DIGESTIBILITY OF DIETARY FIBER BY GROWING PIGS

BY

PEDRO ENRIQUE URRIOLA

DISSERTATION

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Doctoral Committee:

Associate Professor Hans H. Stein, Chair and Director of Research
Professor George C. Fahey, Jr.
Professor James E. Pettigrew
Associate Professor Kelly S. Swanson
Research Leader Brian J. Kerr, Agricultural Research Service, USDA

ABSTRACT: *In vivo* digestibility and *in vitro* digestibility experiments were used to study effects of feeding distillers dried grains with solubles (DDGS) and other high fiber ingredients to growing pigs. The objective of Exp. 1 was to measure the effects on digestibility of AA, energy, and fiber of adding 30% DDGS to a corn soybean meal diet and to measure intestinal transit time. Growing pigs were cannulated at the terminal ileum and in the cecum. Results showed that apparent ileal digestibility (AID) of Lys (74.1%) was reduced ($P < 0.05$) in the diet with 30% DDGS compared with the control diet (78.6%). However, the AID of most other AA was not affected by the inclusion of DDGS. The AID and the apparent total tract digestibility (ATTD) of energy and TDF were lower in the diet with 30% DDGS (81.0 and 55.5%) than in the control diet (86.0 and 60.0%), but that reduction could not be explained by changes in gut transit time, or by changes in concentration of volatile fatty acids (VFA) in ileal, cecal, or fecal matter. The objective of Exp. 2 was to measure the AID and ATTD of total dietary fiber (TDF) in 24 sources of corn DDGS (C-DDGS), sorghum DDGS (S-DDGS) and a blend of corn and sorghum DDGS (SC-DDGS). We observed that, on average, the ATTD of TDF was 47.3%, but it ranged from 29.3 to 57.0%. The ATTD of TDF was correlated (r^2) to the ATTD of crude fiber (0.42), NDF (0.90), and IDF (0.79), but it was not correlated to ATTD of SDF (0.25) or carbohydrates (0.21). These data suggest that the ATTD of TDF needs to be improved to increase utilization of fiber from DDGS as a source of dietary energy. Therefore, in Exp. 3, the effect of the type of dietary fiber and the breed of pigs were studied. Five light Yorkshire pigs (BW: 80.1 ± 11.2 kg; 4 mo old), 5 heavy Yorkshire pigs (BW: 102.1 ± 3.5 kg; 4 mo old), and 5 Meishan pigs (BW: 77.2 ±15.2 kg; 5 mo old) were cannulated in the distal ileum and fed 5 diets with increasing concentration of soluble dietary fiber (SDF). When fed the corn soybean meal diet (SDF = 0%), Meishan pigs, had a greater ($P < 0.05$) ATTD of DM, GE, and carbohydrates (89.2, 89.5, 95.5%)

than light (86.6, 86.6, and 92.4%) and heavy (87.0, 86.5, and 93.0%) Yorkshire pigs. The ATTD of TDF was greater ($P < 0.05$) in Meishan pigs fed DDGS (75.3%) than in light (39.0%) and heavy (55.7%) Yorkshire pigs. The ATTD of TDF ($P < 0.05$) in DDGS was also greater ($P < 0.05$) in heavy than in light Yorkshire pigs. However, the ATTD of TDF was not different among the 3 groups of pigs when fed sugar beet pulp, soybean hulls, and pectin. These results indicate that the ATTD of TDF is greater in Meishan than in Yorkshire pigs in feed ingredients with high concentration of IDF, but in ingredients containing more SDF, no differences were observed. Because, ATTD of GE is dependent on the ATTD of TDF and because *in vivo* digestibility experiments are expensive and time consuming, it is advantageous to develop procedures to measure digestibility of fiber *in vitro*. The objective of Exp. 4 was, therefore, to modify the 3 step *in vitro* digestibility of OM to measure the *in vitro* ATTD of NDF in a subset of samples that was analyzed in Exp. 2. Results indicate that *in vitro* AID (28.5%) and *in vitro* ATTD (37.5%) of NDF were lower than the *in vivo* AID (45.9%) and ATTD (59.3%) values observed in Exp. 2. There were some agreements between values obtained using both procedures. In DDG, the AID and ATTD of DM (30.1 and 42.5%) and NDF (-19.2 and 17.5%) were lower ($P < 0.01$) than in any source of DDGS and this pattern also was observed in Exp. 2. However, the relationships were not strong enough ($R^2 = 0.12$) to predict *in vivo* ATTD of NDF. In conclusion, dietary fiber from DDGS has an intermediate digestibility and does not affect digestibility of the other nutrients in the diet. The ability of pigs to digest fiber varies with age and breed and there are interactions with the type of fiber. A procedure that measures digestibility of fiber is, therefore, necessary.

Key words: Dietary Fiber, Digestibility, Distillers Dried Grains with Solubles, Energy, Pig

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Thanks to my family for their support. Last, to Maria for sharing ideas, happiness, and understanding that I'm just a dreamer.

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LIST OF ABBREVIATIONS

AA	amino acid(s)
ADF	acid detergent fiber
ADFI	average daily feed intake
ADL	acid detergent lignin
AEE	acid hydrolyzed ether extract
AID	apparent ileal digestibility
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
Arg	arginine
Asp	aspartate
ATP	adenosine triphosphate
ATTD	apparent total tract digestibility
BW	body weight
cal	calorie
C-DDGS	distillers dried grains produced from corn
CHO	carbohydrates
CoA	coenzyme A
CP	crude protein (N × 6.25)
Cu	copper
CV	coefficient of variation
Cys	cysteine
d	day(s)

DDG	distillers dried grains
DDGS	distillers dried grains with solubles
$DDGS_{beverage}$	distillers dried grains with solubles from production of beverage
$DDGS_{ethanol}$	distillers dried grains with solubles from ethanol plant
DE	digestible energy
DM	dry matter
DMI	dry matter intake
Eq.	Equation(s)
Exp.	experiment
Fe	iron
g	gram
G:F	gain-to-feed ratio
GE	gross energy
GLM	general linear model
Glu	glutamate
Gly	glycine
h	hour(s)
HCl	hydrochloric acid
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGF	hindgut fermentation
His	histidine
IDF	insoluble dietary fiber
IL	Illinois

Ile	isoleucine
IVDMD	in vitro dry matter disappearance
L	liter
Leu	leucine
Lsmeans	least square means
Lys	lysine
m	meter
M	molar (concentration; preferred over mol/L)
ME	metabolizable energy
Met	methionine
min	minute(s)
Mn	manganese
MO	Missouri
mo	month(s)
mol	mole
N	nitrogen
N	normal (concentration)
n	sample size
Na	sodium
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NaOH	sodium hydroxide
ND	not determined

NDF	neutral detergent fiber
NE	net energy
NFE	nitrogen free extract
NRC	National Research Council
OM	organic matter
P	probability
PPAR	peroxisome proliferator-activated receptor
Pro	proline
r	simple correlation coefficient
r^2	simple coefficient of determination
rpm	revolutions/minute
s	second(s)
s^2	variance (sample)
SAS	Statistical Analysis System
SBH	soybean hulls
SBM	soybean meal
SC-DDGS	distillers dried grains with solubles produced a blend of sorghum and corn
SD	standard deviation (sample)
SDF	soluble dietary fiber
S-DDGS	distillers dried grains with solubles produced from sorghum
SE	standard error
SEM	standard error of the mean
Ser	serine

TDF	total dietary fiber
TN	Tennessee
Thr	threonine
Trp	tryptophan
Val	valine
VFA	volatile fatty acid(s)
vol	volume
vol/vol	volume/volume
vs.	versus
WBC	water binding capacity
wk	week(s)
wt	weight
σ	standard deviation (population)
Zn	zinc

CHAPTER 1

INTRODUCTION

Swine feeding programs have traditionally used starch and fats as a source of dietary energy. Dietary fiber, on the contrary, was usually kept at the lowest concentration possible, because of its negative impacts on digestibility of energy (Bindelle et al., 2008). There is, however, a growing need for using alternative feed ingredients with relatively high concentrations of dietary fiber because prices of cereal grains have increased due to their utilization in production of biofuels. Better knowledge of the physiological effects and the energy value of dietary fiber may increase the ability of nutritionist to successfully introduce alternative ingredients into swine diets.

Research on dietary fiber shows the diversity of structures of carbohydrates that compose dietary fiber and the diversity of physiological responses that these complex carbohydrates exert on the animal (Elia and Cummings, 2007; Englyst et al., 2007). This creates some difficulty in dealing with dietary fiber and, in fact, the definition and classification are the initial challenges (Noblet and Le Goff, 2001). The first chapter of this thesis, therefore, is a review of the literature on dietary fiber, definitions, classifications, and the effects of fiber on energy value of swine diets.

The newest alternative feed ingredients are coming from the production of biofuels (Stein and Shurson, 2009), and the most common by-products that are used are distillers dried grains with solubles (DDGS). There is evidence that dietary fiber from DDGS may reduce the digestibility of AA and energy (Stein et al., 2006; Petersen et al., 2007; Urriola et al., 2009). However, no data are available on the effects of adding DDGS to complete diets based on corn and soybean meal on the physiological responses of the gut, on the transit time, or on VFA

production. Chapter 2, therefore, provides data on the effect of DDGS in complete diets on physiologic responses of the pig.

The concentration and digestibility of crude fiber, ADF, and NDF have been measured in a limited number of sources of DDGS (Guo et al., 2004; Stein et al., 2009). However, there are no data on the concentration, solubility, and digestibility of dietary fiber in DDGS, but because of the importance of dietary fiber for the digestibility of energy in DDGS, such data are needed. The concentration of digestible dietary fiber was measured in a wide range of sources of DDGS and other by-products of ethanol distillation, and data are presented in chapter 3.

Different breeds of pigs, the age of the pigs, and the time pigs are allowed to adapt to dietary fiber may influence the digestibility of dietary fiber (Kemp et al., 1991; Longland et al., 1993; Le Goff et al., 2002). Chinese Meishan pigs fed a basal diet composed of corn, barley, and wheat had a greater digestibility of crude fiber than Dutch Landrace pigs, but no differences were observed when 15% oats were added to the diet (Kemp et al., 1991). Also Meishan pigs did not digest fiber better than modern breeds of pigs that were fed alfalfa meal (Yen et al., 2004). The interactions among breed of pig, age of the pig, and type of dietary fiber may explain the differences in results observed in the literature. However, there are no data on the interactions of breeds of pigs and type of dietary fiber and there are no data on the effect of breed of pig on digestibility of fiber from DDGS. Thus, chapter 4, tested the hypothesis that differences in digestibility of dietary fiber among breeds of pigs is influenced by fiber type that is fed (insoluble vs. soluble) and by pig age.

Fermentation of TDF supplies some of the energy that pigs obtain from DDGS, but on average, only 47.3% of TDF disappears in the intestines of the pig and this value varies among sources of DDGS (Urriola et al., 2010). There are numerous *in vitro* procedures to measure

digestibility of OM and energy (Boisen and Fernandez, 1997; Regmi et al., 2009) and to measure products of fermentation (Coles et al., 2005). These procedures have been applied to measure *in vitro* digestibility of DM, energy, and CP in corn gluten meal (Guo et al., 2004) and purified corn fibers (de Godoy et al., 2009). However, *in vitro* procedures have not been applied to measure the digestibility of fiber in DDGS and compare results to *in vivo* data from pigs. Chapter 5 contains data from 5 experiments that were conducted with the objective of developing a procedure for measuring *in vitro* digestibility of fiber and measuring *in vitro* digestibility of fiber in DDGS.

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CHAPTER 2

Digestibility of dietary fiber by growing pigs: Review of literature

Cereal grains are currently in high demand because of their use in ethanol production (Stein and Shurson, 2009). This trend is expected to continue and less grain will be available for feeding livestock. As a consequence, livestock producers must use more fibrous feed ingredients to keep feed prices at competitive levels (de Lange, 2008). It is important, therefore, to understand the process that allows the pig to utilize dietary fiber as a source of energy. The objective of this literature review is to determine the current state of knowledge of the utilization of dietary fiber by growing pigs and to review methods for analysis of dietary fiber in feed ingredients. A further objective is to identify methods to improve utilization of dietary fiber by growing pigs.

Definition of Dietary Fiber

There are numerous definitions of dietary fiber, but most of them either define dietary fiber as a group of compounds that are identified in analytical methods or as a group of compounds that have specific physiological functions (Food and Nutrition Board-IOM, 2001). In the 19th century, the Weende procedure defined crude fiber as the organic residue that is insoluble in acid and alkaline treatments (Mertens, 2003). This portion of the diet was considered the *de facto* definition of dietary fiber and without real value to the animal (AACC, 2001).

Later, two researchers in separate ways proposed that this indigestible residue may improve human health (Kritchevsky, 1988). Denis Burkitt reported that bowel cancer is rare in humans who consume a “high residue diet”, and Hugh Trowell suggested that high intake of undigested residue helps protect people in developing countries from ischemic heart disease

(Burkitt et al., 1972; Kritchevsky, 1988; Carpenter, 2003). These conclusions triggered interest in dietary fiber, but it became clear that dietary fiber is a heterogeneous group of chemical components with multiple physiological functions and, therefore, difficult to define (Carpenter, 2003).

It is now accepted that an accurate definition of fiber must include the physiological effects of fiber (IOM, 2006). Therefore, an important aspect of the definition is that dietary fiber consists of carbohydrates that are indigestible by mammalian enzymes (AACC, 2001; IOM, 2006). The inclusion of this term is important, but difficult to measure (Englyst et al., 2007).

The current definition of dietary fiber from the American Association of Cereal Chemists (AACC, 2001) includes the following aspects:

1. It is an indigestible portion of the diet.
2. It consists of carbohydrates and lignin.
3. It originates from plants.
    a. It has physiological effects that increase laxation and reduce blood cholesterol and/or blood glucose.

The definition of dietary fiber by the Institute of Medicine separates the definition into three parts (i. e., dietary fiber, functional fiber, and total fiber). Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans, and total fiber is the sum of dietary fiber and functional fiber (IOM, 2006).

The term non-starch polysaccharide (**NSP**) is related to dietary fiber, but does not cover all components that can be classified as dietary fiber (Elia and Cummings, 2007). For example, NSP does not include oligosaccharides and lignin, which were included in the definitions of

dietary fiber by AACC (2001) and by IOM (2006). Use of the term NSP may not be an accurate description of fiber in feed ingredients because dietary fiber is not limited to NSP or plant cell walls (Cho et al., 1997).

There are several issues that need to be addressed in the definition of dietary fiber (Table 2.1.). Most of these issues are important when it comes to labeling the concentration of dietary fiber in human food products, and special claims of physiological effects. Maximum guaranteed values of crude fiber are required in labels of animal feeds (AAFCO, 2008). The value of crude fiber of a feed ingredient does not have a nutritional relevance. Therefore, the most important issue in swine nutrition is to clearly describe the components of dietary fiber that have nutritional and physiological effects in animals and the compounds that contribute to the energy value of the diet. It is also important that analytical procedures are available to accurately determine the concentration of dietary fiber in animal feed and feed ingredients.

Classification of Dietary Carbohydrates and Dietary Fiber

The classification of dietary fiber starts with the classification of dietary carbohydrates. There are two ways to classify carbohydrates, 1) classification and nomenclature suggested by chemistry societies, and 2) classification according to nutritional properties. Chemistry societies separate carbohydrates in three groups: monosaccharides, oligosaccharides (including disaccharides), and polysaccharides (Nelson and Cox, 2008). Monosaccharides and disaccharides, however, are often grouped together as sugars and oligosaccharides are defined as compounds containing between 3 and 9 monosaccharides while polysaccharides contain more than 10 (Cummings and Stephen, 2007).

*C*arbohydrates are polyhydroxy aldehydes or ketones or substances that yield such compounds upon hydrolysis (Lewis, 2000).The term, carbohydrate, includes monosaccharides,

disaccharides, oligosaccharides, and polysaccharides, but also derived substances such as aldiols, carboxylic acids, substances where one or more hydroxyl groups are replaced (e. g., hydrogen, amino group, thiol group), and derivatives of these substances (IUPAC-IUB Commission on Biochemical Nomenclature, 1996).

Monosaccharides are polyhydroxyaldehydes (aldoses) or polyhydroxyketoses (ketoses). Monosaccharides may contain 3 (triose), 4 (tetraose), 5 (pentose), 6 (hexoses), or 7 (sepoheptulose) carbons and tend to form ring structures that are called furanoses (tetrahydrofuran) or pyranoses (tetrahydropyran; Sturgeon, 2003). Monosaccharides also include analogous structures such as acidic monosaccharides (e.g., glucuronic acid and galacturonic acid; IUPAC-IUB Commission on Biochemical Nomenclature, 1996).

The word "sugar" covers monosaccharides, disaccharides, and polyols (Cummings and Stephen, 2007). Polyols are alcohol derivaties of sugars (e. g., isomaltose, lactitol, maltitol, sorbitol) and are, therefore, included in this group (Elia and Cummings, 2007).

Disaccharides are not included as a division in the classification of carbohydrates; instead they are included in the group called oligosaccharides (Sturgeon, 2003). Oligosaccharides are molecules with two or more monosaccharides joined by glycosidic bonds (Sturgeon, 2003). By convention, oligosaccharides are limited to have 2 to 10 monosaccharides (IUPAC-IUB Commission on Biochemical Nomenclature, 1982). However, more recent publications of the IUPAC-IUB Commissions do not specify how many monosaccharides are included in oligosaccharides and polysaccharides (Cummings and Stephen, 2007; IUPAC-IUB Commission on Biochemical Nomenclature, 1996). Polysaccharides are, therefore, macromolecules composed of an undefined number of monosaccharides (Sturgeon, 2003; IUPAC-IUB Commission on Biochemical Nomenclature, 1996). However, oligosaccharides may be separated from

polysaccharides because polysaccharides precipitate in 80% ethanol while oligosaccharides are soluble (Cummings and Stephen, 2007).

Characteristics of Dietary Fiber

Dietary fiber can also be classified by physicochemical properties. The physicochemical properties include: solubility, water holding and water binding capacity, and viscosity (Kritchevsky, 1988). Physiological properties of dietary fiber are cation binding capacity, binding or adsorption of organic molecules, and susceptibility to fermentation (Kritchevsky, 1988; Cho et al., 1997). The physicochemical properties are easier to measure than the physiological properties, but physiological properties are the most important in nutrition.

Solubility. This is the property that describes how dietary fiber mixes homogenously in different solvents (e. g., cold water, hot water, dilute acid, dilute alkali). The solubility of a polysaccharide depends not on the monosaccharide, but on the links among them (Cho et al., 1997). Cellulose and β-glucans are both composed of glucose, but the β(1→4) glycosidic links in cellulose makes it insoluble whereas the β(1→6) links of β-glucans make them soluble and, therefore, easier to access by microbes (Oakenfull, 2001).

Fractionation of the components of dietary fiber based on solubility starts with extraction of dietary fiber in 75% alcohol (Theander and Åman, 1979). The insoluble residue then is treated with hot water and a chelating agent to extract pectins and the second insoluble residue is treated with alkali to obtain hemicelluloses. The final residue after the extraction with alkali is cellulose (Southgate, 2001).

Separation of dietary fiber into soluble and insoluble fractions was the initial step in understanding dietary fiber (Cho et al., 1997). Soluble fiber influence the absorption of lipids and glucose, while insoluble fiber influence bowel movement and is less fermented in the large

intestine than soluble fiber (Cho et al., 1997; Serena et al., 2008). Some soluble fibers, however, are not fermented in the large intestine and do not influence absorption of lipids or glucose, while some insoluble fibers can be fermented almost completely in the large intestine (Cummings and Stephen, 2007).

Water Holding or Water Binding Capacity. These terms refer to the amount of water absorbed within the structure of fiber (Cho et al., 1997), and they differ because water binding capacity describes the amount of water retained in the fiber after stress has been applied. Stress factors such as centrifugation, pH changes, and particle size reduction can increase the amount of water that dietary fiber retains (Cho et al., 1997). Soluble and insoluble fiber can both retain water because water holding capacity comes from the hydroxyl groups that can form hydrogen bonds with water (Oakenfull, 2001). Water holding capacity is greater in pectin, potato pulp, and sugar beet pulp than in seed residues, pea hulls, and intermediate in wheat and barley (Serena and Bach Knudsen, 2007).

Viscosity. This concept is easier to imagine than conceptualize, and there are many ways to measure and define viscosity. Viscosity is the relationship between the flow of matter and the force that moves it (Dikeman and Fahey, 2006). Honey is more viscous than water and flows slower. Absorption of glucose and other nutrients may be reduced by dietary fiber with high viscosity (Nyman, 2003).

Cation Binding Capacity. Dietary fiber can bind minerals and organic molecules (Oakenfull, 2001). It is believed that free carboxyl groups and uronic acids (ionizable groups) are attached to metal ions. This attachment between fiber and minerals may prevent the absorption of minerals such as Ca^{+2}, Mg^{+2}, and Zn^{+2} (Cho et al., 1997). Some of the compounds in dietary fiber that bind minerals are phytates, but lignin and other co-passengers (e. g. steryl ferulates and

steryl glycosidates) may also have effects on mineral absorption (Kritchevsky, 1988; Adlercreutz et al., 2006).

Binding to Organic Molecules. Dietary fiber can also bind to organic molecules such as bile acids (Scheneeman, 1998). Lignin seems to be one of the strongest binding substances in dietary fiber (Kritchevsky, 1988).

Susceptibility to Fermentation. Dietary fiber differs in the degree to which it can be fermented by microbes (Gallager, 2006). The more susceptible to fermentation a dietary fiber source is the more energy the pig obtains as VFA (McBurney and Sauer, 1993). Fermentation of a dietary fiber depends on the access of bacterial enzymes to their substrate, chemical composition of the substrates, solubility, water holding capacity, and porosity of the fiber (Cho et al., 1997; Guillon et al., 1998; Gallaher, 2006; Guillon et al., 2006).

Laxation. The effect of dietary fiber on laxation depends on many of the factors discussed above (Oakenfull, 2001; Cho et al., 1997). Therefore, more than being a single characteristic of fiber, laxation and fermentation are the combination of the previous physicochemical properties of fiber.

Methods to Measure Dietary Fiber

There are many methods available for determination of the concentration of dietary fiber in human food, animal feed, and feed ingredients. All methods include two basic steps; first, digestion of carbohydrates and other non-fiber components of the diet (e. i., protein, fat, water, minerals) and, second, quantification of the undigested residue. The digestion procedure can use chemical compounds (e. g., acid, alkali, and detergents) or use enzymes (amylase, amyloglucosidases, and proteases). Measurement of the indigestible residue can be accomplished by weighing the residue (gravimetric) or by measuring chemical compounds in the residue using

chromatography, gas liquid chromatography, and high performance liquid chromatography. There are newer methods to study the composition and structure of non-starch polysaccharides in the cell wall of plants and it relationship with degradation in the gut (Guillon et al., 2006). These methods include Raman Microspectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), inmunolabeling, fluorescence, and mass spectroscopy, among others (Guillon et al., 2006).

Crude Fiber Analysis. This is a chemical-gravimetric method that is part of the proximate analysis of feed ingredients developed at the Agricultural Experimental Station in Weende, Germany (Grieshop et al., 2001). The method separated carbohydrates into 2 portions, nitrogen free extract and crude fiber. Crude fiber is the residue that is left after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide (Cho et al., 1997; Furda, 2001). At the time of the development of the procedure, it was only known that digestion included acid and alkaline processes, but enzymes were not known (Mertens, 2003). The crude fiber procedure is very robust and repeatable, but there is no relationship between crude fiber and any definition of dietary fiber (Mertens, 2003) because the recovery of cellulose (40 to 100 %), hemicelluloses (15 to 20 %), and lignin (5 to 90 %) is not complete (Grieshop et al., 2001; Metens, 2003). However, the procedure still is used to regulate maximum fiber concentration in animal feed (Mertens, 2003).

Detergent Fiber Procedures. The detergent procedure is a chemical-gravimetric procedure that empirically relates the value from the analysis to the physiological properties of dietary fiber (Van Soest et al., 1991). The procedure separates dietary fiber into neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin fractions (ADL; Robertson and Horvath, 1992). This procedure was an improvement over the crude fiber procedure; however, it does not recover soluble dietary fiber such as pectins, mucilages, gums, and β-

glucans (Grieshop et al., 2001). The lack recovery of soluble dietary fiber components is less concerning in cereal grains such as corn and DDGS that have high concentrations of insoluble fiber (Johnston et al., 2003). Other problems with the detergent procedure include the possible contamination of the residue with starch that reduces robustness and repeatability (Mertens, 2003).

Total Dietary Fiber. The procedure of Prosky is known as the total dietary fiber procedure (**TDF**) AOAC Official Method 985.29 (AOAC Int., 2007) and has been modified to determine soluble and insoluble dietary fiber by AOAC Official Method 991.43 (AOAC Int., 2007). The TDF procedure uses enzymes (e.g., amylase, glucoamylase, and protease) to mimic digestion in the small intestine, then the residue is weighed (Theander and Åman, 1979). The residue also is analyzed for undigested proteins and ash. Therefore, the TDF procedure is more time consuming and less reproducible than the crude fiber and detergent methods (Mertens, 2003). More work is needed to improve the TDF procedure to include low molecular weight indigestible carbohydrates and correct for contaminants of the indigestible residue (Gordon et al., 2007).

Enzymatic Chemical Methods. There are several methods that combine the initial steps of enzymatic digestion with chemical determination of sugars in the undigested residue (Theander and Åman, 1979; Campbell et al., 1997; Grieshop et al., 2001). The Uppsala method calculates TDF as the sum of amylase-resistant polysaccharides, uronic acids, and Klason lignin (AOAC Int., 2007; Grieshop et al., 2001). The digestion step in the AOAC Official Method 994.13 uses a heat-stable α-amylase and amyloglucosidase (AOAC Int., 2007). The residue is divided into soluble and insoluble fractions by 80% ethanol. The neutral sugars released are quantified as alditol acetate derivatives by gas liquid chromatography and uronic acids

chromatographically (Theander and Åman, 1979). The NSP method developed by Englyst and Cummings is similar to the Uppsala method but it excludes lignin and resistant starch from the final value (Englyst et al., 1996; Grieshop et al., 2001). There are other similar methods that are not as common as the two methods mentioned above. These are the methods of Schweizer and Wursh and the method developed in 1969 by Southgate (Cho et al., 1997; Southgate, 2001). These procedures determine neutral sugars colorimetrically, which is easier than GLC, but they are less accurate and, therefore, not used in the current research.

