30	13.90	14.70	8.60	10.00	7.70
NDF, %	22.20	23.50	26.90	21.30	19.80	23.50	18.70	19.60	20.40
TDF, ³ %	23.00	23.40	21.40	24.70	23.20	25.00	20.70	20.90	18.30
Lignin, %	2.20	2.30	2.20	0.80	0.90	1.50	2.00	2.80	2.10
Hemicellulose, ⁴ %	9.70	11.00	13.90	6.00	5.90	8.80	10.00	9.60	12.70
Cellulose, ⁴ %	10.30	10.20	10.80	14.50	13.00	13.20	7.00	7.20	5.60
Titanium, %	0.33	0.33	0.30	0.27	0.33	0.28	0.35	0.38	0.37
Bulk density, g/cm ³	0.37	0.42	0.40	0.64	0.61	0.60	0.67	0.68	0.67

¹The vitamin and trace mineral premix provided the following (per kg of diet): 3,527,392 IU vitamin A, 661,386 IU vitamin D₃, 13,228 IU vitamin E, 1,323 mg vitamin K (menadione dimethylpyrimidinol bisulfite), 2,205 mg riboflavin, 13,228 mg niacin, 8,818 mg pantothenic acid, 13 mg vitamin B₁₂, 119 mg iodine (ethylenediamine dihydroiodide), 119 mg selenium (selenite), 22,046 mg organic zinc, 13,228 mg organic iron, 454 mg organic manganese, and 1,543 mg organic copper.

²EE = ether extract with acid hydrolysis.

³TDF = total dietary fiber.

⁴Hemicellulose was calculated as NDF – ADF and cellulose was calculated as ADF – lignin.

DM (method 930.15; Horwitz, 2006), GE (ASTM D 5865-13; ASTM Standard, 2007), CP (method 992.15; Horwitz, 2006), ether extract with acid hydrolysis (EE; method 922.06; Horwitz, 2006), ADF (Ankom Technology, 2017a), NDF (Ankom Technology, 2017b), TDF (method 991.43; AOAC International, 2005), lignin (method 973.18; Horwitz, 2006), titanium (Wavelength Dispersive X-Ray Fluorescence; ASTM D6906 - 12a; ASTM Standard, 2016), and bulk density (United States Pharmacopeial Convention < 616 > method I; World Health Organization, 2012). Hemicellulose was calculated by the difference of NDF and ADF, and cellulose was calculated by the difference of ADF and lignin.

Calculations

Hemicellulose. Hemicellulose (%) = NDF (%) – ADF (%).

Cellulose. Cellulose (%) = ADF (%) – lignin (%).

Apparent Total Tract Digestibility of Total Dietary Fiber. ATTD of TDF (%) = [(TDF in ingredient/TiO₂ in ingredient – TDF in feces/TiO₂ in feces)/(TDF in ingredient/TiO₂ in ingredient)] × 100.

Total Feces Needed to Prepare the Inoculum Per Run. Feces (g) = 30 mL × number of samples × number of replicates per batch × 0.05 g/mL.

Gas Volume Released at Each Time Point.

$$V = V_h + (25 - V_r),$$

in which V is expressed in milliliters, V_h is the volume of the burette headspace, and V_r is the reading volume record, and V_h ≤ V ≤ V_h + 25.

$$V = V_r,$$

in which $0 < V < V_h$ and V_r was measured the same as the headspace volume.

$$V = V_{r1} + V_{r2} + \dots + V_{rn}$$

in which $V > V_h + 25$, and V needed to be measured at least 2 times. Although V rarely exceeded burette capacity, it did occur for a few samples. Under the conditions when the observer noted that the gas volume was approaching the open end of the burette and more gas was being produced, the observer stopped the valve and recorded the volume as V_{r1} . Then the burette was refilled with water and subsequent gas volume produced was measured and recorded as V_{r2} . The same process continued for the n th time until all gas produced was measured and recorded.

