RESEARCH REPORT
ANIMAL SCIENCE

Title: Evaluation of an *in vitro* digestibility procedure for dietary fiber in high fiber feed ingredients fed to growing pigs – NPB #13-014 revised

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Industry Summary: Use of high fiber feed ingredients in commercial swine diets is a common strategy to decrease feed costs during times of high corn and soybean meal prices. However, the addition of high fiber feed ingredients to diets consistently results in a decrease in nutrient digestibility and subsequent decrease in energy and nutrient utilization efficiency. This reduction in feed efficiency is unpredictable using current methods to analyze dietary fiber such as neutral detergent fiber (NDF) or total dietary fiber (TDF). Therefore, the objective of this project was to develop and validate a "nutritional tool" to measure *in vitro* digestibility of fiber among high fiber feed ingredients, as well as measure changes in gut physiology and function of growing pigs fed diets with high concentration of different types of dietary fiber.

The most relevant high fiber feed ingredient used in North America are distillers dried grains with solubles (DDGS), which dietary fiber is highly insoluble. Therefore, for comparison we selected wheat straw (WS) and soybean hulls (SBH) as 2 other feed ingredients with high concentration of insoluble dietary fiber. We utilized an *in vitro* dry matter digestibility (IVDMD) assay that mimics gastric and small intestine hydrolysis ($IVDMD_h$) and large intestine fermentation ($IVDMD_f$) of pigs.

We observed differences in $IVDMD_h$ among WS (14.5%), SBH (19.7%), and DDGS (55.8%).We also observed differences in $IVDMD_f$ among WS (41.7%), SBH (68.5%), and DDGS (52.7%). These differences in $IVDMD_f$ were also in agreement with our data showing greater asymptotic gas production (A, mL/g DM substrate) for SBH (383.9) than DDGS (238.1) or WS (115.6). In addition to the differences among feed ingredients, we also observed a wide range of $IVDMD_h$ (45.3 to 63.2%) and $IVDMD_f$ (41.4 to 64.2%) among sources of corn DDGS.

To further test this technique, we fed growing pigs with 3 sources of WS, SBH, and DDGS with predicted low, medium, and high $IVDMD_f$. We observed that apparent total tract digestibility (ATTD) of TDF was least in WS (26.7%),

intermediate for DDGS (43.0%), and greatest in SBH (78.9%). These observations were in agreement with predictions from the *in vitro* gas production results, and suggest that this *in vitro* gas production technique is a reliable procedure to measure ATTD of TDF.

An additional objective was to measure the impact of insoluble dietary fiber on gut function (digestion of nutrients), morphology (cell proliferation), and immunity. We observed that feeding feed ingredients with insoluble dietary fiber (WS, SBH, and DDGS) to growing pigs modified morphology of the gut, the composition of the cells, and the balance among immune cells. In pigs fed the WS diet, the area of the gut occupied by mucus producing (goblet) cells was increased in comparison with pigs fed DDGS and SBH diets. However, DDGS caused greater proliferation of all cells. This greater proliferation index is consistent with greater number of enterocytes observed from measuring the fatty acid binding protein (FABP) marker. Finally, feeding all 3 sources of insoluble dietary fiber (WS, DDGS, and SBH) to growing pigs decrease the ratio between pro-inflammatory markers (IL10) and anti-inflammatory markers (IL-12) compared with corn-soybean meal diets.

In conclusion, we observed that large differences exist in ATTD of TDF among sources high fiber feed ingredients. Fortunately, these differences in ATTD of TDF among high fiber feed ingredients can be reasonably predicted *in vitro* by simulation of gastric and small intestine hydrolysis and large intestine fermentation and gas production. Additionally, dietary fiber from these feed ingredients affects gut physiology and function in different fashion that it is not predicted by the concentration of TDF or NDF in the diet. Therefore, more detailed information on the carbohydrate structure and interaction with the gut epithelium is necessary to improve utilization of high fiber feed ingredients fed to growing pigs.

Key Findings:

- Digestibility of dietary fiber affects the concentration of metabolizable energy among sources of corn DDGS
- *In vitro* procedures can be used to measure digestibility and fermentability of fiber among sources of corn DDGS
- Our results are the first to show that there are differences in goblet cells and mucin production among pigs fed diets with insoluble dietary fiber, which suggests that the effect of dietary fiber on gut physiology and function is not only related to the concentration of fiber in the diet (TDF or NDF), but also to the botanical origin of the dietary fiber.
- Feeding feed ingredients with insoluble dietary fiber (WS, SBH, and DDGS) to growing pigs modified morphology of the gut, the composition of the cells, and the balance among immune cells, which will guide future experiments to develop feed formulation and feeding strategies to optimize fiber use in swine diets.

Keywords: dietary fiber, digestibility, energy, gas production, gut physiology, *in vitro*, pig

Scientific abstract: Dietary fiber from distillers dried grains with solubles (DDGS) decreases feed efficiency and nutrient utilization in growing pigs. Current methods that measure the concentration of dietary fiber in feed ingredients such as neutral detergent fiber (NDF) and total dietary fiber (TDF) do not accurately predict utilization of dietary fiber in high fiber feed ingredients by growing pigs. Therefore, the objective of this project was to develop and validate an *in vitro* "nutritional tool" to measure digestibility of fiber among high fiber feed ingredients. A second objective was to measure gut morphology and function of growing pigs fed diets with high concentration of dietary fiber. Wheat straw (WS), soybean hulls (SBH), and corn dried distillers grains with solubles (DDGS) were selected for their high concentration of insoluble dietary fiber and their inherent differences in apparent total tract digestibility (ATTD) of dietary fiber. We selected 16 sources of all 3 ingredients (WS, DDGS, and SBH) to measure *in vitro* dry matter digestibility (IVDMD) after stomach and small intestine hydrolysis ($IVDMD_h$), and after inoculating the hydrolysis residues with fecal inocula of pigs fed corn-soybean meal diets for a fermentation of 72 hours ($IVDMD_f$). Kinetics of gas production were calculated using a monophasic model, where the amount of gas produced during fermentation (A: mL/g DM incubated), time to half asymptote (B: h), and a constant for the sharpness of gas production (C) were the main response variables. The $IVDMD_h$ for WS (14.5%) was less ($P < 0.01$) than that of SBH (19.7%), and SBH was less ($P < 0.01$) than that for corn DDGS (55.8%). The $IVDMD_f$ of WS (41.7%) was less than DDGS (52.7%), which was less ($P < 0.01$) than $IVDMD_f$ for SBH (68.5%). These differences in $IVDMD_f$ were also in agreement with greater amount of gas produced for SBH (383.9 mL) compared with DDGS (238.1 mL) and WS (115.6 mL). In addition to these differences among feed ingredients, we also observed a wide range ($P < 0.05$) of $IVDMD_h$ (45.3 to 63.2%) and $IVDMD_f$ (41.4 to 64.2%) among sources of corn DDGS. The $IVDMD_h$ had a strong negative correlation with TDF ($P < 0.01$, r = -0.98), NDF ($P < 0.01$, r = -0.98), and ADF ($P < 0.01$, r = -0.99); but a mild positive correlation with hemicellulose ($P < 0.01$, r = 0.36). The $IVDMD_f$ was not correlated with TDF ($P = 0.62$), NDF ($P = 0.44$), and ADF ($P = 0.99$), but it was negatively correlated with hemicellulose ($P < 0.01$, r = -0.49). For kinetics of gas production parameters, asymptotic gas production (A) was negatively correlated to hemicellulose concentration ($P < 0.01$, r = -0.55) and positively correlated to $IVDMD_f$ ($P < 0.01$, r = 0.87). Time to reach half asymptotic gas production (B) was positively correlated to TDF ($P < 0.01$, r = 0.43), NDF ($P = 0.02$, r = 0.35), ADF ($P < 0.01$, r = 0.48), and $IVDMD_f$ ($P < 0.01$, r = 0.59), but negatively correlated with hemicellulose ($P < 0.01$, r = -0.70) and $IVDMD_h$ ($P < 0.01$, r = -0.45). The C value was negatively correlated with hemicellulose ($P < 0.01$, r = -0.46) and positively correlated with $IVDMD_f$ ($P < 0.01$, r = 0.84).

In a second experiment, we measured the impact of insoluble dietary fiber on gut function and morphology. There were differences ($P < 0.01$) in ATTD of DM, GE, CP, EE, and TDF among the 3 types of diets based on WS, SBH, and DDGS. The diets containing SBH had greater ($P < 0.01$) ATTD of DM, GE, and TDF than the DDGS diets, which were greater ($P < 0.01$) than observed with the WS diets. The DDGS diets had greater ($P < 0.01$) ATTD of CP than the WS diets, which was greater ($P < 0.01$) than the SBH diets. The WS diets had greater ($P < 0.01$) ATTD of AEE than SBH diets, which was greater ($P < 0.01$) than DDGS diets. In conclusion, these data suggest that among sources of corn DDGS, large differences exist in ATTD of TDF. However, these differences in ATTD of TDF among high fiber feed ingredients can be reasonably predicted using this *in vitro* procedure which simulates gastric and small intestine hydrolysis, as well as large intestine fermentation and gas production. Additionally, dietary fiber from these feed ingredients affects gut physiology and function in different fashion that it is not predicted by the concentration of TDF or NDF in the diet. Therefore, more detailed information on the carbohydrate structure and interaction with gut epithelium is necessary to improve utilization of high fiber feed ingredients fed to growing pigs.

Introduction

High fiber feed ingredients, such as distillers dried grains with solubles (DDGS), are an economical source of nutrients and have been widely used in swine diets (Kerr and Shurson, 2013). Corn DDGS is used to partially replace corn and soybean meal in commercial swine diets, but its highly variable concentration of digestible (DE), metabolizable (ME), and net energy (NE) for swine (Noblet and Perez 1993; Noblet et al., 1994) is a challenge for using accurate energy loading values in feed formulation. This low and variable concentration of NE is the result of several factors. The apparent total tract digestibility (ATTD) of dietary fiber is less compared with other energy yielding nutrients (i.e starch, protein, fat) and dietary fiber contributes relatively less energy than these substrates (Noblet et al., 1994; Noblet and van Milgen, 2004). In addition, dietary fiber modifies gut and metabolic functions (such as transit time, endogenous losses, and maintenance energy) that reduce efficiency of utilization of ME (Noblet and van Milgen 2004).

Nutritional tools such as *in vitro* digestion and fermentation systems that simulate gastrointestinal processes of pigs are useful methods for rapid and inexpensive measurement of the degradation of feed ingredients and their contributions to DE (Boisen and Fernandez, 1997). There are numerous modifications that have been used with the *in vitro* digestibility of dry matter technique (IVDMD). For example, some researchers have used commercially available enzymes (Viscozyme; Boisen and Fernandez, 1997) while others have used microbial inoculum (Williams et al., 2005; Bindelle et al., 2007a,b) to simulate large intestine fermentation processes. The amount of energy and nutrients that disappear *in vivo* during gastrointestinal digestion has been estimated using IVDMD coupled with the gas production technique in swine for hulless and hulled barley (Jha et al., 2011a,b) as well as oats , wheat bran, flax seed meal, sugar beet pulp, pea hulls, and DDGS (Jha and Leterme, 2012; Jha et al., 2015).

The concentration of ME among sources of corn DDGS varies greatly (Pedersen et al., 2007; Anderson et al., 2012; Kerr et al., 2013). The reason for this variability is partly due to variable concentrations of dietary fiber and variable digestibility of dietary fiber among sources of corn DDGS (Urriola et al., 2010). Although IVDMD has been used to measure *in vitro* nutrient utilization of several ingredient, variability in IVDMD and gas production among sources of corn DDGS have not determined.