Campbell et al. (1997) compared values for dietary fiber in several grains as measured by the procedures of Prosky, Englyst, and Thelander. The Uppsala and NSP methods resulted in lower concentrations of dietary fiber than the procedure described by Prosky. The NDF procedure resulted in the lowest concentration of dietary fiber.

Estimation of Dietary Fiber by Difference. Direct measurement of dietary carbohydrates is a tedious procedure and it is more difficult than measurement of other dietary nutrients (e. i., protein, fat, water, and ash). Calculation of carbohydrates by difference is a method to circumvent these problems (FAO, 2002). The underlining assumptions of this method are that all other nutrients have relative low analytical errors and that all values are additive. Total carbohydrates are calculated using Eq [1]:

Total carbohydrates/100 g = 100 – (protein, g + fat, g + water, g + ash, g) [1]

The concentration of carbohydrates in human food is commonly calculated from the values analyzed for other nutrients following Eq. 1 (IOM, 2006). Also, the total carbohydrates can be separated in available or digestible carbohydrates and indigestible carbohydrates or dietary fiber. The Weendee System, developed in 1860, separated carbohydrates in crude fiber

and nitrogen free extract (**NFE**). The Cornell Net Carbohydrate and Protein System (**CNCPS**) separate total carbohydrates in digestible carbohydrates and NDF.

Total carbohydrates g = non fiber carbohydrates – NDF

Other variations of the concept include calculation of indigestible carbohydrates by analyzing starch and sugars along with protein, fat, and water (de Lange, 2008). For practical purposes, the concentration of indigestible nutrients in feed ingredients can be calculated using the following Eq. [2]:

Indigestible carbohydrates, g = DM, g – (ash + starch + sugars + protein + fat) [2]

Calculation of carbohydrates by difference includes, however, the cumulative errors from all other analytical procedures (e. i. protein, fat, ash, DM). Also, these calculations do not take into account other components of the diet that are not analyzed such as polyols, alcohol, and organic acids. Therefore, the value is not reliable for feed ingredients and should be discouraged.

Utilization of Dietary Fiber by Growing Pigs

Fermentation. The environment in the intestine requires that microbes live without oxigen. There are 3 types of microorganism that can live without oxygen, anaerobic phototrophs, anaerobic respires (sulfate reducers, methanogens, acetogens), and fermentative microorganisms (White, 2000; Müller, 2008). Fermentative microorganisms conserve energy in a process in which electrons from redox reactions are transferred to part of the substrate from which energy is derived. The substrate is only partially oxidized during fermentation and only a small amount of energy is conserved for microbial growth (Müller, 2008).

Microbes start breaking down polysaccharides into smaller polysaccharides or the constituent carbohydrates during fermentation of dietary fiber in the pig intestine (Müller, 2008). Depolymerization occurs using a combination of reactions (e. g., hydrolysis, redox,

phosphorylation, and lyases). The monomers then are absorbed into the microbial cell and channeled into the pathways of central metabolism (White, 2000). The final products are excreted from the microbial cell into the intestinal lumen. Other microbes can use the products of the first microbe as a substrate and excrete a second product (anaerobic food chain). Finally, the pig absorbs some of the end products of fermentation of carbohydrates, mainly VFA (Table 2.2).

Absorption of VFA. The absorption of VFA in the pig large intestine is a very efficient process (Barcroft et al., 1944). When VFA were infused in the cecum of growing pigs, less than 1% were excreted in the feces (Jørgensen et al., 1997). Absorption of VFA is proposed to occur by 3 mechanisms 1) diffusion of protonated VFA 2) anion exchange (Wong et al., 2006), and 3) transporter mediated (Kirat and Kato, 2006). Diffusion of protonated VFA is likely the least important of the 3 mechanisms because at physiological pH, only 1% of all VFA in the intestinal lumen is protonated (Cook and Sellin, 1998). If anion exchange is used, VFA are taken up into the enterocyte and HCO_3^- is released to the intestinal lumen (Cook and Sellin, 1998). More recent studies have documented the existence of active transportation of VFA. Active transporters of VFA belong to the monocarboxilate family and MCT-1 is the transporter present in the intestine of pigs (Welter and Claus, 2008). Another transporter expressed in human colonocytes is the sodium-coupled monocarboxylate transporter or SLC5A8 that may be implicated in absorption of VFA, especially butyrate (Thangaraju et al., 2008). The MCT1 transporter has been identified in pig intestinal cells, but is not clear if the SLC5A8 is also present in pig colonocytes.

Absorption of VFA also facilitates absorption of other nutrients from the diet. Water and sodium are absorbed along with VFA (Yen, 2001). Plant lignans, diphenolic compounds similar to endogenous steroid hormones are also co-transported by VFA (Bach Knudsen et al., 2006).

Inulin improves the bioavailability of iron in corn and soybean meal diets in young anemic piglets (Yasuda et al., 2006). It is not clear if inulin increases absorption of Fe by increasing production of VFA and thereby VFA increase absorption of Fe, or if VFA reduce luminal pH and increase solubility of Fe, or if VFA increase the expression of the Fe transporters (Tako et al., 2008).

Metabolism of VFA. The VFA are metabolized in 3 ways: 1) by colon cells that use them as an energy source, 2) by the liver that use propionate for gluconeogenesis, and 3) by adipose tissue and muscle (Wong et al., 2006). The concentration and molar proportions of VFA in portal blood is different from that in intestinal digesta suggesting that VFA are metabolized in the intestinal cells (Argenzio et al., 1974; Marsono et al., 1993). The typical VFA molar proportions in intestinal content is 65:25:10 (acetate:propionate:butyrate). This ratio changes after absorption and passage thought liver to 90:10:0 in hepatic circulation demonstrating selective metabolism of VFA in enterocytes and in liver (Robertson, 2007).

Human colonocytes metabolize 70 to 90% of absorbed butyrate to CO_2 and ketone bodies, and therefore, use butyrate as an energy source instead of glutamine (Kritchevsky 1988; Elia and Cummings, 2007). Butyrate is not only an energy source, but it also regulates cell proliferation and differentiation, which in turn may contribute to prevention of colorectal cancer and other diseases (Cook and Sellin, 1999; Wong et al., 2006).

Most of the acetate and propionate leave the intestine without being metabolized and reach the liver where propionate is metabolized for gluconeogenesis (Wong et al., 2006). Propionate metabolism may inhibit hydroxymethyl glutaryl (HMG) CoA reductase, and therefore, inhibit synthesis of cholesterol (Wong et al., 2006). Most of the acetate is believed to be transported to the adipose tissue and skeletal muscle where it is used in the synthesis of fatty

acids or oxidized and used for synthesis of ATP (Elia and Cummings, 2007). The energy absorbed as VFA accounted for 67 to 74% of the total energy absorbed in the hindgut of pigs fed high fiber diets (Anguita et al., 2006), and the energy from VFA provided 7.1 to 17.6 % of the total available energy. In some cases up to 82% of the energy infused inside the cecum as VFA was retained as body energy (Jørgensen et al., 1997).

The effects of VFA on metabolism of fatty acids and fat distribution are not fully understood (Robertson, 2007), but it is suggested that VFA, and especially propionate, may change adipose tissue lipolysis, change adipocyte size and differenciation, and change body fat distribution. Especially VFA appear to stimulate PPARγ, acetyl CoA carboxylase, and fatty acid synthase (Lee and Hosser, 2002).

Factors Affecting Energy Value of Dietary Fiber

Estimates of production and concentration of VFA in ileal and cecal digesta and in fecal samples vary among experiments (Table 2.3). There are several factors that influence the concentration and production of VFA in the intestine of growing pigs. These factors may be divided into factors inherent to the pig and factors inherent to the diet.

Factors Inherent to Pigs. Factors depending on the pigs are age and breed. Sows can digest greater amount of dietary fiber than growing and finishing pigs (Le Goff and Noblet, 2001), which may be explained by a slower rate of digesta passage in the intestine of sows (Grieshop et al., 2001). However, the ability of the microbial flora of sows to digest fiber is believed to be similar to that of growing pigs (Le Goff et al., 2003), so that the greater fermentability in sows is mainly a function of a greater microbial population and a longer retention time of digesta.

Meishan pigs have a greater ability to digest fiber than pigs from Western breeds (Fevriere et al., 1988; Kemp et al., 1991). Several other native breeds of pigs such as Mukota (Zimbabwe), Mong Cai (Vietnam), Kune-Kune (New Zealand), Schwaebisch Haellisches Schwein and Bunte Bentheime have greater capacity for digestion of dietary fiber than crossbred pigs (Ndindana et al., 2002; Len et al., 2006; Morel et al., 2006; von Heimendahl et al., 2009). However, greater fermentation capacity is not only observed among native breeds of pigs. Among crossbred pigs, high lean growing pigs, were observed to digest more energy than slow growing pigs, which was explained by a greater concentration of cellolytic bacteria in the large intestine of lean growing pigs (Varel et al., 1988). However, other experiments have not observed greater digestibility of dietary fiber in Meishan pigs than in pigs from crossbred pigs (Yen et al., 2004). There is no information about which portion of dietary fiber (insoluble or soluble) native breeds digest better than Western crossbreeds.

Factors Inherent to the Diet. There are several processes that can be used to improve the microbial degradation of dietary fiber in fibrous feedstuffs, which may consequently increase the energy value of the ingredient. These processes include physical processes (e.g., grinding, heating, irradiation, mechanical separation of plant parts) and chemical processes such as hydrolytic and oxidative agents. Sodium hydroxide may increase rumen digestibility of OM from 52% to 76% in barley straw and the digestibility of DM by 22% in other crop residues (Fahey et al., 1993). The disadvantage of NaOH is that it may leak to soil where it is pollutant. Anhydrous NH_3, NH_4OH, thermoammoniation, and urea have been used to treat fibrous materials, but the increment in digestibility is not as great as when using NaOH. In 32 experiments where crop residues were treated, digestibility of DM by ruminants increased by 15% (Fahey et al., 1993). Other chemicals such as $Ca(OH)_2$ and KOH have also been used to treat fibrous crop residues. In

fact KOH had similar effect as NaOH, but the cost of KOH may be too high to make this process profitable (Fahey et al., 1993).

Treatment of fiber with oxidative agents, such as ozone increases IVDMD from 44% to 67%. However, at ground level ozone is also a pollutant and therefore leak to the environment need to be controlled (Fahey et al., 1993). Hydrogen peroxide may increase the apparent rumen digestibility of cellulose from 56.5% to 85.7% (Kerley et al., 1985) and sulfur dioxide can increased *in vitro* digestibility of DM by 80%. However, the extra sulfur in the treated feed may not be tolerable to animals (Fahey et al., 1993). Ammonia fiber expansion (AFEX) may be used as pretreatment of DDGS before enzymatic digestion of cellulose. This procedure combines ammonia and high pressure to increase degradability of fiber, and has been observed to degrade 100% of the cellulose in DDGS (Realf and Abbas, 2004; Bals et al., 2006).

Contribution of Energy from Dietary Fiber

Degradation of dietary fiber varies among feed ingredients, type of fiber, and the interaction among dietary factors (Högberg and Lindberg, 2004; Bindelle et al., 2008). All those factors are taken together as fermentability of dietary fiber. The apparent ileal digestibility (**AID**) of dietary fiber by pigs vary from -10% to 62% (Bach Knudsen and Jorgensen, 2001). The apparent total tract digestibility (**ATTD**) of cellulose vary from 23 to 65% in barley, from 24 to 60% in wheat and wheat by products, from 10 to 84% in rye and in rye fractions, and from 13 to 42% in bran, hulls of wheat, corn, and oats. The ATTD of TDF in DDGS produced from corn is 47.5% and varies among sources of DDGS from 29.3 to 57.0% (Urriola et al., 2009). The ATTD of soluble dietary fiber (92.0%) is greater than the ATTD of insoluble fiber (41.3%; Urriola et al., 2009).

Amount of VFA Produced per g of Fermented Fiber. The quantities of VFA that are produced from fermentation depend on the fiber that is being fermented. As an example, fermentation of oligosaccharides from soybeans yields more gasses (CH_4, H_2) that are not used for energy by the animal than fermentation of other substrates such as resistant starch (Cummings, 1981; Liener, 1994; Topping and Clifton, 2001; Middelbos and Fahey, 2008). However, acetate, propionate, and butyrate are the VFA produced in the largest quantities and, therefore, the only VFA reported in most experiments, but fermentation of branched chain AA yields branched chain VFA (isobutyrate, isovalerate, and valerate; Nelson and Cox; 2008), but the total production of VFA from AA fermentation is usually of minor importance and is, therefore, often disregarded.

Fermentation of resistant starch is suggested to yield more butyrate than fermentation of other fiber components (Topping and Clifton, 2001) and fermentation of specialized fiber yields variable ratios of acetate: propionate: butyrate (Macfarlene and Macfarlene, 1993). As a consequence, the energy that is available for absorption is different among these specialized fibers (Table 2.5). However, for most raw fibrous feed ingredients, the ratio of acetate: propionate:butyrate is relatively constant. It has, therefore, been suggested that for practical purposes, those ratios may be assumed constant among all types of feed ingredients (Wang et al., 2004; de Lange, 2008).

Production of ATP from VFA. The number of moles of ATP produced by each mole of VFA that is oxidized by the animal tissues is 10 ATP for acetate, 18 ATP for propionate, and 28 ATP for butyrate (Table 2.6). The total quantity of ATP produced can be calculated by multiplying the total amount of each VFA by the number of ATP that is produced from each VFA. The energy that is produced from each ATP is similar for all 3 VFA and average

approximately 7.3 kcal/mole of ATP (Nelson and Cox, 2008) so the total kcal yield is calculated by multiplying the total number of moles of ATP by 7.3 kcal.

Fermentation of one mole of glucose yields the following compounds (Cummings 1981): $58(C_6H_{12}O_6) \rightarrow 62CH_3COOH + 22CH_3CH_2COOH + 16CH_3(CH_2)_2COOH + 60.5COO_2 + 33.5CH_4 + 27H_2O$.

If it is assumed that all fermentation of carbohydrates is from glucose as is the case if cellulose is fermented, the fermentation products and the total VFA production can be used to calculate potential absorbed energy (Table 2.6). In this calculation, the potential absorbed energy (1.02 kcal/g) from fermentation of cellulose is approximately 70% of the energy that can be produced if glucose is oxidized (Table 2.7).

In reality, not all sugars in fiber are glucose. Hemicellulose contains also pentoses that have lower energy value than glucose. It is, therefore likely that the actual energy value of fiber is less than 1.02 kcal/g of glucose. It has been suggested that the amount of energy that a pig can utilize from fiber is 60% of the value for glucose (Boisen, 2007), which is equivalent to 0.88 kcal/g of fermented fiber.

The energy value of dietary fiber from a feed ingredient can, therefore, be estimated by measuring the ATTD of TDF in the ingredient and then multiply the resulting value by the total concentration (g/kg DM) of TDF in the feed ingredient. The calculated value, g of digested TDF/kg feed DM is then multiplied by 0.88 or 1.02 kcal/g digested TDF to obtain the final energy from fermentation of the feed ingredient. The method has the limitation that it assumes no interaction between dietary fiber and digestibility of nutrients in the diet.

In conclusion, Utilization of dietary fiber is an important subject for swine nutrition because more high fiber ingredients will be fed to pigs. Current methods to measure dietary fiber

do not measure all components that are defined as dietary fiber, but the TDF procedure appears to be the most accurate procedure available. The most important characteristic of dietary fiber from the perspective of energy utilization is fermentation and the amount of VFA absorbed by the pig. Fermentation of fiber depends on factors inherent to the diet pig and factors inherent to the pig. Soluble dietary fiber is much more fermentable than insoluble dietary fiber, and the energy value of fiber increases with the concentration of SDF. Longer adaptation time to high fiber diets may increase the digestibility of fiber and energy and Meishan pigs may be more efficient in fermenting dietary fiber than western crossbred pigs.

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Table 2.1. Issues of current definition of dietary fiber

Item	Issue
Animal origin	Chitosan and mucopolysaccharides are included in the analysis of dietary fiber. However, IOM and AACC definitions exclude them. When analyzing ileal digesta and fecal samples, mucopolysaccharides are included in the value of dietary fiber (Food and Nutrition Board, 2001).
Lignin	Is not a carbohydrate but by convention is classified as dietary fiber (AACC, 2001; IOM, 2006)
Products of Maillard reaction	These compounds are produced when amino acids and carbohydrates are heated. Does not constitute dietary fiber by definition but some analysis may include this residue (Kritchevsky, 1988). Maillard products are in greater concentration in heated feed ingredients (e.g., DDGS). Late Maillard products may have no energy value and, therefore, they may underestimate the dietary fiber value of heated feed ingredients.
Fatty derivatives	Two hydroxyl acid monomers (C16 and C18) constitute cutin. Cutin is part of plants, is undigestible, and is part of dietary fiber (Cho et al., 1997). Mineral oil is neither digested nor absorbed and causes laxation. These characteristics fit with part of the definition of dietary fiber (Cho et al., 1997). However, it is not common among feed ingredients.

Table 2.1. (cont.)

Item	Issue
Precipitation in ethanol	Raffinose, stachyose, verbacose, fructooligosaccharides, inulin, guar gum, methylcellulose, and polydextrose are resistant to enzymatic digestion. However, these compounds do not precipitate in alcohol and do not appear in the dietary fiber analysis. Therefore, the concentration of fiber in feed ingredients with high concentration of oligosaccharides is underestimated and so is the energy value of the feed ingredient.
Special mono – disaccharides	Small amounts of mono- and disaccharides may not be absorbed in the small intestine, but are soluble in ethanol and fit the definition of dietary fiber (Food and Nutrition Board, 2001).

Table 2.2. Common plant derived carbohydrates and associated compounds in animal nutrition

Class	Sub-group	Principal monomers and component
Monosaccharides		
	Pentoses	Xylose, arabinose
	Hexoses	Glucose, galactose, mannose, fructose
	Acids	Glucoronic, galacturonic
Disaccharides		Sucrose, maltose, celliobiose, trehalose
Oligossacharides		
	Maltodextrins	Maltotriose, α-limiting maltodextrins, resistant maltodextrins
	α-galactosides	Raffinose, stachyose, verbascose
	Non-α-glucan	Inulin (fructose β(2→1), levans (β(2→6)
	Functional	Fructooligosaccharides, trans-galactooligosaccharides, α-galactooligosaccharides, mannanoligosaccharides
Polysaccharides		
	Starch	Glucose α(1→4)
	Resistant starch	Physical inaccessible (R1), native (R2), retrograded (R3), chemical modified (R4)

Table 2.2. (cont.)

Class	Sub-group	Principal monomers and component
Non starch polysaccharides (NSP)		
Cell wall NSP	Cellulose	Glucose β(1→4)
	β-glucan	Glucose β(1→3), Glucose β(1→4)
	Xyloglucans	backbone of xylans and branches of glucose
	Arabinoxylans	chain of xylose β1→4, and branches of arabinose
	Arabinogalactans	Minor group of hemicelluloses
	Galactans	Galactose
Non-cell wall NSP	Pectins	Galacturonic acid backbone with branches of galactose, glucose, xylose with several degrees of methylation
Gums	Galactomannans	Mannose β(1 →4) backbone with branches of α(1→6) galactose
	Glucomannans	Konjac mannans
Synthetic NSP	Polydextrose	Glucose
	Dextrins	Glucose

Table 2.3. Products of fermentation of carbohydrates in the large intestine of mammals

Product	Characteristics
Bacterial cells	The main product of microbial fermentation in the intestine
Volatile fatty acids (acetate, propionate, and butyrate)	The most predominant end-products of microbial fermentation
Branched-chain VFA (isovalerate, valerate, and isobutyrate)	Products of the fermentation of branched chain AA (Iso, Leu, Val)
Ethanol, succinate, and lactate	Produced during fermentation, but are further metabolized by other bacteria into VFA
Gases, such as CO_2, CH_4, H_2, and H_2S	Produced during fermentation
Biogenic amines	Produced from decarboxylation of AA
Phenols and indoles	Produced from fermentation of cyclic substrates such as aromatic amino acids (i. e., Tyr, Phe, and Trp)
Ammonia, urea, and nitrate	Produced during microbial metabolism of protein
Heat	Produced during fermentation of food in the intestine

[1]Modified from Macfarlane (1991).

Table 2.4. Concentration of VFA in ileal, cecal, or fecal samples of growing pigs

Reference	Acetic	Propionic	Butyric	BCVFA[1]	Total
Ehle et al., 1982		------ m*M* g DM digesta^{-1} ------			
Cecum	106	40	20	10	176
Upper colon	97	40	20	15	172
Lower colon	70	32	15	19	136
Feces	27	13	7	5	42
Just et al., 1983		------ mmol d^{-1} ------			
Ileal	N/A	N/A	N/A	N/A	200
Feces					88
		------ mmol kg feed intake^{-1}			
Mc Burney and Sauer, 1993	1,094	309	189	76	1,668
Jensen et al., 1997	530	422	211	117	1,280
Christensen et al., 1999	319	134	64	43	560
Smiricky-Tjardes et al., 2003		------ µmol g DM digesta ------			
Ileal	71	27	24	N/A	122
Högberg and Lindberg, 2004		------ mmol L^{-1} ------			
Ileal	27	12	2.4	N/A	41.4
Anguita et al., 2006		------ mmol kg DM intake^{-1} ------			
Feces	16	6	3	N/A	25
Bindelle et al., 2009		------ mg g of hydrolyzed residue^{-1} ------			
In vitro fermentation	106	42	11	2	161

[1]Branched chain VFA.

Table 2.5. Molar ratios and potential absorbed energy after fermentation of sources of dietary fiber

	Fiber type	Acetate	Propionate	Butyrate	Total
Energy in VFA, kcal/mol		209	367	529	1,105
----Wang et al., 2004----					
Molar ratios	Control	52	23	14	89
	Potato starch	54	22	16	92
	Sugar beet pulp	60	23	12	95
	Wheat bran	55	28	10	93
Energy	Control	10,868	8,441	7,406	26,715
	Potato starch	11,286	8,074	8,464	27,824
	Sugar beet pulp	12,540	8,441	6,348	27,329
	Wheat bran	11,495	10,276	5,290	27,061
---Macfarlane and Macfarlane, 1993					
Molar ratios	Starch	50	22	29	101
	Pectin	84	14	2	100
	Arabinogalactans	50	42	8	100
	Xylan	82	15	3	100
Energy	Starch	10,450	8,074	15,341	33,865
	Pectin	17,556	5,138	1,058	23,752
	Arabinogalactans	10,450	15,414	4,232	30,096
	Xylan	17,138	5,505	1,587	24,230

Table 2.6. Production of moles of ATP per mole of VFA[1]

Item	A Amount, moles	B ATP/moles	C Total ATP (A×B)	D ATP, kcal/mole (Constant)	E Energy, kcal (C×D)	F kcal/mole
Glycolisis						
Glucose $C_6H_{12}O_6$	58	36	2,088	7.3	15,242	262
Fermentation[2]						
Glucose $C_6H_{12}O_6$	58	36		7.3		
Acetic CH_3COOH	62	10	620	7.3	4,526	73
Propionic CH_3CH_2COOH	22	18	396	7.3	2,891	131
Butyric $CH_3(CH_2)_2COOH$	16	28	448	7.3	3,270	204
Carbon dioxide CO_2	60.5	0		7.3	0	
Methane CH_4	33.5	0		7.3	0	
Water H_2O	27	0		7.3	0	

[1]Brody, 1945; Cummings, 1981; Blaxter 1989

[2]$58(C_6H_{12}O_6) \rightarrow 62CH_3COOH + 22CH_3CH_2COOH + 16CH_3(CH_2)_2COOH + 60.5CO_2 + 33.5CH_4 + 27H_2O$

Table 2.7. Summary of energy from VFA as compared to energy from glucose.

Item	GE/58 moles	Kcal/mole	MW	Kcal/g
Glucose	15,242	262	180	1.46
Total VFA	10,687	184	"180"	1.02

CHAPTER 3

Effects of distillers dried grains with solubles on AA, energy, and fiber digestibility and on intestinal marker appearance of a corn soybean meal diet fed to growing pigs[1]

ABSTRACT: The objective of this experiment was to measure the effect of distillers dried grains with solubles (DDGS) on the digestibility of AA, energy, and fiber, on the fermentation of fiber, and on the first appearance of digesta at the end of the ileum, in the cecum, and in the feces of growing pigs fed a corn soybean meal-based diet. Sixteen pigs (initial BW: 38.0 ± 1.6 kg) were prepared with a T-cannula in the distal ileum and a T-cannula in the cecum and allotted to 2 treatments. In period 1, all pigs were fed a corn soybean meal diet. In periods 2, 3, and 4, pigs were fed the control diet or a diet containing corn, soybean meal, and 30% DDGS. First appearance of digesta at the end of the ileum, in the cecum, and over the entire intestinal tract was measured at the end of period 4. The apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) of nutrients were measured and the concentration of VFA was analyzed in ileal, cecal, and fecal samples. The AID of Lys (74.1%) in the DDGS diet was lower ($P < 0.05$) than in the control diet (78.6%), but the AID of most other AA and GE, NDF, and total dietary fiber (TDF) were not different between the 2 diets. The ATTD of GE (81.0%), NDF (57.2%), TDF (55.5%), and DM (81.7%) were lower ($P < 0.05$) in the DDGS diet than in the control diet (86.0, 69.3, 66.0, and 87.2%, respectively). The concentration of VFA in ileal, cecal, and fecal samples was not different between pigs fed the 2 diets. The pH of ileal and cecal digesta from pigs fed the DDGS diet (6.3 and 5.5) was greater ($P < 0.01$) than from pigs fed the control diet (5.8 and 5.3). The ATTD of DM, GE, ADF, NDF, and TDF did not change with

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collection period, but the AID of ADF, NDF, and TDF increased ($P < 0.05$) from period 2 to period 4. The concentration of all VFA, except isobuturate, was greater ($P < 0.05$) in cecal samples from period 4 compared with period 2, and the concentration of all VFA except propionate and isovalerate were greater ($P < 0.05$) in fecal samples collected in period 4 compared with those collected in period 2. The first appearance of digesta at the end of the ileum, in the cecum, and in the feces was not affected by DDGS. In conclusion, pigs fed the diet containing DDGS had a lower digestibility of Lys, GE, ADF, NDF, and TDF than pigs fed the control diet. The digestibility of DM and GE was not influenced by collection period, but the concentration of VFA in cecal digesta and feces increased with the length of time pigs received the diets.