Gastric and Small Intestinal Hydrolysis Disappearance. *In vitro* DM disappearance from simulated gastric and small intestinal hydrolysis (IVDMD_h) was calculated as follows: IVDMD_h (%) = [(dry weight of the sample before hydrolysis – dry weight of residues)/dry weight of the sample before hydrolysis] × 100.

Large Intestine Fermentation Disappearance. *In vitro* DM disappearance from simulated large intestine fermentation (IVDMD_f) was calculated as follows: IVDMD_f (%) = [(dry weight of hydrolyzed residues – dry weight of the residues after fermentation)/dry weight of hydrolyzed residues] × 100.

Apparent Total Tract Disappearance. *In vitro* DM disappearance from simulated total tract digestion (IVDMD_t) was calculated as follows: IVDMD_t (%) = [1 – (1 – IVDMD_h/100) × (1 – IVDMD_f/100)] × 100.

Kinetics of Gas Production. Gas accumulation curves recorded during the 72 h of fermentation were modified according to a monophasic model from Groot et al. (1996): $G = A/[1 + (B^C/t^C)]$, in which G (mL/g DM substrate) denotes the amount of gas produced per gram of DM incubated, A (mL/g DM) represents the asymptotic gas production, B (h) is the time after incubation at which half of the asymptotic amount of gas has been formed, C is a constant determining the sharpness of the switching characteristic of the profile, and t is the incubation time.

Statistical Analyses

The kinetics of gas production parameters were modeled using PROC NLIN of SAS 9.3 (SAS Inst. Inc., Cary, NC). The ATTD of TDF, IVDMD, and fitted gas production kinetic parameters were analyzed using PROC MIXED of SAS 9.3. The comparisons among the 3 IDF ingredients (WS, SBH, and corn DDGS) as well the comparisons among the 3 sources nested under each ingredient were analyzed using the following linear additive model:

$$y_{ijk} = \mu + \tau_i + \alpha_{j(i)} + \beta_k + E_{ijk}$$

in which y is the parameter to be tested (ATTD of TDF, IVDMD, or gas production kinetic parameters A , B , and C), μ is the overall population mean, τ_i is the effect of the i th ($i = 1, 2, 3$) ingredient or diet, $\alpha_{j(i)}$ is the effect of the j th ($j = 1, 2, 3$) sources nested under each ingredient, β_k is the effect of replicate ($k = 8$), and E_{ijk} is the experiment error. The ingredient ($n = 3$) and source ($n = 3$) nested under each ingredient were fixed factors, and the replicate was a random factor. The least squares means among the 3 sources nested under each ingredient were analyzed using the slice effect. Differences were considered significant when $P \leq 0.05$ and a trend when $0.05 < P < 0.1$.

The PROC CORR was used to determine the associations between ATTD of TDF of experimental diets with IVDMD and A . Data were separated into 4 sets of variables: 3 sources of each ingredient (WS, SBH, and corn DDGS) and all 9 sources of the 3 ingredients (WS + SBH + corn DDGS). We used PROC REG (stepwise) to determine the prediction equations of ATTD of TDF for WS, SBH, and corn DDGS from IVDMD_f and A . Variables with $P \leq 0.15$ were retained in the model. Variance inflation and collinearity diagnostics were tested using COLLIN (Kaps and Lamberson, 2004) to avoid selection of highly correlated IVDMD_f and A at the same time. The R^2 , SE, Mallow's Cp, and difference between predicted and measured ATTD of TDF were used to define the accuracy of the prediction equations.

RESULTS

Chemical Composition of Wheat Straw, Soybean Hulls, and Corn Distiller's Dried Grains With Solubles

We selected WS, SBH, and corn DDGS as 3 ingredients with a high concentration of IDF but with different chemical composition (Table 1). Overall, sources of corn DDGS had relatively greater GE, CP, EE, and bulk density than WS or SBH sources. Corn DDGS also had a relatively larger particle size than sources of SBH. Sources of WS had GE and EE comparable to SBH but less CP and bulk density. The concentration of fiber fractions was also different among WS, SBH, and corn DDGS and among sources of the same ingredient. Sources of WS and SBH had relatively greater ADF, NDF, TDF, and cellulose than corn DDGS sources. Sources of SBH also had relatively less NDF than sources of WS. The concentration of TDF varied among sources of each ingredient, WS (77.2 to 82.6%), SBH (75.9 to 80.2%), and corn DDGS (37.5 to 38.2%). Given the insoluble nature of fiber in these ingredients, the concentration of NDF followed a pattern similar to

that for TDF, where the concentration of NDF varied among sources of WS (78.2 to 80.0%), SBH (63.2 to 68.4%), and corn DDGS (32.7 to 35.1%).

