IN VIVO AND IN VITRO DISAPPEARANCE OF ENERGY AND NUTRIENTS IN NOVEL CARBOHYDRATES AND CEREAL GRAINS BY PIGS

BY

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DISSERTATION

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ABSTRACT: In vivo and in vitro experiments were conducted to determine digestibility of GE and nutrients, as well as DE and ME of carbohydrates fed to growing pigs. The objective of Exp. 1 was to determine the DE and ME of 4 novel carbohydrates fed to pigs. The 4 novel carbohydrates were 2 sources of resistant starch (RS 60 and RS 70), soluble corn fiber (SCF), and pullulan. These carbohydrates were produced to increase total dietary fiber (TDF) intake by humans. Maltodextrin (MD) was used as a highly digestible control carbohydrate. The DE and ME for RS 60 (1,779 and 1,903 kcal/kg, respectively), RS 75(1,784 and 1,677 kcal/kg, respectively), and SCF (1,936 and 1,712 kcal/kg, respectively) were less ($P < 0.05$) than for MD (3,465 and 3,344 kcal/kg, respectively) and pullulan (2,755 and 2,766 kcal/kg, respectively), and pullulan contained less ($P < 0.05$) DE and ME than MD. However, there was no difference in the DE and ME for RS 60, RS 75, and SCF. The varying degrees of small intestinal digestibility and differences in fermentability among these novel carbohydrates may explain the differences in the DE and ME among carbohydrates. Therefore, the objectives of Exp. 2 were to determine the effect of these 4 novel carbohydrates and cellulose on apparent ileal (AID) and apparent total tract (ATTD) disappearance, and hindgut disappearance (HGD) of GE, TDF, and nutrients when added to diets fed to ileal-cannulated pigs. The second objective was to measure the endogenous flow of TDF to be able to calculate the standardized ileal disappearance (SID) and standardized total tract (STTD) disappearance of TDF in the 4 novel fibers fed to pigs. Results of the experiment indicated that the AID of GE and DM in diets containing cellulose or the novel fibers was less ($P < 0.05$) than of the maltodextrin diet, but the ATTD of GE and DM was not different among diets. The addition of RS 60, RS 75, and SCF did not affect the AID of acid hydrolysed ether extract (AEE), CP, or ash, but the addition of cellulose and pullulan reduced ($P < 0.01$) the AID of CP. The average ileal and total tract endogenous losses of TDF were calculated to be

25.25 and 42.87 g/kg DMI, respectively. The SID of TDF in diets containing RS 60, SCF, and pullulan were greater ($P < 0.01$) than the SID of TDF in the cellulose diet, but the STTD of the SCF diet was greater ($P < 0.05$) than for the cellulose and pullulan diets. Results of this experiment indicate that the presence of TDF reduces small intestinal disappearance of total carbohydrates and energy which may reduce the DE and ME of diets and ingredients. Therefore, the objective of Exp. 3 was to determine the DE and ME in yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, polished rice, rye, sorghum, and wheat fed to growing pigs and to determine the AID and ATTD of GE, OM, CP, AEE, starch, total carbohydrates, and TDF in these cereal grains fed to pigs. Results indicated that the AID of GE, OM, and total carbohydrates was greater ($P < 0.001$) in rice than in all other cereal grains. The AID of starch was also greater ($P < 0.001$) in rice than in yellow dent corn, dehulled barley, rye, and wheat. The ATTD of GE was greater ($P < 0.001$) in rice than in yellow dent corn, rye, sorghum, and wheat. With a few exceptions, the AID and ATTD of GE and nutrients in Nutridense corn was not different from the values for dehulled oats. Likewise, with a few exceptions, the AID, ATTD, and HGD of GE, OM, total carbohydrates, and TDF in yellow corn, sorghum, and wheat were not different from each other. The AID of GE and AEE in dehulled barley was greater ($P < 0.001$) than in rye. The ATTD of GE and most nutrients was greater ($P < 0.001$) in dehulled barley than in rye. Dehulled oats had the greatest ($P < 0.001$) ME (kcal/kg DM) whereas rye had the least ME (kcal/kg DM) among the cereal grains. Results of the experiment indicate that the presence of TDF and RS may reduce small intestinal digestibility of starch in cereal grains resulting in reduced DE and ME in these grains. Digestibility experiments involving animals are time consuming and expensive. Therefore, the objective of Exp. 4 was to correlate DM and OM digestibility obtained from 3 in vitro procedures with ATTD of GE and with the concentration of

DE in 50 corn samples that were fed to growing pigs. The second objective was to develop a regression model that can predict the ATTD of GE or the concentration of DE in corn. The third objective was to evaluate the suitability of using the Daisy^{II} incubator as an alternative to the traditional water bath when determining in vitro DM and OM digestibility. Results indicated that corn samples incubated with Viscozyme for 48 h in the Daisy^{II} incubator improved ($P < 0.001$) the ability of the procedure to detect small differences in the ATTD of GE or to detect small differences in the concentration of DE in corn. Likewise, compared with using cellulase or fecal inoculum, the variability in the ATTD of GE and the variability in the DE in corn was better ($R^2 = 0.56$; $P < 0.05$ and $R^2 = 0.53$; $P < 0.06$, respectively) explained if Viscozyme was used than if cellulase or fecal inoculum was used. A validated regression model that predicted the DE in corn was developed using Viscozyme and with the corn samples incubated in the Daisy^{II} incubator for a 48 h.