Key words: digestibility, distillers dried grains with solubles, energy, fiber, pig

INTRODUCTION

The apparent total tract digestibility (**ATTD**) of energy and the apparent ileal digestibility (**AID**) of AA are less in distillers dried grains with solubles (**DDGS**) than in corn (Stein et al., 2006; Pedersen et al., 2007; Urriola et al., 2009a). This may be a result of the fiber concentration in DDGS because DDGS contains approximately 3 times more dietary fiber than corn (Stein and Shurson, 2009). The reason dietary fiber reduces digestibility of energy and AA is that fiber has a low digestibility, induces an increase in endogenous nutrient losses, and increases the rate of passage (Grieshop et al., 2001; Souffrant, 2001). Dietary fiber in DDGS consists mainly of insoluble dietary fiber (Urriola et al., 2009b) that may increase the water binding capacity and the bulkiness of the diet (Potkins et al., 1991; Cherbut et al., 1994). The AID and ATTD of dietary fiber varies among sources of DDGS (Urriola et al., 2009b), but there is no information

on the effects of DDGS on digestibility of AA, energy, and fiber in mixed diets containing corn, soybean meal, and DDGS.

The digestibility of energy may change as pigs adapt to the presence of fiber in the diet (Longland et al., 1993) and fermentation of fiber increases over time. This results in greater production of VFA and greater absorption of energy (Castillo et al., 2007), but there are no data on the time it takes for pigs to adapt to the presence of DDGS in the diet.

The first objective of this experiment was to test the hypothesis that AID and ATTD of energy and nutrients is lower in a diet containing 30% DDGS than in a corn soybean meal diet. The second objective was to test the hypothesis that the digestibility of nutrients and energy will increase if pigs are allowed to adapt to the presence of DDGS in the diet. The third objective was to test the hypothesis that digesta from diets containing DDGS will appear sooner at the end of the ileum, in the cecum, and in the feces than the digesta from pigs fed no DDGS.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

Animals, Housing, and Diets

Sixteen growing barrows (initial BW: 38.0 ± 1.6 kg) that were the offspring of line 337 boars mated to C-22 females (Pig Improvement Company, Hendersonville, TN) were surgically prepared with a T-cannula with an inner diameter of 1.6 cm in the distal ileum (Stein et al., 1998). Another T-cannula with an inner diameter of 2.1 cm was inserted in the mid-cecum. The ileal cannula was exteriorized immediately behind the last rib, while the cecal cannula was exteriorized approximately 10 cm caudal to the ileal cannula. Following surgeries, pigs were

allowed to recover for 30 d and a corn soybean meal diet was provided on an ad libitum basis during this time. All pigs were housed in individual pens (1.2 × 1.5 m) that had a nipple drinker, a feeder, and a fully slatted tri-bar floor. The room temperature was between 20 and 22°C throughout the experiment.

Two diets were formulated to contain similar concentrations of total Lys without using crystalline Lys. The control diet was based on corn and soybean meal and the DDGS diet contained corn, soybean meal, and 30% DDGS (Table 3.1). Because of the greater concentration of GE, CP, and dietary fiber in DDGS than in corn and soybean meal, the concentration of these components was greater in the DDGS diet than in the control diet. Titanium dioxide (Chicago Sweeteners, Chicago, IL) was included at 3 g/kg in both diets as an indigestible marker. Vitamins and minerals were included in both diets to meet or exceed nutrient requirements of growing pigs (NRC, 1998).

Experimental Design and Sample Collection

Pigs were randomly allotted to 2 treatment groups with 8 pigs per treatment in a randomized complete block design. Feed was provided to each pig at a daily level of 3.4 times the maintenance requirement for energy (i.e., 106 kcal of ME per kg $BW^{0.75}$; NRC, 1998). The calculated ME of the control diet and the DDGS diet was 3,336 $kcal \cdot kg^{-1}$ and 3,350 $kcal \cdot kg^{-1}$, respectively.

The daily feed allotments were divided into 2 equal meals that were provided at 0800 and 1700. Pigs were fed experimental diets during four 9-d periods. During the first period, all pigs were fed the control diet. During the following 3 periods, pigs on each treatment group were fed 1 of the 2 treatment diets, and the same diet was provided during all 3 periods.

Feces were collected via grab sampling in the morning of d 5 of each period. The pH of the fecal samples was measured immediately after collection using a pH meter (Model Accumet Basic, Fisher Scientific, Pittsburgh, PA). Twenty grams of each fecal sample were mixed with 2*N* HCl in a 1:1 ratio and stored at -20°C until analyzed for concentrations of VFA. The remaining feces were stored in plastic bags at -20°C. One sample of cecal digesta were collected every 2 h from 0700 to 1700 h on d 6 and 7 of each collection period and ileal digesta were collected continuously from 0730 to 1630 h on d 8 and 9. The procedures for collection and storage of cecal and ileal digesta were similar to the procedure for ileal digesta described by Cervantes-Pahm and Stein (2008). However, from each bag of cecal and ileal digesta, a subsample of 10% was collected and mixed with 2*N* HCl at a 1:1 ratio. These samples were combined within pig and collection period and stored at -20°C until analyzed for VFA. The pH of the first cecal and the first ileal sample collected from each pig after 1000, 1200, 1400, and 1600 h on each collection d was also measured as described for the fecal samples.

After the conclusion of period 4, pigs were fed their respective diets for another 3 d to measure the time it takes for digesta to appear at the end of the ileum, in the cecum, and in the feces. On d 3 of this last period, the morning meal of each pig was mixed with 5 g/kg of chromic oxide. Pigs were allowed to eat their meal and the start of eating was considered time zero. The ileal cannula of each pig was opened 1 h after the morning meal was fed to observe if green digesta were present in the cannula. The cannula was closed again if no green digesta were present and the cannula was opened every 15 min thereafter until green digesta were detected. The time of first appearance of green digesta was recorded. From that time, the cecal cannula was opened every 15 min and the time for the first appearance of green color in the cecal digesta

was recorded. During the following 36 h, feces were scored every 30 min from all pigs, and the first time green feces appeared was recorded.

Sample Processing and Chemical Analysis

At the conclusion of each experimental period, samples were thawed and mixed within animal and a sub-sample was collected for chemical analysis. Ileal and fecal samples were lyophilized and ground prior to analysis.

Samples of corn, soybean meal, DDGS, diets, ileal digesta, and feces were analyzed for DM (method 930.15; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), and total dietary fiber (**TDF**; method 985.29; AOAC Int., 2007). Energy was also analyzed in these samples using a bomb calorimeter (Model 6300, Parr Instuments, Moline, IL). Amino acids were analyzed on an amino acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc, Pleasaton, CA) using ninhydrin for post-column derivatization and norleucine as the internal standard. Before analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C (method 982.30 E[a]; AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E[b]; AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E[c]; AOAC Int., 2007). All diets and ileal digesta samples were analyzed for titanium (Myers et al., 2004). Water binding capacity (**WBC**) was measured by weighing 1 g of sample into a centrifuge tube. This sample was then mixed with 30 mL of distilled water (Robertson et al., 2000). After stirring, samples were allowed to settle and were centrifuged for 20 min at 3,000 rpm. The supernatant was removed and sample weights were recorded. Values for WBC were expressed as the amount of water retained by the pellet (g/g DM).

The ileal, cecal, and fecal samples that were preserved in HCl were thawed and stirred, and 1 mL was mixed with 9 mL of distilled water. One mL of this mixture was added to 4 mL of 25% metaphosphoric acid and vortexed. Samples were analyzed for VFA following the procedure described by Erwin et al. (1961). Briefly, a gas chromatograph (Hewlett-Packard 5890A series II; Hewlett-Packard, Palo Alto, CA) was standardized with a glass column (180 cm x 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was used as the carrier with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were set at 125, 175, and 180°C, respectively.

Calculations

The AID of CP, AA, ADF, NDF, TDF, DM, and energy were calculated for each diet as previously described (Stein et al., 2007), and the ATTD of ADF, NDF, TDF, DM, and energy was also calculated using this equation. The fermentation of DM, energy, ADF, NDF, and TDF in the large intestine was calculated by subtraction of the amount (g) of ileal digested nutrient or DM from the amount (g) of total tract digested nutrient or DM. Fermentation of energy was calculated by subtracting the amount (kcal) of ileal digested energy from the amount of total tract digested energy (kcal).

The ileal flow of DM, ADF, NDF, and TDF was calculated using the following equation:

$$Flow_{nutrient} = Nutrient_{digesta} \times (Marker_{diet} / Marker_{digesta}),$$

where $Flow_{nutrient}$ is the flow of ADF, NDF, or TDF (g/kg DMI), $Nutrient_{digesta}$ is the concentration of DM, ADF, NDF, and TDF in ileal digesta (g/kg DM), $Marker_{diet}$ is the concentration of titanium in the diet (g/kg DM), and $Marker_{digesta}$ is the concentration of titanium in ileal digesta (g/kg DM). The flow over the entire intestinal tract was calculated using the same equation and the ileal and fecal flow of energy (kcal/kg DMI) and the ileal, cecal, and fecal flow

of VFA were also calculated using this equation. The first appearance of digesta in the intestinal tract of pigs was calculated as the difference between the time that the green marker was fed and the time that it appeared in ileal, cecal, or fecal samples.

Statistical Analyses

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normal distribution of the data, equal variances, and to identify outliers. An observation was considered an outlier if the value was more than 3 SD away from the mean. The AID data for periods 2, 3, and 4 were analyzed by the MIXED procedure of SAS. The REPEATED statement was used to model the effect of collection period on AID and ATTD values using the individual pig as the subject from which repeated observations were recorded (Littell et al., 1998). Digestibility values from period 1 were used as the covariate for each pig to correct for differences in digestibility among pigs. Main effects were period and DDGS. The interaction between period and DDGS was included in the initial model and considered significant at $P \leq 0.05$, but if it was not significant, the interaction was removed and only main effects were included in the final model. Least square means were calculated using the LSMEAN statement of SAS. The contrast option was used to identify linear and quadratic effects of collection period on digestibility values. Pig was the experimental unit for all analyses. Differences among main effects were considered significant if $P < 0.05$ and they were considered a trend if the P – value was between 0.05 and 0.10. Data for first appearance of the digesta at the end of the ileum, in the cecum, and in the feces were also analyzed by the MIXED procedure of SAS using pig as a random variable. Period and DDGS were fixed effects and the interaction between period and DDGS was included in the model, but removed from the final model if not significant.

RESULTS

All pigs were successfully cannulated at the distal ileum and in the mid-cecum. Pigs recovered from surgery without complications. The BW of pigs at the start of period 1, 2, 3, and 4 was 71.7 ± 6.4, 75.4 ± 6.6, 84.8 ± 10.0, and 95.4 ± 8.3 kg, respectively. The final BW at the end of period 4 was 103.7 ± 9.2 kg. The analyzed nutrient concentration in the diets was similar to calculated values (Table 3.2). The control diet had a lower concentration of GE (3,897 kcal/kg), CP (14.8%), ADF (2.85%), NDF (11.3%), and TDF (12.4%) than the diet containing 30% DDGS (GE, 4,294 kcal/kg; CP, 18.5%; ADF, 4.65%; NDF, 15.1%; and TDF, 17.0%). Whenever effects of period were analyzed, no differences between period 2 and 3 were observed. Therefore, values for period 3 are not reported.

Apparent Ileal Digestibility, Apparent Total Tract Digestibility, and Hindgut Fermentation

The AID of Lys and Asp were lower ($P < 0.05$) in the DDGS diet than in the control diet (Table 3.3), and the AID for Leu and Ala were greater ($P < 0.05$) in the DDGS diet than in the control diet. There was also a tendency ($P < 0.10$) for the digestibility of Met and Tyr to be greater in the DDGS diet than in the control diet, but there were no differences in the AID of CP or any other AA between the 2 diets, and there was no effect of period on the AID of CP or AA.

The AID of GE, NDF, and TDF were not affected by inclusion of 30% DDGS in the diet (Table 3.4). The AID of DM in the DDGS diet (71.2%) was lower ($P < 0.01$) than the AID of DM in the control diet (74.0%). In contrast, the AID of ADF in the DDGS diet (33.8%) was greater ($P < 0.01$) than in the control diet (13.1%). The ATTD of DM (81.7%), GE (81.0%), NDF (57.2%), and TDF (55.5%) were also less ($P < 0.05$) in the DDGS diet than in the control diet (87.2, 86.0, 69.3, and 66.0, respectively). The AID of ADF, NDF, and TDF increased (linear, $P < 0.05$) from period 2 (21.0, 38.5, and 32.2%, respectively) to period 4 (29.4, 44.7, and

39.8, respectively). However, there was no effect of period on the ATTD of DM, GE, ADF, NDF, and TDF.

The hindgut fermentation of DM (11.5%), GE (8.9%), ADF (21.0%), NDF (18.1%), and TDF (20.5%) were less ($P < 0.05$) in the DDGS diet than in the control diet (13.9, 13.2, 41.1, 26.8, and 29.9%, respectively). The hindgut fermentation of ADF, NDF, and TDF was reduced (linear, $P < 0.05$) from period 2 (36.0, 27.2, and 31.1%, respectively) to period 4 (28.2, 20.7, and 23.2%, respectively).

Ileal and Total Tract Flow of DM, GE, ADF, NDF, and TDF

The ileal flow of DM, ADF, NDF, and TDF was greater ($P < 0.01$) in pigs fed the DDGS diet (273, 35, 105, and 126 g/kg DMI) than in pigs fed the control diet (239, 29, 75, 92 g/kg DMI; Table 3.5). The ileal flow of GE was also greater ($P < 0.01$) in pigs fed the DDGS diet (1,371 kcal/kg DMI) than in pigs fed the control diet (1,220 kcal/kg DMI). The ileal flow of ADF and TDF decreased (linear, $P < 0.05$) from period 2 (33 and 114 g/kg DMI) to period 4 (30 and 102 g/kg DMI), and there was a tendency (linear, $P < 0.10$) for a decrease in the ileal flow of NDF and DM from period 2 to period 4.

The total tract flow of DM, ADF, NDF, and TDF were greater ($P < 0.01$) in pigs fed the DDGS diet (169, 39, 120, and 140 g/kg DMI) than in pigs fed the control diet (118, 31, 84, and 103 g/kg DMI). The total tract flow of GE was also greater in pigs fed the DDGS diet (926 kcal/kg DMI) than in pigs fed the control diet (633 kcal/kg of DMI).

Ileal, Cecal, and Fecal pH and concentration of VFA

The pH of ileal digesta (Table 3.6) from pigs fed the DDGS diet (6.3) was greater ($P < 0.05$) than the pH of ileal digesta from pigs fed the control diet (5.8). Likewise, the pH of cecal digesta from pigs fed the DDGS diet (5.5) was greater ($P < 0.05$) than the pH of cecal digesta

from pigs fed the control diet (5.3). There was, however, no effect of collection period on the pH of feces and the pH of feces in period 4 was not different from the pH of feces in period 2.

The concentration of VFA in ileal, cecal, and fecal samples was not different between the control diet and the DDGS diet, but the ileal concentrations of isobutyrate, isovalerate, and valerate were below detection levels and are, therefore, not reported. The concentration of acetate (416 mmol/kg), propionate (162 mmol/kg), butyrate (66 mmol/kg), isovalerate (0.8 mmol/kg), and valerate (17.6 mmol/kg) measured in cecal samples in period 2 increased linearly ($P < 0.05$) to 443, 196, 76, 9.8, and 26.4 mmol/kg in period 4. The concentration of acetate (596 vs. 350 mmol/kg), isobutyrate (79 vs. 10 mmol/kg), butyrate (83 vs. 52 mmol/kg), and valerate (33 vs. 21 mmol/kg) also increased (linear $P < 0.01$) in fecal samples from period 2 to period 4, but the concentration of propionate in fecal samples was not affected by collection period.

First Appearance of Digesta

The time from feed was ingested until it first appeared in ileal digesta in pigs fed the control diet (238 min) was not different from the time it took for digesta to appear at the end of the ileum in pigs fed the DDGS diet (225 min; Table 3.7). Likewise, the time for the first appearance of digesta in the cecum of pigs fed the control diet (263 min) was not different from that of pigs fed the DDGS diet (277 min). The time it took for digesta to appear in the feces of pigs fed the control diet (1,603 min) was not different from the time it took for pigs fed the diet containing DDGS (1,674 min).

DISCUSSION

Inclusion of DDGS in a corn soybean meal diet increases the concentration of dietary fiber in the diet. The effects of dietary fiber on energy and nutrient digestibility may be

influenced by the physicochemical characteristics of dietary fiber. In DDGS, most of the TDF is insoluble (Urriola et al., 2009b).

The AID of Lys may be reduced by heating and by the addition of solubles to the distilled grains during the production of DDGS (Stein and Shurson, 2009). The AID of Lys in the DDGS diet was lower than the AID of Lys in the control diet, which agrees with the observation that the AID of Lys in DDGS is lower than the AID of Lys in corn (Stein et al., 2006). Dietary fiber may reduce the digestibility of AA (Shultze et al., 1994). The DDGS diet contained more TDF (17.0%) than the control diet (12.4%), but there was no difference in the AID of most AA between the 2 diets. The reason for this observation may be that insoluble dietary fiber has only minor effects on the digestibility of dietary AA (Zhu et al., 2005) and on the basal endogenous losses of AA (Leterme et al., 1996). The DDGS diet also contained more ether extract than the control diet and dietary ether extract may increase the digestibility of AA because high fat digesta moves through the intestinal tract more slowly than low fat digesta (Cervantes-Pahm and Stein, 2008). This may be the reason why the digestibility of only Lys was less in the DDGS diet than in the control diet.

Insoluble fiber have minimal effect on the ileal digestion and absorption of nutrients and energy, which has been demonstrated in several experiments that used different sources of insoluble dietary fiber (Wang et al., 2002; Serena et al., 2008b). It has also been shown that the AID and the ATTD of acid hydrolyzed fat is not affected by dietary levels of NDF provided as wood cellulose (Kil et al., 2007). Results of this experiment showing that there is no effect of DDGS on the AID of GE and most AA in a corn soybean meal diet, therefore, is in agreement with previous results.

The lower ATTD of DM, GE, NDF, and TDF in the DDGS diet than in the control diet may be due to the lower ATTD of NDF and TDF in DDGS than in soybean meal. Dietary fiber in soybean meal is more soluble and contains highly fermentable oligosaccharides such as stachyose and raffinose (Bach Knudsen, 1997; Karr-Lilienthal et. al., 2005), whereas corn fiber is mainly insoluble and composed of cellulose and arabinoxylans that are more resistant to hindgut fermentation (Bach Knudsen, 1997; Guillon et al., 2007). It is, therefore, likely that the increased concentration of corn fiber in the DDGS diet is the reason for the lower ATTD of NDF and TDF in the DDGS diet than in the control diet.

It was expected that the fiber in DDGS would stimulate bowel movement and reduce the time it took for first digesta appearance at the end of the ileum, in the cecum, and in the feces (Bastianelli et al., 1996; Scheneeman, 1998; Bindelle et al., 2008). However, in the current experiment, the first appearance of digesta in pigs fed the DDGS diet and the control diet was not different, despite the greater concentration of TDF in DDGS than in corn and soybean meal. The time it took for digesta to appear at the end of the ileum for pigs fed both diets is similar to previously reported data for growing-finishing pigs fed diets containing no DDGS (Ehle et al., 1982; Kim et al., 2007; Wilfart et al., 2007). The reason for this observation may be that DDGS contains more fat than corn and soybean meal (Spiehs et al., 2002; Stein and Shurson, 2009) and the presence of fat in the small intestine increases the secretion of cholecystokinin, which may reduce gastric emptying (Cervantes-Pahm and Stein, 2008). The lack of an effect of DDGS on marker appearance at the end of the ileum, in the cecum, and over the entire tract indicates that the effects of TDF and fat in DDGS neutralize each other so that the net effect is that first appearance of digesta at the end of the ileum, in the cecum, and in the feces is not changed when DDGS is included in the diet.

It was expected that the AID and the ATTD of nutrients, especially TDF, would increase with time as has been shown in previous experiments (Longland et al., 1993; Castillo et al., 2007). This effect was observed for the AID of ADF, NDF and TDF, but not for the ATTD of DM, GE, ADF, NDF, or TDF. This observation indicates that fermentation in the small intestine increases if fiber is fed for a longer time, but this increase is followed by a reduction in hindgut fermentation so the end result is that the ATTD of fiber is not changed. We are not aware of any other data that have shown this effect of time on the fermentation of fiber in pigs fed DDGS containing diets.

This experiment used pigs that had a T-cannula installed in the distal ileum and another T-cannula was installed in the cecum. This allowed for collection of digesta from the distal ileum and from the cecum in the same pigs. The pigs that were used in this experiment tolerated the procedure well and did not seem to have any discomfort from the 2 cannulas. Previous experiments used 2 sets of pigs to collect ileal and cecal digesta for measurements of VFA concentration (Htoo et al., 2007). The ileal cannula is needed for the measurement of ileal digestibility of AA and also allows for measurement of VFA in the ileal digesta. The cecal cannula allows for measurements of VFA in cecal contents, which is an important indicator of cecal fermentation as it has been demonstrated that there is a substantial synthesis of VFA in the cecum (Htoo et al., 2007; Serena et al., 2008a).

Fermentation of branched chained AA yields branched chained fatty acids (Macfarlane et al., 1992). The concentration of the branched chained fatty acids was below the detection limit in ileal digesta, but concentrations of these fatty acids in cecal digesta and in fecal samples was greater than in ileal digesta, which indicates fermentation of undigested protein in the cecum and colon. There were, however, no differences between the 2 treatment groups, which indicate that

the greater concentration of CP in the DDGS-containing diet than in the control diet did not increase the synthesis of branched chained fatty acids in the cecum and colon. The concentration of VFA in feces that were obtained in the current experiment is within the range of reported values for pigs fed diets based on cereal by products (McBurney and Sauer, 1993; Jensen et al., 1997), but no differences among treatments were observed.

In conclusion, results of this experiment indicate that inclusion of 30% DDGS in a corn soybean meal diet does not affect ileal digestibility of energy or most AA, but the ileal digestibility of Lys is reduced if DDGS is included in the diet. The total tract digestibility of energy and fiber is also reduced if DDGS is used in the diet because dietary fiber in DDGS is partially resistant to hindgut fermentation and the flow through the intestinal tract of DM and energy increases if DDGS is included in the diet. The pH of the feces and the digesta passage rate are, however, not influenced by the presence of DDGS in the diet. The AID of AA did not change as pigs were fed their diet for a longer time indicating that a 7-d adaptation period is sufficient for measuring AA digestibility. Likewise, the digestibility of energy was not influenced by the time the diet was fed to the pigs, but the ileal digestibility of ADF, NDF, and TDF increased as pigs were fed their respective diets for a longer period. However, this increase was followed by a reduction in hindgut fermentation of fiber, and the ATTD of ADF, NDF, and TDF was, therefore, not influenced by the time diets were fed to the pigs.

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Table 3.1. Composition of experimental diets, as-fed basis

	Distillers dried grains with solubles, %	
Item	0	30
Ingredient, %		
Ground corn	78.75	54.60
Soybean meal (48% CP)	18.00	12.50
Distillers dried grains with solubles	-	30.00
Cornstarch	1.00	1.00
Ground limestone	0.75	1.00
Dicalcium phosphate	0.80	0.20
Salt	0.40	0.40
Vitamin mineral premix[1]	0.30	0.30
Calculated concentration		
ME, kcal·kg^{-1}	3,336	3,350
CP, %	15.10	18.70
Lys, standardized ileal digestible, %	0.66	0.66
Ether extract, %	3.30	5.60
NDF, %	9.10	19.00
Total dietary fiber, %	10.00	16.20

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg;

biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.2. Analyzed nutrient composition of experimental diets, as-fed basis

	Distillers dried grains with solubles, %	
Item	0	30
GE, kcal/kg	3,897	4,294
CP, %	14.8	18.5
Acid hydrolyzed ether extract, %	3.07	7.42
ADF, %	2.85	4.65
NDF, %	11.3	15.1
Total dietary fiber, %	12.4	17.0
Water binding capacity, g/g	1.81	1.96
Indispensable AA, %		
Arg	0.89	0.99
His	0.39	0.48
Ile	0.59	0.69
Leu	1.43	1.94
Lys	0.74	0.77
Met	0.26	0.35
Phe	0.71	0.88
Thr	0.53	0.66
Trp	0.18	0.19
Val	0.73	0.87

Table 3.2. (cont.)