Apparent Total Tract Digestibility of Total Dietary Fiber

The ATTD of TDF in SBH (78.9%) was greater ($P < 0.01$) than that in corn DDGS (43.0%), which was also greater ($P < 0.01$) than that in WS (26.7%; Table 3). There were no differences in the ATTD of TDF among sources of WS or SBH. However, among sources of corn DDGS, we observed a range in ATTD of TDF between 36.0 and 49.8%.

In Vitro DM disappearance and Gas Production

The $IVDMD_h$, $IVDMD_f$, and $IVDMD_t$ were different among WS, SBH, and corn DDGS. As a result of the greater concentrations of CP, EE, and starch in corn DDGS, we observed greater ($P < 0.01$) $IVDMD_h$ in DDGS than in SBH and WS. Greater ($P < 0.01$) $IVDMD_h$ was also observed in SBH than in WS. The $IVDMD_f$ was greatest ($P < 0.01$) in SBH, intermediate in DDGS, and the least ($P < 0.01$) in WS. The small $IVDMD_f$ combined with small $IVDMD_h$ of WS resulted in the least ($P < 0.01$) $IVDMD_t$, compared with SBH and corn DDGS, but was not different between corn DDGS and SBH. Differences of $IVDMD_h$ ($P = 0.05$), $IVDMD_f$ ($P < 0.05$), and $IVDMD_t$ ($P < 0.05$) among sources of WS were also observed. Although differences of $IVDMD_h$ were observed among sources of SBH ($P < 0.01$) and corn DDGS ($P < 0.01$), there were no differences in $IVDMD_f$ and $IVDMD_t$ among sources of SBH or corn DDGS. The differences in $IVDMD_f$ were consistent with the asymptote of the gas production curve (A; mL of gas produced), where SBH had greater ($P < 0.01$) A than corn DDGS, which, in turn, had greater ($P < 0.01$) A than WS. There were no differences in asymptotic gas production among sources of WS, SBH, or corn DDGS.

Prediction of Apparent Total Tract Digestibility of Total Dietary Fiber

In the current study, ATTD of TDF had a positive correlation ($P < 0.05$) with both $IVDMD_f$ and A for WS + SBH + corn DDGS (Table 4). However, ATTD of TDF was not correlated with $IVDMD_f$ or A for WS, SBH, or corn DDGS when the ingredients were analyzed separately. To test the accuracy of using $IVDMD_f$ and A as predictors of ATTD of TDF, prediction equations were separated into 4 data sets among and within WS, SBH, and corn DDGS. Equations [1] to [4] (Table 5) were the prediction equations for ATTD of TDF in WS, SBH, corn DDGS, and WS + SBH + corn DDGS based on

their $IVDMD_f$ or A. As indicated by the coefficient of prediction (R^2) and P -value, the ATTD of TDF could be predicted by $IVDMD_f$ and A for WS + SBH + corn DDGS ($P < 0.05$) but not for WS, SBH, or corn DDGS. When analyzing results for each ingredient separately, the sample size was too small ($n = 3$) to develop accurate prediction equations because there were a limited number of parameters to be used as predictors. The prediction equations for ATTD of TDF among sources of SBH were not possible because the difference in ATTD of TDF among sources was small. Among sources of WS and corn DDGS, the ATTD of TDF could be predicted from $IVDMD_f$ using Eq. [1] and [3] (Table 5). However, these predictions were not significant ($P > 0.05$) even though the R^2 values were high, because the differences in measured values were small and may have been due to the small sample size. When WS, SBH, and corn DDGS were combined, ATTD of TDF could be predicted using Eq. [4] (Table 5), which contained the asymptotic gas production (A) with relatively high precision ($R^2 = 0.82$, SE = 10.68, C(p) = 3.13). This prediction equation had the least R^2 (0.82) but the greatest differences (5.1 to 45.7%) between predicted and measured ATTD of TDF among the 4 equations.