In conclusion, this present work used the pig as a model for human gastrointestinal function and evaluates carbohydrates from 2 different nutritional perspectives – humans and animals. The addition of novel carbohydrates reduced the digestibility of energy in the diets without necessarily reducing the digestibility of other nutrients. Thus, supplementation of novel carbohydrates in the diets may be beneficial for the management of diabetes. Aside from diabetic management, cereal grains such as rye and sorghum, may also help in BW management because of there low caloric value, but for undernourished individuals, dehulled oats, dehulled barley, and rice are the ideal grains. From an animal nutrition standpoint, high concentration of dietary fiber is undesirable because it reduces feed efficiency. Therefore, the inclusion of feed ingredients that have a high concentration of dietary fiber is often limited in animal diets.

Although in vivo determination is ideal, in vitro procedures are useful tools to determine caloric value of food and feed ingredients.

Key words: cereal grains, energy, in vitro digestibility novel carbohydrates, total dietary fiber, pigs

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May the road rise up to meet you.

May the wind always be at your back.

May the sun shine warm upon your face,

and rains fall soft upon your fields.

And until we meet again,

May God hold you in the palm of His hand.

~ Irish Blessing~

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ADFI	average daily feed intake
ADL	acid detergent lignin
AEE	acid hydrolyzed ether extract
AID	apparent ileal digestibility (disappearance)
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ATTD	apparent total tract digestibility (disappearance)
BW	body weight
CP	crude protein (N × 6.25)
Cu	copper
CV	coefficient of variation
d	day(s)
DE	digestible energy
DM	dry matter
DMI	dry matter intake
Eq.	Equation(s)
Exp.	experiment
g	gram
GE	gross energy
GLM	general linear model
h	hour(s)
HCl	hydrochloric acid
HGD	hindgut disappearance

IL	Illinois
IEL	ileal endogenous loss
IVDMD	in vitro dry matter digestibility (disappearance)
IVOMD	in vitro organic matter digestibility (disappearance)
Kcal	kilocalories
L	liter
LSmeans	least square means
m	meter
*u*m	micrometer
M	molar (concentration)
Mcal	Megacalorie
MD	maltodextrin
ME	metabolizable energy
min	minute(s)
Mn	manganese
MO	Missouri
Mol	mole
N	nitrogen
N	normal (concentration)
n	sample size
Na	sodium
NaOH	sodium hydroxide
NRC	National Research Council
OM	organic matter
P	probability

RS	resistant starch
r^2	simple coefficient of determination
R^2	multiple coefficient of determination
rpm	revolutions/minute
s	second(s)
SAS	Statistical Analysis System
SCF	soluble corn fiber
SCFA	short-chain fatty acid(s)
SD	standard deviation (sample)
SEM	standard error of the mean
SID	standardized ileal digestibility (disappearance)
STTD	standardized total tract digestibility (disappearance)
TDF	total dietary fiber
TN	Tennessee
TTEL	total tract endogenous loss
WBC	water binding capacity
wt	weight

CHAPTER 1

INTRODUCTION

Carbohydrates are ubiquitous in plants (BeMiller, 2007). Because of their abundance in nature, carbohydrates in plants are the major source of energy for man and animals. Carbohydrates exist in diverse structures, molecular sizes, physical, and chemical properties (Kritchevsky, 1988; BeMiller, 2007). The capability of man and monogastric animals to utilize different forms of carbohydrates as an energy source is limited by the digestive capacity of the gastrointestinal tract. Dietary carbohydrates that are digested by enzymes secreted by animals provide more energy than dietary carbohydrates that escape digestion but undergo fermentation in the hind gut (Englyst and Englyst, 2005). For this reason, not all carbohydrates in their natural form can be utilized with equal efficiency by man and monogastric animals as an energy source.

Recently, studies in human and animal nutrition have focused on the portion of carbohydrates that is not digested by enzymes secreted by animals. The study of dietary fiber has gained interest in both fields for different reasons. Epidemiological studies have shown that the increase in the incidence of metabolic diseases observed during recent years in Western countries is associated with a low intake of dietary fiber (Kritchevsky, 1988). Subsequent studies have shown that greater intake of dietary fiber results in several health benefits including diabetic control (Wolever and Jenkins, 2001), control of cardiovascular disease (Jenkins et al., 2001), and reduced incidence of colon cancer (Slavin, 2001). When the definition of dietary fiber was broadened to include physiological or functional properties of carbohydrates and after the analytical method for dietary fiber was established (De Vries, 2004), the list of potential sources of dietary fiber that can be incorporated into food increased (Southgate, 2001). Novel carbohydrates that act as dietary fiber were also developed in an attempt to increase the daily

dietary fiber intake to the recommended 25-30 g from the average of 15 g (IOM, 2005). However, by definition, a source of dietary fiber should have low caloric value and must have physiological effects such as the attenuation of blood glucose concentration. Therefore, Chapter 2 provides caloric values for 4 novel carbohydrates using the pig as a model for human digestion and Chapter 3 provides data on the effects of these 4 novel carbohydrates on GE and nutrient digestibility when added to the diet of pigs.