	Distillers dried grains with solubles, %	
Item	0	0
Dispensable AA, %		
Ala	0.83	1.15
Asp	1.35	1.46
Cys	0.27	0.35
Glu	2.67	3.12
Gly	0.59	0.72
Pro	0.95	1.22
Ser	0.64	0.80
All AA	13.8	16.6

Table 3.3. Effects of period and distillers dried grains with solubles (DDGS) on apparent ileal digestibility of AA by growing pigs[1]

Period	2		4			*P*-value[2]	
DDGS	0%	30%	0%	30%	SEM	DDGS	Period[3]
CP, %	75.6	74.2	75.0	72.7	2.1	0.79	0.81
Indispensable AA							
Arg	87.9	86.1	87.9	86.7	0.8	0.12	0.20
His	83.1	81.3	82.7	81.1	1.1	0.17	0.47
Ile	79.3	78.2	78.5	78.1	1.1	0.74	0.51
Leu	82.0	83.6	81.8	84.1	0.9	< 0.01	0.56
Lys	78.6	74.1	77.1	73.3	1.6	0.01	0.40
Met	82.2	82.8	83.6	85.1	1.0	0.08	0.16
Phe	81.1	81.4	80.5	81.2	1.0	0.21	0.56
Thr	70.4	69.8	68.1	68.9	2.1	0.45	0.63
Trp	81.6	80.0	76.6	75.8	1.9	0.70	0.05
Val	76.9	75.7	75.8	75.0	1.4	0.71	0.54
Mean	80.6	80.0	79.8	79.9	1.1	0.79	0.55
Dispensable AA							
Ala	76.3	78.5	76.0	78.8	1.3	< 0.01	0.36
Asp	78.2	74.8	76.8	74.1	1.5	0.04	0.64
Cys	74.4	72.6	70.9	71.9	2.0	0.89	0.28
Glu	84.8	82.9	83.0	82.4	1.1	0.27	0.55
Gly	65.5	65.8	59.8	62.2	3.0	0.32	0.23

Table 3.3. (cont.)

Period	2		4			*P*-value[2]	
DDGS	0%	30%	0%	30%	SEM	DDGS	Period[3]
Pro	78.8	77.9	76.9	77.0	1.8	0.79	0.55
Ser	78.2	77.5	77.0	78.2	1.4	0.36	0.96
Tyr	82.0	82.9	80.8	82.7	1.1	0.07	0.55
Mean	79.5	78.4	77.6	77.7	1.4	0.96	0.52
All AA	79.5	78.6	78.1	78.2	1.3	0.86	0.55

[1]In period 1, all pigs were fed the control diet and the digestibility values obtained in period 1 were used as a covariate to correct the values for periods 2, 3, and 4.

[2]There was no interaction between DDGS and period. Therefore, only main effects are reported.

[3]Data for period 3 were not different from period 2 for any of the variables that were measured.

Table 3.4. Effects of distillers dried grains with solubles (DDGS) and period on apparent ileal digestibility, apparent total tract digestibility, and hindgut fermentation of DM, energy, ADF, NDF, and total dietary fiber (TDF) by growing pigs[1,2]

Item	DDGS, %				Period[3]			
	0	30	SEM	*P*-value	2	4	SEM	P-value
Apparent ileal digestibility, %								
DM	74.0	71.2	1.0	< 0.01	72.0	73.5	0.9	0.31
GE	72.7	72.0	1.1	0.47	71.9	73.2	0.9	0.46
ADF	13.1	33.8	1.9	< 0.01	21.0	29.4	2.3	0.03
NDF	42.1	39.1	1.5	0.17	38.5	44.7	1.9	0.05
TDF	35.4	35.0	1.6	0.85	32.2	39.8	2.0	0.02
Apparent total tract digestibility, %								
DM	87.2	81.7	0.4	< 0.01	86.4	86.1	0.5	0.07
GE	86.0	81.0	0.6	< 0.01	84.4	84.3	0.5	0.13
ADF	56.1	54.8	2.5	0.60	57.5	59.0	2.0	0.51
NDF	69.3	57.2	1.9	< 0.01	66.0	65.6	1.6	0.16
TDF	66.0	55.5	1.9	< 0.01	63.7	63.4	1.6	0.15
Hindgut disappearance, %								
DM	13.9	11.5	0.8	0.03	14.2	12.2	0.9	0.14
GE	13.2	8.9	0.9	< 0.01	12.4	11.0	1.1	0.21
ADF	41.1	21.0	2.7	< 0.01	36.0	28.2	3.1	0.05
NDF	26.8	18.1	1.9	< 0.01	27.2	20.7	2.3	0.03
TDF	29.9	20.5	2.0	< 0.01	31.1	23.2	3.4	0.01

Table 3.4. (cont.)

[1]In period 1, all pigs were fed the control diet and the digestibility value from period 1 for each pig was used as covariate to correct the values for periods 2, 3, and 4.

[2]There was no interaction between DDGS and period. Therefore, only main effects are reported.

[3]Data for period 3 were not different from period 2 for any of the variables that were measured.

Table 3.5. Effects of period and distillers dried grains with solubles (DDGS) on ileal and total tract flow (g or kcal/kg of DMI) of DM, energy, ADF, NDF, and total dietary fiber (TDF) by growing pigs[1,2]

Item	DDGS, %				Period[3]				
	0	30	SEM	*P*-value	2	4	SEM	Linear	Quadratic
Ileal									
DM	239	273	6	< 0.01	262	239	7	0.08	0.07
GE	1,220	1,371	36	0.01	1,317	1,254	44	0.38	0.58
ADF	29	35	1	< 0.01	33	30	1	0.03	0.17
NDF	75	105	2	< 0.01	93	84	3	0.06	0.26
TDF	92	126	3	< 0.01	114	102	3	0.03	0.41
Total tract									
DM	118	169	4	< 0.01	138	136	5	0.66	< 0.01
GE	633	926	19	< 0.01	739	746	24	0.34	< 0.01
ADF	31	39	1	< 0.01	36	33	1	0.29	0.23
NDF	84	120	3	< 0.01	102	96	4	0.48	0.13
TDF	103	140	4	< 0.01	122	115	5	0.44	0.10

[1]In period 1, all pigs were fed the control diet and digestibility values were used as covariate to correct the values of periods 2, 3, and 4.

[2]There was no interaction between DDGS and period. Therefore, only main effects are reported.

[3]Data for period 3 were not different from period 2 for any of the variables that were measured.

Table 3.6. Effects of period and distillers dried grains with solubles (DDGS) on the pH and concentration of VFA (mmol × kg^{-1} digesta DM) in the ileal digesta, cecal digesta, and feces of growing pigs[1,2]

Item	DDGS, %				Period[3]				
	0	30	SEM	P-value	2	4	SEM	Linear	Quadratic
Ileal digesta									
pH	5.8	6.3	0.04	< 0.01	6.1	6.1	0.1	0.47	< 0.01
Acetate	550	553	37	0.96	540	533	36	0.48	0.40
Propionate	82	45	4	0.55	162	196	20	0.75	0.48
Cecal digesta									
pH	5.3	5.5	0.02	< 0.01	5.4	5.4	0.1	0.69	0.16
Acetate	443	500	37	0.18	416	443	37	< 0.01	0.71
Propionate	213	194	43	0.53	162	196	44	< 0.01	0.43
Isobutyrate	6.8	4.5	3.6	0.60	2.3	11.3	4.1	0.22	0.40
Butyrate	76	83	7	0.53	66	76	7	0.01	0.65
Isovalerate	5.9	5.9	1.8	0.69	0.8	9.8	10.2	< 0.01	0.13
Valerate	24.5	23.5	2.6	0.81	17.6	26.4	2.8	0.03	0.69
Feces									
pH	5.9	5.8	0.1	0.46	5.9	5.8	0.1	0.18	0.86
Acetate	461	458	24	0.91	350	596	28	< 0.01	0.89
Propionate	127	149	20	0.46	149	131	25	0.87	0.48
Isobutyrate	36	41	2	0.17	10	79	3	< 0.01	0.19
Butyrate	72	66	5	0.41	52	83	5	< 0.01	0.26

Table 3.6. (cont.)

Item	DDGS, %				Period[3]				
	0	30	SEM	P-value	2	4	SEM	Linear	Quadratic
Isovalerate	25	20	2	0.15	20	27	3	0.06	0.96
Valerate	28	24	3	0.27	21	33	3	< 0.01	0.40

[1]In period 1, all pigs were fed the control diet and digestibility values were used as covariate to correct the values of periods 2, 3, and 4.

[2]There was no interaction between DDGS and period. Therefore, only main effects are reported.

[3]Data for period 3 were not different from period 2 for any of the variables that were measured.

Table 3.7. Effect of distillers dried grains with solubles (DDGS) on the time (min) of first appearance of digesta at the end of the ileum, in the cecum, and over the total tract in growing pigs

	DDGS			
Item	0%	30%	SEM	*P*-value
Ileal	238	225	18	0.46
Cecal	263	277	22	0.99
Total tract	1,603	1,674	920	0.14

CHAPTER 4

Digestibility of dietary fiber in distillers co-products fed to growing pigs[1]

ABSTRACT: The objective of this work was to measure the apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) of dietary fiber in different sources of distillers dried grains with solubles (DDGS) and to calculate hindgut fermentation of dietary fiber in DDGS fed to growing pigs. Diets, ileal digesta, and fecal samples from pigs fed corn or diets containing 1 of 28 sources of distillers co-products were analyzed for fiber. Of the 28 sources of co-products, 24 sources were corn DDGS (C-DDGS), 1 source was sorghum DDGS (S-DDGS), 1 source was DDGS from a blend of sorghum and corn (SC-DDGS), 1 source was C-DDGS from beverage production ($DDGS_{beverage}$), and a source of corn distillers dried grain (DDG) was also included in the experiment. Total dietary fiber (TDF) and DM were analyzed in all DDGS sources, ileal digesta, and fecal samples. Hindgut fermentation was calculated by subtracting values for AID from values for ATTD. In 10 sources of DDGS and in ileal and fecal samples from pigs fed those sources, crude fiber, ADF, NDF, insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) were also determined. Concentrations of CP, ether extract, and ash were also analyzed in these samples and the organic residue (OR) was calculated by subtracting the concentration of CP, ether extract, and water from OM. Results showed that the AID and the ATTD of TDF differed ($P < 0.01$) among sources of C-DDGS. The average AID of TDF in 10 sources of C-DDGS (21.5%) was not different from the AID of TDF in corn (16.5%), but the ATTD and the hindgut fermentation of TDF in the 10 sources of C-DDGS (44.5 and 23.0%, respectively) were greater ($P < 0.05$) than in corn (23.1 and 6.6%, respectively). The AID of

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crude fiber, NDF, IDF, SDF, and TDF were not different between C-DDGS and S-DDGS, but the AID of ADF was greater ($P < 0.01$) in S-DDGS (57.4%) than in C-DDGS (36.8%). The ATTD of OR in S-DDGS (72.5%) and SC-DDGS (68.4%) were less ($P < 0.05$) than in C-DDGS (77.1%), but the ATTD of ADF, NDF, IDF, SDF, and TDF were not different among the 3 sources of DDGS. The AID, ATTD, and hindgut fermentation of TDF were not different between DDGS from an ethanol plant and DDGS from a beverage plant. The average AID, ATTD, and hindgut fermentation of TDF in the 24 sources of C-DDGS was 23.0, 47.3, and 24.4%, respectively. It is concluded that the AID and ATTD of fiber differ among sources of DDGS and those differences may contribute to differences in the digestibility of energy in DDGS.

Key words: dietary fiber, digestibility, distillers co-products, distillers dried grains with solubles, pigs

INTRODUCTION

Dietary fiber is the sum of carbohydrates and lignin that are resistant to digestion by mammalian enzymes in the small intestine, but they may be partially or completely fermented in the hindgut (AACC, 2001; IOM, 2006). Methods to measure dietary fiber include the crude fiber analysis (Mertens, 2003), the ADF and NDF procedures (Van Soest, 1963), and the total dietary fiber (**TDF**) procedure, which may separate dietary fiber into insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF;** Prosky et al., 1992). An alternative to analyzing samples for dietary fiber is to calculate the concentration of organic residue (**OR**) by subtracting CP, ash, moisture, ether extract, sugar, and starch from 100 (de Lange, 2008).

The greater concentration of dietary fiber in distillers dried grains with solubles (**DDGS**) compared with corn and soybean meal may be one of the primary reasons for the lower digestibility of energy in DDGS than in corn (Stein and Shurson, 2009). The efficiency of energy utilization in fibrous feed ingredients such as DDGS in pigs is affected by the digestibility of dietary fiber and the production of VFA (Bindelle et al., 2008). Values for the apparent total tract digestibility (**ATTD**) of ADF and NDF in DDGS have been reported (Guo et al., 2004; Stein et al., 2009), but no values for the ATTD of TDF, IDF, SDF, or OR are available. Likewise, hindgut fermentation of fiber in DDGS has not been measured, but fiber fermentation can provide energy to pigs. The first objective of this study, therefore, was to measure the apparent ileal digestibility (**AID**), the ATTD, and the hindgut fermentation of dietary fiber and OR in DDGS and to compare these values to the AID and ATTD and hindgut fermentation in corn and distillers dried grain (**DDG**). The second objective was to determine the relationship between the ATTD of TDF and the ATTD of crude fiber, ADF, NDF, IDF, SDF, and OR.

MATERIALS AND METHODS

Samples

Samples of DDGS, diets containing DDGS, ileal digesta, and feces from 3 experiments (Stein et al., 2006; Urriola et al., 2009; Pahm et al., 2008) designed to measure AID and ATTD of nutrients in DDGS were used. The diets contained 66.7% DDGS or DDG. Corn was included in 1 of the experiments and the only source of dietary fiber in the diets was DDGS, DDG, or corn (Table 4.1). In each of the 3 experiments, pigs were allotted to Youden square designs with 7 or 8 replicates per sample. Ileal digesta and fecal samples were collected according to standard procedures described by Stein et al. (2006), Urriola et al. (2009), and Pahm et al. (2008) in Exp.

1, 2, and 3, respectively. Experiment 1 was designed to compare the digestibility of nutrients in 10 sources of DDGS produced from corn (**C-DDGS**) to the digestibility of nutrients in corn grain (Table 4.2). In Exp. 2, the digestibility of 8 sources of C-DDGS was compared with the digestibility of nutrients in 1 source of DDGS produced from sorghum (**S-DDGS**) and in 1 source of DDGS produced from a blend of sorghum and corn (**SC-DDGS;** Table 4.3). In Exp. 3, the digestibility of nutrients in DDGS produced by 6 dry-grind ethanol plants (**$DDGS_{ethanol}$**) was compared with the digestibility of nutrients in 1 source of DDG and 1 source of DDGS from a beverage plant (**$DDGS_{beverage}$**; Table 4.4).

Chemical Analyses

Ingredients that were used in Exp 1, 2, and 3 were analyzed for DM (method 930.15; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007), starch (method 979.10; AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), and TDF (method 985.29; AOAC Int., 2007). Ingredients used in Exp. 2 were also analyzed for crude fiber (method 978.10; AOAC Int., 2007), and IDF (method 985.29; AOAC Int., 2007), and the concentration of SDF in these ingredients was calculated as the difference between TDF and IDF.

Diets, ileal digesta, and feces from Exp. 1 were also analyzed for DM and TDF, and the concentration of chromium in these samples was analyzed after nitric acid – perchloric acid wet ash sample preparation (method 990.08; AOAC Int., 2007). Diets, ileal digesta, and fecal samples from Exp. 2 were analyzed for DM, CP, ether extract, ash, starch, TDF, chromium, crude fiber, ADF, NDF, and IDF, and the concentration of SDF was calculated. Diets, ileal digesta, and feces from Exp. 3 were analyzed for TDF, DM, and chromium.

Calculations

The AID and ATTD of TDF were calculated for samples used in all 3 experiments according to Stein et al. (2007). For samples used in Exp. 2, the concentration of OR in the diets were calculated using the following equation:

OR_{diet} (%) = OM – [CP + ether extract + (100 – DM) + $starch_{added}$ + $sucrose_{added}$],

where $starch_{added}$ and $sucrose_{added}$ were the added cornstarch and sucrose that were included in the diet. Starch and sucrose were assumed to be 100% digestible in the small intestine. Therefore, the calculations of OR in ileal digesta and feces were as follows:

$$OR_{ileal\ or\ feces}\ (\%) = OM – [CP + \text{ether extract} + (100 – DM)].$$

The hindgut fermentation of nutrients was calculated using the following equation (Högberg and Lindberg, 2004):

$$\text{Hindgut fermentation}\ (\%) = ATTD – AID.$$

For samples used in Exp. 2, the AID, ATTD, and the hindgut fermentation of crude fiber, ADF, NDF, IDF, and SDF were also calculated using this equation.

Statistical Analysis

In all 3 experiments, the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normal distribution of the data and equal variances, and to identify outliers. An observation was considered an outlier if the value was more than 3 SD away from the grand mean. No outliers were identified in any of the 3 experiments. Data were analyzed by ANOVA using the MIXED procedure of SAS (Littell et al., 1998). The pig was considered as the experimental unit. Pig and period were random effects and DDGS source was considered a fixed effect. The LSMeans procedure in SAS was used to calculate mean values. The CONTRAST option of SAS was used to compare the digestibility of DDGS and corn in Exp. 1,

C-DDGS, S-DDGS, and SC-DDGS in Exp. 2, and $DDGS_{ethanol}$, $DDGS_{beverage}$, and DDG in Exp. 3. In all analyses, the differences were considered significant if $P < 0.05$. The PROC REG procedure of SAS was used to determine the relationship between the ATTD of TDF and the ATTD of IDF, SDF, ADF, NDF, crude fiber, and OR that were estimated in Exp. 2.

RESULTS

Exp. 1

The concentration of TDF varied from 18.6 to 31.4% among the 10 sources of DDGS and starch concentration was between 5.2 and 7.9% (Table 4.2). The AID of TDF (12.6 to 25.9%) also varied ($P < 0.01$) among the 10 sources of DDGS (Table 4.5). There was no difference between the mean AID of TDF in DDGS (21.5%) and the AID of TDF in corn (16.5%). The ATTD of TDF (30.5 to 52.4%) also varied ($P < 0.01$) among the 10 DDGS sources. The mean ATTD of TDF was greater ($P < 0.05$) in the 10 DDGS sources (44.5%) than in corn (23.1%). There was no difference among DDGS sources in hindgut fermentation of TDF, but the mean hindgut fermentation of TDF in C-DDGS (23.1%) was greater ($P < 0.01$) than in corn grain (6.6%).

Exp. 2

There was variation in the concentration of crude fiber (6.1 to 7.4%), ADF (9.7 to 12.9%), NDF (37.4 to 44.4%), IDF (28.3 to 33.8%), SDF (0.0 to 1.6%), and TDF (28.7 to 34.9%) among the 8 sources of C-DDGS (Table 4.3). The concentration of TDF in S-DDGS (32.2%) was similar to the average for C-DDGS (31.8%) and for SC-DDGS (35.8%). The AID of crude fiber (13.7 to 42.8%), ADF (28.2 to 47.0%), NDF (37.5 to 52.1%), IDF (5.9 to 33.6%), SDF (56.4 to 81.7%), TDF (19.6 to 38.2%), and OR (38.4 to 67.0%) were different ($P < 0.01$)

among the 8 sources of C-DDGS (Table 4.6). The AID of ADF in S-DDGS (57.4%) was greater ($P < 0.01$) than the mean AID of ADF in C-DDGS (36.8%), and the AID of crude fiber in S-DDGS (38.6%) tended ($P = 0.07$) to be greater than the mean AID of crude fiber in C-DDGS (31.0%). However, the AID of OR in S-DDGS (41.6%) was lower ($P < 0.01$) than in C-DDGS (58.6%), but the AID for NDF, IDF, SDF, and TDF were not different in S-DDGS compared with C-DDGS. The AID of NDF (37.9%), IDF (4.8%), and TDF (15.9%) were lower ($P < 0.01$) in SC-DDGS than in C-DDGS, but the AID of crude fiber, ADF, and SDF were not different between SC-DDGS and C-DDGS.

The ATTD of crude fiber (36.3 to 51.2%), ADF (51.8 to 64.3%), NDF (51.6 to 65.8%), IDF (29.3 to 51.0%), SDF (89.4 to 95.3%), TDF (39.4 to 56.4%), and OR (72.4 to 81.3%) were different ($P < 0.01$) among the 8 sources of C-DDGS. There were no differences in the ATTD of crude fiber, ADF, NDF, IDF, SDF, and TDF between S-DDGS and the mean of the 8 sources of C-DDGS. However, the ATTD of OR was less ($P < 0.05$) in S-DDGS (72.5%) and SC-DDGS (68.4%) than in the 8 sources of C-DDGS (77.1%). The ATTD of IDF (28.6%) in SC-DDGS was lower ($P = 0.05$) than in C-DDGS and there was a tendency ($P < 0.10$) for a lower ATTD of NDF (51.5%) and TDF (39.2%) in SC-DDGS than in C-DDGS, but for crude fiber, ADF, and SDF, no differences between SC-DDGS and C-DDGS were observed.

Hindgut fermentation of crude fiber (0.1 to 23.9%), ADF (12.9 to 28.1%), NDF (6.5 to 20.3%), IDF (8.2 to 31.2%), SDF (13.6 to 35.2%), TDF (11.1 to 30.9%), and OR (9.5 to 39.2%) were different ($P < 0.01$) among the 8 sources of C-DDGS. Hindgut fermentation of ADF was less ($P < 0.05$) in S-DDGS and SC-DDGS (3.3 and 12.3%) than in the 8 sources of C-DDGS (21.7%). The hindgut fermentation of OR, however, was greater ($P < 0.01$) in S-DDGS and SC-DDGS (30.9 and 35.5%) than in C-DDGS (18.5%).

Exp. 3

The concentration of TDF varied from 28.6 to 32.4% among the 6 sources of $DDGS_{ethanol}$, and was lower than the concentration of TDF in $DDGS_{beverage}$ (38.5%) and in DDG (43.9%; Table 4.4). The AID (11.4 to 30.8%) and the ATTD (29.3 to 57.0%) of TDF were different ($P < 0.01$) among the 6 sources of $DDGS_{ethanol}$, but there were no differences between the AID and ATTD of TDF in $DDGS_{beverage}$ and $DDGS_{ethanol}$ (Table 4.7). The AID of TDF in DDG (0.7%) was lower ($P < 0.01$) than the AID of TDF in $DDGS_{ethanol}$ (18.5%). However, the ATTD of TDF in DDG (43.8%) was not different from the ATTD of TDF in $DDGS_{ethanol}$ (48.0%) and $DDGS_{beverage}$ (46.4%). Hindgut fermentation of TDF was greater ($P = 0.05$) in DDG (43.1%) than in $DDGS_{ethanol}$ (29.5%) and $DDGS_{beverage}$ (33.2%), but there were no differences in the hindgut fermentation of TDF among $DDGS_{ethanol}$ sources.

Correlation among Methods to Measure Dietary Fiber

There was a good relationship between the ATTD of TDF and the ATTD of NDF ($r^2 = 0.90$), IDF ($r^2 = 0.79$), and ADF ($r^2 = 0.71$; Figure 1). There was less relationship between the ATTD of TDF and the ATTD of crude fiber ($r^2 = 0.42$), and there was a poor relationship between the ATTD of TDF and the ATTD of SDF ($r^2 = 0.24$), and OR ($r^2 = 0.21$).

DISCUSSION

Data from all 3 experiments indicate that dietary fiber in DDGS is composed of a fraction that is fermented before the end of the ileum and a fraction that is fermented in the hindgut. The fraction that is fermented before the end of the ileum may be considered a fast fermentable fraction because the transit time from mouth to ileum averages 2.9 h (Wilfart et al., 2007a; Wilfart et al., 2007b). In contrast, the fiber that is fermented in the large intestine may be

considered a slow fermentable fraction of fiber. The fast fermentable fraction of dietary fiber was present in greater concentrations in sources of DDGS that had the greatest AID values, whereas the slow fermentable fraction was present in greater concentrations in DDGS sources with greater values for hindgut fermentation. This observation explains the average differences in the AID of TDF among sources of C-DDGS in Exp. 1, 2, and 3 (21.5, 28.9, and 18.5%, respectively; average = 23.0%). The average ATTD of TDF was relatively constant among sources of C-DDGS in Exp. 1, 2, and 3 (44.5, 49.5, and 48.0 %, respectively; average = 47.3%). Therefore, hindgut fermentation averaged 23.0, 20.6, and 29.5 with the average of 24.4% in the 3 experiments.

The differences in the ATTD of dietary fiber among sources of C-DDGS may be a result of differences in the digestibility of nutrients in the corn grain that was used to produce DDGS (Stein and Shurson, 2009). The digestibility of TDF may also be affected by post-harvest processing of corn (Fahey et al., 1993), but the effect of the ethanol plant processing on fiber digestibility in C-DDGS has yet to be determined. However, the greater ATTD of TDF in DDGS compared with corn that was observed in Exp. 1 indicates that processing of the corn during ethanol production (e. g., grinding, heating, and fermentation) may modify the structure of dietary fiber, which may make it more digestible than corn fiber (Le Gall et al., 2009).

The average AID of TDF of the 24 sources of C-DDGS (23.0%) is close to the average AID of TDF (24.0%) that was measured in diets containing a wide variety of feed ingredients (Bach Knudsen and Jørgensen, 2001). The average ATTD of TDF in C-DDGS observed in the present experiments (47.3%) is also comparable to values measured in growing pigs fed corn-bran (48%), but less than values observed when growing pigs are fed sugar beet pulp (Graham et al., 1986; Le Goff et al., 2002).

The differences in the AID and ATTD of TDF among sources of C-DDGS indicate that the digestibility of energy may also vary among these sources. Guo et al. (2004) observed differences in the ATTD of NDF, but no difference in the ATTD of GE among 4 sources of C-DDGS. However, this observation is in contrast with Pedersen et al. (2007) and Stein et al. (2009), who reported that the ATTD of GE in 14 sources of C-DDGS varied between 73.9 and 82.8%. The differences in the digestibility of TDF among sources of C-DDGS measured in the present experiments may be the reason for the reported differences in energy digestibility.

The greater AID and ATTD of SDF compared with IDF indicates that the soluble fraction of dietary fiber is much more fermentable than the insoluble fraction. This observation indicates that ethanol processes that increase the concentration of SDF in DDGS may also increase the AID and ATTD of TDF, which in turn is expected to increase the digestibility of energy. Extrusion increases the soluble portion of TDF in wheat, oats, and rice bran (Gualberto et al., 1997), which may explain why the ATTD of energy in DDGS increases after extrusion (Beltranena et al., 2009).