The dietary fiber contained in DDGS is composed mainly on insoluble arabinoxylans, cellulose, and lignin (Jaworski, 2013; Pedersen et al., 2014). The effects of insoluble dietary fiber on gut physiology and digestibility of energy yielding nutrients are different from those of soluble dietary fiber (e.g. pectins, gums). Fermentability and solubility of dietary fiber are 2 properties that are generally related, but in most cases, are also 2 independent properties. Wheat straw, soybean hulls, and DDGS are all primarily insoluble dietary fiber sources. However, the ATTD of these 3 ingredients is different. Therefore, energy contribution from these 3 sources of insoluble fiber is different and is poorly predicted solely on the basis of the concentration of total dietary fiber (TDF) or neutral detergent fiber (NDF). The hypothesis of this study was that different fibrous ingredients with variable types of dietary fiber and chemical composition have different patterns of enzymatic hydrolysis and large intestine fermentation patterns.

Dietary fiber affects gut morphology and function. The most observed change when feeding dietary fiber to pigs is an increase in gut fill and gut mass (Kass et al. 1980; Agyekum et al., 2011; Asmus et al., 2014). Weight of small intestine, cecum, and colon of pigs fed a diet with 43.95% NDF from alfalfa meal increased by 30, 22, and 44% (Kass et al., 1980). This increase is due to gut fill, but also epithelial cell proliferation and differentiation with an increase in

number of goblet cells (Ito et al., 2009) and an increase in villus height and crypt depth (Serena et al., 2008). It is possible that dietary fiber modulates gastrointestinal epithelial proliferation and differentiation that may favor a secretory lineage of cells at the expense of absorptive cells (enterocytes). However, most of the experiments measuring changes in gut morphology or function have being limited to measuring gut fill or villi:crypt responses, which provide minimal mechanistic information. In addition, these experiments have utilized sources of dietary fiber for which solubility and the extent of fermentation were confounded, and subsequently it is difficult to identify the portion of dietary fiber responsible for these changes.

Objectives

The objectives of this study were to measure *in vitro* hydrolysis and *in vitro* fermentation characteristics of feed ingredients with insoluble dietary fiber including wheat straw (WS), soybean hulls (SBH), and corn DDGS and to estimate the variability in IVDMD and gas production as well as the concentration of net energy supplied from dietary fiber. In addition, we compared results of the *in vitro* digestibility assays with *in vivo* measurements of apparent total tract digestibility of dietary fiber, and measured the impact of diets with insoluble dietary fiber on gut morphology and function to estimate differential impact of feeding these high fiber feed ingredients.

Materials & Methods

To address these objectives, this study was divided into 3 major components. The first component was to measure *in vitro* DM digestibility and gas production of various sources of corn DDGS, wheat straw, and soybean hulls. The second component was to conduct an *in vivo* experiment to measure the effect of insoluble dietary fiber with variable extent of fermentation on apparent total tract digestibility of nutrients. The final component was to measure gut physiology changes in pigs fed diets containing these 3 sources of dietary fiber.

Experiment 1

Experimental Samples

A total of 48 samples were collected from various sources, including 16 WS samples, 16 SBH samples, and 16 corn DDGS samples during May to July 2013. The WS samples were obtained from University of Minnesota Beef Barn (St. Paul, MN), Cargill Animal Nutrition (Elk River, MN), Dairyland Laboratories, Inc. (Arcadia, WI), and University of Minnesota West Central Research and Outreach Center (Morris, MN). The SBH samples were obtained from Nutrena (Minneapolis, MN), Archer Daniels Midland (Mankato, MN, Mexico, MO, Quincy, IL, Des Moines, IA and Fosteria, OH), AGP Ag Processing Inc. (Dawson, MN), Bunge (Council Bluffs, IA and Decatur, IN), and Consolidated Grain & Barge Soybean Processing (Mount Vernon, IN). Corn DDGS samples were obtained from previous study (Kerr et al., 2013) and Highwater Ethanol (Lamberton, MN). The 3 ingredients were chosen based on their differences in chemical composition (Table 1) and dietary fiber solubility and fermentability. We considered each sample within each ingredient as one source of the ingredient.

Enzymatic Hydrolysis

All the samples were ground to pass through a 1 mm mesh screen with Wiley No. 4 Laboratory Mill (Arthur H. Thomas, Philadelphia, PA). After grinding, all samples were subjected to a 2-step enzymatic hydrolysis *in vitro* procedure to mimic digestion of nutrients in the gastric and small intestine using pepsin and pancreatin solutions (Boisen and Fernández, 1997). Briefly, 2 g of each sample was weighed into each 500 mL conical flask, and then all the flasks with samples were placed in a 39° C water bath to be heated. Each sample was treated with a phosphate buffer solution (100 mL, 0.1 *M*, pH = 6.0) and a HCl solution (40 mL, 0.2 *M*, pH = 2.0). The pH was adjusted to 2.0 by adding 1 *M* HCl or 1 *M* NaOH. Two milliliters of a chloramphenicol (C0378; Sigma-Aldrich Corp., St. Louis, MO) solution (0.5 g/100 mL ethanol) were added to prevent bacterial growth during hydrolysis. Then each sample was treated with 4 mL fresh porcine pepsin (P7000, 421 units/mg solids; Sigma-Aldrich Corp.) solution (100 mg/mL 0.2 *M* HCl) at 39° C and incubated in a water bath for 2 h. During heating, all bottles were shaken gently for 5 seconds by hand every 15 min. Afterward, 40 mL phosphate buffer (0.2 *M*, pH = 6.8) and 20 mL of 0.6 *M* NaOH were added into the solution. The pH was adjusted to 6.8 with 1 *M* HCl or 1 *M* NaOH. Four mL of fresh porcine pancreatin (P1750, 4 × USP specifications; Sigma-Aldrich Corp.) solution (100 mg/mL 0.2 *M* phosphate buffer) was added to each flask and hydrolysis was continued for 4 h using the same conditions as used for the pepsin hydrolysis.

After enzymatic hydrolysis, residues were collected by filtration through a filter paper (Diameter 15 cm; pore size 40 µm; VWR International, Radnor, PA), 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 enzymatic hydrolysis was repeated 4 to 7 times, depending on the digestibility of each sample, to obtain sufficient sample residue for the subsequent *in vitro* fermentation. The hydrolyzed residues were pooled for each sample, and pooled samples were used for *in vitro* fermentation determinations.

In Vitro Fermentation

The rate of *in vitro* fermentation of the hydrolyzed residues was assessed by a cumulative gas production technique (Bindelle et al., 2007a,b; Bindelle et al., 2009; Jha et al., 2011a,b; Jha and Leterme, 2012; Jha et al., 2015). Briefly, each hydrolyzed residue (200 mg) was weighed and incubated at 39° C in a 125 mL serum bottle with 30 mL buffer solution, including macro and microminerals (Menke and Steingass, 1988), and a fecal inoculum. Five growing pigs (19 to 21 wk age, 68.5-83.4 kg, Hampshire × Yorkshire) from the swine herd located at the University of Minnesota Saint Paul Campus Swine Research Facility were used as donors of fecal inoculum. Pigs had the same genetic background and were fed a standard commercial diet without antibiotics (Maverick Nutrition Inc., Austin, MN). Fecal samples were randomly collected from 3 out of the 5 pigs immediately after pigs defecated. Immediately after collecting feces, samples were placed it in a Ziploc bag, air was removed and the bags were tightly sealed. Bags were then placed in an incubation container with 39° C water and delivered to the laboratory. The process from the initial time of feces collection until feces were delivered to the laboratory was 30 to 60 min. The inoculum was prepared by diluting blended feces in a buffer solution, followed by filtering through folded cheesecloth (Fig. 1). The final inoculum concentration was 0.05 g feces per mL of buffer. Each of the 30 mL inoculum aliquots was transferred into bottles containing the hydrolyzed residues, and bottles were sealed with a rubber stopper and placed in the incubator. Throughout the whole process, we attempted to maintain an anaerobic environment by adding CO_2 gas to the inoculum preparation until the incubation, as well as adding reducing elements to the buffer solution.

The gas produced during fermentation was measured at 0, 2, 5, 8, 12, 16, 20, 24, 30, 36, 48 and 72 h through an inverted 25 mL burette with its stopcock end attached toa vacuum and its open end submerged into a 39° C water bath (Fig. 2). Before assembling the burette apparatus, the unmarked headspace volume of the burette was determined. To measure gas volume of each time point, the inverted burette was filled with water to remove the air. Then, the serum bottle was quickly transferred from the incubating water bath to the water bath with the burette, one attached 20 gauge needle was inserted through the stopper, the valve was opened to release all of the gas into the burette, and the volume displaced by the gas produced in the bottle using burette calibration marks was immediately recorded. Once the measurement was recorded, the bottles were immediately transferred back into the incubating water bath. After *in vitro* fermentation, residues were collected by filtration and then dried and weighed as described for the hydrolyzed residues.

Physicochemical Analysis

All the samples were analyzed in University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Chemical analyses (Table 1) were performed according to standard procedures (AOAC 2006) with specific methods as follows: DM (method 930.15), ADF (method 973.18), NDF (method 2002.04), and total dietary fiber (TDF) (method 991.43).

Calculations

Hemicellulose

Hemicellulose, % = NDF, % - ADF, % [1]

Total Feces Needed to Prepare the Inoculum Per Run

Feces, g = 30 mL × No. of samples × No. of replicates per run × 0.05 g/mL [2]

Gas Volume Released from Each Time Point

V, mL = $V_h + (25 - V_r)$, [3]

where, V_h was the volume of the burette headspace, V_r was the reading volume record, $V_h \leq V \leq V_h + 25$;

when $0 < V < V_h$, $V = V_r$, V_r was measured similar to the headspace volume; when $V > V_h + 25$, closed the valve before the gas went beyond the open end of the burette, recorded Vr_1, and then repeated the procedure and took Vr_2, $V = Vr_1 + Vr_2$.

(Based on our experience, V was rarely beyond the burette capacity.)

Gastric and Small Intestine Hydrolysis Disappearance

The $IVDMD_h$ during the pepsin and pancreatin hydrolysis 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

[4]

Large Intestine Fermentation Disappearance

The $IVDMD_f$ during the fecal microbial fermentation was calculated as follows: $IVDMD_f$, % = [(dry weight of hydrolyzed residues – dry weight of the residues after fermentation)/ dry weight of hydrolyzed residues] × 100

[5]

Apparent Total Tract Disappearance

$IVDMD_t$, % = $[1 - (1 - IVDMD_h/100) \times (1 - IVDMD_f/100)] \times 100$ [6]

Kinetics of Gas Production

Gas accumulation curves recorded during the 72 h of fermentation were modified according to monophasic model from Groot et al., (1996):

$$G = A / (1 + (B^C/t^C)), \quad [7]$$

where G (ml/g DM substrate) denotes the amount of gas produced per gram of dry matter 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 characteristics of the profile.

Volatile Fatty Acid and Energy Production of corn DDGS

$$\text{VFA production, mmol} = (A/200) \times VFA_{r,} \text{ mmol}, \quad [8]$$

where, VFAr was referenced VFA production (Jha et al., 2015), acetate is 3.92, propionate is 1.61, and butyrate is 0.57, A + P + B = 3.92 + 1.61 + 0.57 = 6.1

Total energy production, kcal = VFA production, mmol × energy production, cal/mmole VFA; [9]

raw DDGS substrate, cal/g DM = total energy production, kcal × $IVDMD_h$ [10]

Statistical Analyses

The kinetics of gas production parameters were modeled using PROC NLIN procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC). The IVDMD and modeled gas production kinetic parameters were analyzed using the PROC MIXED procedure of SAS (Version 9.3; SAS Inst. Inc.) with the ingredients (n = 3), and sources (n = 16) nested under each ingredient as fixed factors, and batch as a random factor, using the following linear additive mode:

$$Y_{ijk} = \mu + \tau_i + \alpha_{j(i)} + \beta_k + \varepsilon_{ijk}, \quad [11]$$

where Y is the parameter to be tested (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, $\alpha_{j(i)}$ is effect of the j^{th} (j = 1, 2, 3,……,16) sources nested under each ingredient, β_k is effect of batch (k = 6 for $IVDMD_h$, k = 3 for $IVDMD_f$, and k = 3 for A, B and C), ε_{ijk} = experiment error. Least Square Means differences of $IVDMD_h$ and $IVDMD_f$ among different ingredients and different sources nested under each ingredient were adjusted by Tukey, and $P < 0.05$ was used to denote significant differences. The $IVDMD_t$ was not statistically compared, because $IVDMD_f$ values were calculated from pooled replicates of the same sample after enzymatic hydrolysis. Correlations among sample chemical composition, IVDMD and kinetics of gas production were analyzed using PROC CORR procedure in SAS (Version 9.3; SAS Inst. Inc.).