The primary carbohydrate in cereal grain is starch. Starch in corn, wheat, rice, and other cereals are very digestible and contributes 70 to 80% of the caloric intake in man and animals (BeMiller, 2007). However, cereal grains also contain non-starch polysaccharides, which are a component of TDF. It is also recognized that a portion of the starch in grain resists digestion by mammalian enzymes (Sajilata et al., 2006). The concentration of this portion, called resistant starch, in a carbohydrate source varies depending on botanical source, the structure and the conformation of the starch granule, and processing (Livesay et al., 1990; Goldring, 2004). Resistant starch and TDF in cereal grains are usually fermented in the hindgut with subsequent absorption of SCFA, but the energetic contribution from fermented carbohydrates is less than from digested carbohydrates. The presence of TDF and resistant starch may, therefore, affect the energy and nutrient digestibility of cereal grains. Chapter 4 reports data on the digestibility of GE, OM, CP, AEE, starch, total carbohydrates, and TDF in 8 cereal grains, and the DE and ME of these 8 cereal grains are also presented.

There is a different reason for studying dietary fiber in animal nutrition. Increasedethanol production has diverted the supply of corn, the major source of energy for livestock in the U.S., from meat production to ethanol production. Corn supply has, therefore, become limited. The by-products of ethanol production have high concentrations of protein and fat, however, they

also contain substantial amounts of dietary fiber (Stein and Shurson, 2009). Feed ingredients that have high concentrations of dietary fiber usually have low energy and nutrient digestibility when fed to pigs. Evaluation of feed ingredients to determine their energy value is, therefore, important.

Because in-vivo studies are expensive and time consuming, in vitro procedures are sometimes used as alternative methods to estimate energy digestibility and the caloric value of feed ingredients. Chapter 5 presents results from several experiments aimed at identifying in vitro procedures that may be used to predict the DE in corn.

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CHAPTER 2

CARBOHYDRATES AND THE USE OF PIGS AS MODELS FOR HUMAN GASTROINTESTINAL FUNCTION.

The main energy source for most humans and non-carnivorous animals is dietary carbohydrate of plant origin. The primary classification of carbohydrates is based on their chemical properties, i.e., degree of polymerization, type of linkages, and characteristics of the individual monomers (Cummings and Stephen, 2007). Using this classification, carbohydrates are grouped into sugars, oligosaccharides, and polysaccharides (FAO, 1998). However, this classification does not provide information about the physiological attributes or nutritional contributions of these groups of carbohydrates when included in human or animal diets. To overcome this limitation, dietary carbohydrates may be classified based on small intestinal digestibility (FAO, 2003; Cummings and Stephen, 2007; Englyst et al., 2007) or based on their ability to influence blood glucose concentration (glycemic or non-glycemic carbohydrates; Englyst and Englyst, 2005).

CLASSIFICATION OF DIETARY CARBOHYDRATES

Available Carbohydrates

The term "available carbohydrates" is equivalent to that of "glycemic carbohydrates" (Englyst et al., 2007) and represents the portion of carbohydrates digested by animal enzymes and that participates in intermediary metabolism (FAO, 2003). Some monosaccharides, such as glucose, fructose, and galactose, are easily absorbed from the small intestine via energy-dependent transporters (Englyst and Hudson, 2000). Other monosaccharides, such as arabinose, xylose, and mannose, are passively absorbed in the small intestine, but the presence of these monosaccharides in food is quantitatively small (Englyst and Hudson, 2000; IOM, 2001).

Disaccharides, such as sucrose (glucose and fructose) and maltose (2 glucose units), are digested by the enzymes sucrase and maltase, respectively, to their monosaccharide constituents, before absorption (Englyst and Hudson, 2000). Sucrase and maltase are enzymes that specifically break α-(1-2) and α-(1-4) glycosidic linkages, respectively. Lactase, on the other hand, breaks the β-(1-4) linkage in lactose, releasing galactose and glucose prior to absorption (Englyst and Hudson, 2000).

The glucose absorbed from these disaccharides is rapidly reflected in an increase in blood glucose concentration. Thus, monosaccharides and disaccharides are also called glycemic carbohydrates because of the immediate increase in the blood glucose level after the consumption of these carbohydrates (Englyst and Englyst, 2005). The consumption of available or glycemic carbohydrates is a concern because of the increased incidence of diabetes and obesity (Lunn and Buttriss, 2007).

Starch, the principal carbohydrate in most diets, is also considered an available carbohydrate. Starch is unique among carbohydrates because it occurs in nature as granules and it is composed of amylose and amylopectin polymers (BeMiller, 2007). Most cereal starches contain about 25% amylose and 75% amylopectin (BeMiller, 2007). Amylose is predominantly a linear chain of glucose residues linked by α-(1-4) glycosidic bonds, although a few α-(1-6) bonds may occur as side chains (BeMiller, 2007; Cummings and Stephen, 2007). Amylopectin is a large, highly branched polymer composed of both α-(1-4) and α-(1-6) glycosidic linkages (Cummings and Stephen, 2007). Starch that is composed predominantly of amylopectin is referred to as waxy starch (BeMiller, 2007).