The main difference between the detergent fiber procedures (ADF and NDF) and the TDF procedure is that the detergent procedures do not include soluble fiber, while the TDF procedure accounts for both the soluble and the insoluble fractions of dietary fiber (Campbell et al., 1997). Therefore, it is expected that values for TDF represent more accurately the total fiber fraction in a feed ingredient than values for ADF and NDF (Campbell et al., 1997; Cho et al., 1997; Mertens, 2003).

The reason the AID of TDF in DDG is lower than the AID of TDF in DDGS may be that most of the SDF is captured in the solubles fraction of the wet distillers' grains, and because no solubles are added to the DDG, the concentration of SDF in DDG is lower than in DDGS (Pahm

et al., 2008). This results in a lower AID of TDF in DDG because the AID of SDF is greater than the AID of IDF.

The fact that there were no differences in the AID and ATTD of TDF between $DDGS_{ethanol}$ and $DDGS_{beverage}$ is in agreement with observations showing that there is no difference in the AID of AA between these 2 sources of DDGS (Pahm et al., 2008). This indicates that the production processes used in beverage plants have no greater influence on the digestibility of nutrients in DDGS than the processes used in fuel ethanol plants (Pahm et al., 2008). These results also indicate that the digestibility of energy between these sources of DDGS most likely is similar.

The high relationship between the ATTD of TDF and the ATTD of NDF, ADF, or IDF, is most likely a result of the fact that most of the fiber in DDGS is insoluble (Bach Knudsen, 1997). The procedures used for fiber analysis that measure concentrations of insoluble fiber give values that are close to the concentration of TDF (Mertens, 2003). The ATTD of SDF was much greater than the ATTD of IDF, but the concentration of SDF is low in DDGS. As a result, the relationship between the ATTD of TDF and the ATTD of SDF is low. A strong correlation relationship between the ATTD of TDF and the ATTD of OR was expected because TDF and OR represent the entire fraction of fiber in DDGS. However, results showed that there was no relationship between the ATTD of TDF and the ATTD of OR, which indicates that the procedure that was used to calculate OR did not give an accurate estimate of the concentration of fiber in DDGS.

There are limitations when using ileal T-cannula for measuring digestibility of dietary fiber in pigs because only a portion of the total digesta and fecal output are collected, which may result in relatively large variations among pigs. However, use of a T-cannula is one of the few

methods available for collection of ileal digesta, and without the cannula, it would not be possible to calculate ileal digestibility of fiber in the DDGS sources. In the present work, we attempted to overcome the inherent limitations of use of T-cannulas by using a relatively large number of replications and by allotting pigs to a Youden square design, which is believed to reduce variability (Kuehl, 2000; Kim and Stein, 2009). We also standardized all feeding and collection procedures among pigs.

In conclusion, the AID and ATTD of dietary fiber and OR varies among sources of C-DDGS and this difference is believed to influence the digestibility of energy. The greater AID and ATTD in DDGS than in corn indicates that fiber digestibility is improved by the processing or fermentation in the ethanol plants. However, less than 50% of TDF in DDGS is digested over the entire intestinal tract, which means that more than 50% of the TDF in DDGS passes through the pig without being digested.

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Table 4.1. Ingredient composition (%) of experimental diets, as-fed basis

Ingredient, % of diet	DDGS[1]	DDG[1]	Corn
DDGS[1]	66.70	-	-
DDG[1]	-	66.70	-
Corn	-	-	97.00
Cornstarch	27.00	27.00	-
Sucrose	3.00	3.00	-
Soybean oil	1.00	1.00	-
Limestone	1.35	1.35	0.80
Dicalcium phosphate	-	-	1.05
Chromic oxide	0.30	0.30	0.30
Salt	0.30	0.30	0.50
Vitamin premix[2]	0.10	0.10	0.10
Micromineral premix[3]	0.25	0.25	0.25
Total	100.00	100.00	100.00

[1]DDGS = distillers dried grains with solubles; DDG = distillers dried grain.

[2]Provided the following quantities of vitamins per kg of complete diet: vitamin A, 10,990 IU as vitamin A acetate; vitamin D3, 1,648 IU as D-activated animal sterol; vitamin E, 55 IU as alpha tocopherol acetate; vitamin K3, 4.4 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 3.3 mg as thiamine mononitrate; riboflavin, 9.9 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; vitamin B12, 0.044 mg; D-pantothenic acid, 33 mg as calcium pantothenate; niacin, 55 mg; folic acid, 1.1 mg; and biotin, 0.17 mg.

[3]Provided the following quantities of minerals per kg of complete diet: Cu, 26 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 0.31 mg as potassium iodate; Mn, 26 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 130 mg as zinc oxide.

Table 4.2. Analyzed composition of corn and distillers dried grains with solubles (DDGS) and diets containing each source of DDGS used in Exp. 1 (as-fed basis)[1]

		Sources of distillers dried grains with solubles										
Item	Corn	1	2	3	4	5	6	7	8	9	10	Mean
DDGS												
DM	85.4	89.2	88.7	86.8	88.9	89.2	87.1	88.6	90.8	90.0	89.4	88.9
CP	7.9	27.6	27.9	27.2	29.0	26.7	24.6	26.6	28.4	29.1	27.3	27.4
Starch	-	7.0	7.9	5.2	5.6	7.0	6.9	6.4	5.4	7.4	6.1	6.5
TDF[2]	-	30.4	31.1	30.2	30.3	29.6	31.3	29.3	31.4	29.9	29.2	29.2
Diets												
TDF[2]	8.0	19.9	18.8	21.5	21.8	18.4	21.2	19.7	20.0	20.3	19.6	20.1

[1]Samples from Stein et al. (2006).

[2]TDF = total dietary fiber.

Table 4.3. Analyzed composition of distillers dried grains with solubles (DDGS) produced from corn, sorghum (S-DDGS), and from a blend of sorghum and corn (SC-DDGS) used in Exp. 2 (as-fed basis)[1]

	Source of corn-DDGS										
Item	1	2	3	4	5	6	7	8	Mean	S-DDGS	SC-DDGS
DDGS											
DM	90.5	90.6	90.5	90.5	89.7	89.6	89.0	87.5	89.7	91.6	92.7
CP	29.4	28.7	27.4	27.3	27.5	27.3	31.9	28.0	28.4	32.7	30.6
Starch	7.8	9.1	5.2	8.2	6.5	6.6	6.5	6.2	7.0	7.1	6.0
Crude fiber	6.1	6.8	7.4	6.5	6.4	6.6	6.3	6.5	6.6	9.8	8.1
ADF	10.8	11.0	12.0	12.0	10.0	11.5	10.6	11.1	11.1	22.8	16.5
NDF	36.2	40.4	43.2	34.6	35.9	36.8	36.3	37.5	37.6	40.7	39.5
IDF[2]	28.7	32.5	33.8	30.0	31.0	31.1	28.3	30.5	30.7	34.1	35.4
SDF[2]	0.0	0.8	1.1	1.6	0.8	1.6	1.5	1.3	1.1	1.2	0.4
TDF[2]	28.7	33.3	34.9	31.6	31.8	32.7	29.8	31.8	31.8	32.2	35.8
OR	44.5	48.2	47.1	49.3	46.8	46.5	44.1	45.6	46.5	45.6	46.9
Diets											
Crude fiber	3.4	4.2	5.8	4.3	4.2	4.5	4.2	4.2	4.4	6.1	5.7
ADF	6.9	7.2	8.7	7.2	6.7	7.3	7.7	7.5	7.4	15.3	11.0
NDF	21.3	23.5	28.3	25.0	23.5	24.1	24.7	24.1	24.3	25.9	26.3

Table 4.3. (cont.)

	Source of corn-DDGS										
Item	1	2	3	4	5	6	7	8	Mean	S-DDGS	SC-DDGS
IDF	20.2	22.1	27.1	20.8	21.4	20.4	24.7	21.4	22.2	22.9	25.6
SDF	0.0	1.1	0.3	1.1	0.6	2.9	1.7	1.3	1.3	4.1	2.6
TDF	20.2	23.2	27.4	21.9	22.0	23.3	27.4	22.7	23.5	26.9	28.2
OR	33.6	33.8	34.5	35.7	33.6	33.5	32.3	31.7	33.6	32.6	33.5

[1]Samples from Urriola et al. (2009).

[2]IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TDF = total dietary fiber.

Table 4.4. Analyzed composition of distillers dried grains with solubles (DDGS) used in Exp. 3 (as-fed basis)[1]

	$DDGS_{ethanol}$ source								
Item	1	2	3	4	5	6	Mean	$DDGS_{beverage}$	DDG
DDGS									
DM	88.9	87.0	88.4	88.7	86.9	87.8	88.0	89.7	88.5
CP	26.4	25.4	24.7	29.0	24.7	25.5	26.0	26.3	26.1
Starch	11.4	7.3	7.0	8.1	7.4	9.6	8.0	7.3	3.8
TDF[2]	31.7	29.5	31.5	32.4	28.6	30.6	30.7	38.5	43.9
Diets									
TDF	21.7	21.9	23.3	21.3	21.2	22.7	22.0	28.3	28.1

[1]Samples from Pahm et al. (2008).

[2]TDF = total dietary fiber.

Table 4.5. Apparent ileal digestibility (AID), apparent total tract digestibility (ATTD), and hindgut fermentation (HGF) of total dietary fiber in corn and 10 sources of distillers dried grains with solubles (DDGS) produced from corn and fed to growing pigs[1], Exp. 1

		Source of corn DDGS											DDGS		DDGS vs. corn[2]	
Item	Corn	1	2	3	4	5	6	7	8	9	10	Mean	SEM	*P*-value	SEM	*P*-value
AID, %	16.5	25.2	19.7	24.8	25.6	12.6	25.9	21.9	19.7	24.6	14.7	21.5	2.6	< 0.01	17.7	0.23
ATTD, %	23.1	45.1	46.1	52.4	50.0	43.9	49.0	44.2	47.1	36.8	30.5	44.5	4.8	< 0.01	7.8	0.05
HGF, %	6.6	19.9	26.4	27.6	24.4	31.3	23.1	22.3	27.5	12.2	15.8	23.0	5.8	0.22	16.3	0.03

[1]Least square means of 8 pigs per diet.

[2]Contrast of corn vs. all DDGS sources.

Table 4.6. Apparent ileal digestibility (%), apparent total tract digestibility (%), and hindgut fermentation (%) by growing pigs of DM, crude fiber, ADF, NDF, insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total dietary fiber (TDF), and organic residue (OR) in distillers dried grains with solubles (DDGS) produced from corn (C-DDGS), sorghum (S-DDGS), or from a blend of sorghum and corn (SC-DDGS)[1], Exp. 2

Item	C-DDGS source									S-DDGS	SC-DDGS	SEM	*P*-value	Contrasts[2]	
	1	2	3	4	5	6	7	8	Mean					S vs. C DDGS	SC vs. C DDGS
Apparent ileal digestibility															
Crude fiber	13.7	19.2	42.8	35.3	34.0	36.2	31.7	34.7	31.0	38.6	30.7	5.5	< 0.01	0.07	0.95
ADF	35.0	28.2	47.0	40.0	32.6	40.8	36.5	34.1	36.8	57.4	41.4	4.0	< 0.01	< 0.01	0.12
NDF	41.7	37.5	52.1	48.8	45.7	45.1	45.5	50.4	45.9	49.9	37.9	4.2	< 0.01	0.18	< 0.01
IDF	5.9	9.0	33.6	26.7	13.9	21.1	20.5	29.3	20.0	27.7	4.8	6.9	< 0.01	0.14	< 0.01
SDF	81.7	62.1	70.2	56.5	59.6	63.8	56.4	64.5	64.4	65.9	63.4	3.6	< 0.01	0.59	0.73
TDF	29.0	19.6	38.2	32.8	21.8	28.3	25.5	35.9	28.9	33.4	15.9	5.9	< 0.01	0.30	< 0.01
OR	66.5	65.5	67.0	65.1	67.0	53.9	38.4	45.0	58.6	41.6	32.9	2.6	< 0.01	< 0.01	< 0.01

Table 4.6. (cont.)

Item	C-DDGS source									S-DDGS	SC-DDGS	SEM	*P*-value	Contrasts[2]	
	1	2	3	4	5	6	7	8	Mean					S vs. C DDGS	SC vs. C DDGS
Apparent total tract digestibility, %															
Crude fiber	37.6	38.0	50.6	47.2	48.1	36.3	51.2	45.1	44.3	41.6	39.9	4.0	< 0.01	0.88	0.76
ADF	63.1	51.8	62.2	61.7	54.7	53.7	64.3	56.5	58.5	60.7	53.7	4.1	< 0.01	0.13	0.64
NDF	61.0	54.3	60.7	57.9	62.3	51.6	65.8	60.8	59.3	59.3	51.5	3.6	< 0.01	0.42	0.06
IDF	37.1	30.9	45.8	41.8	41.7	29.3	51.0	45.0	40.3	41.3	28.6	4.2	< 0.01	0.30	0.05
SDF	95.3	92.7	92.1	91.7	92.6	89.4	91.3	91.1	92.0	90.9	90.6	1.5	< 0.01	0.78	0.59
TDF	55.0	41.1	52.8	49.4	49.5	39.4	56.4	52.0	49.5	48.8	39.2	4.6	< 0.01	0.49	0.06
OR	81.3	78.4	76.8	74.6	81.3	74.5	77.6	72.4	77.1	72.5	68.4	2.3	< 0.01	0.03	< 0.01

Table 4.6. (cont.)

Item	C-DDGS source									S-DDGS	SC-DDGS	SEM	*P*-value	Contrasts[2]	
	1	2	3	4	5	6	7	8	Mean					S vs. C DDGS	SC vs. C DDGS
Hindgut fermentation															
Crude fiber	23.9	18.8	7.8	11.9	14.1	0.1	19.5	10.4	13.3	3.0	9.2	8.3	< 0.01	0.44	0.21
ADF	28.1	23.6	15.2	21.7	22.1	12.9	27.8	22.4	21.7	3.3	12.3	6.7	< 0.01	0.01	0.04
NDF	19.3	16.8	8.6	9.1	16.6	6.5	20.3	10.4	13.4	9.4	13.6	6.2	< 0.01	0.76	0.51
IDF	31.2	21.9	12.2	15.1	27.8	8.2	30.5	15.7	20.3	13.6	23.8	8.7	< 0.01	0.98	0.77
SDF	13.6	30.6	21.9	35.2	33.0	25.6	34.9	26.6	27.6	25.0	27.2	4.4	< 0.01	0.35	0.52
TDF	26.0	21.5	14.6	16.6	27.7	11.1	30.9	16.1	20.6	15.4	23.3	7.5	< 0.01	0.84	0.73
OR	14.8	12.9	9.8	9.5	14.3	20.6	39.2	27.4	18.5	30.9	35.5	2.9	< 0.01	< 0.01	< 0.01

[1]Least square means of 8 pigs per diet.

[2]Contrasts of S-DDGS vs. C-DDGS and SC-DDGS vs. C-DDGS.

Table 4.7. Apparent ileal digestibility (AID), apparent total tract digestibility (ATTD), and hindgut fermentation (HGF) by growing pigs of total dietary fiber in 6 sources of corn distillers dried grains with solubles produced from ethanol plants ($DDGS_{ethanol}$), corn distillers dried grains with solubles produced at a beverage plant ($DDGS_{beverage}$), and in corn distillers dried grains (DDG)[1], Exp. 3

	$DDGS_{ethanol}$ source											*P* – values, contrasts	
Item	1	2	3	4	5	6	Mean	$DDGS_{beverage}$	DDG	SEM	*P* – value	$DDGS_{beverage}$ vs. $DDGS_{ethanol}$	DDG vs. $DDGS_{ethanol}$
AID	13.0	12.8	24.0	11.4	30.8	18.8	18.5	13.2	0.7	4.9	< 0.01	0.29	< 0.01
ATTD	29.3	51.8	52.4	41.3	56.0	57.0	48.0	46.4	43.8	3.5	< 0.01	0.19	0.60
HGF	16.3	39.0	28.4	29.9	25.2	38.2	29.5	33.2	43.1	6.02	0.19	0.59	0.05

[1]Least square means of 8 pigs per diet.

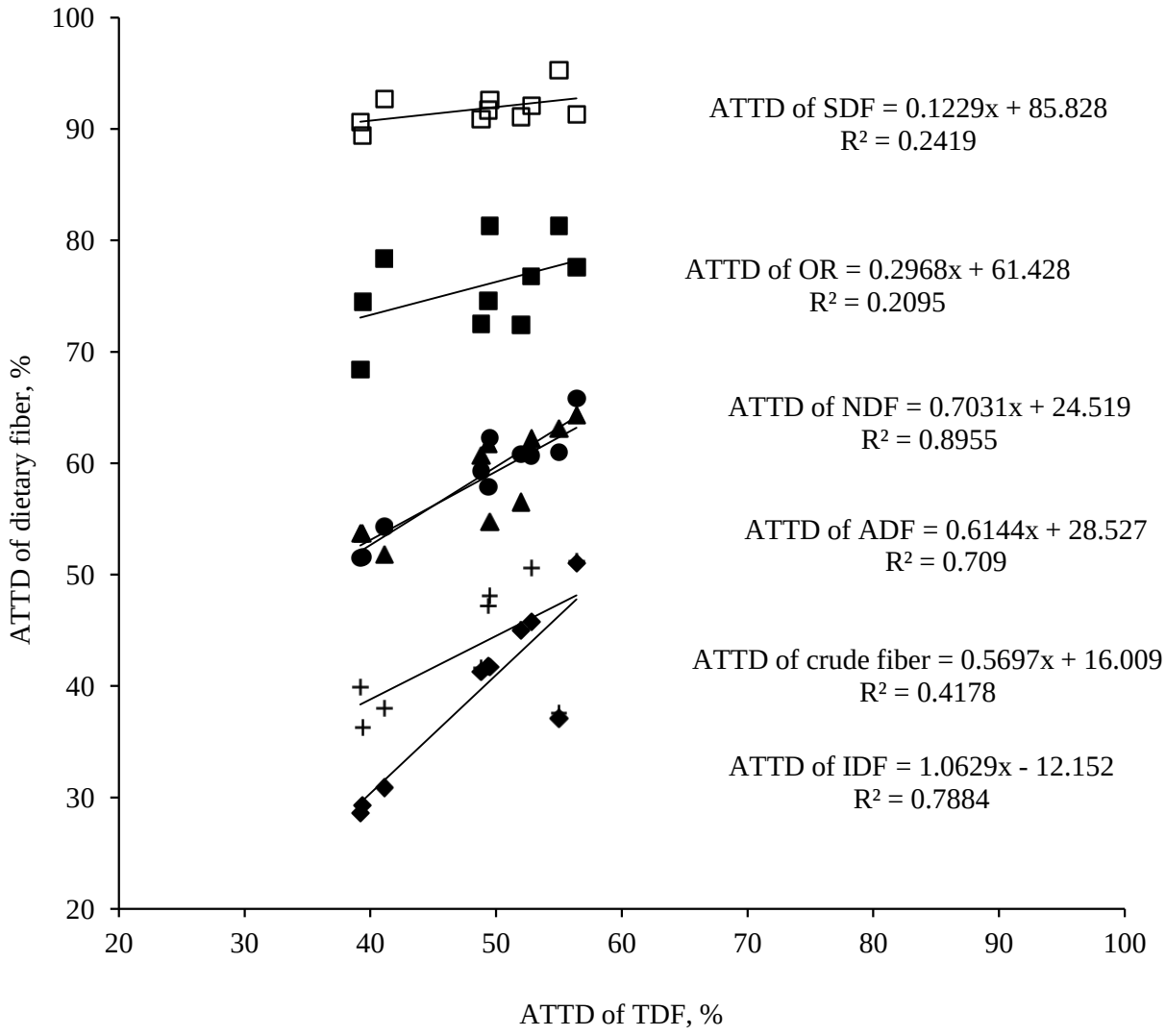

Figure 4.1. Relationship between the apparent total tract digestibility (ATTD) of total dietary fiber (TDF) and the ATTD of crude fiber (+), ADF(▲), NDF (●), insoluble dietary fiber (IDF; ◊), soluble dietary fiber (SDF; □), and organic residue (OR; ■) in distillers dried grains with solubles fed to growing pigs.

CHAPTER 5

Comparative digestibility of energy and nutrients in fibrous feed ingredients in Meishan and Yorkshire pigs

ABSTRACT: The objective of this experiment was to test the hypothesis that differences in the digestibility of total dietary fiber (TDF) among breeds of the pigs is influenced by the type of fiber that is being fed (insoluble vs. soluble) and also by age of the pig. Five light Yorkshire pigs (BW: 80.1 ± 11.2 kg; 4 months old), 5 heavy Yorkshire pigs (BW: 102.1 ± 3.5 kg), and 5 Meishan pigs (BW: 77.2 ±15.2 kg; 5 months old) were surgically prepared with a T-cannula in the distal ileum. A corn-soybean meal diet (control) was formulated with 5 $g \cdot kg^{-1}$ of titanium dioxide as an indigestible marker. Three additional diets were formulated by replacing 30% of the control diet with 30% of distillers dried grains with solubles (DDGS), soybean hulls (SBH), or sugar beet pulp (SBP) and one diet was formulated by replacing 15% of the control diet with 15% pectin. Each group of pig was allotted to a 5 × 5 Latin square design and pigs were fed the 5 experimental diets during five 14-d periods. Fecal samples were collected on d 12 and ileal digesta were collected on d 13 and 14 of each period. The apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of GE and nutrients in each ingredient were calculated using the substitution method. Hindgut fermentation was calculated as the difference between ATTD and AID. When fed the control diet, Meishan pigs, had a tendency ($P < 0.10$) for a greater AID of GE and CP (78.6 and 80.3%) than light (77.0 and 78.9%) and heavy Yorkshire pigs (75.7 and 76.9%). and they had a greater ($P < 0.05$) ATTD of DM, GE, and carbohydrates (89.2, 89.5, 95.5%) than light (86.6, 86.4, and 92.4%) and heavy (87.0, 86.6, and 93.0%) Yorkshire pigs. The ATTD of DM, GE, CP, carbohydrates, and TDF in DDGS (75.4, 76.3, 81.3, 78.0, and 75.3%;

respectively) was greater ($P < 0.01$) by Meishan pigs than by light (55.7, 58.5, 66.7, 49.2, and 39.0%) and heavy (59.8, 62.9, 70.0, 51.1, 55.7%) Yorkshire pigs. There were no differences among the 3 groups of pigs in the ATTD of energy or nutrients in SH, SBP, and pectin. The ATTD of CP, was, however, lower ($P < 0.05$) in Meishan pigs (-15.2%) than heavy (-4.9%) and light (10.4%) Yorkshire pigs. There were no differences among the 3 groups of pigs in hindgut fermentation of nutrients in diets or in ingredients. In conclusion, Meishan pigs have a greater ATTD of DM, GE, and some nutrients in corn-soybean meal diets and in DDGS than Yorkshire pigs.

Key words: breeds, digestibility, insoluble dietary fiber, pig, soluble dietary fiber

INTRODUCTION

Modern pigs have a relative poor capacity to digest dietary fiber and the apparent total tract digestibility (**ATTD**) of fiber is less than 50% in some feed ingredients when fed to growing pigs (Urriola et al., 2010). However, native breeds of pigs such as Meishan (Kemp et al., 1991), Alentejano (Freire et al., 1998). Mong-Cai (Len et al., 2006), and Mukota (Ndindana et al., 2002) may have a greater capacity to digest dietary fiber than modern crossbred pigs. Meishan pigs fed a diet based on corn, wheat, and barley had greater ATTD of crude fiber and energy than Dutch Landrace pigs (Kemp et al., 1991). This may be a result of a larger hindgut and a more active microflora in Meishan pigs compared with Dutch Landrace pigs, which in turn may increase the fermentation of fiber and subsequently the absorption of VFA. However, if 15% oats were added to the basal diet no differences between the 2 groups of pigs were observed (Kemp et al., 1991).

Diets based on corn and 35% dehydrated alfalfa meal were, however, not digested better by 20 kg Meishan pigs than by 20 kg white composite breeds (Yen et al., 2004). It is, therefore, possible that the type of dietary fiber as well as the breed of the pig influences ATTD of dietary fiber, but this hypothesis has only been investigated with pigs fed sugar beet pulp (von Heimendahl et al., 2009). Sugar beet pulp, however, has a variable concentration of soluble dietary fiber (**SDF**) and insoluble dietary fiber (**IDF**; Sunvold et al., 1995). Dietary fiber in distillers dried grains with solubles (**DDGS**) is mostly IDF (Urriola et al., 2010). The objective of this experiment, therefore, was to test the hypothesis that the source of dietary fiber (SDF vs. IDF) and the breed of pigs (Meishan pigs vs. Yorkshire pigs) influence the ATTD of energy, fiber, and other nutrients.

MATERIALS AND METHODS

Animals and Housing

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. Five Meishan pigs (initial BW: 77.2 ± 15.2 kg; 5 months old) and 10 Yorkshire pigs were surgically prepared with a T-cannula in the distal ileum following the technique by Stein et al. (1998). Five of the Yorkshire pigs had the same age as the Meishan pigs (5 months) and weighed 102.1 ± 3.5 kg at time of surgery (heavy Yorkshires). The other 5 Yorkshire pigs had an initial BW that was close to that of the Meishan pigs (80.1 ± 11.2 kg), but these pigs were only 4 months old (light Yorkshires). Following surgeries, pigs were allowed to recover for 10 d and a corn soybean meal diet was provided on an ad libitum basis during this time. All pigs were housed in individual pens (1.8 ×

2.7 m) that had a nipple drinker and a feeder. The floors of the pens were half concrete and half concrete slats. The room temperature was kept between 20 and 22°C throughout the experiment.