DISCUSSION

The objective of this experiment was to test the hypothesis that in vivo ATTD of TDF among and within sources of ingredients with high concentrations of IDF can be predicted by an in vitro DM disappearance and gas production procedure. We selected an in vitro model developed by Boisen and Fernández (1997) and modified by Bindelle et al. (2007) for its simplicity and relative frequency of use in previously published papers. In addition, this in vitro model was used previously to predict the ATTD of NDF of rice bran, tofu residue, and water spinach (Dung and Udén, 2002) with relative success. However, none of these procedures were developed to predict ATTD of NDF in the large intestine of pigs fed diets containing ingredients with high concentrations of IDF such as corn DDGS, SBH, and WS, nor have studies been published that have evaluated the accuracy of predicting ATTD of TDF among high-fiber ingredients.

We selected ATTD of TDF because this measurement is a more reliable predictor of the carbohydrate disappearance in the large intestine of monogastric species than ATTD of NDF (Mertens, 2003). Portions of soluble dietary fiber, such as pectins, gums, and glucans, are not recovered in the analysis of NDF (Mertens, 2003). Therefore, estimates of degradation of dietary fiber in the large intestine may not be accurate when using the NDF procedure. Also, most agri-

Table 3. Apparent total tract digestibility (ATTD) of total dietary fiber (TDF), IVDMD, and kinetics of gas production of 3 insoluble dietary fiber ingredients: wheat straw (WS), soybean hulls (SBH), and corn distiller's dried grains with solubles (DDGS)¹

Item	ATTD of TDF, %	IVDMD _h ² %	IVDMD _f ³ %	IVDMD _t ⁴ %	A ⁵	B ⁶	C ⁷
WS	26.7 ^c	13.3 ^c	23.3 ^c	33.5 ^b	121 ^c	21.3 ^{ab}	1.98 ^a
SBH	78.9 ^a	18.9 ^b	84.6 ^a	88.9 ^a	412 ^a	20.0 ^b	2.15 ^a
Corn DDGS	43.0 ^b	53.7 ^a	69.6 ^b	86.1 ^a	317 ^b	22.6 ^a	1.45 ^b
SEM	2.24	0.24	1.83	1.46	11.7	1.03	0.12
<i>P</i> -value	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	<0.01
WS							
1	24.2	13.8 ^A	18.7 ^B	30.0 ^B	100	18.8 ^B	2.03
2	29.4	12.8 ^B	24.3 ^{AB}	33.9 ^{AB}	141	23.3 ^A	1.94
3	26.4	13.4 ^{AB}	26.8 ^A	36.5 ^A	122	21.9 ^{AB}	1.97
SEM	1.75	0.16	1.40	0.94	9.81	0.93	0.09
<i>P</i> -value	0.52	0.05	0.03	0.02	0.19	0.05	0.91
SBH							
4	79.0	17.0 ^C	86.8	92.4 ^x	407	20.2	2.31
5	75.0	20.5 ^A	84.1	88.1 ^{xy}	412	18.7	2.10
6	82.7	19.0 ^B	82.9	86.2 ^y	419	21.0	2.05
SEM	1.49	0.17	1.37	1.03	9.29	0.88	0.08
<i>P</i> -value	0.12	<0.01	0.43	0.06	0.83	0.42	0.39
Corn DDGS							
7	43.1 ^{AB}	52.0 ^B	70.0	86.6	335	26.0 ^A	1.43
8	49.8 ^B	52.1 ^B	71.0	86.1	313	21.4 ^B	1.46
9	36.0 ^A	56.9 ^A	67.7	85.8	305	20.4 ^B	1.47
SEM	1.49	0.19	1.45	1.13	9.46	0.90	0.08
<i>P</i> -value	<0.01	<0.01	0.58	0.96	0.33	<0.01	0.98

^{a-c}Means among ingredients with different superscripts differ ($P \leq 0.05$).