Digestion of starch in pigs and humans starts when the food is mixed with salivary amylase secreted in the mouth (Englyst and Hudson, 2000). Starch digestion in the mouth is

short and limited because salivary amylase is deactivated by the low pH of the stomach as the food is swallowed (Englyst and Hudson, 2000). Starch digestion, however, predominantly occurs in the small intestine where it is hydrolyzed to maltose, maltotriose, and isomaltose (also called α-dextrins) by pancreatic and intestinal α-amylase and isomaltase (Groff and Gropper, 2000). Maltase, which is an intestinal brush border enzyme, hydrolyzes maltose and maltotriose to its glucose monomers whereas isomaltase (also called α-dextrinase) hydrolyzes the α-(1-6) glycosidic linkage of isomaltose to produce 2 glucose molecules (Groff and Gropper, 2000). Although enzymes can completely digest starch, the rate and extent of starch digestion varies depending on several factors including (1) the nature of crystallinity of the starch granule or the source of starch, (2) the amylose:amylopectin ratio, and (3) the type and extent of processing of the starch (Cummings et al., 1997; Englyst and Hudson, 2000; Svihus et al., 2005). Because of the different factors that affect starch digestibility, starch can be classified further based on its rate of digestion and glucose appearance in the blood, as rapidly available starch and slowly available starch (Englyst et al., 2007).

Dietary Fiber or Unavailable Carbohydrates

Dietary fiber is defined as carbohydrates that are not digested or are poorly digested in the small intestine, but are completely or partially fermented in the large intestine (De Vries, 2004). The concept of small intestinal indigestibility is also sassociated with the terms "unavailable carbohydrates" and "non-glycemic carbohydrates" (Englyst et al., 2007). Non-starch polysaccharides, resistant starch, non-digestible oligosaccharides, and sugar alcohols belong to the group of carbohydrates that are classified as dietary fiber (Englyst and Englyst, 2005; Englyst et al., 2007).

Non-starch Polysaccharides. Originally, dietary fiber only referred to intrinsic polysaccharide components present in plant cell walls (Englyst et al., 2007). Eighty to 90% percent of the carbohydrates present in plant cell walls are non-starch polysaccharides (Cummings et al., 1997) and differ from available carbohydrates in that they do not have α-(1-4) glycosidic linkages, which is a characteristic of available carbohydrates (Englyst et al., 2007). However, the current definition of dietary fiber recognizes that carbohydrates, other than those present in plant cell walls, provide similar physiological effects as plant cell wall carbohydrates (De Vries, 2004). Thus, dietary fiber includes non-starch polysaccharides that are plant cell wall as well as non-cell wall components.

Cell Wall Components. Cellulose and hemicelluloses are the most common non-starch polysaccharides in cell walls. Cellulose comprises 10 to 30% of the non-starch polysaccharides in food and is a linear, unbranched chain of glucose units with β-(1-4) linkages (Cummings and Stephen, 2007; BeMiller, 2007). The linear and unbranched structure of glucose chains in cellulose enables the chains to pack closely and form microfibrils that provide structural integrity to the plant cells and tissues (Cummings and Stephen, 2007; Englyst et al., 2007). Because of the nature of the glycosidic linkages, cellulose is not digested by endogenous enzymes secreted by the animal (BeMiller, 2007).

Hemicelluloses differ from cellulose in that they area branched chain polysaccharides composed of different types of hexoses and pentoses (Cummings and Stephen, 2007). The most common hemicelluloses in annual plants, including cereal grains, is xylan (BeMiller, 2007). Xylan consists of a xylose backbone that may be linear or highly branched (BeMiller, 2007). Side chains are present in the linear or branched core structure and are usually composed of arabinose, mannose, galactose, and glucose (Cummings and Stephen, 2007). Some

hemicelluloses also contain uronic acids that are derived from glucose (glucuronic acid) or from galactose (galacturonic acid; Southgate and Spiller, 2001). The presence of uronic acids gives hemicelluloses the ability to form salts with metal ions such as calcium and zinc (Cummings and Stephen, 2007).

Lignin is not a carbohydrate, but it is closely associated with plant cell walls and is included in the analysis for dietary fiber (Lunn and Buttriss, 2007). Lignin is formed by cross linkage of phenylpropane polymers of coumaryl, guaiacyl, coniferyl, and sinapyl alcohols (Kritchevsky, 1988). As the plant matures, lignin penetrates the plant polysaccharide matrix and forms a 3-dimensional structure within the matrix of the cell wall (Southgate, 2001). Lignin is resistant to enzymatic and bacterial degradation. As a consequence, plants with a high concentration of lignin are poorly digested (Southgate, 2001; Wenk, 2001).

Analysis of total dietary fiber using the enzymatic-gravimetric method (AOAC methods 985.29, 991.42, and 993.16) and its modification (AOAC method 991.43) includes lignin as part of total dietary fiber (Cho et al., 1997). Exclusion of lignin and other acid insoluble components derived from protein and carbohydrates (Klason lignin) from the measurement of total dietary fiber is possible using enzymatic-chemical methods as developed by Theander and Åman (1982) and Englyst and Hudson (1993).