Experiment 2

The specific objective of this experiment was to measure the impact of insoluble dietary fiber from WS, SBH, and DDGS on nutrient digestibility and gut morphology and physiology. All experimental procedures involving animals were approved by the University of Minnesota Institutional Animal Care and Use Committee and were conducted at the University of Minnesota Southern Research and Outreach Center (Waseca, MN).

Sample Collection and Diet Formulation

Sources of each high fiber feed ingredient (i.e., WS, SBH, and DDGS) were collected from 3 locations (Table 2.2). Sources of WS were collected at the University of Minnesota St. Paul Campus Beef Barn (St. Paul, MN), UMore Park (Rosemount, MN), and Southern Research and Outreach Center (Waseca, MN). Sources of SBH were obtained from

Archer Daniels Midland (Mexico, MO; Des Moines, IA; Valdosta, GA). Sources of DDGS were obtained from Heron Lake BioEnergy, LLC (Heron Lake, MN), Big River Resources (Dyersville, IA), and Commonwealth Agri Energy (Hopkinsville, KY).

Nine test diets were formulated using 9 test ingredients, and WS, SBH, and DDGS were the only sources of dietary fiber supplied to the experimental diets. The concentration of NDF was analyzed in the test ingredients and utilized to establish the dietary inclusion rate of each test ingredient to provide 17% NDF in all diets. We chose this NDF concentration because it is the typical level of NDF that is obtained when 30% DDGS is added to corn and soybean meal diets. Additional ingredients that were free of dietary fiber were added to balance amino acids and phosphorus content of the diets. Diets were formulated to meet the standardized ileal digestible requirements for amino acids and standardized total tract digestible phosphorus for growing pigs (NRC, 2012). Titanium dioxide (TiO2) was added (0.5%) as an indigestible marker.

In Vivo Animal Experiment

Thirty four growing barrows and 2 gilts (BW 84 ± 7 kg, Large White × Danish Landrace) were housed individually in metabolism cages and allotted to 4 blocks with 9 pigs in each block. The experiment used a changeover design, where 9 growing pigs from each block were fed the 9 experimental diets in 2 consecutive 13-d periods. Each period consisted of 10-d for adaptation to the diets and 3-d for sample collection. In each collection period, feces and urine of each pig were collected.

Pigs were provided feed twice daily (0800 and 1600 h) an amount that was equivalent to 2.5% of their respective body weight. Water was available ad libitum in nipple drinkers located at the front of the metabolism cage. Pigs were weighed at the beginning and end of each period, before the morning meal. Feces and urine from each pig were collected separately twice daily at 0800 and 1600 h. A screen and funnel were placed under the cage to separate feces from urine, and a bucket containing 30 mL 6 N HCl was used to collect urine under the funnel. Hydrochloric acid was added to the urine to prevent N losses by evaporation of ammonia. About 200 g of feces per day were collected in sealable plastic bags and kept frozen 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 storage and shipment to the analytical laboratory. Urine weight and volume were recorded. A total of 5% volume of each urine collection was sampled for each period and were stored at -20˚ C for further analysis.

In Vitro Dry Matter Disappearance (IVDMD) and Gas Production

Enzymatic hydrolysis and *in vitro* fermentation followed the same procedures utilized in Exp. 1. The main difference between Exp. 1 and Exp. 2, was that feces from pigs fed each of the 9 experimental diets were collected on the morning of the *in vitro* assay and used for the fermentation and gas production measurements.

Physicochemical Analysis

All ingredients, diets, feces, and urine samples were analyzed in Midwest Laboratories (Omaha, NE). The analysis methods were as follows: DM (method 930.15, AOAC 2006), GE (ASTM D 5865-13), CP (method 992.15, AOAC 2006), ether extract with acid hydrolysis (AEE) (method 922.06, AOAC 2006), ADF (Ankom Technology), NDF (Ankom Technology), total dietary fiber (TDF) (method 991.43), lignin (method 973.18, AOAC 2006), titanium (WDXRF), bulk density (USP <616> method I), viscosity (Perten, AACC international).

Calculations

Hemicellulose. Hemicellulose, % = NDF, % - ADF, % [1]

Cellulose. Cellulose, % = ADF, % - Lignin, % [2]

Apparent Total Tract Digestibility of the Diet Nutrients. ATTD, % = [(Nutrient in ingredient/TiO_2 in ingredient - Nutrient in feces/ TiO_2 in feces)/ (Nutrient in ingredient/ TiO_2 in ingredient)] × 100 [3]

Digestible Energy of Diets. DE, kcal/kg = GE, kcal/kg × ATTD of GE, % [4]

Metabolizable Energy of the Diets. ME, kcal/kg = DE, kcal/kg – Total energy in urine, kcal/kg [5]

Nitrogen retention, % = (Nitrogen intake, g/d – nitrogen output in feces, g/d – nitrogen output in urine, g/d)/(nitrogen intake, g/d)

Total Feces Needed to Prepare the Inoculum Per Run. Feces, g = 30 mL × No. of samples × No. of replicates per run × 0.05 g/mL [6]

***In vitro* dry matter disappearance and kinetics of gas production** *In vitro* gastric and small intestine hydrolysis disappearance, large intestine fermentation disappearance, apparent total tract *in vitro* disappearance, and kinetics of gas production were calculated using similar procedures as in Exp. 1.

Statistical Analysis

The kinetics of gas production parameters were modeled using PROC NLIN procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC). The ATTD of nutrients, energy values, nitrogen retention, harvest characteristics, IVDMD, and modeled gas production kinetic parameters were analyzed using the PROC MIXED procedure of SAS (Version 9.3; SAS Inst. Inc.) with the ingredients (n = 3), and sources (n = 3) nested under each ingredient as fixed factors and batch as a random factor, using the following linear additive mode:

$$Y_{ijk} = \mu + \tau_i + \alpha_{j(i)} + \beta_k + \varepsilon_{ijk}, \quad [12]$$

where Y is the parameter to be tested (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, $\alpha_{j(i)}$ is effect of the j^{th} (j = 1, 2, 3) sources nested under each ingredient, β_k is effect of replicates, ε_{ijk} = experiment error. Least Square Means differences of $IVDMD_h$, and $IVDMD_f$ among different ingredients and different sources nested under each ingredient were adjusted by Tukey, and $P < 0.05$ was used to denote significant differences. The $IVDMD_t$ was not statistically compared, because $IVDMD_f$ values were calculated from pooled replicates of the same sample after enzymatic hydrolysis. PROC REG, stepwise regression was used to develop prediction equations for ATTD of energy and nutrients, as well as DE and ME content of the diets. The variables with $P \leq 0.15$ were retained in the model. The r^2, the SE of the estimate, and the Mallows statistic [C(p)] were used to define best fit equation.

Volatile Fatty Acid and Energy Production from corn DDGS

Values of VFA production during *in vitro* fermentation were obtained from Jha et al., (2015). The production of acetate, propionate, and butyrate were utilized to calculate energy from VFA (Table 1.7).

Results:

Experiment 1

In Vitro Dry Matter Disappearance among Different Ingredients and Sources

Gastric and small intestine hydrolysis disappearance, $IVDMD_h$, from corn DDGS was greater than SBH ($P < 0.01$), which was also greater ($P < 0.01$) than WS (Table 1.2). Disappearance of DM from fermentation, $IVDMD_f$, of SBH was greater ($P < 0.01$) than corn DDGS, which was intermediate to WS. Calculated apparent total tract disappearance rate ($IVDMD_t$) of SBH and DDGS were numerically similar, and both values were greater than WS. There were differences ($P < 0.01$) in $IVDMD_h$ among sources of each ingredient (Table 1.3). The greatest range in $IVDMD_h$ was among sources of corn DDGS. There were no differences ($P = 0.23$) in $IVDMD_f$ among sources of each of the ingredients.

Kinetics of Gas Production

When expressed on per g DM of substrate incubated basis, the asymptotic gas production (A) was greatest ($P < 0.01$) for SBH (383.9 mL); while corn DDGS (238.1 mL) was intermediate, and WS (115.6 mL) was the least (Table 1.4). Corn DDGS (1.5 h) reached half asymptotic amount of gas production (B) sooner ($P < 0.01$) than WS (1.6 h), which was sooner ($P < 0.01$) than SBH (2.1 h). Among all the 3 ingredients, SBH had the greatest ($P < 0.01$) C value, following by corn DDGS and WS (Fig. 1.3). There were also differences among sources of each ingredient ($P < 0.01$) for all the 3 gas production kinetic parameters (Table 1.5).

Correlations among sample chemical composition, IVDMD, and kinetics of gas production

The $IVDMD_h$ had a strong negative correlation with TDF ($P < 0.01$, r = -0.98), NDF ($P < 0.01$, r = -0.98), and ADF ($P < 0.01$, r = -0.99); but a mild positive correlation with hemicellulose ($P < 0.01$, r = 0.36; Table 6). The $IVDMD_f$ was not correlated with TDF ($P = 0.62$), NDF ($P = 0.44$), and ADF ($P = 0.99$); but it was negatively correlated with hemicellulose ($P < 0.01$, r = -0.49). For kinetics of gas production parameters, asymptotic gas production (A) was negatively correlated to hemicellulose concentration ($P < 0.01$, r = -0.55) and positively correlated to $IVDMD_f$ ($P < 0.01$, r = 0.87). Time to reach half asymptotic gas production (B) was positively correlated to TDF ($P < 0.01$, r = 0.43), NDF ($P = 0.02$, r = 0.35), ADF ($P < 0.01$, r = 0.48), and $IVDMD_f$ ($P < 0.01$, r = 0.59); but negatively correlated with hemicellulose ($P < 0.01$, r = -0.70) and $IVDMD_h$ ($P < 0.01$, r = -0.45). The C value was only negatively correlated with hemicellulose ($P < 0.01$, r = -0.46) and positively correlated with $IVDMD_f$ ($P < 0.01$, r = 0.84).

Experiment 2

Apparent Total Tract Digestibility of Nutrients There were differences ($P < 0.01$) in ATTD of DM, GE, CP, EE, and TDF among the 3 types of diets based on WS, SBH, and DDGS (Table 2.3). The SBH diets had greater ($P < 0.01$) ATTD of DM, GE, and TDF than DDGS diets, which was greater ($P < 0.01$) than WS diets. The DDGS diets had greater ($P < 0.01$) ATTD of CP than WS diets, which was greater ($P < 0.01$) than SBH diets. The WS diets had greater ($P < 0.01$) ATTD of AEE than SBH diets, which was greater ($P < 0.01$) than DDGS diets.

We observed differences in ATTD of some nutrients among sources of each ingredient. Among sources of WS, we observed the greatest ATTD of GE in source 3. The greater ATTD of GE in this WS source appeared to be due to greater ATTD of AEE, and not due to differences in ATTD of DM, CP, or TDF. Among sources of SBH, we observed that source 4 had the greatest ATTD of CP and AEE, but there were no differences in ATTD of DM, GE, or TDF among sources of SBH. Among sources of DDGS, there were only differences in the ATTD of TDF, where source 9 had the least ATTD of TDF and source 8 had the greatest. There were no differences in ATTD of any other nutrients or GE among sources of DDGS.

Energy Value and Nitrogen Retention of the Diets

Diets with WS had lower ($P < 0.01$) DE than SBH and DDGS diets (Table 2.4). The WS diets also had lower ME and DE:GE than DDGS diets, which were lower ($P < 0.01$) than SBH diets. The DDGS diets had a lower ($P < 0.01$) ME:DE than SBH diets and WS diets, and the SBH diets had greater ($P < 0.01$) ME:GE than WS diets and DDGS diets. For N retention, the DDGS diets was lower ($P < 0.01$) than SBH and WS diets. There were no differences in DE, ME, DE:GE, ME:DE, or N retention among sources of WS, SBH, or DDGS.