Four feed ingredients with different concentration of IDF and SDF were used (Table 5.1). The 4 ingredients were distillers dried grains with solubles (**DDGS;** Lincolnland Agri-Energy, Palestine, IL), soybean hulls (**SBH**; Archer Daniels Midland, Decatur), sugar beet pulp (**SBP**; Siemer Milling Company, Teutopolis, IL), and fruit derived pectin (TIC Gums, Belcamp, MD). Five diets were formulated (Table 5.2). The control diet was based on corn and soybean meal and contained 5 g/kg of titanium dioxide (Chicago Sweeteners, Chicago, IL) as an indigestible marker. Three additional diets were formulated by replacing 30% of the control diet with 30% DDGS, SBH, or SBP. The last diet was formulated by replacing 15% of the control diet with 15% citrus pectin. Vitamins and minerals were included in all diets to meet or exceed the nutrient requirements of growing pigs (NRC, 1998).

Experimental Design and Sample Collection

Pigs within each group (i. e., Meishans, light Yorkshire, and heavy Yorkshire) were randomly allotted to a 5 × 5 Latin square design with 5 diets and five 14-d periods (Kim and Stein, 2009). Feed was provided to each pig at a daily level of 2 times the maintenance requirement for energy (i.e., 106 kcal ME per kg $BW^{0.75}$; NRC, 1998). The daily feed allotments were divided into 2 equal meals that were provided at 0800 and 1700.

Fecal samples were collected on d 12 of each period via grab-sampling and stored at -20ºC. Ileal digesta were collected on d 13 and 14 of each period following procedures described by Cervantes-Pahm and Stein (2008). Pig BW was recorded at the beginning of the experiment and at the end of each period to calculate feed allowance for the following period.

Chemical Analyses

At the end of each period, samples were thawed and mixed within animal and diet and a sub-sample was collected for chemical analysis and stored at –20°C. Ileal and fecal samples were dried to a constant weight in a forced air oven and ground through a 1mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). Samples of corn, soybean meal, DDGS, SBH, SBP, and pectin, and of all diets, ileal samples, and fecal samples were analyzed for DM (method 930.15, AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007), acid hydrolyzed fat (**AEE**; method 996.01, AOAC Int., 2007), ash (method 942.15; AOAC Int., 2007), and total dietary fiber (**TDF**; method 985.29; AOAC Int., 2007). Diets and ingredients were also analyzed for NDF (Holst, 1973), ADF (method 973.18; AOAC Int., 2007), ADL (method 973.18 (A-D), AOAC Int., 2007), and IDF (method 985.29; AOAC Int., 2007). The concentration of GE in ingredients and diets and in ileal and fecal samples was determined using an adiabatic bomb calorimeter (model 6300, Parr Instruments, Moline, IL). Benzoic acid was used as the internal standard. All diets, ileal samples, and fecal samples were also analyzed for titanium concentration following the procedure by Myers et al. (2004).

Water binding capacity was measured by weighing 1 g of sample into a centrifuge tube and mixing with 30 mL of distilled water (Robertson et al., 2000). After stirring, samples were allowed to settle and were centrifuged (3,000 g; 20 min). The supernatant was removed and the samples were weight. The WBC values are expressed as the amount of water retained by the pellet (g/g).

Calculations

The concentration of SDF in diets and ingredients was calculated as the difference between TDF and IDF. Hemicellulose in each sample was calculated as the difference between

NDF and ADF and cellulose was calculated as the difference between ADF and ADL (Van Soest et al., 1991). The concentration of carbohydrates (**CHO**) in diets, ileal samples, and fecal samples was calculated according to the following equation:

$$\text{CHO} = 100 - [\text{CP} + \text{AEE} + \text{ash} + (100 - \text{DM})]$$

Apparent ileal digestibility (**AID**) and ATTD of DM, energy, CP, AEE, ash, TDF, and CHO were calculated in all diets (Equation 2; Stein et al., 2007). The AID and ATTD of DM, energy, CP, AEE, ash, TDF, and CHO in DDGS, SBH, SBP, and pectin were subsequently calculated by the difference procedure (Fan and Sauer, 1995) using the following equation:

$$\text{AD}_{\text{nutrient}} = \frac{(\text{AD}_{\text{assay}} - \text{AD}_{\text{control}}) \times \text{Nutrient}_{\text{control}}}{(1 - \text{Nutrient}_{\text{control}})}$$

where $AD_{nutrient}$ is the AID or ATTD of a nutrient in the ingredient (%), AD_{assay} is the AID or ATTD of the nutrient in the assay diet (%), $AD_{control}$ is the AID or ATTD of the nutrient in the control diet, and $Nutrient_{control}$ is the contribution of the nutrient from the control diet to the assay diet (decimal %).

The hindgut fermentation of nutrients, DM, and energy was calculated according to the following equation (Urriola et al., 2010):

$$\text{Hindgut fermentaion}_{\text{Nu}} = \text{ATTD}_{\text{Nu}} - \text{AID}_{\text{Nu}}$$

where $ATTD_{Nu}$ is the amount of apparent total tract digestible nutrient (g), DM (g), or energy (kcal/kg) and AID_{Nu} is the amount of ileal digestible nutrient (g), DM (g), or energy (kcal/kg).

Statistical Analysis

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to confirm normal distribution of the data, equal variances, and to identify outliers. An observation was considered an outlier if the value was more than 3 SD away from the mean. No other outliers

were identified, however, one observation was removed because the pig was sick during the adaptation period. Data were analyzed within each diet by ANOVA using the MIXED procedure of SAS. The group of pigs was the fixed effect and random effects were the period and the pig nested within group of pigs. Least square means of the 3 groups of pigs within each diet were calculated using the LSMEAN statement of SAS, the pdiff option was used to separate means, and multiple comparisons were adjusted by Tukey. A similar model was used to calculate and analyze the digestibility values of DM, energy, and nutrients in ingredients. The differences were considered significant if $P < 0.05$ and a trend if $P > 0.05 < 0.10$. The pig was the experimental unit for all analyses.

RESULTS

All pigs were successfully cannulated at the distal ileum and quickly recovered after surgery. The BW at the beginning of period 1 was 90.0 ± 12.4 kg (light Yorkshires), 116.7 ± 6.2 kg (heavy Yorkshires), and 82.5 ± 11.6 kg (Meishans). All pigs gained BW during the experiment and the initial BW of the last period was, 116.8 ± 12.0 kg (light Yorkshires), 131.0 ± 17.5 kg (heavy Yorkshires), and 92.2 ± 11.1 kg (Meishans).

The concentration of GE varied from 5,086 kcal/kg in DDGS to 3,140 kcal/kg in pectin, while varied CP in soybean meal (47.6%) to SBH (8.1%). The concentration of AEE varied from 16.6% in DDGS to 0.9% in pectin (Table 5.1). The concentration of TDF varied from 67.0% in SBP to 8.4% in corn, but the concentration of SDF was greatest in pectin (41.2%) and no SDF was detectable in DDGS. The WBC varied from 17.3 g/g in pectin to 1.9 g/g in corn.

The concentration of SDF varied among diets (Table 5.2), and was increased with the addition of high fiber feed ingredients from 0% in the control diet to 2.6% in the diet with DDGS, 1.8% in the diet with SBH, 3.8% in the diet with SBP, and 9.7% in the diet with 15% pectin. All diets

contained IDF, the greatest level was in the diet with SBH (25.3%) and the least in the diet with pectin (8.5%). The WBC of the SBP diet was greatest (4.0 g/g) while the control diet had the least WBC (2.2 g/g).

Apparent Ileal Digestibility of DM, GE, and Nutrients in Diets

When pigs were fed the control diet, Meishan pigs had a tendency ($P < 0.10$) for a greater AID of GE and CP (78.6 and 80.3%) than light (77.0 and 78.9%) and heavy (75.7 and 76.9%) Yorkshire pigs (Table 5.3). There were, however, no differences in the AID of DM, AEE, ash, CHO, and TDF among Meishan, light Yorkshire, and heavy Yorkshire pigs. When pigs were fed the diet containing 30% DDGS, there were no differences in the AID of DM, GE, AEE, ash, CHO, or TDF among Meishan, light Yorkshire, and heavy Yorkshire pigs. When pigs were fed the diet containing 30% SBP, heavy Yorkshire pigs had lower ($P < 0.05$) AID of CP (64.3%) than Meishan pigs (68.8%), whereas the AID of CP in light Yorkshire pigs (66.9%) was not different from the other 2 groups, and there were no differences in the AID of DM, CP, GE, or other nutrients. When pigs were fed the diet containing pectin, Meishan pigs tended ($P < 0.10$) to have a greater AID of CP (74.9%) than light Yorkshire (71.3%) and heavy Yorkshire pigs (72.0%), but there were no differences in the AID of DM, GE, or other nutrients among the 3 groups of pigs. There was also a tendency ($P < 0.10$) for a lesser AID of ash in Meishan pigs (-18.2%) than in heavy Yorkshire pigs (-6.6%), whereas the AID of ash in light Yorkshire pigs (-14.5) was not different from that of the other 2 groups.

Apparent Total Tract Digestibility of DM, Energy, and Nutrients in Diets

When pigs were fed the control diet Meishan pigs had a greater ($P \leq 0.05$) ATTD of DM, GE, and CHO (89.2, 89.5, 95.5%) than light (86.6, 86.4, 92.4%) and heavy (87.0, 86.6, and 93.0%) Yorkshire pigs (Table 5.4). When pigs were fed the diet containing 30% DDGS, Meishan

pigs also had a greater ($P < 0.01$) ATTD of DM, GE, CHO, and TDF (84.0, 83.5, 90.4, and 66.5) than light (78.1, 77.3, 84.5, and 45.8%) and heavy (79.3, 78.8, 84.9, and 55.3%) Yorkshire pigs. The ATTD of CP (83.7%) in Meishan pigs was greater ($P < 0.01$) than in light Yorkshire pigs (76.9%), but not different from that of heavy Yorkshire pigs (78.4%). When pigs were fed the SBP diet, Meishan pigs tended ($P < 0.10$) to have a greater ATTD of GE (84.7%) than heavy Yorkshire pigs (81.4%), but no differences were observed for the ATTD of DM and nutrients. There were also no differences among the 3 groups of pigs in the ATTD of DM, GE, or nutrients in the diets containing SBH or pectin.

Hindgut Fermentation of DM, Energy, and Nutrients in Diets

When fed the DDGS diet, Meishan pigs, had a greater ($P < 0.01$) hindgut fermentation of DM and CHO (15.1 and 20.2%) than light (10.1 and 14.0%) and heavy (10.7 and 13.5%) Yorkshire pigs (Table 5.5). There was also a tendency ($P < 0.10$) for a greater hindgut fermentation of GE for Meishan pigs (12.9%) than for light (8.3%) and heavy (9.3%) Yorkshire pigs. There were no differences among the 3 groups of pigs in hindgut fermentation of DM, GE, or nutrients when pigs were fed the control, SBH, SBP, or pectin diets. Values for the hindgut fermentation of AEE were negative for all diets indicating that there was influx of AEE in the hindgut of pigs regardless of the group of pigs or diet that was fed.

Apparent Ileal Digestibility of DM, GE, and Nutrients in Feed Ingredients

The AID of DM, GE, and nutrients in DDGS and SBH were not different among the 3 groups of pigs (Table 5.6). The AID of CP in SBP was greater ($P < 0.05$) in Meishan (37.9%) and light (33.1%) than heavy (22.7%) Yorkshire pigs, but the AID of DM, GE, or other nutrients were not different among the 3 groups of pigs. The AID of CP in pectin also tended to be greater ($P < 0.10$) in Meishan pigs (60.7%) than in light Yorkshire (16.7%) and heavy Yorkshire pigs

(23.1%), but other differences among the 3 groups of pigs were not observed. The AID of ash in SBH, SBP, and pectin was negative for all 3 groups of pigs.

Apparent Total Tract Digestibility of DM, Energy, and Nutrients in Ingredients

The ATTD of DM, GE, CP, and CHO in DDGS (Table 5.7) by Meishan pigs (75.4, 76.3, 81.3, and 78.0%) were greater ($P < 0.01$) than in light Yorkshire pigs (55.7, 58.5, 66.7, and 49.2%) and heavy Yorkshire pigs (59.8, 62.9, 70.0, and 51.1%). The ATTD of TDF in DDGS was greater ($P < 0.01$) in Meishan pigs (75.3%) than in heavy (55.7%) and light Yorkshires pigs (39.0%), and the ATTD of TDF was also greater ($P < 0.05$) in heavy than in light Yorkshire pigs. There were no differences in the ATTD of AEE and ash in DDGS among the 3 groups of pigs. The ATTD of CP in SBP by heavy Yorkshire pigs (-4.9%) was less ($P < 0.05$) than in light Yorkshire pigs (10.4%) and Meishan pigs had an ATTD of CP (-15.2%) that was less than both heavy and light Yorkshires pigs. There were no differences in ATTD of DM, energy, and nutrients in SBH and in pectin among the 3 groups of pigs.

Hindgut fermentation of DM, Energy, and Nutrients in Ingredients

Regardless of the ingredient, no differences in hindgut fermentation of DM, GE, and nutrients among Meishan, light Yorkshire, and heavy Yorkshire pigs were observed (Table 5.8). Negative fermentation values were calculated for AEE in all 3 groups of pigs for DDGS and SBH.

DISCUSSION

The ATTD of TDF in DDGS is only 46% (Urriola et al., 2010), which is the reason for the relatively low ATTD of DM and GE in corn based co-products (Stein and Shurson, 2009). In contrast, the ATTD of TDF in SBP and pectin are 71.8 and 90%, respectively (Graham et al., 1986; Drochner et al., 2004), and the ATTD of hemicellulose and cellulose in SBH is 58.9 and

82.1%, respectively (Kornegay, 1981). The TDF in DDGS is mainly IDF, whereas, SBH, SBP, and pectin contain greater concentrations of SDF, which is likely the reason for the less ATTD of TDF in DDGS than in SBH, SBP, and pectin because the ATTD of IDF is much less than the ATTD of SDF (Urriola et al., 2010).

The ATTD of crude fiber in 28 kg Meishan pigs (47.8%) is greater than in Dutch Landrace pigs of similar BW (36.4%; Kemp et al., 1991), but 20 kg Meishan pigs had ATTD of crude fiber that was not different from the ATTD of crude fiber by 20 kg White composite crossbred pigs (Yen et al., 2004). Other native breeds of pigs such as Mong Cai (Vietman) and Mukota (Zimbabwe) also have greater ATTD of NDF, ADF, and crude fiber than modern crossbred pigs (Ndindana et al., 2002; Len et al., 2006; Len et al., 2009), but the ATTD of dietary fiber in Kune-Kune and Haellisches Schwein or Bunte Bentheimer pigs is not different from that of modern crossbred pigs (Morel et al., 2006; von Heimendahl et al., 2009). The reason for these different results among experiments may be that the ATTD of fiber is influenced not only by the breed of pigs, but also by the type of fiber and the age of the pigs.

In the present experiment, Meishan and Yorkshire pigs, had similar ATTD of GE and nutrients when fed diets based on SBH, SBP, and pectins. This observation is consistent with the fact that there are no differences in ATTD of NDF in SBP among Schwaebisch Haellisches Schwein, Bunte Bentheimer, and modern crossbred pigs (von Heimendahl et al., 2009). However, Meishan pigs had a greater ATTD of TDF than Yorkshire pigs when pigs were fed diets with a greater proportion of IDF (control and DDGS diets). This observation indicates that Meishan pigs have a greater capacity to digest IDF than Yorkshire pigs, whereas there is no difference between the 2 breeds in the capacity to digest SDF. This is not a surprise because the

ATTD of SDF in modern breeds of pigs is close to 90% (Serena et al., 2008; Urriola et al., 2010) as was also observed for the ATTD of TDF in pectin in this experiment.

The ATTD of TDF and energy is influenced by the age of the pig (Le Goff and Noblet, 2001). To separate the effects of BW and age we attempted to compare the ATTD of TDF in Meishan pigs to Yorkshire pigs that had either a BW or an age that was similar to the Meishan pigs. The fact that Meishan pigs and heavy Yorkshire pigs had a greater ATTD of TDF than light Yorkshire pigs when fed DDGS, but not when fed SBH, SBP, or pectin, indicates that age related differences in the ATTD of fiber are caused by differences in the ATTD of IDF, whereas age does not influence the ATTD of SDF. This observation is in agreement with Le Goff and Noblet (2001) and with Jørgensen et al. (2007) who reported that the ATTD of GE and dietary fiber were not different among modern crossbred growing pigs, finishing pigs, or sows when fed diets based on SBP. However, if diets that contained IDF from wheat bran or corn bran were fed, sows had greater ATTD of GE and fiber than growing and finishing pigs and finishing pigs had a grater ATTD of GE and fiber than growing pigs.

Greater ATTD of IDF by older pigs than by younger pigs may be explained by differences in feed intake, differences in the size of the intestines, and differences in the capacity of microbes to ferment IDF (Varel and Yen, 1988; Dierick et al., 1989; Varel et al., 1997). In the current experiment, feed intake was equalized among the 3 groups of pigs so feed intake did not contribute to the observed differences among pigs. However, Meishan pigs, have larger intestines in proportion to BW than White composite crossbred pigs (Yen et al., 2004), which may have contributed to the increased ATTD of IDF and GE. The greater ATTD of IDF in Meishan pigs than in Yorkshire pigs may also have been due to a greater capacity of the microflora in Meishan pigs to digest fiber. However, the concentrations of total viable bacteria

and cellulitic bacteria in the feces of Meishan pigs at 13, 17, and 19 wk of age were not different from that of White composite crossbred pigs (Yen et al., 2004) and the capacity to ferment fiber is not different between sows and growing pigs (Le Goff et al., 2003). Meishan pigs have greater intestinal concentrations of Bacteroides and Firmicutes than Yorkshire pigs (Guo et al., 2008) and these differences contribute to an increase in digestibility of dietary energy in mice (Turnbaugh et al., 2006).

Hindgut fermentation was calculated as the difference between ATTD and AID and differences in hindgut fermentability among breeds of pigs were not observed regardless of the diets being fed. However, dietary fiber is defined as the CHO that are resistant to digestion by mammalian enzymes (AACC, 2001). Therefore, any disappearance of fiber in the small intestine may be considered a result of fermentation in the small intestine, but results of this experiment showed that there were no differences among breeds in the AID of dietary fiber. We are not aware of any previous data that compared the AID of TDF among different breeds of pigs.

The negative hindgut fermentation of AEE for all diets and for DDGS and SBH is most likely a consequence of synthesis of fatty acids in the hindgut, because the presence of CHO in the hindgut allows microbes to synthesize fatty acids. No synthesis of fat takes place in the hindgut, in low fiber diet, but in high fiber diets, fat is synthesized in the hindgut, which results in lower values for the ATTD than for the AID of AEE. This, in term, results in negative values for hindgut fermentation of AEE (Kil, 2008).

The negative values for the AID of ash in SBH, SBP, and pectin, but not in DDGS, indicate that ingredients that contain significant quantities of SDF may draw minerals into the stomach and (or) small intestines. This observation is in agreement with data showing that AID of ash in SBP (-116%) is much lower than in wheat bran (-3.9%; Graham et al., 1986).

The AID of TDF in DDGS that was measured in this experiment agrees with the value of 24% that has previously been reported (Urriola et al., 2010). The AID for TDF in all diets are also within the range for AID of TDF (-10 to 62%) that has previously been reported (Bach Knudsen and Jørgensen, 2001).

Because the small intestinal as well as the hindgut disappearance of TDF is a result of fermentation, values for the ATTD of TDF represent the total fermentation of fiber. The energy contribution from fermentation in the small intestine and in the hindgut is a result of absorption of VFA and, therefore, the total energy contribution from fermentation of fiber can be calculated from the ATTD of fiber. However, the energy value of dietary fiber depends not only on the VFA produced after fermentation, but also on the effects of fiber on the digestibility of other nutrients and on the endogenous losses of nutrients (Elia and Cummings, 2007; Bindelle et al., 2009). Dietary fiber may increase endogenous losses of nutrients (Bindelle et al., 2009) and the negative values for AID and ATTD of AEE in SBH and pectin indicate that the fiber in these ingredients promote a net secretion of AEE into the gut in addition to the net synthesis of AEE in the hindgut. Endogenous losses of AEE may increase with increasing levels of dietary fiber (Kil, 2008) and the digestibility of AEE, therefore, may decrease with addition of dietary fiber (Bach Knudsen and Hansen, 1991; Dégen et al., 2009). The reason no negative AID and ATTD values for AEE were observed for DDGS is most likely that the concentration of AEE in DDGS is greater than in the other ingredients because the influence of the endogenous losses of AEE are much greater in diets with low concentration of AEE than in diets with greater concentration of AEE (Stein et al., 2007; Kil, 2008).

In conclusion, Meishan pigs had a greater ATTD of TDF than Yorkshire pigs when fed the control or the DDGS diet, which have a high concentration of IDF. There were, however, no

differences among the 3 group of pigs when pigs where fed diets containing SBH, SBP, or pectin, which contain more SDF. This indicates that Meishan pigs are more efficient in fermenting IDF than Yorkshire pigs. It is possible that this difference is a result of differences in the microbial population, but further research is needed to measure the influence of breed on intestinal microbial populations.

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Table 5.1. Composition of feed ingredients, as is basis

Item	Ingredients[1]					
	Corn	SBM	DDGS	SBH	SBP	Pectin
GE, kcal/kg	3,881	4,203	5,086	3,771	3,857	3,140
DM, %	87.5	89.7	90.0	85.2	88.4	79.8
CP, %	8.3	47.6	29.3	8.1	10.6	10.6
Acid hydrolyzed fat, %	2.2	1.4	16.6	1.6	1.4	0.9
Ash, %	1.2	7.2	4.6	6.0	4.1	2.8
Total dietary fiber, %	8.4	14.3	31.5	57.2	67.0	45.0
Soluble dietary fiber	0.4	1.1	0.0	6.2	5.4	41.2
Insoluble dietary fiber	8.5	13.2	31.5	51.0	61.6	3.8
NDF	10.9	8.8	31.0	42.0	57.4	-
Hemicellulose[2]	8.9	2.9	20.6	19.4	16.1	-
ADF	2.0	5.9	10.4	22.7	41.3	-
Cellulose[2]	1.7	5.4	8.9	21.0	39.9	-
ADL	0.3	0.5	1.5	1.7	1.4	-
Water holding capacity, g/g	1.9	4.4	3.0	6.5	5.5	17.3

[1]SBM = soybean meal, DDGS = distillers dried grains with solubles, SBH = soybean hulls, SBP = sugar beet pulp.

[2]Soluble dietary fiber was calculated as the difference between total dietary fiber and insoluble dietary fiber; hemicelluloses were calculated as the difference between NDF and ADF, and cellulose was calculated as the difference between ADF and ADL.

Table 5. 2. Composition of experimental diets, as-fed basis

	Diet[1]				
Item	Control	DDGS	SBH	SBP	Pectin
Ingredient composition					
Ground corn, %	76.7	53.7	53.7	53.7	65.2
Soybean meal, 48%	18.0	12.6	12.6	12.6	15.3
Distillers dried grains with solubles, %	-	30.0	-	-	-
Soybean hulls, %	-	-	30.0	-	-
Sugar beet pulp, %	-	-	-	30.0	-
Pectin, %	-	-	-	-	15.0
Soybean oil, %	2.0	1.4	1.4	1.4	1.7
Ground limestone, %	1.5	1.1	1.1	1.1	1.3
Monocalcium phosphate, %	0.90	0.60	0.60	0.60	0.80
Titanium dioxide[2], %	0.50	0.35	0.35	0.35	0.43
Vitamin and micromineral premix[3]	0.40	0.30	0.20	0.30	0.30
Total	100.0	100.0	100.0	100.0	100.0
Analyzed composition					
GE, kcal/kg	3,904	4,303	3,874	3,926	3,813
DM	87.8	89.5	88.4	89.5	88.6
CP	14.9	18.7	13.9	12.5	14.7
Acid hydrolyzed fat	5.3	8.7	4.1	3.8	4.0
Ash	5.4	5.0	5.0	4.9	4.7

Table 5.2. (cont.)

	Diet				
Item	Control	DDGS	SBH	SBP	Pectin
Total dietary fiber	10.5	16.0	27.1	27.9	18.8
Soluble dietary fiber[4]	0	2.6	1.8	3.8	9.7
Insoluble dietary fiber	10.5	13.4	25.3	24.1	8.5
Water binding capacity, g/g	2.2	2.3	3.2	4.0	3.4

[1]DDGS = distillers dried grains with solubles, SBH = soybean hulls; SBP = sugar beet pulp.

[2]Chicago Sweeteners, Chicago, IL.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4] Soluble dietary fiber was calculated as the difference between total dietary fiber and insoluble dietary fiber; hemicelluloses were calculated as the difference between NDF and ADF, and cellulose was calculated as the difference between ADF and ADL.