Non-cell Wall Component. Carbohydrates that are not components of the plant cell wall but are considered dietary fiber are pectins, gums, non-digestible oligosaccharides, and resistant starches. Commercially available pectin is usually extracted from citrus peel or apple pomace although other sources of pectin are also available (Fernandez, 2001). A key feature of pectins is that they are composed primarily of linear polymers of galacturonic acids that are linked together by α-(1-4) linkages (BeMiller, 2007). Pectins may also contain side chains of rhamnose,

galactose, and arabinose (Cummings and Stephen, 2007). The uronic acids have carboxyl groups, some of which are naturally occurring methyl esters that are responsible for the gelling property of pectin (BeMiller, 2007). The composition and properties of pectin vary depending on the source, handling, extraction procedures, and subsequent treatments (BeMiller, 2007). Pectins are classified according to degree of esterification as high methoxy and low methoxy pectins. The commercial value of pectin is based on its ability to form gels in the preparation of jams and jellies (Fernandez, 2001).

Gums are natural plant polysaccharides, but may also be produced by fermentation. Naturally occurring gums can be formed as exudates from plants or shrubs that are physically damaged or they can be a part of the seed endosperm (BeMiller, 2007). An example of an exudate gum is gum Arabic and an example of a gum from seed endosperm is guar gum. Xanthan gum and Pullulan are examples of gums produced from fermentation. Although each of these gums is used in the food industry as thickeners and emulsifiers, each gum has its own unique property brought about by differences in its carbohydrate structure and moiety.

Gum Arabic (or gum Acacia) is a heterogeneous material that consists mainly of a branched β-(1-3) linked galactose backbone with ramified side chains composed of arabinose, rhamnose, galactose, and glucuronic acid linked through the 1-6 positions (Osman et al., 1995, Williams and Phillips, 2001). Guar gum is a galactomannan that consists of a linear β-(1-4) mannose backbone, with some of the mannose units having a single galactose unit as a side chain (BeMiller, 2007). The microbial polysaccharide, xanthan gum, produced by *Xanthomonas campestris,* is a β-glucan with a backbone structure similar to that of cellulose (BeMiller, 2007). A trisaccharide side chain is attached to the third carbon of every other glucose unit in the backbone chain and the trisaccharide side chain is composed of a glucose unit between 2

mannose units (BeMiller, 2007). The trisaccharide side chain wraps around the β glucan backbone, which renders the molecule stiff and highly viscous (BeMiller, 2007). Pullulan, on the other hand, is produced by *Aureobasidium pullulans* (Wolf et al., 2003). It is a homopolysaccharide composed of repeating units of a maltotriose with an α-(1-6) terminal glucose unit (Shingel, 2004). Despite its starch-like structure, it is a slowly digestible polysaccharide (Wolf et al., 2003).

Oligosaccharides are compounds that consist of monosaccharide units joined by glycosidic linkages (BeMiller, 2007). The distinction between oligosaccharides and polysaccharides is not clear. Some authorities define oligosaccharides as carbohydrate polymers having 3 to 10 monosaccharide units (IOM, 2005) whereas others define oligosaccharides as polymers containing 2 to 19 monosaccharide units (FDA, 1993). However, analytical separation of oligosaccharides from polysaccharides is often based on solubility in 80% v/v ethanol (Englyst and Englyst, 2005). On the basis of this procedure, the terms "non-digestible oligosaccharide", "resistant oligosaccharide", and "resistant short-chain carbohydrates" are synonymous and refer to any carbohydrate that resists pancreatic and small intestinal digestion and is soluble in 80% ethanol (Englyst et al., 2007). This analytical definition includes fructo-oligosaccharides, galacto-oligosaccharides, and mannan-oligosaccharides.

Fructo-oligosaccharides or fructans are carbohydrates that are composed mainly of fructose monosaccharides with varying degrees of polymerization (BeMiller, 2007). Fructo-oligosaccharides are classified on the basis of how they are produced. Inulin is a storage carbohydrate in several fruits and vegetables including onions, Jerusalen artichoke, wheat, and chicory (Englyst et al., 2007). The chain length of inulin varies from 2 to 60 with an average degree of polymerization of 12 (Roberfroid, 2005). Commercial hydrolysis of inulin from

chicory produces inulin-type fructans which are linear polymers mainly composed of β linked 2-1 fructose units which are often terminated with sucrose at the reducing end (BeMiller, 2007). A glucose molecule and side chains having β-(2-6) linkages may also be present in some inulin-type fructans (Meyer, 2004; Roberfroid, 2005).

Levans are β-linked 2-6 fructans synthesized by some bacteria and fungi that secrete levansucrase (Franck, 2006). Levansucrase catalyzes transglycosylation reactions that convert sucrose to levans that may contain β-(2-1)- linked side chains (BeMiller, 2007). Fructans with a high degree of polymerization (>10^7 Da) are mainly the levan type (Franck, 2006), but they are not commercially produced (Meyer, 2004). Aside from being a source of dietary fiber, fructans are prebiotics. They promote the growth of bifidobacteria (Franck, 2006) and lactobacillus (Mul and Perry, 1994) and reduce the growth of harmful bacteria such as clostridia (Franck, 2006), thus contributing to improved intestinal health.