In Vitro Dry Matter Disappearance (IVDMD) of Fiber Sources

In agreement with observations from Exp. 1, the $IVDMD_h$ was greatest ($P < 0.01$) in DDGS, followed by SBH, and least in WS. The $IVDMD_f$ was greatest ($P < 0.01$) for SBH, followed by DDGS, and least for WS. There were no differences in $IVDMD_h$ or $IVDMD_f$ among sources of WS, SBH, or DDGS.

Kinetics of Gas Production

The asymptotic gas production (A) of SBH was greater ($P < 0.01$) than DDGS, which was greater ($P < 0.01$) than WS (Table 2.6). There were no differences ($P > 0.05$) among ingredients on the time necessary to reach half of asymptotic gas production (B), or the shape of the gas curve (C). Furthermore, there were no differences ($P > 0.05$) observed among different sources nested under each ingredient for all the kinetics of gas production parameters.

Correlations of apparent total tract digestibility (ATTD) of energy and nutrients, as well as energy content of pig feed with IVDMD and kinetics of gas production

There was no correlation between $IVDMD_h$ and the ingredient concentrations of DM, GE, TDF, ME, DE:GE, or ME:DE. There was an increase in $IVDMD_h$ in each ingredient as the concentration of CP increased. However, as the concentration of AEE increased, $IVDMD_h$ decreased. The $IVDMD_f$ was affected by the concentration of nutrients in the feed ingredients, where the greater the concentration of DM, GE, TDF, DE, ME, and DE:GE, the greater $IVDMD_f$. However, $IVDMD_f$ decreased with greater concentration of AEE, and tended to decrease with the concentration of CP. The A value was increased as the concentration of DM and TDF increased, and was decreased by greater concentration of AEE. The A value had a good correlation with DE, ME, DE:GE, and ME:DE. Likewise, the B value was well correlated with DE, ME, and DE:GE, but not with ME:DE. There were no correlations between C and any of the nutrients in the feed ingredients.

Immune and gut morphology and modulating effect of insoluble dietary fiber

There were significant ($P < 0.01$) differences in gene expression profiles between pigs fed the diets with various sources of insoluble fiber (Figure 2.1). Compared with wheat straw, feeding diets with DDGS increased ($P < 0.01$)

expression of CCL1, CCL17, CCR2, CCR3, CCR4, CCR7, CD40LG, CXCR2, FASLG, IL13, IL15, IL17A, IL17F, IL27, IL4, IL-5, IL6R, IL9, OSM, IL33, LOC100519468, TNF, and TNFRSF11B. The expression of gene IL23A was less ($P < 0.01$) in WS than in DDGS. Compared with SBH, feeding diets with DDGS increased ($P < 0.01$) expression of all genes that were included in our PCR array profile. Furthermore, compared with SBH, feeding diets with WS induced greater ($P < 0.01$) expression of CCL1, CCL17, CCR2, CCR3, CCR4, CCR7, CD40LG, CXCR2, FASLG, and IL13. Likewise, expression of IL17A, IL17F, IL23A, IL27, IL4, IL-5, IL6R, IL9, OSM, IL33, LOC100519468, TNF, and TNFRSF11B was greater ($P < 0.01$) in ileum tissue of pigs fed WS compared with SBH. However, there was only a tendency for greater ($P = 0.1$) expression of IL15 in ileum tissue of pigs fed WS compared with SBH.

Feeding diets with high concentrations of insoluble dietary fiber modified gut morphology as well. Mucosal area occupied by mucin producing cells (goblet cells) was greater ($P < 0.01$) in mucosa of pigs fed WS compared with those fed DDGS, and mucosal area occupied by mucin tended to be greater ($P = 0.06$) in pigs fed DDGS compared with SBH.

DISCUSSION

This study aimed to explore *in vitro* hydrolysis and fermentation characteristics of feed ingredients with high concentration of insoluble dietary fiber (i.e., WS, SBH and corn DDGS). *In vitro* digestion systems such as *in vitro* gas production (Bindelle et al., 2007) were developed to predict ATTD of TDF using a combination of raw materials with variable concentration of insoluble and soluble dietary fiber. However, DDGS contains almost no soluble fiber. Therefore, it is not known if the current *in vitro* procedures are useful to predict ATTD of TDF. In addition, the majority of literature that studied physiological effects of dietary fiber use a combination of soluble dietary fiber included at different levels. Therefore, the second objective of this research was to measure gut physiology parameters among pigs fed diets with similar concentrations of TDF, but of variable ATTD of TDF. Based on these objectives, feed ingredients were selected with the criteria of high concentration of insoluble fiber, but with distinct ATTD of TDF. These 3 ingredients allowed us to test the *in vitro* gas production procedure among sources of ingredients with insoluble fiber and to measure physiology and morphology responses in the gut of pigs fed insoluble fiber independently of viscosity and other parameters.

The $IVDMD_h$ was negatively correlated with TDF, NDF and ADF concentration of these samples, which agrees with results from previous studies (Jha et al., 2015). Also, since the gastric and small intestine segments of the gastrointestinal tract mainly digest proteins and lipids, differences in concentration of CP and EE of the ingredients explains the differences in $IVDMD_h$ among ingredients (Yang et al., 2010; Jha et al., 2015). Our $IVDMD_f$, results showed that SBH was more fermentable than corn DDGS, which was more fermentable than WS, but was not correlated to the concentration of TDF, NDF and ADF. This indicates that the concentration of TDF, NDF and ADF are poor predictors of fiber fermentability responses in the large intestine. However, increasing hemicellulose concentration of the samples reduced fiber fermentability ($P < 0.01$, $r = -0.49$), which indicates fiber type and fiber fraction may affect fiber fermentability. The impact of hemicellulose to $IVDMD_f$ may be due to its insolubility, which is similar to the effect of high dietary inclusion of lignin (Table 1) in WS that reduces large intestine fermentability (Bach Knudsen, 2001). Similarly, the relatively high soluble pectin content in SBH (Karr-Lilienthal et al., 2005) may be a primary factor for its greater fermentability.

The kinetics of gas production had a strong positive correlation with $IVDMD_f$, which means greater $IVDMD_f$ relates to greater gas production, slower time to reach half asymptotic gas production, and greater slope. Hemicellulose concentration of the samples was negatively correlated with all the kinetics of gas production, which confirms that dietary fiber type and fraction impact large intestine fermentation pattern.

Despite its ability to reasonably predict dry matter and fiber digestibility, the current *in vitro* procedure has limitations. The greatest issue is the inherent variability in $IVDMD_f$ estimates which did not allow us to observe differences in $IVDMD_f$ among sources within the same ingredients. Improvements to the current procedure will be made by improving consistency of fecal characteristics and handling from donor pigs, which may improve the impact of fecal dry matter on calculations of $IVDMD_f$. There were differences among batches that we presumed were a result of using different pigs selected as fecal donors, but also there was error in the procedure introduced by weighing errors associated with relatively low inoculation substrate weight (0.2 g) compared with the filter paper weight (about 2.2 g). The impact of fecal dry matter and substrate weight affects the calculation of $IVDMD_f$, but has less impact on measured gas production because blanks were used to correct the data.

The current *in vitro* procedure is capable of detecting differences in fermentability of fiber among feed ingredients of high concentration of insoluble fiber. However, not all responses in the animal are derived from production of volatile fatty acids (Serena et al., 2008; Yang et al., 2010). We observed differences in expression of genes related to immune function among pigs fed insoluble dietary fiber (WS, corn DDGS, and SBH). These data suggest that the immune system in the gastrointestinal tract of pigs responds to various types of insoluble fiber in the diet. These are the first data to describe differences in gene expression of pigs fed diets with insoluble dietary fiber with different fermentability characteristics. In addition to the differences of feeding these diets on gene expression of the immune system, there were also differences in the concentration of mucin production. We observed that the mucosa of pigs fed (WS, DDGS, and SBH) was covered with goblet celsl (producing mucin). This observation suggests a response in secretion of mucin to protect the mucosa from potential injury from fiber or products of fiber degradation, and not only in changes in digesta viscosity (Högberg and Lindberg, 2004; Ito et al., 2009). This increase in mucin production and protection is not only the result of high level of dietary fiber in the diets of pigs fed WS, DDGS, and SBH, but that there were differences among these 3 ingredients. Pigs fed WS had a greater number of goblet cells than pigs fed DDGS and SBH. These are the first data that suggest that there are differences in goblet cells and mucin production among pigs fed diets with insoluble dietary fiber. Therefore, these data suggest that the effect of dietary fiber on gut physiology and function is not only related to the concentration of fiber in the diet (concentration of TDF or NDF), but also to the botanical origin of the dietary fiber (Bach Knudsen, 2001).

In conclusion, fiber type and fiber fraction affect both *in vitro* stomach and small intestine hydrolysis patterns, as well as large intestine fermentation patterns in growing pigs. Among WS, SBH and corn DDGS, corn DDGS had greatest $IVDMD_h$, while SBH has greatest $IVDMD_f$ and kinetics of gas production, whereas WS had the lowest $IVDMD_h$ and $IVDMD_f$. Energy variation was observed from calculated VFA production of different corn DDGS sources. Similar results from several different studies indicate that this *in vitro* procedure is a promising method to evaluate IVDMD and even energy utilization of high fiber feed ingredients for growing pigs. Our results are the first to show that different insoluble fiber sources alter intestinal gene expression, cell proliferation and type, and immune responses, which represent

the beginning of our understanding of the multiple physiological responses from feeding high fiber diets beyond their effects on energy and nutrient digestibility.

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Table 1.1. Analyzed composition of wheat straw, soybean hulls, and corn distillers dried grains with solubles (DM basis[1])

Source	TDF[2], %	NDF, %	ADF, %	Hemicellulose[3], %
Wheat straw				
1	85.4	73.1	55.4	17.7
2	89.3	75.2	54.6	20.6
3	90.5	76.8	54.4	22.4
4	87.9	71.8	54.4	17.3
5	96.8	82.2	54.3	27.9
6	91.8	77.9	59.7	18.2
7	NA[4]	77.6	56.9	20.7
8	95.9	79.4	55.8	23.6
9	99.8	83.4	53.1	30.2
10	89.6	77.0	55.0	22.0
11	81.8	69.0	54.0	15.1
12	93.8	78.5	55.9	22.6
13	90.6	78.5	54.3	24.2
14	88.7	74.9	52.8	22.1
15	87.4	71.6	51.5	20.0
16	92.9	76.6	56.1	20.5
Soybean hulls				
17	76.7	64.0	49.0	15.0
18	81.7	67.0	51.4	15.6
19	82.1	67.0	51.9	15.1
20	78.8	65.0	49.0	16.0
21	80.6	67.0	51.6	15.3
22	80.1	66.6	49.7	16.9
23	74.6	60.9	48.6	12.3
24	79.0	63.7	48.6	15.2
25	78.7	67.4	50.4	17.0
26	76.4	62.8	47.8	14.9
27	80.3	67.2	50.5	16.7
28	77.4	65.0	48.5	16.5
29	77.7	65.6	49.9	15.7
30	78.8	65.5	48.0	17.5
31	80.4	67.7	48.7	19.0
32	78.8	65.3	49.4	15.9
Corn DDGS				
33	32.4	34.0	9.9	24.1
34	31.3	28.8	10.3	18.5
35	35.3	38.6	13.9	24.7
36	33.9	38.2	12.5	25.8
37	32.5	35.7	13.4	22.3
38	33.9	36.5	12.1	24.4
39	30.8	33.3	10.5	22.8
40	37.8	44.0	14.0	30.0
41	36.1	31.6	10.1	21.5
42	36.0	33.9	10.6	23.3
43	35.6	30.5	9.4	21.1
44	33.8	31.6	9.0	22.6