^{A-C}Means within different sources of the same ingredient with different superscripts differ ($P \leq 0.05$).

^{xy}Means within different sources of the same ingredient with different superscripts tended to differ ($0.05 < P < 0.1$).

¹Data were expressed as means. There were 8 observations of each measurement.

²IVDMD_h = in vitro DM disappearance from simulated gastric and small intestinal hydrolysis.

³IVDMD_f = in vitro DM disappearance from simulated large intestine fermentation.

⁴IVDMD_t = in vitro DM disappearance from simulated total tract digestion.

⁵A = asymptotic gas production (mL/g DM substrate).

⁶B = the time after incubation at which half of the asymptotic amount of gas has been formed (h).

⁷C = a constant determining the sharpness of the switching characteristic of the profile.

industrial byproducts, such as corn DDGS, contain a substantially greater proportion of IDF compared with soluble fiber (Serena and Bach Knudsen, 2007). As a result, it is necessary to evaluate the accuracy of the in vitro procedure for estimating ATTD of TDF among feed ingredients with relatively high concentrations of IDF. In addition to solubility, apparent viscosity and composition (e.g., lignin content) also affect the fermentability of fiber (Nyman et al., 1986). Therefore, the in vitro procedure was evaluated using multiple feed ingredients with a diverse content of fiber and chemical composition, but there are no data comparing the accuracy of the in vitro procedure among sources of a specific feed ingredient containing high concentrations of IDF, such as DDGS. Therefore, we selected 3 sources of IDF from ingredients with different chemical composition (WS, SBH, and corn DDGS).

In agreement with our hypothesis, the current in vitro model was useful in predicting the ATTD of TDF among all 3 feed ingredients with relatively high concentrations of IDF. First, the ATTD of TDF in WS and SBH were in agreement with expected values based on measurements of the ATTD of NDF and nonstarch polysaccharides (Chabeauti et al., 1991) as well as the high degree of lignification of WS (Jung et al., 1997). Also, we expected a high (>80%) ATTD of TDF in SBH based on previous observations (Urriola and Stein, 2012) and high ATTD of NDF (Kornegay, 1978, 1981). This high ATTD of TDF and NDF in SBH may be a result of the relatively high solubility of fiber in SBH (6 to 17%; Cole et al., 1999) compared with WS (1 to 2%; Panthapulakkal et al., 2006; Alemdar and Sain, 2008) and corn DDGS (0 to 5%; Urriola et al., 2010). The main soluble fiber component in SBH

Table 4. Correlations of apparent total tract digestibility (ATTD) of total dietary fiber (TDF) with IVDMD and asymptotic gas production in wheat straw (WS), soybean hulls (SBH), and corn distiller's dried grains with solubles (DDGS)¹

Item	Ingredient	ATTD of TDF
IVDMD _f ²	WS	0.29
	SBH	-0.28
	Corn DDGS	0.98
	WS + SBH + corn DDGS	0.86*
A ³	WS	0.99
	SBH	0.59
	Corn DDGS	0.30
	WS + SBH + corn DDGS	0.91*

¹Pearson correlation coefficients (*r*) are reported; there were 3 samples for each ingredient.

²IVDMD_f = in vitro DM disappearance from simulated large intestine fermentation.

³A = asymptotic gas production (mL/g DM substrate).

**P* ≤ 0.05.

is pectin, which is highly fermentable (Snyder and Kwon, 1987; Urriola and Stein, 2012). The values for disappearance of DM during in vitro fermentability using fecal inocula (IVDMD_f) for WS and SBH were in agreement with the in vivo determined ATTD of TDF. Intermediate (>30 and <60%) ATTD of TDF was expected for corn DDGS, and observations from this experiment agree with those previously reported (Urriola et al., 2010; Gutierrez et al., 2014).