Two groups of galacto-oligosaccharides are well studied. The best known galacto-oligosaccharides (also referred to as α-galactosides) are from legumes and include raffinose, stachyose, and verbascose (Cummings and Stephen, 2007; Martinez-Villaluenga et al., 2008). Raffinose is a trisaccharide composed of a unit of galactose linked to sucrose via an α-(1-6) glycosidic linkage. Stachyose is composed of 2 galactose units linked to sucrose; whereas, verbascose is composed of 3 galactose units linked to sucrose via an α-(1-6) linkage (Cummings and Stephen, 2007). Galacto-oligosaccharides are mostly present in legume seeds such as peas and beans (Cummings and Stephen, 2007).

The second group of galacto-oligosaccharides (also referred to as transgalacto-oligosaccharide) is commercially produced by transglycosylation using lactose as the substrate (Meyer, 2004). Reactions catalyzed by β-galactosidase convert lactose to β-(1-6) linked

galactose units connected to a terminal glucose unit via an α-(1-4) linkage. Degree of polymerization can vary from 2 to 5 (Meyer, 2004).

Some plants, such as barley, secrete α-galactosidase which is involved not only in the metabolism of raffinose but also with leaf development and stress tolerance (Chrost et al., 2007). But because α-galactosidase is not secreted by animals, these oligosaccharides are fermented in the colon where they may exert a prebiotic effect (Meyer, 2004).

Mannan-oligosaccharides are polymers of mannose. Most of the mannan-oligosaccharides used in the animal industry are derived from yeast cell walls (Zentek et al., 2002). Yeast cell wall is composed of a network of mannans, β-glucans, and chitin (Cid et al., 1995). The mannose units are located in the outer surface of the cell wall and are attached to the inner β-glucan component of the cell wall through β-(1-6) and β-(1-3) glycosidic linkages (Cid et al., 1995). Mannan-oligosaccharides are not digestible by gastric and intestinal enzymes (Zentek et al., 2002) and when fed to animals, mannan-oligosaccharides function as prebiotics, and as immune modulators. Mannan- oligosaccharides may also aid in gastrointestinal pathogenic resistance by acting as alternative receptors for bacteria (i.e., *E. coli*) that have a mannan specific lectin (Mul and Perry, 1994; Swanson et al., 2002).

Resistant starch is "starch and the derivatives of starch that are not digested in the small intestine of healthy people" (Brown, 2004). They differ from slowly digestible starch in that slowly digestible starch is completely digested in the small intestine, whereas resistant starch is fermented in the large intestine and colon (Champ, 2004). Resistant starch is naturally present in all starch-containing foods, but the amount of resistant starch in food and feed depends on the source of the starch, the processing techniques used in the preparation of food and feed, and the storage conditions of the starch prior to consumption (Livesey, 1990; Brown, 2004; Goldring,

2004). Resistant starch has 4 classifications. Resistant starch 1 refers to starches that are physically inaccessible to digestive enzymes because it is enclosed in an indigestible matrix (BeMiller, 2007). Whole or partly milled grains contain resistant starch that belongs to this type (Brown, 2004). Resistant starch 2 refers to native (uncooked) starch granules that resist digestion because of the granules' conformation or structure (Brown, 2004). Processing of this type of starch can make the starch susceptible to enzymatic hydrolysis; however, high amylose starch is unique because its granules are not affected by processing and, therefore, so it retains the ability to resist hydrolysis by digestive enzymes (Brown, 2004). Resistant starch 3 refers to retrograded starches, which are starches that have been gelatinized and cooled to allow for crystalline formation that resist digestion (Brown, 2004). Starch that has been modified by certain chemical reactions to reduce its enzymatic susceptibility to digestive enzymes is referred to as resistant starch 4 (Brown, 2004).

PHYSICO-CHEMICAL PROPERTIES OF DIETARY FIBER

The unique properties that differentiate dietary fiber from digestible polysaccharides are influenced by the chemical composition and the physical structure of the fiber. The physico-chemical properties that are relevant to human and animal nutrition include solubility, water holding and water binding capacity, viscosity, and fermentability. These physico-chemical properties of dietary fiber are responsible for the physiological effects that improve human well-being but reduce animal production efficiency.

Solubility. Dietary fiber may be classified as soluble and insoluble fiber (Cho et al., 1997). Solubility of dietary fiber not only refers to the ability of the dietary fiber to dissolve in water (Oakenfull, 2001), but it can also be defined as its ability to dissolve in dilute acid, dilute base, or a buffer or enzyme solution that mimics the enzyme solution existing in the

gastrointestinal tract (Cho et al., 1997). Soluble fiber may be separated from total dietary fiber by precipitation in ethanol after enzyme digestion (Cho et al., 1997).

Solubility of a dietary fiber is greatly influenced by the linkages between and among monosaccharide units that make up dietary fiber (Oakenfull, 2001). The linkages provide the physical structure that dictates the hydration property of dietary fiber. The β-(1-4) linkage among glucose units in cellulose allows for an ordered crystalline structure preventing the entrance of water molecules in the structure, thus making cellulose insoluble (Oakenfull, 2001). However, the presence of β- (1-3) branching in β-glucan does not allow for the formation of an ordered crystalline structure similar to that of cellulose, thus making β-glucan a soluble fiber (Oakenfull, 2001).