45	32.8	31.1	8.6	22.5
46	31.1	39.6	11.6	28.0
47	32.4	31.0	8.9	22.1
48	44.1	30.5	15.0	15.5
	CP, %	Ether extract, %	Lignin, %	Cellulose, %
Wheat straw	3.8	2.1	7.1	45.8
Soybean hulls	11.4	1.8	2.0	45.3
Corn DDGS	31.3	10.0	2.4	12.7

[1]TDF, NDF, ADF and hemicellulose data were obtained for all the 48 samples used in this study. Crude protein, ether extract, lignin and cellulose data were from samples analyzed from a different batch and were not the same as used in the current study.
[2]Total dietary fiber
[3]Calculated as NDF-ADF
[4]Not applicable

Table 1.2. *In vitro* dry matter digestibility of ingredients

Ingredient	N[1]	IVDMD[2]$_h$, %	N	IVDMD[3]$_f$, %
Wheat Straw	77	14.5 ± 0.3[a]	94	41.7 ± 8.1[a]
Soybean hulls	69	19.7 ± 0.3[b]	88	68.5 ± 8.1[c]
Corn DDGS	89	55.8 ± 0.3[c]	90	52.7 ± 8.1[b]
P-value		< 0.01		< 0.01

[1]N = number of observations

[2]$IVDMD_h$ = Gastric and small intestine hydrolysis disappearance expressed as least squares means (LSM) ± standard error (SE)

[3]$IVDMD_f$ = Large intestine fermentation disappearance expressed as LSM ± SE

[a,b,c]Means are different ($P < 0.05$)

Table 1.3. *In vitro* dry matter digestibility of all wheat straw, soybean hulls, and DDGS samples

Source	N[1]	IVDMD[2]$_h$, %	N	IVDMD[3]$_f$, %
Wheat straw				
1	4	17.0 ± 0.6	6	47.4 ± 9.8
2	5	14.8 ± 0.6	6	38.8 ± 9.8
3	5	14.9 ± 0.6	6	39.5 ± 9.8
4	4	15.8 ± 0.6	5	39.9 ± 10.2
5	6	11.8 ± 0.5	6	40.8 ± 9.8
6	5	12.6 ± 0.6	6	44.6 ± 9.8
7	4	13.8 ± 0.6	5	46.4 ± 10.2
8	4	12.4 ± 0.6	6	36.8 ± 9.8
9	5	12.7 ± 0.6	6	40.6 ± 9.8
10	5	17.2 ± 0.6	6	37.9 ± 9.8
11	5	18.0 ± 0.6	6	37.3 ± 9.8
12	5	11.9 ± 0.6	6	44.6 ± 9.8
13	4	11.2 ± 0.6	6	39.3 ± 9.8
14	5	15.5 ± 0.6	6	48.0 ± 9.8
15	5	18.3 ± 0.6	6	42.8 ± 9.8
16	6	13.6 ± 0.5	6	43.4 ± 9.8
Soybean hulls				
17	4	21.0 ± 0.6	6	49.0 ± 9.8
18	4	18.5 ± 0.6	5	73.4 ± 10.2
19	4	17.6 ± 0.6	6	66.1 ± 9.8
20	5	20.4 ± 0.6	5	69.8 ± 10.2
21	5	18.3 ± 0.6	5	61.8 ± 10.2
22	4	19.6 ± 0.6	5	65.8 ± 10.2
23	5	20.3 ± 0.6	6	76.9 ± 9.8
24	3	20.9 ± 0.7	6	83.2 ± 9.8
25	4	16.7 ± 0.6	6	71.6 ± 9.8
26	4	23.0 ± 0.6	6	58.8 ± 9.8
27	4	18.2 ± 0.6	6	75.8 ± 9.8
28	4	20.3 ± 0.6	6	64.3 ± 9.8
29	5	20.6 ± 0.6	5	68.4 ± 10.2
30	4	20.2 ± 0.6	6	65.4 ± 9.8
31	5	19.3 ± 0.6	5	79.9 ± 10.2
32	5	21.0 ± 0.6	4	65.9 ± 10.6
Corn DDGS				
33	5	57.5 ± 0.6	6	49.8 ± 9.8
34	7	53.8 ± 0.5	5	64.2 ± 10.2
35	5	51.8 ± 0.6	6	61.2 ± 9.8
36	5	51.6 ± 0.6	5	54.9 ± 10.2
37	6	54.7 ± 0.5	6	51.1 ± 9.8
38	5	53.2 ± 0.6	6	57.3 ± 9.8
39	6	54.2 ± 0.5	5	54.8 ± 10.2
40	4	45.3 ± 0.6	6	56.1 ± 9.8
41	6	57.8 ± 0.5	6	49.9 ± 9.8
42	6	56.1 ± 0.5	6	48.0 ± 9.8
43	6	58.5 ± 0.5	6	47.9 ± 9.8
44	5	59.4 ± 0.6	5	44.3 ± 10.2
45	6	61.2 ± 0.5	6	51.3 ± 9.8
46	4	57.3 ± 0.6	5	60.0 ± 10.2

47	7	63.2 ± 0.5	6	41.4 ± 9.8
48	6	56.1 ± 0.5	5	51.6 ± 10.2
P-value		< 0.01		0.23

[1]N = number of observations
[2]$IVDMD_h$ = Gastric and small intestine hydrolysis disappearance expressed as least squares means (LSM) ± standard error (SE)
[3]$IVDMD_f$ = Large intestine fermentation disappearance expressed as LSM ± SE

Table 1.4. Fitted kinetics parameters on the gas accumulation for wheat straw, soybean hulls, and DDGS

	Per g DM substrate incubated			
Ingredient	N[1]	A, [2]	B[3]	C[4]
Wheat Straw	48	115.6[a]	1.6[b]	6.9[a]
Soybean hulls	48	383.9[c]	2.1[c]	16.0[c]
Corn DDGS	48	238.1[b]	1.5[a]	12.0[b]
SE[5]		8.9	0.1	0.6
P-value		< 0.01	< 0.01	< 0.01

[1]N = number of observations
[2]A = mL/g DM substrate, represents the asymptotic gas production
[3]B = time (hours) after incubation at which half of the asymptotic amount of gas has been formed
[4]C = constant that determines the sharpness of the switching characteristic of the profile
[5]SE = standard error
[a,b,c]Means are different ($P < 0.05$)

Table 1.5. Fitted kinetics parameters on the gas accumulation for wheat straw, soybean hulls, and DDGS

	Per g DM substrate incubated			
Source	N[1]	A[2]	B[3]	C[4]
Wheat straw				
1	3	95	2.2	5.1
2	3	116	1.5	6.5
3	3	113	1.6	6.8
4	3	101	2.3	5.4
5	3	123	1.5	7.2
6	3	110	1.5	7.0
7	3	118	1.5	7.0
8	3	110	1.6	6.6
9	3	102	1.9	5.6
10	3	124	1.4	8.5
11	3	105	1.7	6.3
12	3	138	1.3	9.7
13	3	123	1.6	6.9
14	3	131	1.4	7.6
15	3	120	1.5	7.3
16	3	121	1.5	7.1
Soybean hulls				
17	3	352	2.1	15.1
18	3	382	2.2	16.5
19	3	401	2.1	16.3
20	3	372	2.0	16.4
21	3	414	2.1	17.4
22	3	387	2.1	16.1
23	3	385	2.1	16.5
24	3	371	2.2	15.5
25	3	392	2.0	16.6
26	3	391	2.1	16.4
27	3	374	2.3	15.4
28	3	396	2.2	15.6
29	3	385	2.1	16.1
30	3	368	2.0	14.6
31	3	380	2.1	15.5
32	3	393	2.1	15.7
Corn DDGS				
33	3	246	1.4	12.7
34	3	272	1.5	13.5
35	3	219	1.4	13.1
36	3	281	1.5	14.2
37	3	265	1.4	16.0
38	3	229	1.5	10.5
39	3	239	1.5	11.8
40	3	240	1.3	13.9
41	3	221	1.5	11.3
42	3	221	1.5	10.6
43	3	236	1.6	10.8
44	3	227	1.6	10.4
45	3	235	1.6	10.4

46	3	227	1.5	10.1
47	3	219	1.6	10.5
48	3	234	1.5	11.7
SE[5]		14	0.1	0.9
P-value		< 0.01	< 0.01	< 0.01

[1]N = number of observations
[2]A = mL/g DM substrate, represents the asymptotic gas production
[3]B = time (hours) after incubation at which half of the asymptotic amount of gas has been formed
[4]C = constant that determines the sharpness of the switching characteristic of the profile
[5]SE = standard error

Table 1.6. Correlations among sample chemical composition, *in vitro* dry matter digestibility, and kinetics of gas production

Item	Statistic	$IVDMD^1_h$, %	$IVDMD^2_f$, %	A[3]	B[4]	C[5]
TDF[6], %	r[7]	-0.98	-0.07	-0.13	0.43	-0.23
	P-value	< 0.01	0.62	0.39	< 0.01	0.12
NDF, %	r	-0.98	-0.11	-0.19	0.35	-0.28
	P-value	< 0.01	0.44	0.19	0.02	0.06
ADF, %	r	-0.99	< 0.01	-0.06	0.48	-0.16
	P-value	< 0.01	0.99	0.69	< 0.01	0.29
Hemicellulose[7], %	r	0.36	-0.49	-0.55	-0.70	-0.46
	P-value	0.01	< 0.01	< 0.01	< 0.01	< 0.01
$IVDMD_h$, %	r			0.06	-0.45	0.15
	P-value			0.69	< 0.01	0.31
$IVDMD_f$, %	r			0.87	0.59	0.84
	P-value			< 0.01	< 0.01	< 0.01

[1]$IVDMD_h$ = gastric and small intestine hydrolysis disappearance
[2]$IVDMD_f$ = large intestine fermentation disappearance
[3]A = mL/g DM substrate, represents the asymptotic gas production
[4]B = time (hours) after incubation at which half of the asymptotic amount of gas has been formed
[5]C = constant that determines the sharpness of the switching characteristic of the profile
[6]Total dietary fiber
[7]Calculated as NDF-ADF
[8]r: Person correlation coefficients

Table 1.7. Production of volatile fatty acids (acetate, propionate, and butyrate) and calculated energy content in dietary fiber among sources of corn distillers dried grains with solubles

Corn DDGS sources	A[1], mL	VFA[2], mmol/g DM substrate fermented	Total energy[3], cal/g DM substrate fermented	$IVDMD^{4}_{h}$, %	Calories/g DM raw DDGS substrate
33	246	7.5	2,102	57.5	1,209
34	272	8.3	2,325	53.8	1,251
35	219	6.67	1,872	51.8	969
36	281	8.57	2,401	51.6	1,239
37	265	8.08	2,265	54.7	1,239
38	229	6.98	1,957	53.2	1,041
39	239	7.28	2,043	54.2	1,107
40	240	7.31	2,051	45.3	929
41	221	6.74	1,889	57.8	1,092
42	221	6.74	1,889	56.1	1,060
43	236	7.2	2,017	58.5	1,180
44	227	6.93	1,940	59.4	1,152
45	235	7.17	2,008	61.2	1,229
46	227	6.93	1,940	57.3	1,112
47	219	6.67	1,872	63.2	1,183
48	234	7.14	2,000	56.1	1,122

[1]A = mL/g DM substrate, represents the asymptotic gas production
[2]Referenced from Jha et al. (2015).
[3]Referenced from Christensen et al. (1999).
[4]$IVDMD_h$ = gastric and small intestine hydrolysis disappearance.
[5]NRC (2012). For corn DDGS 6% < oil < 9%, NE = 2343 kcal/kg, ME = 3396 kcal/kg, DE = 3582 kcal/kg, GE = 4710 kcal/kg.