Use of in vitro disappearance of DM during the fermentation step (IVDMD_f) appears to be of similar benefit to using the measured asymptotic gas production value (A) to predict ATTD of TDF ($R^2 = 0.86$ vs. $R^2 = 0.91$) among all 3 high-fiber feed ingredients. This may be because most equations developed to predict ATTD of TDF used only IVDMD_f or A as parameters and the combination of IVDMD_f or A did not provide greater precision of prediction. Therefore, there appears to be minimal benefit of providing additional information from the rate of gas production (B) or the shape of the gas production curve (C) in predicting ATTD of TDF. Although in Eq. [2-1] (Table 5; $y = 13.80 + 3.26 \times B$), B was used as a predictor for TDF digestibility of SBH, the *P*-value was not significant. In Eq. [4-1] (Table 5; $y = -48.84 + 0.16 \times A + 28.05 \times C$), the accuracy of prediction was improved by adding C to Eq. [4] (Table 5), resulting in a significant *P*-value. Therefore, B or C may be used in some of these prediction equations, but A or IVDMD_f were the main predictor variables for TDF digestibility. These observations suggest that a portion of dietary fiber in all 3 feed ingredients remains indigestible, regardless of the kinetics of degradation of dietary fiber, and represents the recalcitrant

portion of dietary fiber (de Vries et al., 2013). The kinetics of degradation of dietary fiber along the gastrointestinal tract of pigs may have important implications due to the interaction of dietary fiber with digestibility of other nutrients such as AA and lipids, but there are no data to confirm this hypothesis.

Despite good agreement between the in vivo and in vitro observations among the feed ingredients evaluated in this study, the accuracy of prediction was different among the 3 sources for each of these 3 ingredients. Differences in the ATTD of TDF among sources of WS or SBH were relatively small. This is likely a result of the small range in TDF content among the 3 sources of each of these 2 ingredients. There are 2 reasons that may explain this observation. Ingredients such as WS and SBH represent 2 examples of ingredients with low (<30%) or high (>80%) ATTD of TDF, respectively, and it appears that there is little variability among sources of these ingredients. In fact, the magnitude of ATTD of TDF and IVDMD_f was similar and suggests that WS had low degradability under both evaluation conditions (in vitro and in vivo) whereas SBH had high degradability using both the in vivo and in vitro methods. Likewise, WS was not processed whereas processing conditions among soybean processing plants may be similar.

In contrast, the range in ATTD of TDF among sources of corn DDGS (36.0 to 49.8%) was greater than the range between WS and SBH. In fact, a larger range in ATTD of TDF (29.3 to 57.0%) has been reported (Urriola et al., 2010; Gutierrez et al., 2014). Therefore, it appears that many factors affect ATTD of TDF among different sources of corn DDGS (with intermediate ATTD of TDF), such as source of corn, differences in processing methods used in various ethanol plants, or interactions among nutrients. We observed a correlation between particle size of corn DDGS and ATTD of TDF ($R^2 = 0.78$), which is consistent with results from a previous experiment that measured ATTD of GE (Liu et al., 2012). Other conditions such as retention time or incubation time also appear to affect degradability of dietary fiber in corn DDGS but not in WS or SBH. The difference between in vitro DM fermented (69.6%) and in vivo ATTD of TDF (43.0%) was greater in DDGS than observed in WS or SBH. This observation agrees with previous studies that determined the ATTD of TDF among breeds of pigs, where longer retention time increased the ATTD of TDF in DDGS but it did not affect ATTD of TDF of soluble dietary fiber, such as pectins (Udén and Van Soest, 1982; Urriola and Stein, 2012).

Limitations in accuracy of the in vitro procedure to predict ATTD of TDF may be due to multiple factors. Feed ingredients such as DDGS, compared with purified sources of fiber (e.g., pectin, inulin, maltodextrin), contain mixed proportions of fiber and nutrients

Table 5. Prediction of the apparent total tract digestibility (ATTD) of total dietary fiber (TDF) of wheat straw (WS), soybean hulls (SBH), and corn distiller's dried grains with solubles (DDGS) from in vitro DM disappearance from simulated large intestine fermentation (IVDMD_f) and asymptotic gas production (A; mL/g DM substrate)