The solubility of dietary fiber does not provide much information about the carbohydrate composition, physical structure, or the degree of polymerization, but it is important because soluble and insoluble fibers differ in their physiological effect and overall contribution in human health and animal production. Soluble fiber results in increased digesta viscosity, which is responsible for reducing post-prandial insulin and blood glucose increases in humans and dogs (Dikeman and Fahey, 2006), whereas insoluble dietary fiber results in increased rate of digesta passage in the gastrointestinal tract and increased fecal mass (Chesson, 2006).

Water Holding and Water Binding Capacity. The physiological property of fiber is affected by the interaction between fiber and water. Fiber binds water through different mechanisms such as ionic interactions, hydrogen bonding, and enclosure of water involving capillary action (Chaplin, 2003). Because of these different binding mechanisms, soluble and insoluble fibers are capable of binding water (Oakenfull, 2001). The intensity of binding and the amount of water bound are largely dictated by the morphological structure and composition of

fiber. The binding strength and the amount of water bound, therefore, vary among fiber sources (Cadden, 1987; Chaplin, 2003).

The ability of dietary fiber to hold water may be expressed in different ways. The expression, "water holding capacity", describes the quantity of water that can be bound in fiber without the application of any external force, whereas "water binding capacity" **(WBC)** or the preferred term "water retention capacity" describes the quantity of water retained in a hydrated fiber after the application of an external force (Robertson et al., 2000). In the literature, however, these terms are used interchangeably (Ang, 1991; Leterme et al., 1998; Chaplin, 2003).

Several methods can be used to measure the capability of fiber to hold water. Water holding capacity is measured by filtration (Chaplin, 2003) or by a Baumann apparatus (Auffret et al., 1994). Water binding capacity can be measured by centrifugation, suction pressure, or the use of a dialysis tubing immersed in simulated gut contents (Stephen and Cummings, 1979; Cadden, 1987; Chaplin, 2003). These different methods evaluate different mechanisms of water binding. Measured values for WBC of fiber, therefore, depend on the method that was used to measure it. A European collaborative study has recommended standardized methods to evaluate WBC and other hydration properties of fiber (Robertson et al., 2000). This method is based on centrifugation, but modifications in terms of sample weight or centrifugal speed are needed to minimize sample loss, which could affect the results (Robertson et al., 2000).

The WBC of fiber is an appropriate measure of "bulk" (Kyriazakis and Emmans, 1995) because the swelling property of fiber is positively correlated with WBC (Auffret et al., 1993). Soluble fiber usually has greater WBC than insoluble fiber (Auffret et al., 1994; Robertson et al., 2000). Cellulose and lignin are generally associated with low water holding capacity and hemicelluloses are generally associated with high water holding capacity (Shelton and Lee,

2000). Monosaccharide components of hemicelluloses that are positively correlated with water holding capacity include arabinose and xylose (Holloway and Greig, 1984).

Viscosity. Viscosity refers to the ability of dietary fiber, particularly soluble dietary fiber, to thicken or forms gels in solution (Dikeman and Fahey, 2006). Insoluble fiber is usually not associated with viscosity although insoluble fiber may influence viscosity through its ability to absorb water (Takahashi et al., 2009).

The viscosity induced by dietary fiber is usually affected by the inclusion rate of dietary fiber, but the effect is not linear (Dikeman and Fahey, 2006). At low concentration of soluble dietary fiber, the molecules in solution are separated and free to flow, but at a critical concentration, molecular movement becomes limited and physical entanglement of the dietary fiber molecules occur (Oakenfull, 2001). Thus, the viscosity of a solution with soluble dietary fiber increases rapidly with increasing concentration of pectin (Buraczewska et al., 2007). Measurement of viscosity involving dietary fiber in solution depends on the shear rate or the stirring rate of the liquid (Oakenfull, 2001). Greater shear rates result in low viscosity measurements (Dikeman and Fahey, 2006). In most studies, viscosity is measured using only 1 shear rate but because different shear rates provide different viscosity values, comparison of viscosity values, whether in solution or in digesta, is not possible (Dikeman and Fahey, 2006). To overcome this limitation, measurements of viscosity along different shear rates is recommended to generate viscosity profiles for different dietary fibers (Dikeman and Fahey, 2006).

Aside from shear rate, the viscosity of dietary fiber in solution or in digesta is affected by other factors such as molecular weight and particle size. At equal inclusion rates, high molecular weight guar gums produced more viscous solutions than low molecular weight guar gums

(Dikeman and Fahey, 2006). Larger particle size also contributes to greater apparent viscosity in pig cecal contents because its removal reduced viscosity (Takahashi and Sakata, 2002).

Fermentability. The susceptibility of dietary fiber to microbial degradation varies depending on the accessibility of dietary fiber to the microbial population in the hind gut (Oakenfull, 2001). The solubility and the WBC greatly influence the fermentation rate of dietary fiber. Upon absorption of water, dietary fiber swells, which increases the surface area of the polysaccharide for microbial action (Canibe and Bach Knudsen, 2001). Because soluble fiber has a higher WBC and, therefore, a greater degree of swelling than insoluble fibers, soluble fiber is fermented at a faster rate than insoluble fiber (Auffret et al., 1993; Auffret et al., 1994; Oakenfull, 2001). Fermentation of soluble dietary fiber, therefore, is mainly in the proximal colon whereas fermentation of insoluble fiber is sustained until the distal colon (Cho et al., 1997).