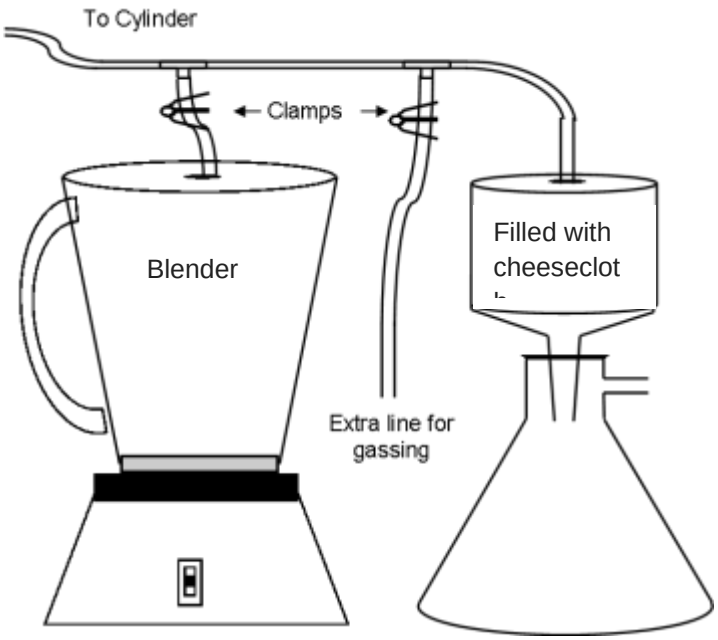

Figure 1.1. Arrangement of blender assembly, filter funnel and flask for filtering swine feces. The entire system should be filled with CO_2 prior to addition of swine feces (Modified according to van Soest, 2015).

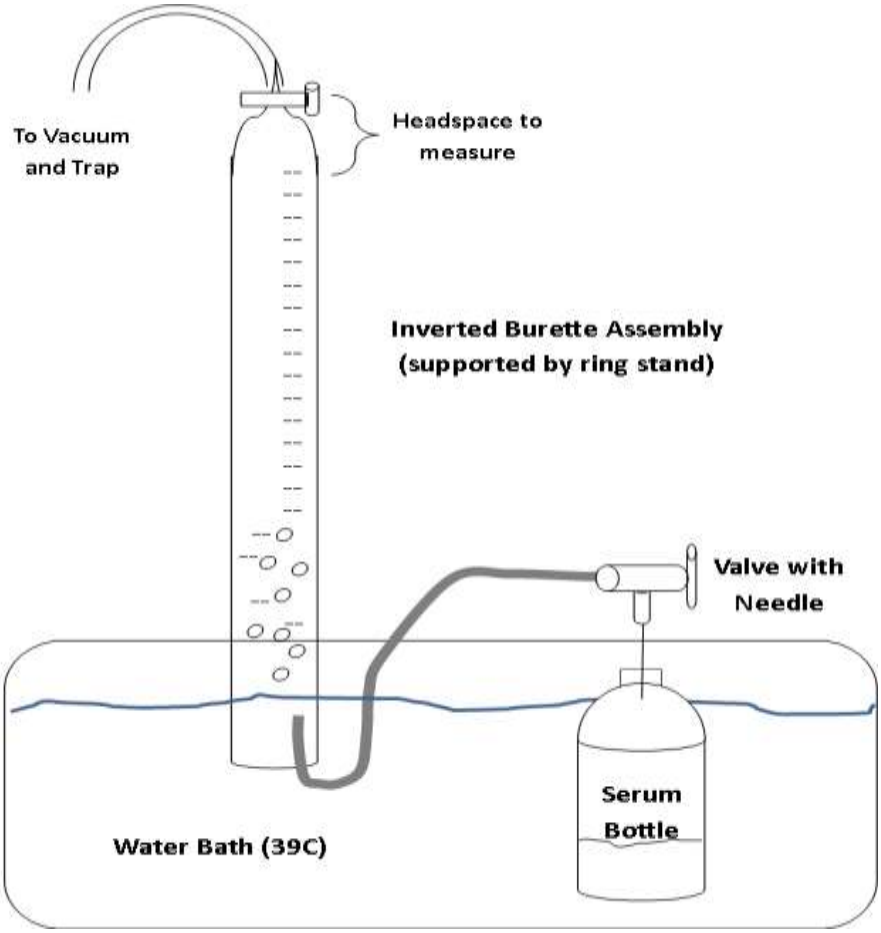

Figure 1.2. Arrangement of inverted burette assembly for gas measurement.

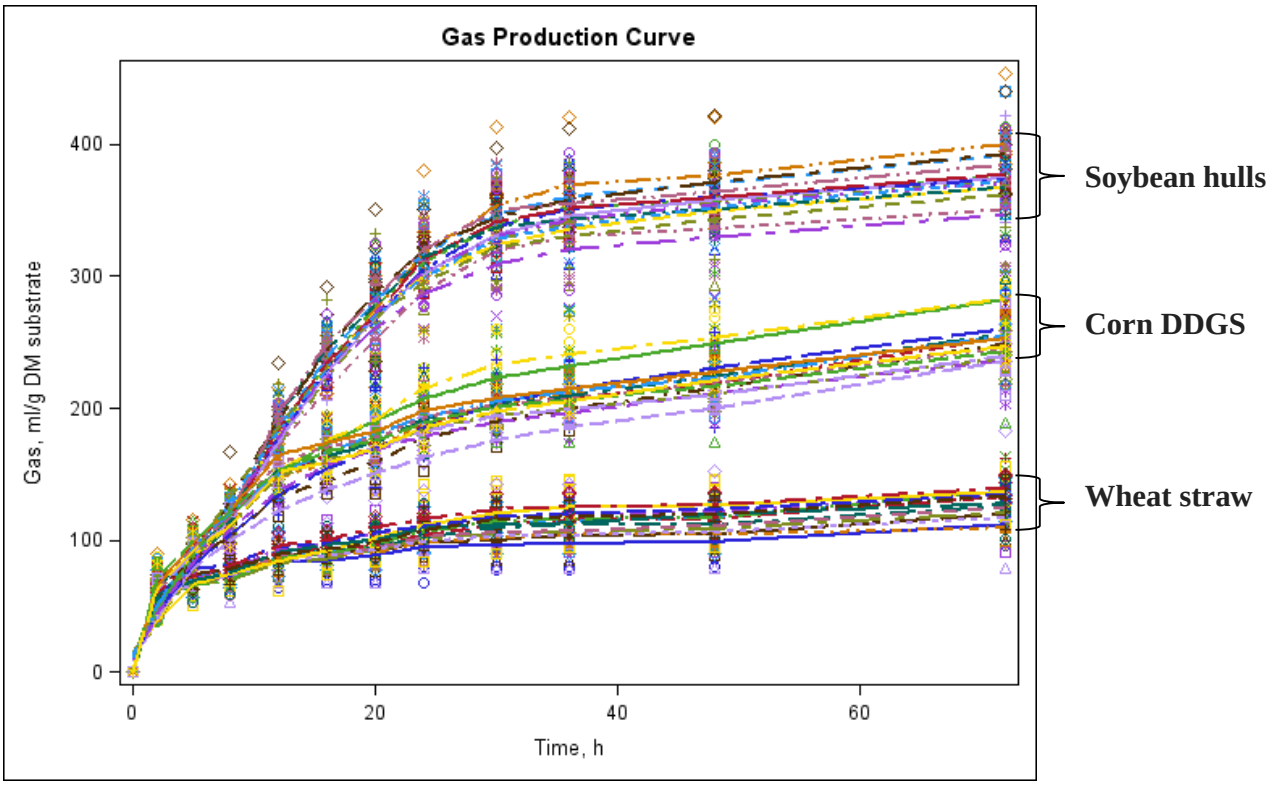

Figure 1.3. Gas production curve responses to incubation time.

Table 2.1. Analyzed composition of wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS), DM basis

Fiber source	GE, kcal/kg	CP, %	EE[1], %	ADF, %	NDF, %	TDF[2], %	Lignin, %	Hemicellulose[3], %	Cellulose[4], %
WS									
1	4,050	4.1	2.2	53.0	79.1	77.2	6.6	26.0	46.4
2	4,160	6.2	2.5	53.0	80.0	82.6	7.6	27.0	45.4
3	4,032	3.8	2.8	52.7	78.2	80.0	7.5	25.5	45.2
SBH									
4	4,070	11.5	2.4	51.0	68.4	80.2	3.5	17.4	47.5
5	4,065	12.6	2.8	48.2	63.2	75.9	3.9	15.1	44.3
6	3,998	11.3	2.8	50.2	66.4	75.9	4.9	16.2	45.2
DDGS									
7	4,879	29.9	9.8	13.8	32.7	38.2	1.8	18.8	12.0
8	4,842	31.6	9.0	17.4	35.1	37.5	4.6	17.7	12.8
9	4,776	30.7	8.2	14.5	33.8	37.8	2.6	19.2	11.9

[1]Ether extract with acid hydrolysis

[2]Total dietary fiber

[3]Calculated as NDF-ADF

[4]Calculated as ADF-lignin

Table 2.2. Composition and nutrient concentration of experimental diets

Ingredient, %	WS			SBH			DDGS		
	1	2	3	4	5	6	7	8	9
Wheat straw (WS)	23.0	23.0	23.0	0	0	0	0	0	0
Soybean hulls (SBH)	0	0	0	30.0	30.0	30.0	0	0	0
Corn distillers dried grains with solubles (DDGS)	0	0	0	0	0	0	55.0	55.0	55.0
Plasma spray-dried	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Corn starch	61.1	61.1	61.1	56.7	56.7	56.7	34.7	34.7	34.7
Casein	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Fish meal, menhaden	6.7	6.7	6.7	3.8	3.8	3.8	0	0	0
Titanium dioxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate 18.5%	0	0	0	0.3	0.3	0.3	0	0	0
Limestone	1.4	1.4	1.4	0.4	0.4	0.4	0.3	0.3	0.3
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Grow-finish vitamin and mineral premix[1]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
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, %	13.0	13.6	12.4	13.2	13.7	13.1	22.2	24.0	23.0
EE[2], %	2.9	2.6	3.1	2.4	2.5	2.3	6.3	6.3	6.0
ADF, %	12.4	12.5	13	15.3	13.9	14.7	8.6	10.0	7.7
NDF, %	22.2	23.5	26.9	21.3	19.8	23.5	18.7	19.6	20.4
TDF[3], %	23	23.4	21.4	24.7	23.2	25	20.7	20.9	18.3
Lignin, %	2.2	2.3	2.2	0.8	0.9	1.5	2.0	2.8	2.1
Hemicellulose[4], %	9.7	11.0	13.9	6.0	5.9	8.8	10.0	9.6	12.7
Cellulose[5], %	10.3	10.2	10.8	14.5	13.0	13.2	7.0	7.2	5.6
Titanium, %	0.33	0.33	0.30	0.27	0.33	0.28	0.35	0.38	0.37
Bulk density, g/100cm³	0.37	0.42	0.40	0.64	0.61	0.60	0.67	0.68	0.67
Viscosity, centipoise	1989	2142	2455	532	607	817	311	301	310

[1]The vitamin and trace mineral premix (ANS Swine G-F premix) provided the following (per kg of diet): vitamin A 3,527,392 I.U., vitamin D_3 661,386 I.U., vitamin E 13,228 I.U., vitamin K (MPB) 1,323 mg, riboflavin 2,205 mg, niacin 13,228 mg, pantothenic Acid 8,818 mg, vitamin B_{12} 13 mg, iodine (EDDI) 119 mg, selenium (sodium selenite) 119 mg, SQM organic zinc 22,046 mg, SQM organic iron 13,228 mg, SQM organic manganese 454 mg, SQM organic copper 1,543 mg.