Table 5.3. Apparent ileal digestibility of DM, energy, and nutrients in experimental diets

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Control							
Light Yorkshires	76.7	77.0	78.9	68.2	34.0	80.7	25.8
Heavy Yorkshires	77.1	75.7	76.9	68.6	39.4	81.3	23.8
Meishans	79.0	78.6	80.3	67.4	34.1	83.6	20.4
SEM	1.0	0.9	1.0	2.6	2.5	1.3	6.6
P-value	0.28	0.09	0.10	0.95	0.25	0.30	0.85
Distillers dried grains with solubles							
Light Yorkshires	68.0	69.0	73.2	64.5	31.3	70.4	21.6
Heavy Yorkshires	68.6	70.0	71.8	70.0	27.8	71.4	33.8
Meishans	68.9	70.7	75.9	72.9	27.7	70.2	28.0
SEM	0.9	1.0	1.5	2.9	3.5	1.3	5.3
P-value	0.76	0.50	0.21	0.17	0.70	0.78	0.31
Soybean hulls							
Light Yorkshires	58.3	59.2	66.0[b]	55.5	11.8	60.5	49.3
Heavy Yorkshires	58.2	59.5	66.7[ab]	55.5	18.0	59.7	50.6
Meishans	58.3	59.9	71.0[a]	59.8	10.5	59.4	44.6
SEM	1.6	1.5	1.3	3.1	3.6	1.6	5.6
P-value	0.99	0.94	0.04	0.56	0.32	0.88	0.73

Table 5.3. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Sugar beet pulp							
Light Yorkshires	59.7	62.9	66.9[ab]	62.6	-47.8	65.9	46.1
Heavy Yorkshires	60.4	62.1	64.3[b]	61.7	-38.1	66.9	44.1
Meishans	61.7	64.4	68.8[a]	67.6	-54.8	68.5	37.4
SEM	1.7	1.6	1.2	3.6	9.1	1.8	5.4
P-value	0.68	0.60	0.02	0.47	0.45	0.62	0.52
Pectin							
Light Yorkshires	65.7	67.8	71.3	53.0	-14.5	71.5	26.0
Heavy Yorkshires	66.8	68.5	72.0	57.0	-6.6	72.0	28.2
Meishans	67.7	69.2	74.9	66.1	-18.2	73.5	29.8
SEM	0.85	0.9	1.5	4.8	3.2	0.9	4.8
P-value	0.11	0.28	0.06	0.16	0.06	0.31	0.71

Table 5.4. Apparent total tract digestibility of DM, energy, and nutrients in experimental diets

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Control							
Light Yorkshires	86.6[b]	86.4[b]	85.2	63.1	47.6	92.4[b]	54.4
Heavy Yorkshires	87.0[ab]	86.6[b]	85.0	62.1	50.9	93.0[b]	52.0
Meishans	89.2[a]	89.5[a]	87.4	64.9	47.2	95.5[a]	58.0
SEM	0.6	0.8	1.1	2.0	1.9	0.4	1.8
P-value	0.02	0.03	0.24	0.62	0.36	< 0.01	0.11
Distillers dried grains with solubles							
Light Yorkshires	78.1[b]	77.3[b]	76.9[b]	57.1	45.6	84.5[b]	45.8[c]
Heavy Yorkshires	79.3[b]	78.8[b]	78.4[ab]	62.8	48.3	84.9[b]	55.3[b]
Meishans	84.0[a]	83.5[a]	83.7[a]	63.8	47.2	90.4[a]	66.5[a]
SEM	1.0	1.0	1.1	2.4	3.2	1.0	2.2
P-value	< 0.01	< 0.01	< 0.01	0.15	0.24	< 0.01	< 0.01
Soybean hulls							
Light Yorkshires	81.3	79.5	70.7	43.3	49.8	87.9	78.6
Heavy Yorkshires	82.2	80.1	71.3	42.5	53.2	88.7	77.7
Meishans	85.4	84.1	76.2	45.7	52.0	92.0	84.1
SEM	1.7	1.8	2.0	4.5	1.3	2.0	2.6
P-value	0.25	0.19	0.13	0.88	0.23	0.36	0.20

Table 5.4. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Sugar beet pulp							
Light Yorkshires	78.1	83.3	73.5	45.0	38.8	91.0	78.1
Heavy Yorkshires	75.6	81.4	70.1	40.3	39.6	91.1	76.8
Meishans	79.3	84.7	76.7	44.0	31.7	92.4	79.3
SEM	2.0	0.9	2.2	1.6	3.6	0.5	1.9
P-value	0.43	0.08	0.15	0.12	0.27	0.12	0.66
Pectins							
Light Yorkshires	85.7	84.3	81.0	52.8	41.8	92.3	72.9
Heavy Yorkshires	86.5	85.4	81.0	53.9	44.4	93.1	73.5
Meishans	87.6	86.7	84.0	52.4	41.2	94.3	71.6
SEM	1.0	1.1	1.7	4.7	4.8	0.7	3.3
P-value	0.40	0.38	0.39	0.97	0.88	0.14	0.92

Table 5.5. Hindgut fermentation of DM, energy, and nutrients in experimental diets

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Control							
Light Yorkshires	9.9	9.5	6.3	-5.2	13.6	11.8	28.7
Heavy Yorkshires	10.0	10.9	8.0	-6.5	11.5	11.7	28.2
Meishans	10.3	10.7	7.1	-2.6	13.1	12.0	37.5
SEM	1.2	1.2	1.6	3.0	3.0	1.3	6.6
P-value	1.2	0.66	0.75	0.66	0.87	0.99	0.54
Distillers dried grains with solubles							
Light Yorkshires	10.1[b]	8.3	3.7	-7.4	14.2[a]	14.0[b]	24.1
Heavy Yorkshires	10.7[b]	9.3	6.7	-6.9	20.5[a]	13.5[b]	21.5
Meishans	15.1[a]	12.9	7.9	-9.1	19.5[a]	20.2[a]	38.5
SEM	0.9	1.3	2.0	2.4	2.8	1.2	6.4
P-value	< 0.01	0.06	0.35	0.78	0.27	< 0.01	0.17
Soybean hulls							
Light Yorkshires	23.0	20.3	4.7	-12.2	38.0	27.4	29.2
Heavy Yorkshires	24.0	20.7	4.6	-13.1	35.2	29.0	27.0
Meishans	27.1	24.2	5.2	-14.1	41.4	32.5	39.5
SEM	2.2	2.4	2.3	5.2	3.0	2.3	6.4
P-value	0.41	0.48	0.98	0.97	0.37	0.31	0.37

Table 5.5. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Sugar beet pulp							
Light Yorkshires	24.3	20.4	6.6	-17.7	86.6	25.0	32.0
Heavy Yorkshires	23.3	20.2	8.5	-21.5	77.7	24.2	32.8
Meishans	23.4	20.3	7.9	-23.6	86.5	23.9	41.9
SEM	2.0	1.9	2.5	4.5	9.6	2.0	6.1
P-value	0.92	1.00	0.86	0.65	0.75	0.91	0.46
Pectins							
Light Yorkshires	20.0	16.5	9.7	-0.25	56.3	20.8	46.9
Heavy Yorkshires	19.7	16.9	8.9	-3.1	50.8	21.0	45.2
Meishans	19.2	16.7	7.4	-14.3	59.5	20.8	39.9
SEM	1.2	1.2	2.3	6.5	5.5	1.0	4.6
P-value	0.89	0.97	0.77	0.30	0.55	0.98	0.55

Table 5.6. Apparent ileal digestibility of DM, energy, and nutrients in ingredients

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Distillers dried grains with solubles							
Light Yorkshires	45.7	54.0	66.9	62.4	17.2	26.4	20.4
Heavy Yorkshires	47.6	55.8	64.0	70.6	2.3	31.1	41.7
Meishans	48.6	58.9	72.6	75.6	2.0	25.1	31.4
SEM	2.8	2.9	4.5	4.6	11.4	6.2	9.3
P-value	0.76	0.50	0.39	0.17	0.47	0.78	0.31
Soybean hulls							
Light Yorkshires	13.3	18.1	8.5	-14.3	-31.5	16.9	72.2
Heavy Yorkshires	12.8	19.0	12.3	-14.3	-14.1	14.3	74.7
Meishans	13.4	20.5	36.0	13.5	-35.1	13.5	63.3
SEM	5.2	5.0	7.0	20.5	10.0	4.9	10.6
P-value	0.99	0.94	0.40	0.56	0.32	0.88	0.73
Sugar beet pulp							
Light Yorkshires	17.9	29.7	33.1[a]	27.5	-293.9	31.8	55.2
Heavy Yorkshires	20.3	27.0	22.7[b]	20.7	-255.5	34.3	52.4
Meishans	24.7	34.5	37.9[a]	64.6	-321.6	40.3	43.1
SEM	5.5	5.2	3.7	26.4	35.8	5.9	7.6
P-value	0.68	0.60	0.04	0.47	0.45	0.59	0.52

Table 5.6. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Pectins							
Light Yorkshires	-1.9	9.3	16.7	-327.4	-506.9	20.3	28.3
Heavy Yorkshires	5.8	14.3	23.1	-224.1	-419.5	23.5	32.5
Meishans	16.5	24.7	60.7	31.5	-546.7	32.3	38.9
SEM	5.7	6.6	12.4	126.6	33.9	5.5	9.0
P-value	0.11	0.28	0.06	0.16	0.06	0.31	0.71

Table 5.7. Apparent total tract digestibility of DM, energy, and nutrients in ingredients

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Distillers dried grains with solubles							
Light Yorkshires	55.7[b]	58.5[b]	66.7[b]	53.6	36.2	49.2[b]	39.0[c]
Heavy Yorkshires	59.8[b]	62.9[b]	70.0[b]	62.5	47.2	51.1[b]	55.7[b]
Meishans	75.4[a]	76.3[a]	81.3[a]	64.0	42.8	78.0[a]	75.3[a]
SEM	3.3	2.9	2.4	3.8	8.3	0.1	2.0
P-value	< 0.01	< 0.01	< 0.01	0.15	0.65	< 0.01	< 0.01
Soybean hulls							
Light Yorkshires	-2.8	61.1	2.1	-68.3	52.1	-2.3	99.5
Heavy Yorkshires	-13.6	63.2	5.4	-73.9	61.4	-12.2	97.8
Meishans	-10.8	76.4	33.0	52.9	58.1	-9.9	109.9
SEM	8.12	5.9	10.8	29.7	3.7	8.7	4.8
P-value	0.25	0.19	0.13	0.88	0.23	0.36	0.20
Sugar beet pulp							
Light Yorkshires	17.9	29.7	10.4[b]	27.5	-293.9	31.8	55.2
Heavy Yorkshires	20.3	27.0	-4.9[a]	20.7	-255.5	34.3	52.4
Meishans	24.7	34.5	-15.2[a]	64.6	-321.6	40.3	43.1
SEM	5.5	5.2	3.7	26.4	35.8	5.9	7.6
P-value	0.68	0.60	0.04	0.47	0.45	0.59	0.52

Table 5.7. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Pectins							
Light Yorkshires	74.7	64.2	45.3	-215.1	-24.9	85.6	88.5
Heavy Yorkshires	80.0	72.2	45.2	-185.7	5.6	90.3	89.6
Meishans	87.5	81.1	70.0	-225.4	34.4	97.6	86.1
SEM	6.5	8.3	14.1	124.3	73.0	4.0	6.1
P-value	0.41	0.38	0.39	0.97	0.88	0.14	0.92

Table 5.8. Hindgut fermentation of DM, energy, and nutrients in experimental feed ingredients

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Distillers dried grains with solubles							
Light Yorkshires	16.8	19.5	9.0	-0.3	25.7	13.9	38.1
Heavy Yorkshires	14.2	9.2	7.3	-3.9	28.9	13.5	22.9
Meishans	21.0	16.6	7.8	-6.8	12.5	18.3	37.5
SEM	14.0	10.3	3.68	3.0	10.5	5.1	11.4
P-value	0.94	0.77	0.95	0.35	0.53	0.76	0.58
Soybean hulls							
Light Yorkshires	53.3	43.0	-6.4	-54.0	83.7	59.4	27.3
Heavy Yorkshires	56.5	44.2	-6.9	-59.6	75.5	64.3	23.1
Meishans	66.8	55.9	-3.3	-66.4	93.2	75.1	46.6
SEM	7.2	8.0	12.4	34.2	8.5	7.1	12.0
P-value	0.41	0.48	0.98	0.97	0.37	0.31	0.37
Sugar beet pulp							
Light Yorkshires	57.6	43.8	5.0	28.3	304.1	53.4	32.3
Heavy Yorkshires	54.3	43.3	8.2	44.1	268.9	51.5	33.3
Meishans	54.5	43.7	12.4	15.8	303.7	49.3	48.3
SEM	6.5	6.3	8.7	33.3	37.8	6.4	7.8
P-value	0.92	1.0	0.84	0.65	0.75	0.90	0.30

Table 5.8. (cont.)

Item	DM	GE	CP	AEE	Ash	CHO	TDF
Pectins							
Light Yorkshires	76.6	54.9	28.6	112.3	482.0	65.3	60.2
Heavy Yorkshires	74.1	57.9	22.1	38.4	423.4	66.9	57.1
Meishans	71.1	56.5	9.3	-256.9	516.2	65.2	47.2
SEM	8.2	8.8	18.9	169.6	58.8	5.8	8.5
P-value	0.89	0.97	0.77	0.30	0.55	0.98	0.55

CHAPTER 6

Evaluation of *in vitro* procedures to measure digestibility of fiber in distillers dried grains with solubles

ABSTRACT: Four experiments were conducted to develop and evaluate an assay for measuring *in vitro* digestibility of dietary fiber in distillers dried grains with solubles (DDGS). Exp. 1 was conducted to validate the 3 step *in vitro* digestibility assay in our laboratory. *In vitro* apparent ileal digestibility (AID) and *in vitro* apparent total tract (ATTD) digestibility of OM in 4 diets and corn (83.7 and 93.1%) were not different from values analyzed at a reference laboratory (82.4 and 92.4%) indicating that we were able to repeat the assay. Exp. 2 was conducted with the objective of increasing the amount of sample that was used for the *in vitro* digestibility assay from 0.5 g to 2.0 or 4.0 g. Results of this experiment showed that *in vitro* ATTD of DM was not different among the 3 sample sizes (85.1, 83.7, 83.3% for 0.5, 2.0, and 4.0 g, respectively). Exp. 3 was conducted to measure *in vitro* AID and ATTD of DM and NDF in DDGS from the production of ethanol ($DDGS_{ethanol}$), DDGS from beverage ($DDGS_{beverage}$), and distillers dried grains (DDG). There were differences ($P < 0.01$) in the AID of DM (45.2 to 56.7%) among sources of $DDGS_{ethanol}$, and the AID of DM in $DDGS_{ethanol}$ was greater ($P < 0.01$) than values for $DDGS_{beverage}$ (37.7%). Likewise, the ATTD of DM (48.5 to 62.4%) and NDF (32.5 to 52.2%) were different ($P < 0.01$) among sources of $DDGS_{ethanol}$, but all values were greater ($P < 0.01$) than in $DDGS_{beverage}$ (44.4 and 24.8%). In DDG, the AID and ATTD of DM (30.1 and 42.5%) and NDF (-19.2 and 17.5%) were less ($P < 0.01$) than in any source of $DDGS_{ethanol}$. The objective of Exp. 4, was to measure *in vitro* AID and ATTD of DM and NDF in 10 sources of DDGS using purified enzymes or fecal inoculum. The DDGS samples were produced from corn

(n = 8; C-DDGS), 1 source of DDGS produced from sorghum (S-DDGS) and 1 source of DDGS produced from a combination of sorghum and corn (SC-DDGS). The AID of DM (41.2 to 55.5%) was different ($P < 0.01$) among sources of DDGS. The ATTD of DM (47.0 to 58.7%) and NDF (31.4 to 39.9%) were also different ($P < 0.01$) among sources of C-DDGS. Values for hindgut disappearance of DM and NDF obtained after fecal inoculation (23.0 and 54.3%) were greater ($P < 0.05$) than values obtained using purified enzymes (6.3 and 5.6%). In conclusion modifications to the 3 step *in vitro* digestibility assay allowed us to measure the *in vitro* AID and ATTD of DM and NDF in DDGS, but results obtained with the fecal inoculum indicate that the procedure needs to be further improved.

Key words: digestibility, enzymes, inoculum, in vitro assay, NDF, pig

INTRODUCTION

The *in vivo* apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of energy, ADF, NDF, and total dietary fiber (**TDF**) in corn distillers dried grains with solubles (**DDGS**) has been reported (Guo et al., 2004; Stein et al., 2009; Urriola et al., 2010). Some of the energy that pigs obtain from DDGS is from fermentation of TDF, but on average, only 46% of TDF disappears in the gastro intestinal tract of the pig (Urriola et al., 2010). However, there are differences among sources of DDGS in the AID and ATTD of fiber and it is, therefore, necessary to measure the digestibility of fiber in different sources of DDGS.

Procedures that measure *in vitro* digestibility were developed to overcome shortcomings of measuring *in vivo* digestibility of DM and energy (Boisen and Fernandez, 1997). However, at this point, no assay that measures *in vitro* AID or ATTD of fiber in monogastric animals have been identified, but it is possible that assays that were developed for measuring *in vitro*

digestibility of OM can be modified to measure *in vitro* digestibility of fiber. Analysis for NDF is less expensive and faster than analysis for TDF and the ATTD of TDF in DDGS is closely correlated with the ATTD of NDF (Urriola et al., 2010). *In vitro* digestibility of NDF may, therefore, be used to predict *in vivo* ATTD of TDF. However, this hypothesis has not been tested and the objective of the present work was to measure *in vitro* digestibility of NDF.

The 3 step *in vitro* digestibility assay that was described by Boisen and Fernandez (1997) used purified enzymes instead of microbial inoculum, because purified enzymes are more convenient to use (Coles et al., 2005). However, microbial inoculum contains a diversity of microbes that may be more efficient in fermenting fiber than purified enzymes (Moughan, 1999). The second objective, therefore, was to compare values for *in vitro* digestibility of fiber by growing pigs using fecal inoculum and purified enzymes.

MATERIALS AND METHODS

Exp. 1. Digestibility of OM

The objective of Exp. 1 was to measure *in vitro* AID and ATTD of OM in 4 samples of corn-soybean meal diets and in one sample of corn using the 3 step *in vitro* digestibility assay described by Boisen and Fernandez (1997) and to compare values measured at the Univ. of Illinois to values measured at a reference laboratory (Eurofins Steins Laboratorium, Hjaltesvej 8, 7500, Holstebro, Denmark) that routinely measured *in vitro* digestibility of OM in ingredients and diets.

The 5 samples consisted of ground corn, a basal diet containing corn and soybean meal, a high lipid basal diet containing corn, soybean meal, and 8% corn oil, a diet that was formulated

with 25% corn and 75% of the basal diet, and a diet that was formulated with 25% corn and 75 % of the high lipid diet (Kil, 2008).

The *in vitro* assay of Boisen and Fernandez (1997) has 3 steps that aim at mimicking the digestibility in the stomach, small intestine, and large intestine, respectively. All samples were ground in a Wiley Mill Model 4 (Thomas Scientific; Swedesboro, NJ) to pass through a 5 mm screen. Four replicates of each sample (0.5g ± 0.001) were then weighed into four 125 mL conical flasks. Twenty five mL of phosphate buffer (0.1 *M*; pH 6.0) was added to each flask and stirred continuously using a magnetic stirrer. Ten mL of 0.2 *M* HCl was added and the final pH was adjusted to 2 with 1 *M* HCl or 1 *M* NaOH. One mL of freshly prepared pepsin solution that contained 25 mg of pepsin (P7000, Sigma Aldrich, St. Louis, MO) was added to each flask and samples were placed in a shaking water bath (Thermo Fisher Scientific, Rochester, NY) for 2 h at 39°C.

In the second step, a combination of 10 mL of phosphate buffer (0.2 *M*, pH 6.8) and 5 mL of NaOH (0.6 *M*) was added to each flask and the pH was adjusted to 6.8 using 1 *M* HCl or 1 *M* NaOH. A solution with 100 mg of pancreatin (P1790, Sigma Aldrich) was added to each flask and incubated in a shaking water bath at 39°C. After incubation for 4 h, the residues of 2 flasks were filtered into coarse Gooch crucibles (d:3 cm; pore size: 40-90 μ; Thermo Fisher Scientific) containing 0.4 g of celite 545 as filtration aid (Sigma Aldrich).

The other 2 flasks of each sample were used in the third step. Ten mL of 0.2 *M* EDTA solution was added to each flask and pH was adjusted to 4.8 using 30% acetic acid. The slurry was mixed with Viscozyme L (V2010, Sigma-Aldrich) and placed in a shaking water bath for 18 h at 39°C. The undigested residue in the flasks were collected in Gooch crucibles with celite 545 (Sigma Aldrich) at the end of the 18 h incubation period. The undigested material collected in

the crucibles was washed with 10 mL of ethanol (96%) and acetone (99.5%). All samples were ashed at 600°C for 2 h and the weight was recorded to measure ash concentration in the residues (method 930.15; AOAC Int., 2007). Four extra flasks had no sample, but all reagents and enzymes were added to these flasks. The OM of the residue collected in these flask after incubation was used to correct the final weight (Blank OM).

Calculations and Statistical Analyses. The *in vitro* AID and ATTD of OM was calculated using equation [1]:

$$\text{AID or ATTD of OM} = \left(\frac{[(\text{Sample OM}) - (\text{Residue OM-Blank OM})]}{(\text{Sample OM})}\right) \times 100 \quad [1]$$

Five diets were analyzed in this experiment and that number was calculated using the freeware program PIFACE (Lenth, 2006; Lenth, 2007). The desired power was 0.8, an alpha 0.05, and σ of 1.0. The value for σ was calculated from the SEM values reported in previous experiments (Noblet and Jaguelin-Peyraud , 2007; Regmi et al., 2009).

The UNIVARIATE procedure of SAS (SAS Inst, Inc., Cary, NC) was used to determine normal distribution of the data, equal variances, and to identify outliers. An observation was considered an outlier if the value was more than 3 SD away from the mean of the sample, but no outlier was identified using this method. Only 1 value for each diet was available from the analysis at the reference laboratory. Therefore, only the mean values are compared among laboratories. *In vitro* AID and ATTD values were analyzed as one way ANOVA using the Proc GLM procedure of SAS (SAS Institute, Inc., Cary, NC). The differences were considered significant if $P < 0.05$ and a trend if $P > 0.05 < 0.10$.

Exp. 2. Evaluation of Sample Initial Weight and Filtration Method

The weight of the residue that is collected after the 3 steps in the original *in vitro* assay by Boisen and Fernandez (1997) is too small for analysis of NDF. Therefore, to generate enough

residue to measure NDF, it was necessary to either incubate multiple subsamples of each diet or increase the amount of sample that was used in the assay. The objective of this experiment, therefore, was to compare *in vitro* ATTD of DM for 0.5, 2.0, or 4.0 g of sample. The second objective was to compare *in vitro* ATTD of DM of samples that were filtered in Gooch crucibles or in filter paper.

The sample used for this experiment was a corn-soybean meal diet that contained 30% DDGS. In step 1, 30 subsamples, were divided into 3 weight groups with 10 subsamples containing 0.5 g, 10 subsamples containing 2.0 g and 10 subsamples containing 4.0 g. The concentrations of buffers, acid, sodium hydroxide, EDTA, and enzymes were 4 and 8 times greater for the samples containing 2.0 and 4.0 g, respectively than the samples containing 0.5 g (Bindelle et al., 2007). Therefore, the analyses were performed in 500-mL plastic bottles (Thermo Fisher Scientific). Five of the 10 samples for each weight group were filtered using Gooch crucibles and the other 5 were filtered using hardened quantitative ash free filter paper (Grade 541, Whatman Inc. Piscataway, NJ). The weight of each filter paper was recorded before filtration. The paper was dried at 105°C for 2 h, transferred to a dessicator, and the weight was recorded to the nearest 0.1 mg. Three blank samples were also used to correct for the weight of reagents and enzymes. Filter papers and Gooch crucibles containing the residues were dried at 105°C until they reached a constant weight.

Calculations and Statistical Analyses. The weights of papers and Gooch crucibles were recorded and used to calculate *in vitro* AID and ATTD using Eq. [1]. Outliers and normal distribution of data were detected as described for Exp. 1. Data for IVDMD were analyzed by one way ANOVA using the proc GLM procedure of SAS to calculate the effects of initial weight and filtration method and the interaction of initial weight × filtration method was also introduced

in the initial model. However, the interaction was not significant and, therefore, removed from the final model. The differences were considered significant if $P < 0.05$ and a trend if $P > 0.05 < 0.10$.

Exp 3. In vitro Ileal and Total Tract Digestibility of NDF in DDGS from Ethanol or Beverage Production and in Distillers Dried Grains

The objective of this experiment was to measure *in vitro* AID and ATTD of DM and NDF in DDGS from ethanol production (**$DDGS_{ethanol}$**), DDGS from beverage production (**$DDGS_{beverage}$**), and in distillers dried grains (**DDG**). Six sources of $DDGS_{ethanol}$, 1 source of $DDGS_{beverage}$, and 1 source of DDG were obtained from the experiment by Pahm et al. (2008).

Samples were analyzed for *in vitro* AID and ATTD of DM and NDF following the modifications to the 3 step *in vitro* digestibility assay that were validated in Exp 2. Six replicates of 4 g of each sample were incubated for 2 h in pepsin and 4 h in pancreatin. Three replicates of each sample were filtered in Gooch crucibles and dried at 105°C. These replicates represented *in vitro* AID and the other 3 replicates of each sample were incubated for 18 h with Viscozyme. These replicates represented *in vitro* ATTD. Samples were analyzed for NDF after correction for ash (Holst, 1973) at the Agricultural Experimental Station Chemical Laboratory (University of Missouri, Colombia, MO). Six bottles had no sample (blank), but all reagents and enzymes were added to these bottles. Three of these bottles were incubated with pepsin and pancreatin, filtered, and dried and the final weight was subtracted from the weight of the residue plus filter paper obtained for the bottles that contained DDGS or DDG for measurement of *in vitro* AID. The other 3 bottles were also incubated with viscozyme and the weights of those samples were used to correct the weight of the residue and filter paper in the bottles that contained DDGS and DDG for measurement of *in vitro* ATTD.

Calculations and Statistical Analyses. The concentration of NDF in 3 samples of celite was also analyzed to correct the final *in vitro* AID and ATTD of NDF.

$$AID\ or\ ATTD\ = \frac{\left[\left(\frac{Sample_{NDF}}{100}\right) \times Sample_{wt}\right] - \left[\left(\frac{Residue_{NDF}}{100}\right) \times (Residue_{wt} - Blank_{wt})\right]}{\left[\left(\frac{Sample_{NDF}}{100}\right) \times Sample_{wt}\right]}$$

The Proc UNIVARIATE procedure of SAS was used to identify outliers. *In vitro* AID and ATTD data were analyzed by one way ANOVA using the Proc GLM procedure of SAS. Least squares means were calculated and the contrast option was used to compare digestibility values for $DDGS_{ethanol}$ and $DDGS_{beverage}$ and to compare the digestibility values for $DDGS_{ethanol}$ and DDG. The differences were considered significant if $P < 0.05$ and a trend if $P > 0.05 < 0.10$.