ATTD of TDF	Equation ¹	P-value	R ²	SE ²	C(p)	Range of differences between predicted and measured ATTD of TDF, %
WS	[1] $y = 11.68 + 0.12 \times A$	0.08	0.98	0.46	NA ³	0.4–2.6
SBH	[2] NA ⁴	NA	NA	NA	NA	NA
	[2-1] $y = 13.80 + 3.26 \times B^5$	0.10	0.97	0.82	NA	0.3–0.8
Corn DDGS	[3] $y = -199.83 + 3.49 \times \text{IVDMD}_f$	0.12	0.96	1.87	NA	0.3–4.2
WS + SBH + corn DDGS	[4] $y = -2.82 + 0.16 \times A$	<0.01	0.82	10.68	3.13	32.8–45.7 for WS, 15.9–22.3 for SBH, and 5.1–27.6 for corn DDGS
	[4-1] $y = -48.84 + 0.16 \times A + 28.05 \times C^6$	<0.01	0.97	4.79	1.10	0.7–4.2 for WS, 1.4–8.4 for SBH, and 4.0–15.4 for corn DDGS

¹ There were 3 samples of each ingredient (WS, SBH, and corn DDGS), and there was no Mallow's Cp in Eq. [3] due to limited number of samples.

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

³ NA = not applicable because the stepwise selection resulted in no variable being significant at $P < 0.05$.

⁴ Stepwise selection did not include any factors ($P > 0.10$). Therefore, factors were forced in the subsequent equation.

⁵ B = the time after incubation at which half of the asymptotic amount of gas has been formed (h).

⁶ C = a constant determining the sharpness of the switching characteristic of the profile.

(protein, lipids, and starch) that are interconnected in the structure of the plant cell walls (Grundy et al., 2016). Therefore, degradation of DM in the in vitro fermentation procedure may be composed of disappearance of protein and starch. When the ATTD of TDF is calculated, the TDF procedure corrects for CP (Prosky et al., 1988) in the diet and feces. Consequently, there is disagreement between ATTD of TDF values and in vitro disappearance of DM or gas production. Furthermore, ATTD of TDF is not entirely the result of disappearance of fiber along the gastrointestinal tract of pigs but can also be a result of disappearance of nondietary material (Montoya et al., 2015b, 2016). As a result, secretion of mucin and synthesis of microbial cell walls present in the feces of pigs contribute to the analytical error in determining TDF (Montoya et al., 2016). These endogenous factors may also contribute to energy and short-chain fatty acid production in the large intestine of pigs (Montoya et al., 2015a) but are not accounted for in the in vitro system. Consequently, there is disagreement between in vitro and in vivo values of fiber disappearance. Finally, dietary fiber has also been shown to modify the digesta passage rate and digestibility of nutrients (Wilfart et al., 2007). However, current in vitro digestibility systems are static (2 h for step 1, 4 h for step 2, and 72 h for step 3) and do not model the impact of dietary components based on digesta transit time, which results in a discrepancy between in vivo and in vitro digestibility values (Williams et al., 2005). These discrepancies between in vitro procedures, TDF analytical procedures, and the impact of fiber on other gastrointestinal physiological functions offer an opportunity for research and improvement of accuracy.

In conclusion, the results of this study indicate that it is possible to predict ATTD of TDF through IVDMD_f or the asymptotic gas production value (A) for high-

fiber ingredients with high concentrations of IDF such as WS, SBH, and corn DDGS. Developing prediction equations for each ingredient was more accurate and practical than combining data from different high-fiber ingredients. Using static values for ATTD of TDF among sources of high-fiber feed ingredients with low or high ATTD of TDF such as WS and SBH, respectively, seems reasonable based on the appearance that there is minimal variability in nutrient and fiber composition among sources of these ingredients. However, large variability among sources of feed ingredients with intermediate ATTD of TDF, such as corn DDGS, justify the use of in vitro assays. For corn DDGS, using dynamic prediction values based on in vitro digestibility of DM or asymptotic gas production will improve nutritionists' ability to enhance caloric and nutritional efficiency when formulating swine diets using DDGS.

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