[2]Ether extract with acid hydrolysis

[3]Total dietary fiber

[4]Calculated as NDF-ADF

[5]Calculated as ADF-lignin

Table 2.3. Apparent total tract digestibility of nutrients of diets containing wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS)[1]

Item	DM, %	GE, %	CP, %	AEE[2], %	TDF[3], %
WS	75.8 ± 0.5^{c}	75.9 ± 0.6^{c}	81.4 ± 0.5^{b}	79.0 ± 0.9^{a}	26.7 ± 1.8^{c}
SBH	88.8 ± 0.5^{a}	88.0 ± 0.5^{a}	77.6 ± 0.5^{c}	68.3 ± 0.9^{b}	78.9 ± 1.5^{a}
DDGS	80.4 ± 0.5^{b}	79.4 ± 0.5^{b}	85.1 ± 0.5^{a}	59.0 ± 0.9^{c}	43.0 ± 1.5^{b}
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
WS					
1	75.2 ± 0.9	77.1 ± 0.9^{AB}	81.2 ± 0.7	80.4 ± 1.4^{AB}	24.2 ± 2.6
2	74.3 ± 1.0	72.9 ± 1.1^{A}	81.2 ± 0.7	74.7 ± 1.5^{A}	29.4 ± 3.6
3	78.0 ± 0.9	77.8 ± 0.9^{B}	81.9 ± 0.7	81.9 ± 1.4^{B}	26.4 ± 2.8
SBH					
4	88.8 ± 0.9	88.1 ± 0.9	79.4 ± 0.7^{B}	73.2 ± 1.4^{B}	79.0 ± 2.6
5	87.8 ± 0.9	87.0 ± 0.9	76.4 ± 0.7^{A}	65.1 ± 1.4^{A}	75.0 ± 2.6
6	89.7 ± 0.9	88.9 ± 0.9	77.1 ± 0.7^{AB}	66.5 ± 1.4^{A}	82.7 ± 2.6
DDGS					
7	80.2 ± 0.9	79.3 ± 0.9	85.2 ± 0.7	58.9 ± 1.4	43.1 ± 2.6^{AB}
8	80.8 ± 0.9	79.6 ± 0.9	85.8 ± 0.7	58.2 ± 1.4	49.8 ± 2.6^{B}
9	80.3 ± 0.9	79.2 ± 0.9	84.4 ± 0.7	59.8 ± 1.4	36.0 ± 2.6^{A}
P-value	0.10	0.04	0.03	< 0.01	< 0.01

[1]Data are expressed as least squares means (LSM) ± standard error (SE).

[2]Ether extract with acid hydrolysis

[3]Total dietary fiber

[a,b,c]Means with different superscripts are significantly different between ingredients ($P < 0.05$).

[A,B,C]Means with different superscripts are significantly different between different sources nested under each ingredient ($P < 0.05$).

Table 2.4. Energy values and nitrogen retention in experimental diets containing wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS)[1]

Item	DE, kcal/kg	ME, kcal/kg	DE:GE, %	ME:DE, %	N retention, %
WS	3175 ± 25[a]	3098 ± 26[a]	76.2 ± 0.6[a]	97.5 ± 0.2[b]	59.3 ± 1.4[b]
SBH	3610 ± 23[b]	3516 ± 24[c]	88.0 ± 0.5[c]	97.4 ± 0.2[b]	58.4 ± 1.3[b]
DDGS	3552 ± 23[b]	3361 ± 24[b]	79.4 ± 0.5[b]	94.7 ± 0.2[a]	41.9 ± 1.4[a]
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
WS					
1	3207 ± 40	3147 ± 41	77.1 ± 0.9	98.1 ± 0.3	64.1 ± 2.3
2	3072 ± 50	2990 ± 52	73.7 ± 1.2	97.2 ± 0.4	56.6 ± 2.5
3	3245 ± 40	3158 ± 41	77.8 ± 0.9	97.3 ± 0.3	57.0 ± 2.3
SBH					
4	3618 ± 40	3518 ± 41	88.1 ± 0.9	97.2 ± 0.3	60.6 ± 2.3
5	3570 ± 40	3465 ± 41	87.0 ± 0.9	97.1 ± 0.3	56.3 ± 2.3
6	3642 ± 40	3566 ± 41	88.9 ± 0.9	97.9 ± 0.3	58.1 ± 2.3
DDGS					
7	3582 ± 40	3383 ± 41	79.3 ± 0.9	94.4 ± 0.3	39.8 ± 2.3
8	3566 ± 40	3375 ± 41	79.6 ± 0.9	95 ± 0.3	44.0 ± 2.5
9	3507 ± 40	3324 ± 41	79.2 ± 0.9	94.8 ± 0.3	41.9 ± 2.5
P-value	0.10	0.09	0.15	0.09	0.16

[1]Data are expressed as least squares means (LSM) ± standard error (SE).

[a,b,c] Means with different superscripts are significantly different between ingredients ($P < 0.05$).

Table 2.5. *In vitro* dry matter disappearance (IVDMD) of wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS)[1]

Item	N[2]	IVDMD[3]$_h$, %	N	IVDMD[4]$_f$, %
WS	24	13.3 ± 3.0[a]	24	22.4 ± 1.7[a]
SBH	21	30.5 ± 3.3[b]	24	83.5 ± 1.9[c]
DDGS	18	59.9 ± 3.6[c]	21	77.1 ± 2.1[b]
P-value		< 0.01		< 0.01
WS				
1	8	13.8 ± 4.9	8	18.7 ± 2.9
2	8	12.8 ± 5.2	8	21.6 ± 3.1
3	8	13.4 ± 4.9	8	26.8 ± 2.9
SBH				
4	6	43.9 ± 6.1	8	83.9 ± 3.7
5	7	28.7 ± 5.2	8	83.8 ± 3.1
6	8	19.1 ± 4.9	8	82.9 ± 2.9
DDGS				
7	5	62.9 ± 6.1	7	81.4 ± 3.7
8	7	55.5 ± 5.6	7	77.4 ± 3.4
9	6	61.2 ± 6.1	7	72.3 ± 3.7
P-value		0.10		0.33

[1]Data are expressed as least squares means (LSM) ± standard error (SE).

[2]Number of observations

[3]$IVDMD_h$ = gastric and small intestine hydrolysis disappearance

[4]$IVDMD_f$ = large intestine fermentation disappearance

[5]IVDMDt = apparent total tract disappearance

[6]Not applicable

[a,b,c] Means with different superscripts are significantly different between ingredients ($P < 0.05$).

Table 2.6. Fitted kinetics parameters on the gas accumulation parameters for wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS)[1]

Item	N[2]	A[3]	B[4]	C[5]
		Per g DM substrate incubated		
WS	22	445 ± 34[a]	0.9 ± 0.2	20.5 ± 1.7
SBH	23	731 ± 34[c]	1.4 ± 0.2	18.6 ± 1.6
DDGS	23	648 ± 34[b]	1.3 ± 0.2	21.2 ± 1.6
P-value		< 0.01	0.27	0.51
WS				
1	7	386 ± 61	0.9 ± 0.4	15.9 ± 2.9
2	7	436 ± 61	1.0 ± 0.4	19.7 ± 2.9
3	8	514 ± 57	0.9 ± 0.4	25.9 ± 2.7
SBH				
4	7	663 ± 61	1.7 ± 0.4	17.1 ± 2.9
5	8	748 ± 57	1.3 ± 0.4	17.8 ± 2.7
6	8	782 ± 57	1.2 ± 0.4	20.9 ± 2.7
DDGS				
7	8	712 ± 57	0.9 ± 0.4	25.4 ± 2.7
8	8	669 ± 57	1.0 ± 0.4	20.6 ± 2.7
9	7	563 ± 61	2.1 ± 0.4	17.6 ± 2.9
P-value		0.27	0.33	0.1

[1]Data are expressed as least squares means (LSM) ± standard error (SE).

[2]Number of observations

[3]A = mL/g DM substrate, represents the asymptotic gas production

[4]B = time (hours) after incubation at which half of the asymptotic amount of gas has been formed

[5]C = constant that determines the sharpness of the switching characteristic of the profile

[a,b,c]Means with different superscripts are significantly different between ingredients ($P < 0.05$).

Table 2.7. Correlations of apparent total tract digestibility (ATTD) of energy and nutrients, as well as energy content of high fiber ingredients with IVDMD and kinetics of gas production

	$IVDMD_h$		$IVDMD_f$		A		B		C	
	r	*P*-value	r	*P*-value	r	P-value	r	P-value	r	P-value
Dry matter	-0.01	0.96	0.78	< 0.01	0.76	< 0.01	0.82	< 0.01	-0.05	0.71
Gross energy	-0.09	0.50	0.71	< 0.01	0.71	< 0.01	0.81	< 0.01	-0.04	0.75
Crude protein	0.60	< 0.01	-0.21	0.09	-0.18	0.14	-0.58	< 0.01	0.22	0.07
Acid ether extract	-0.77	< 0.01	-0.64	< 0.01	-0.48	< 0.01	-0.14	0.26	0.07	0.57
Total dietary fiber	-0.06	0.66	0.80	< 0.01	0.77	< 0.01	0.86	< 0.01	-0.08	0.52
Digestible energy	0.44	< 0.01	0.79	< 0.01	0.77	< 0.01	0.58	< 0.01	0.04	0.74
Metabolizable energy	0.22	0.08	0.74	< 0.01	0.75	< 0.01	0.65	< 0.01	0.04	0.75
DE:GE	-0.09	0.47	0.69	< 0.01	0.70	< 0.01	0.80	< 0.01	-0.04	0.74
ME:DE	0.00	0.99	0.20	0.10	0.08	0.51	0.24	0.05	-0.04	0.77

$IVDMD_h$ = gastric and small intestine hydrolysis disappearance

$IVDMD_f$ = large intestine fermentation disappearance

A = mL/g DM substrate, represents the asymptotic gas production

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

C = constant that determines the sharpness of the switching characteristic of the profile

Table 2.8. Prediction equations of apparent total tract digestibility (ATTD) of energy and nutrients, as well as energy content of diets from dietary chemical composition, *in vitro* dry matter disappearance (IVDMD), and kinetics of gas production

Equation[1]	R^2	SE[2]	C(p)[3]
(1) ATTD_GE = 48.79 + 0.02 × A[4] + 13.65 × B[5] + 0.53 × ADF	0.80	2.67	14.26
(2) ATTD_GE = 47.50 + 0.64 × $IVDMD^{6}_{h}$ + 0.01 × A + 5.24 × B – 1.47 × CP + 2.43 × ADF	0.85	2.27	4.08
(3) ATTD_DM = 53.73 + 0.01 × A + 9.05 × B + 1.91 × ADF + 1.24 × hemicellulose – 26.74 × BD[7] – 0.01 × VS[8]	0.87	2.29	5.07
(4) ATTD_DM = 43.94 + 0.01 × A + 5.28 × B + 0.92 × ADF + 0.90 × NDF – 0.01 × VS	0.88	2.14	3.57
(5) ATTD_TDF = -173.03 + 0.04 × A + 49.49 × B + 2.66 × ADF + 2.85 × TDF + 4.60 × lignin + 77.12 × BD	0.91	7.40	9.21
(6) ATTD_TDF = -210.99 + 0.03 × A + 39.54 × B + 1.26 × NDF + 5.28 × TDF + 102.53 × BD	0.92	7.22	7.74
(7) DE = 2502.40 + 0.66 × A + 951.23 × BD	0.74	119.17	4.35
(8) DE = 2423.43 + 45.98 × NDF + 17.64 × TDF – 0.34 × VS	0.82	99.12	4.09
(9) ME = 2385.03 + 0.89 × A + 367.66 × B	0.64	129.95	5.74
(10) ME = 2690.66 + 1.35 × A – 10.71 × C[11]	0.70	122.61	10.97

[1]Odd numbers represent equations that only contain inputs that correlated with responses; Even numbers represent equations considering all possible inputs

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

[3]C(p) = Mallows statistic

[4]A = mL/g DM substrate, represents the asymptotic gas production

[5]B = time (hours) after incubation at which half of the asymptotic amount of gas has been formed

[6]$IVDMD_h$ = gastric and small intestine hydrolysis disappearance

[7]Bulk density, g/100cm³

[8]Viscosity, centipoise

[9]TDF = total dietary fiber

[10]EE = ether extract with acid hydrolysis

[11]C = constant that determines the sharpness of the switching characteristic of the profile

Table 3.1. Differential expression of genes in ileum tissue from pigs feed diets with wheat straw (WS), soybean hulls (SBH), and corn distillers dried grains with solubles (DDGS)