Exp 4. In Vitro Digestibility of DM and NDF in DDGS using purified enzymes or fecal inoculum

The objective of this experiment was to measure *in vitro* ATTD of DM and NDF in DDGS using either purified enzymes or fecal inoculums. The *in vitro* AID and ATTD of TDF was measured only on samples that were incubated with purified enzymes. Ten DDGS samples were obtained from the experiment by Urriola et al. (2009). The 10 DDGS sources used in this experiment consisted of 1 source of DDGS that was produced from sorghum (S-DDGS), 8 sources of DDGS that were produced from corn (C-DDGS), and in 1 source of DDGS that was produced from a blend of corn and sorghum DDGS (SC-DDGS).

Six samples of 4 g of each of the 10 sources of DDGS were added to a 500-mL bottle and incubated with pepsin for 2 h and with pancreatin for 4 h. After incubation, 20 bottles were incubated with Viscozyme for 18 h and the residues were collected in filter paper and dried to a constant weight at 105°C. These values were used to calculate *in vitro* AID and ATTD of DM

and NDF using purified enzymes following modifications to the 3 step *in vitro* digestibility assay that was used in Exp. 2 and 3.

The residue of the other 40 bottles was collected in filter paper, dried to a constant weight, and pooled within source of DDGS and used for *in vitro* ATTD using fecal inoculum. One gram of substrate from the pooled residues was added to thirty 100-mL serum bottles (Wheaton Industries, Inc., Millville, NJ) for the fecal inoculums. Three replicates of 1 g of Pectin (TIC Gums, Belcamp, MD) and Solka-floc (International Fiber Corporation, St. Louis, MO) were added to 6 serum bottles and another 3 bottles had no substrate and were used as blank. Bottles were placed overnight in a chamber filled with CO_2 (Coy Laboratory Products, Grass Lake, MI) to ensure that all O_2 was removed from the substrate and the head space of the bottles.

Fecal inoculum was prepared by pooling feces from 3 growing pigs that were consuming a corn-soybean meal diet. Pigs had not been fed diets containing antibiotic growth promoters for 10 wk before the start of the experiment. Fecal samples were collected within 30 min of the preparation of the inoculum by grab sampling of fresh feces and samples were immediately placed in plastic bags that were sealed after all air was excluded. Fecal samples were kept under anaerobic conditions under a stream of CO_2 that was reduced in a heated cupper column. Equal parts of each fecal sample were pooled and diluted 1:10 (wt/vol) in a warmed (39°C) anaerobic diluting solution (Bryant and Burkey, 1953). Feces and diluting solution were blended in a 500-mL blender (Waring Laboratory Products; Torrington, CT) in an anaerobic chamber. The mixture was then filtered in 4 layers of cheesecloth and transferred to CO_2 purged 100-mL serum bottles that were sealed. Bottles with fecal inoculum were removed from the anaerobic chamber and kept in a water bath at 39°C until used for inoculation.

An enriched media was prepared using the procedure by Bourquin et al. (1993). All components of the enriched media, except vitamins and VFA solutions, were mixed and autoclaved. The vitamins and VFA solutions were added to the autoclaved media by injection and sterilized with syringe 22 µm filters (Thermo Fisher Scientific). Fifty two milliliters of media were dispensed to each bottle containing the residue from the enzymatic incubation inside the anaerobic chamber. The bottles were sealed and removed from the anaerobic chamber and were injected with 8 mL of fecal inoculum and allowed to ferment for 18 h at 39°C in a shaking water bath. Samples were then quantitatively transferred to 500-mL bottles, mixed with 240 mL of 95% ethanol and allowed to precipitate. Samples were filtered through Whatman 541 paper and washed sequentially with 78% ethanol, 95% ethanol, and acetone. The residues recovered in the filter papers were dried at 105°C to a constant weight.

Calculations and Statistical Analyses. The disappearances of DM and NDF after *in vitro* fermentation were calculated using Eq. 1 and data were analyzed by one way ANOVA using the Proc GLM procedure of SAS. The contrast option of the GLM procedure was used to compare the digestibility values for S-DDGS and C-DDGS and to compare the digestibility values for SC-DDGS and C-DDGS. The values for ATTD of DM and NDF using purified enzymes and values for ATTD of DM and NDF using fecal inoculum were compared using a t-test. The Proc UNIVARIATE procedure of SAS was used to identify the normal distribution of residuals and outliers in the final model. The differences were considered significant if $P < 0.05$ and a trend if $P > 0.05 < 0.10$.

RESULTS

Exp. 1. Digestibility of OM

The average AID of OM in the 5 complete diets that were analyzed at the reference laboratory (82.4%) was not different from the value analyzed in the laboratory at the University of Illinois (83.7%; Table 6.1). The average ATTD of OM in the 5 complete diets (92.4%) was also not different from the values analyzed in our laboratory (93.1%). There was, however, greater variation in *in vitro* AID (SEM = 1.5) than *in vitro* ATTD (SEM = 0.6).

Exp. 2. Evaluation of Sample Initial Weight and Filtration Method

In vitro ATTD of DM was not different when 0.5 g of sample was used (85.1%) than if 2.0 g (83.7%) or 4.0 g (83.3%) were used (Table 6.2). The *in vitro* ATTD of DM that was measured using the Gooch crucibles (84.2%) was not different from the value that was observed when the residue was filtered in filter paper (83.9%). There was no interaction between filtration method and the initial amount of sample.

Exp 3. In vitro Ileal and Total Tract Digestibility of NDF in DDGS from Ethanol or Beverage Production and in Distillers Dried Grains

The concentration of NDF among sources of $DDGS_{ethanol}$ varied from 39.2 to 56.5%, and the concentration of NDF in $DDGS_{beverage}$ (43.2%) was within this range (Table 6.3). The concentration of NDF in DDG (39.0%) was close to the lowest value for $DDGS_{ethanol}$. The *in vitro* AID of DM (45.2 to 56.7%) and NDF (21.9 to 40.4%) were different ($P < 0.01$) among sources of $DDGS_{ethanol}$. Likewise, the ATTD of DM (48.5 to 62.4%) and NDF (32.5 to 52.2%) were different ($P < 0.01$) among the 6 sources of $DDGS_{ethanol}$. These variations among sources of $DDGS_{ethanol}$ were also observed in the calculation of hindgut disappearance of DM (2.9 to 5.9%) and NDF (7.4 to 16.3%). The *in vitro* AID of DM (37.7%) and NDF (12.3%) were less ($P <$

0.01) for $DDGS_{beverage}$ than the mean for $DDGS_{ethanol}$ (49.3 and 27.0%, respectively). Likewise, the ATTD of DM and NDF (44.4 and 24.8%) were less ($P < 0.01$) in $DDGS_{beverage}$ than the mean for $DDGS_{ethanol}$ (53.8 and 39.9%). The hindgut disappearance of DM and NDF were not different in $DDGS_{beverage}$ and $DDGS_{ethanol}$.

The *in vitro* AID and ATTD of DM in DDG (30.1 and 42.5%) were less ($P < 0.01$) than the mean AID and ATTD for $DDGS_{ethanol}$. Likewise, the AID and ATTD of NDF in DDG (-19.2 and 17.5%) were less ($P < 0.01$) than in any source of $DDGS_{ethanol}$. However, hindgut disappearance of DM and NDF in DDG (12.4 and 36.7%) were greater ($P < 0.01$) than in all sources of $DDGS_{ethanol}$.

Exp 4. In Vitro Digestibility of DM and NDF in DDGS using Enzymes or Inoculum

In vitro digestibility after incubation with enzymes. The *in vitro* AID of DM (41.2 to 55.5%), NDF (25.3 to 34.5%), and TDF (24.3 to 29.5%) were different ($P < 0.01$) among sources of C-DDGS (Table 6.4). Likewise, the ATTD of DM (47.0 to 58.7%), NDF (31.4 to 39.9%), and TDF (32.0 to 38.4%) were different ($P < 0.01$) among sources of C-DDGS. The *in vitro* AID of DM (34.8%) and NDF (23.3%) were less ($P < 0.01$) in S-DDGS compared with the mean for C-DDGS (46.4 and 29.9%, respectively), but it was not different for *in vitro* AID of TDF. The *in vitro* ATTD of DM (41.1%), NDF (29.0%), and TDF (29.8%) were less ($P < 0.01$) in S-DDGS than the mean for C-DDGS (57.2, 35.0, and 34.8; %, respectively).

The *in vitro* AID and ATTD of DM in SC-DDGS (39.5 and 47.4%) were less ($P < 0.01$) than the mean value in C- DDGS and they were not different for TDF. The *in vitro* AID and ATTD of NDF in SC-DDGS (27.8 and 33.7%) were less ($P < 0.01$) than in any source of C-DDGS. However, hindgut disappearance of DM and NDF in SC-DDGS were not different from that of C-DDGS.

In vitro digestibility after incubation with inoculum. The ATTD of DM using fecal inoculum (67.4 to 78.6%) was different ($P < 0.01$) among sources of C-DDGS. The ATTD of NDF was also different ($P < 0.01$) among sources of C-DDGS. The ATTD of DM was less ($P < 0.01$) in S-DDGS (55.7%) than in C-DDGS, but there were no differences in ATTD of NDF. The ATTD of DM in SC-DDGS (62.5%) was less ($P < 0.01$) than in C-DDGS, but the ATTD of NDF (82.1%) was greater ($P < 0.01$) than in C-DDGS.

The *in vitro* hindgut disappearance of DM varied among sources of C-DDGS from 21.1 to 32.6%. The mean *in vitro* hindgut disappearance of DM in C-DDGS was greater ($P < 0.05$) than values for S-DDGS (21.3%), but not different from the value for SC-DDGS (23.0%). Values of *in vitro* hindgut disappearance of DM in C-DDGS using fecal inocula (25.3%) were greater ($P < 0.05$) than values that were obtained using purified enzymes (6.3%). The *in vitro* hindgut disappearance of DM of Solka-floc and pectin was 18.3 and 84.8%, respectively. Because disappearance of DM in pectin samples was 84.8% the residue that remained after *in vitro* fermentation was too small for analysis of NDF.

DISCUSSION

The 3 step *in vitro* digestibility assay is used to measure AID and ATTD of OM (Boisen and Fernandez, 1997). Samples are analyzed in a batch system allowing for more consistent results across diets and the consistent results obtained with this procedure allow for routine measurement of ATTD of OM in swine diets (Boisen, 2007). The 3 step *in vitro* digestibility assay was modified to measure *in vitro* digestibility of energy in barley and wheat (Regmi et al., 2008; Regmi et al., 2009), but the assay has not been used to measure *in vitro* digestibility of fiber.

The *in vitro* digestibility of OM is routinely analyzed at the reference laboratory that was used in Exp. 1. Results of the experiment showed that the *in vitro* digestibility of OM that was measured in our laboratory did not differ from the values reported by the reference laboratory. This observation is in agreement with Noblet and Jaguelin-Peyraud (2007) who observed a high repeatability of the procedure. The reason that the 3 step *in vitro* assay is repeatable may be that it is a batch digestibility system, which has the advantage of analyzing multiple samples at the same time reducing the variation among samples (Coles et al., 2005).

One of the disadvantages of the 3-step *in vitro* assay is that the amount of sample left in the residue after the 3 steps is too small for analysis of undigested NDF or TDF. Analysis of NDF requires 0.5 g of sample and analysis of TDF requires 1.0 g of sample (AOAC Int., 2007). It is, therefore, necessary to modify the procedure to obtain a quantity of residue that is large enough for analysis of NDF and TDF.

The fact that there was no difference among 0.5, 2.0, and 4.0 g in the ATTD of DM indicates that a sample size up to at least 4 g can be used in this assay provided that the concentration of reagents and enzymes are kept proportional to the concentration of substrate. This observation makes it possible to generate residue samples that are sufficiently large to analyze NDF. The procedure has also been modified to use 2.0 g of sample for analysis of *in vitro* gas production (Bindelle et al., 2007).

The values for ileal and total tract *in vitro* digestibility varied among sources of DDGS. This observation is in agreement with *in vivo* data from Guo et al. (2004), Stein et al. (2009), and Urriola et al. (2010) suggesting that digestibility of fiber in corn varies among samples or that ethanol production affects the digestibility of fiber. The *in vivo* ATTD of TDF in $DDGS_{beverage}$ was not different from the ATTD in $DDGS_{ethanol}$ (Urriola et al. 2010). However, *in vitro* AID and

ATTD of NDF in $DDGS_{beverage}$ was lower than in $DDGS_{ethanol}$. We do not have an explanation for this discrepancy. There is agreement, however, between *in vivo* and *in vitro* digestibility of fiber in DDG. The *in vivo* AID of NDF is low (0.7%) and the *in vitro* digestibility of NDF is also very low (-19.2%) and in both cases, calculated hindgut disappearance of NDF or TDF in DDG is greater than in $DDGS_{ethanol}$.

The advantage of the batch *in vitro* digestibility assay is that data obtained with this system are more repeatable than data obtained in continuous digestibility assays. However, in continuous digestibility assays, the conditions of pH, concentration of substrate and enzymes may be modified to mimic conditions in the gut that may be affected by fiber such as rate of passage of digesta (Coles et al., 2005; Meunier et al., 2008). In the last step of the procedure, purified enzymes are used in the last step of *in vitro* digestibility of fiber. Purified enzymes are used rather than fecal inoculums for convenience of easy access, availability, and consistency of the products (Coles et al., 2005). Also, a large proportion of fecal microbes require anaerobic environment for proper growth during incubation (White, 2000). Despite these difficulties, we observed that disappearance of DM and NDF were greater when using the fecal inoculums than the values obtained using purified enzymes. Greater disappearance of DM and NDF suggests that inoculums are more effective in degrading dietary fiber than purified enzymes.

In conclusion the 3 step in vitro digestibility assay does not predict in vivo digestibility of dietary fiber and it requires modifications before the procedure can be used to measure fiber digestibility in DDGS. Fecal inoculums are a better option than purified enzymes to study disappearance of DM during *in vitro* hindgut digestibility.

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Table 6.1. *In vitro* total tract digestibility of OM in corn and complete diets, Exp. 1

Item	Ileal digestibility, %	Total tract digestibility, %
Exp. 1, validation of original procedure[1]		
Reference laboratory in Denmark	82.4	92.4
University of Illinois	83.7	93.1
SEM	1.5	0.6
P-value	0.55	0.43

[1]Least squares means of 5 samples.

[2]Least squares means of 5 observations.

Table 6.2. Evaluation of initial sample weight and filtration method, Exp. 2

Item	Total tract digestibility of OM, %
Initial sample Weight	
0.5	85.1
2.0	83.7
4.0	83.3
SEM	2.6
P-value	0.37
Filtration method	
Gooch crucible	84.2
Filter paper	83.9
SEM	2.4
P-value	0.10

[1]Least squares means of 5 samples.

[2]Least squares means of 5 observations. No interaction was observed.

Table 6.3. Concentration of DM and NDF and *in vitro* ileal, total tract, and hindgut disappearance in distillers dried grains with solubles (DDGS)[1]

Item	$DDGS_{ethanol}$ source							$DDGS_{beverage}$	DDG	SEM	*P* – value	*P* – values, contrasts	
	1	2	3	4	5	6	Mean					$DDGS_{beverage}$ vs. $DDGS_{ethanol}$	DDG vs. $DDGS_{ethanol}$
Concentration of nutrients in DDGS, % as-is													
DM	88.9	87.0	88.4	88.7	86.9	87.8	88.0	89.7	88.5	-	-	-	-
NDF	44.2	56.5	42.9	39.8	39.2	45.5	44.7	43.2	39.0	-	-	-	-
In vitro ileal digestibility, %													
DM	45.2	48.9	48.9	50.3	56.7	46.0	49.3	37.7	30.1	1.1	< 0.01	< 0.01	< 0.01
NDF	21.9	40.4	22.2	23.8	31.5	22.4	27.0	12.3	-19.2	1.6	< 0.01	< 0.01	< 0.01
In vitro total tract digestibility, %													
DM	48.5	53.7	54.7	53.9	62.4	49.6	53.8	44.4	42.5	1.5	< 0.01	< 0.01	< 0.01
NDF	32.8	52.2	38.5	38.2	45.2	32.5	39.9	24.8	17.5	1.8	< 0.01	< 0.01	< 0.01

Table 6.3. (cont.)

Item	$DDGS_{ethanol}$ source							$DDGS_{beverage}$	DDG	SEM	*P* – value	*P* – values, contrasts	
	1	2	3	4	5	6	Mean					$DDGS_{beverage}$ vs. $DDGS_{ethanol}$	DDG vs. $DDGS_{ethanol}$
In vitro hindgut disappearance, %													
DM	3.5	4.7	5.9	3.6	5.7	2.9	4.4	6.7	12.4	1.6	0.01	0.22	< 0.01
NDF	11.4	11.8	16.3	14.4	13.7	7.4	12.5	13.4	36.7	1.9	< 0.01	0.95	< 0.01

[1]Least square means of 3 observations per source of DDGS or DDG.

[2] $DDGS_{beverage}$ = DDGS produced at a beverage plant; $DDGS_{ethanol}$ = DDGS produced at biofuels plant.

Table 6.4. *In vitro* ileal, total tract, and hindgut disappearance of DM and NDF in distillers dried grains with solubles (DDGS) measured using purified enzymes or fecal inoculum, Exp. 4[1]

Item	C-DDGS[2]									S-DDGS[2]	SC-DDGS[2]	SEM	*P*-value	Contrasts[3]	
	1	2	3	4	5	6	7	8	Mean					S vs. C DDGS	SC vs. C DDGS
In vitro ileal digestibility, %															
DM	47.6	41.2	44.8	55.5	46.6	43.0	46.0	46.4	46.4	34.8	39.5	0.5	< 0.01	< 0.01	< 0.01
NDF	29.5	29.6	31.7	31.9	25.3	30.3	26.1	34.5	29.9	23.3	27.8	1.3	< 0.01	< 0.01	0.02
TDF	24.3	27.7	25.1	27.9	27.3	29.5	26.9	31.3	27.5	26.7	19.6	1.7	0.27	0.77	0.01
In vitro total tract digestibility after incubation with purified enzymes, %															
DM	53.7	47.0	50.4	58.7	51.9	50.9	55.7	53.2	57.2	41.1	47.4	1.2	< 0.01	< 0.01	< 0.01
NDF	34.9	35.3	34.5	33.9	31.4	35.8	34.6	39.9	35.0	29.0	33.7	1.8	< 0.01	< 0.01	0.28
TDF	35.6	32.4	32.0	34.3	34.0	35.2	38.4	36.8	34.8	29.8	27.9	0.5	< 0.01	< 0.01	< 0.01
In vitro hindgut disappearance after incubation with purified enzymes, %															
DM	6.1	5.8	5.6	3.2	5.3	7.9	9.7	6.7	6.3	6.3	7.9	1.3	< 0.01	0.99	0.06
NDF	5.4	5.7	2.7	2.0	6.1	5.5	8.5	5.4	5.2	5.6	6.0	2.0	0.01	0.75	0.53

Table 6.4. (cont.)

Item	C-DDGS[1]									S-DDGS	SC-DDGS	SEM	*P*-value	Contrasts[3]	
	1	2	3	4	5	6	7	8	Mean					S vs. C DDGS	SC vs. C DDGS
In vitro total tract digestibility after incubation with fecal inoculum, %															
DM[4]	68.8	68.2	69.6	78.0	70.2	67.4	78.6	75.4	72.0	55.7	62.5	3.1	0.01	< 0.01	< 0.01
NDF[4]	73.8	74.8	72.7	75.8	68.7	76.7	80.3	78.8	75.2	77.5	82.1	2.6	< 0.01	0.35	< 0.01
In vitro hindgut disappearance after incubation with fecal inoculum, %															
DM[4]	21.1	24.9	24.8	22.9	23.6	24.1	32.6	28.2	25.3	21.3	23.0	1.4	< 0.01	0.04	0.24
NDF[4]	44.3	44.2	41.0	44.5	43.4	47.0	54.2	44.8	45.4	53.7	54.3	1.4	< 0.01	< 0.01	< 0.01

[1]Least square means of 3 observations per source of DDGS.

[2]C-DDGS = DDGS produced from corn; S-DDGS = DDGS produced from sorghum; SC-DDGS = DDGS produced from a blend of sorghum and corn.

[3]Contrast of C-DDGS vs. S-DDGS and C-DDGS vs. SC-DDGS.

[4]All values obtained with fecal inoculum were greater ($P < 0.01$) than values obtained after incubation in enzymes.

GENERAL CONCLUSIONS

Swine feeding programs may take advantage of alternative feed ingredients. These feed ingredients are high in dietary fiber which consists of plant derived carbohydrates and lignin that are undigested by mammalian enzymes. The most important characteristics of dietary fiber from the perspective of energy utilization are the amount of VFA absorbed by the pig and the effect of dietary fiber on digestibility of nutrients.

Lysine was the only AA which AID was reduced with addition of DDGS to a corn soybean meal diet. The AID of other AA and the AID of energy did not change with addition DDGS. The concentration of both, dietary fiber and dietary fat, in corn soybean meal diets increase with addition DDGS. In DDGS, fiber and fat may interact affecting AID of AA. One of the mechanism that dietary fiber reduces AID of nutrients in by reducing the time nutrients stay in contact with enzymes and the absorptive mucosa. Addition of DDGS did not changed the time the marker is excreted in ileal digesta, cecal digesta, and feces, suggesting that reduction in AID of Lys may be not due to reduction of contact with digestive enzymes or absorptive mucosa. Therefore, these results suggest that the effect of Maillard reaction on AID of Lys is greater than the effect of dietary fiber.

The energy that the pig will obtain from dietary fiber in DDGS is as VFA that are produced from fermentation of fiber. In DDGS, ATTD of TDF is the main factor affecting the energy value of dietary fiber from DDGS. Therefore, it is necessary to measure ATTD of TDF among a wide variety of sources of DDGS. The AID of TDF among 24 sources of DDGS was 23.0% while the ATTD was 47.3% and there is a wide range of digestibility values (29.3 to 57.0%). These data, along with data of the first experiment, demonstrate that almost half of TDF in DDGS is excreted in feces and it is, therefore, necessary to increase ATTD of TDF in DDGS.

There are two ways that ATTD of TDF may be increased in DDGS. The ability of the pigs to digest fiber may be increased or the fiber in DDGS may be modified to be more accessible to fermentation in the pig intestine.

In the third experiment, the ATTD of TDF of Meishan pigs was compared with the ATTD of TDF in Yorkshire pig that had similar BW than Meishan pigs (Light Yorkshires) or with Yorkshire pigs that were the same age as the Meishan pigs (Heavy Yorkshires). Data show that there is an interaction between the groups of pigs and the type of dietary fiber on ATTD of TDF. The ATTD of TDF is greater in Meishan pigs fed DDGS than in light and in heavy Yorkshire pigs. These data demonstrate that ATTD of IDF, but not SDF, is affected by the breed of pigs. Also, ATTD of IDF is influenced by age of pigs. Heavy pigs have a greater capacity to digest IDF than light pigs. This observation suggests that IDF may be fed to finishing pigs and sows, but not for nursery and young growing pigs.

The results of Exp. 3, suggest that if dietary fiber from DDGS is the main fiber that will be fed by pig farmers in the future, then Meishan pigs may be used in farm breeding programs to increase ATTD of fiber. However, to improve digestibility of fiber by means of breeding programs may be more time consuming than to modify the type of fiber in DDGS.

There are numerous chemical and physical methods to increase ATTD of TDF. Sorting through all these methods will require the use of *in vivo* digestibility procedures. There are several shortcoming of measuring *in vivo* ATTD of TDF. Therefore, the last experiment measured *in vitro* ATTD fiber in DDGS. The ATTD of NDF and the ATTD of TDF were correlated ($R^2 = 0.90$) among sources of DDGS. There are advantages of measuring NDF rather than TDF. Therefore, the *in vitro* assay was designed to measure *in vitro* ATTD of NDF. The results of the last set of experiments suggest, that the 3 step *in vitro* digestibility assay is very

repeatable and that the sample size can be increased up to at least 4 g. These modifications allow measuring *in vitro* ATTD of NDF. Fecal inoculum is, however, a better option than using purified enzymes to study *in vitro* ATTD of fiber. Future modifications to the 3 step *in vitro* digestibility assay are needed be to measure *in vitro* ATTD of TDF. These modifications may include measuring *in vitro* AID and ATTD of TDF, and comparing *in vitro* data with *in vivo* data.

In conclusion, the present work suggests that fiber from DDGS can be fed to swine without deleterious effects on digestibility of AA. However, digestibility of dietary fiber DDGS need to be improved to maximize the energy digestibility. The digestibility of fiber can be measured and improved using a variety of methods.

AUTHOR'S BIOGRAPHY

Pedro E. Urriola is a native of Venezuela where he got a Veterinary degree. In September 2004, he came to Minnesota to study MS in Animal Sciences with Dr. Gerald Shurson. Courses in Minnesota included Statistical Analysis of Experiments and Experimental Design. His MS Thesis title was "*In vivo* digestibility of amino acids and *in vitro* prediction". One manuscript was published and the second manuscript of the MS thesis is in the process of licensing from the University of Minnesota to VAST, a company that buys distillers dried grains with solubles for farmers in the US.

Pedro E. Urriola worked at Cargill Animal Nutrition in Elk River, MN, after completion of his MS. He was assigned to conduct digestibility experiments, summarize data, and present reviews of literature to the group at the Innovation Center. He started the PhD program in Illinois in spring 2007 and took 26 course credits including Microbial Biochemistry and Regulation of Metabolism. At Illinois has conducted a total of 12 experiments and 4 of these experiments are included in his dissertation.

Professional affiliations include: American Society of Animal Sciences, International Pig Veterinarian Society. Honors and awards include: ASAS Midwest Meeting Graduate Student Competition (1^{st} place) and Gamma Sigma Delta Honor Society.

Pedro E. Urriola married to Maria G. Pieters in 2005. On August 16, 2009 Pedro and Maria welcomed Abigail P. Urriola.