Gene	WS vs. DDGS	*P* value	DDGS vs. SBH	*P* value	WS vs. SBH	*P* value
CCL1	-2.88	0.01	38.82	< 0.01	13.46	0.03
CCL17	-3.53	< 0.01	28.41	< 0.01	8.05	0.04
CCR2	-2.89	< 0.01	13.70	< 0.01	4.74	0.03
CCR3	-2.70	0.01	27.21	< 0.01	10.08	0.04
CCR4	-4.54	< 0.01	19.89	< 0.01	4.38	0.01
CCR7	-3.25	< 0.01	9.11	< 0.01	2.80	0.02
CD40LG	-1.70	0.02	7.52	< 0.01	4.43	0.01
CXCR2	-4.58	< 0.01	18.62	< 0.01	4.07	0.02
FASLG	-1.69	0.01	13.18	< 0.01	7.78	< 0.01
IL13	-3.04	0.01	11.55	< 0.01	3.79	0.05
IL15	-2.36	0.02	6.92	< 0.01	2.93	0.10
IL17A	-3.72	< 0.01	31.17	< 0.01	8.37	0.01
IL17F	-2.72	< 0.01	26.31	< 0.01	9.68	< 0.01
IL23A	12.20	0.01	33.88	< 0.01	413.19	0.01
IL27	-2.99	< 0.01	24.23	< 0.01	8.11	0.02
IL4	-4.26	< 0.01	38.33	< 0.01	8.99	< 0.01
IL-5	-3.29	< 0.01	26.75	< 0.01	8.12	0.01
IL6R	-1.71	0.03	14.80	< 0.01	8.65	0.01
IL9	-2.52	0.01	44.31	< 0.01	17.57	0.02
OSM	-4.03	0.01	29.78	< 0.01	7.39	< 0.01
IL33	-3.21	< 0.01	12.97	< 0.01	4.04	0.01
LOC100519468	-2.58	< 0.01	21.90	< 0.01	8.48	< 0.01
TNF	-3.21	< 0.01	11.61	< 0.01	3.62	0.02
TNFRSF11B	-3.42	< 0.01	21.43	< 0.01	6.27	0.01

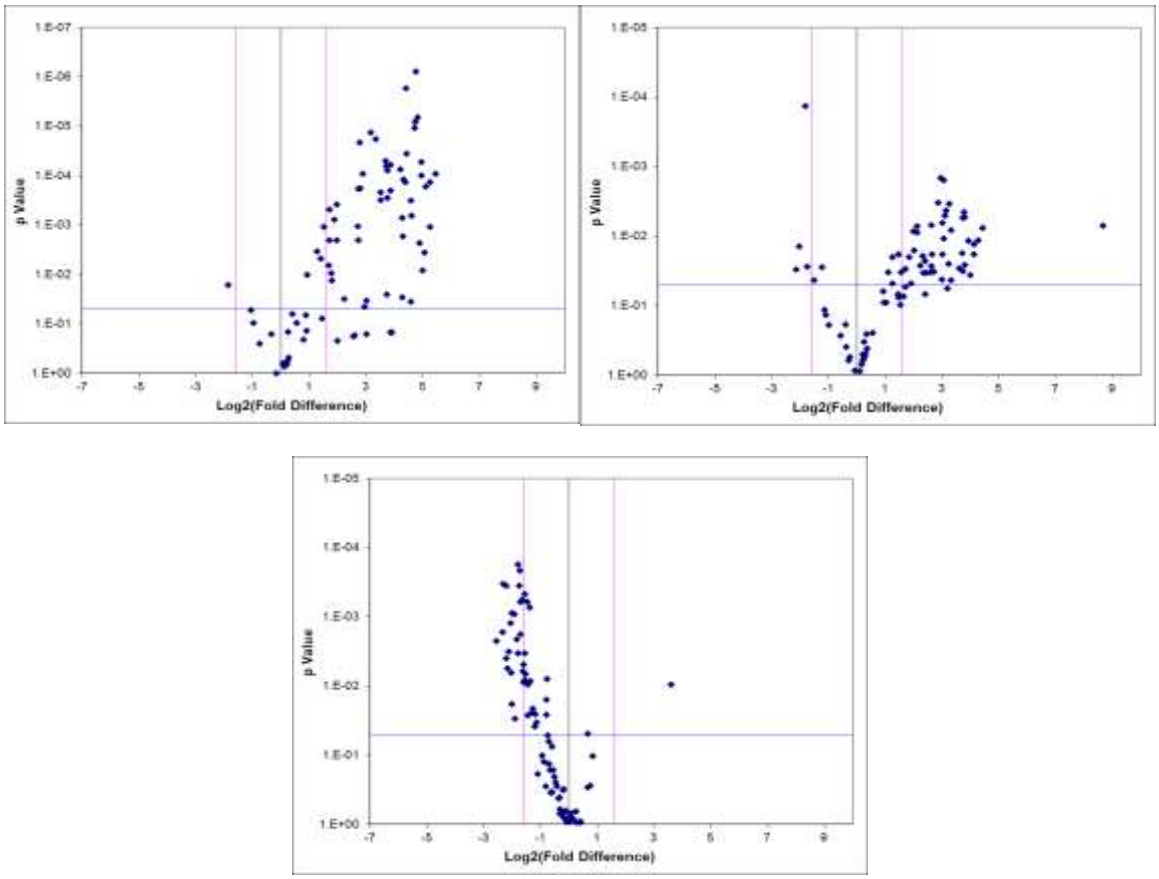

Figure 2.1. Volcano plots of the log differences (X axis) to *P*-value (Y axis) of inflammatory-associated gene expression in pooled ileum samples (5 per group) of pigs between wheat straw and distillers dried grains with solubles (Panel A), distillers dried grains with solubles and soybean hulls (Panel B), and wheat straw vs. soybean hulls (Panel C)

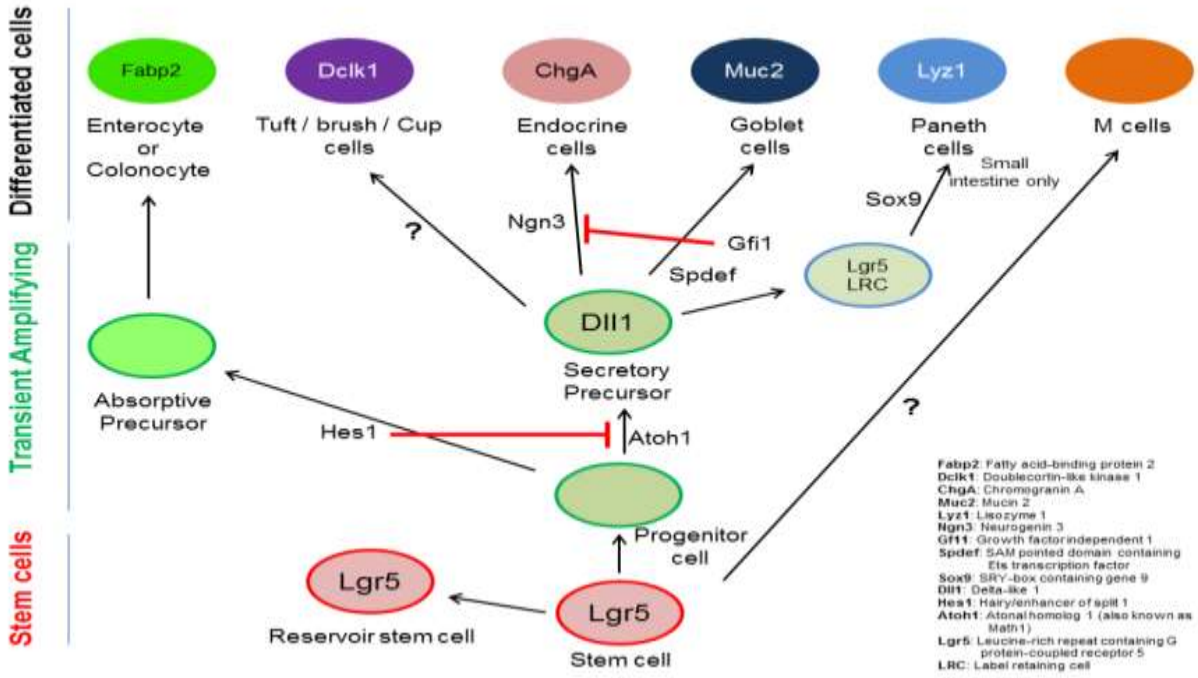

Figure 2.2. Schematic representation of mediators of intestinal cell differentiation

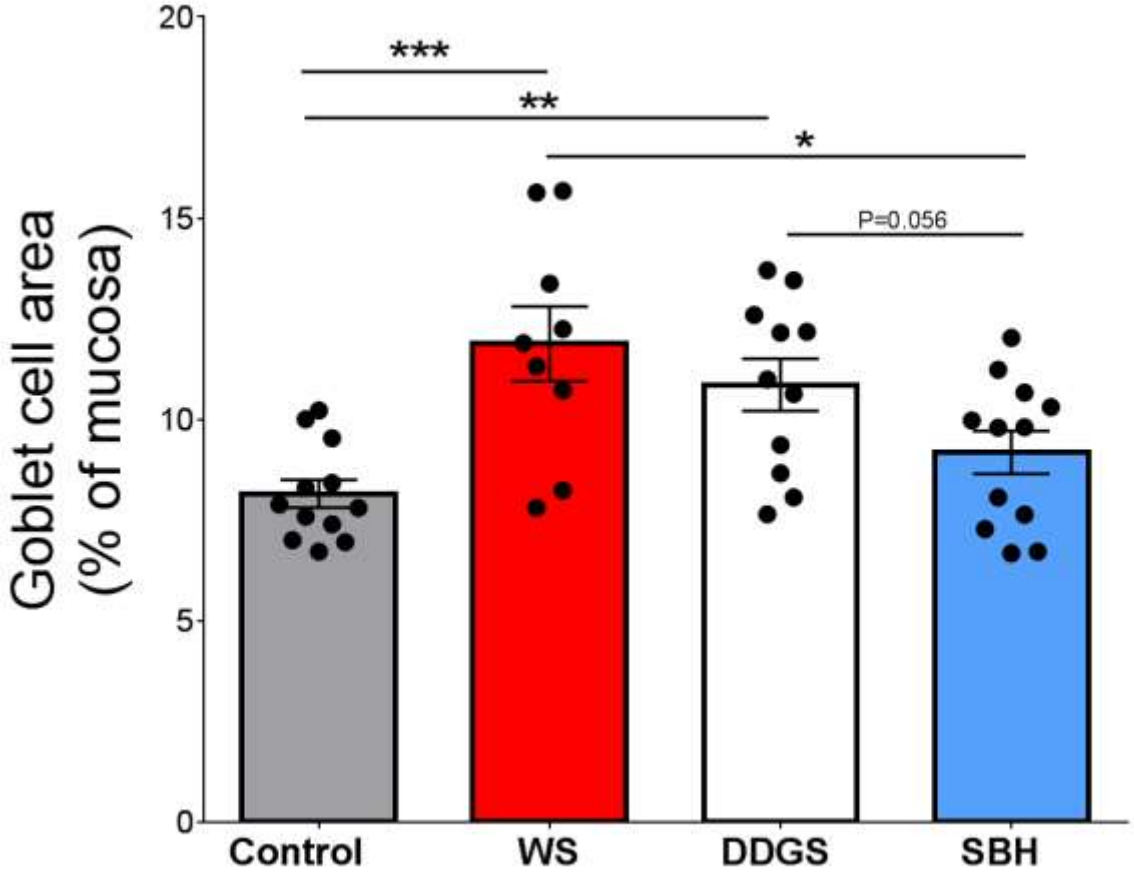

Figure 2.3. Goblet cell area (% of mucosa) of growing pigs fed a corn and soybean meal diet (control), and diets with wheat straw (WS), corn distillers dried grains with solubles (DDGS), and soybean hulls (SBH).