Increase in fecal weight is mainly a function of fermentability of the fiber (Stephen and Cummings, 1979). Fermentable carbohydrates support microbial growth, which may contribute to an increase in fecal output by increasing fecal microbial mass (Cho et al., 1997). Undegraded residues from poorly fermented dietary fiber also contribute to an increase in fecal output (Stephen and Cummings, 1979). Therefore, for dietary fiber that is composed of both soluble and insoluble fiber, the increase in fecal output is attributable to increases both in microbial fecal mass and in undegraded fiber residues (Cho et al., 1997). For purposes of laxation, official guidelines recommend dietary fiber that is coarsely ground (Jenkins et al., 1999). However, fecal output was similar between coarsely ground and finely ground wheat bran, but coarsely ground wheat bran resulted in frequency of bowel movement than finely ground wheat bran (Jenkins et al., 1999). Finely ground wheat bran, however, was fermented to a greater extent

than coarsely ground wheat bran because the concentration of butyrate was greater with finely ground wheat bran than coarsely ground wheat bran (Jenkins et al., 1999). This suggests that particle size may affect the fermentability and the laxative effect of dietary fibers.

The major products of fiber degradation are acetate, propionate, butyrate, carbon dioxide, methane, and hydrogen (Lunn and Buttriss, 2007). The concentration of each of the short-chain fatty acids varies depending on the chemical and physical structure of the dietary fiber (Lunn and Buttriss , 2007). However, acetate is the most abundant short-chain fatty acid, comprising about 60% of the total short chain fatty acid produced in the hindgut (Lunn and Buttriss, 2007) whereas butyrate is the least abundant (Lunn and Buttriss, 2007).

EFFECTS OF DIETARY FIBER ON NUTRITION AND HEALTH

Effects of Dietary Fiber on Human Nutrition and Health

The central theme of the "dietary fiber hypothesis' proposed by Trowell, Burkitt, and Painter revolved around the observation that metabolic disorders that exist in the United States are rare in Africa and that the absence of dietary fiber was the cause of the occurrence of these metabolic disorders (Kritchesvsky, 1988). Results of research that followed have shown that dietary fiber may have protective effects against certain medical conditions including coronary heart disease, diabetes, colorectal cancer, and obesity (Lunn and Buttriss, 2007). The current recommendation for adequate intake of total dietary fiber is at 35 g/d for men and 25 g/d for women (IOM, 2005). This recommendation is based on observed levels that will protect against coronary heart disease (Slavin, 2005).

Effects of Dietary Fiber on Cardiovascular Disease. Cardiovascular disease, which includes coronary heart disease, stroke, and hypertension, is the leading cause of mortality and morbidity in the United States (Anderson et al., 2009). High intakes of dietary fiber, particularly,

soluble fibers such as pectin, psyllium, and oat β-glucan, reduce serum cholesterol and low density lipoprotein cholesterol values (Anderson et al., 2009), but this effect is not observed when insoluble fibers such as wheat bran and cellulose are consumed (Lunn and Buttriss, 2007). There are 2 well supported mechanisms by which soluble and viscous dietary fibers can reduce cholesterol concentration in the blood. Soluble dietary fibers that increase intestinal lumen viscosity may reduce the absorption of bile acids, resulting in greater excretion of bile acids in the feces (Marlett, 2001). The fermentation of dietary fiber in the colon produces short-chain fatty acids including propionate. Propionate reduces cholesterol synthesis by inhibiting HMG-Coa reductase, the rate-limiting step in cholesterol synthesis, thus contributing to the reduction in the overall cholesterol concentration in the body which subsequently results in the reduction in the risk of cardiovascular disease (Anderson et al., 2009).

Effects of Dietary Fiber on Diabetes. Diabetes is a metabolic disorder that is characterized by either the absence of insulin (Type 1) or the insensitivity of the liver and muscles to the presence of circulating insulin (Type 2; Cameron-Smith and Collier, 2001). In both types of diabetes, the control of blood glucose is important to delay other disorders that are associated with the disease such as damage to the retina and kidney failure (Cameron-Smith and Collier, 2001). Continuous exposure to insulin also enhances insulin resistance of muscle tissues (Cameron-Smith and Collier, 2001). Dietary regimens that could reduce postprandial blood glucose and insulin responses could, therefore, be helpful in ameliorating insulin resistance. Viscous fibers, such as pullulan and resistant starch were reported to reduce postprandial insulin and blood glucose responses by delaying glucose absorption (Kendall et al., 2008; Knapp et al., 2008). Including viscous fiber in the diets of diabetic individuals may, therefore, be helpful in controlling fluxes in blood glucose and insulin concentration that could reduce insulin resistance

(Lunn and Buttriss, 2007). Other advantages in increasing intake of dietary fiber include reductions in plasma triglyceride and low-density-lipoprotein cholesterol concentrations (Anderson et al., 2009).

Effects of Dietary Fiber on Weight Management. Epidemiological studies have shown that there is a strong negative association between fiber intake and obesity (Anderson et al., 2009). There is also an association between higher intakes of dietary fiber and lower body fat percentage (Lunn and Buttriss, 2007). However, despite this strong correlation of dietary fiber with weight management, popular weight loss diets such as Atkins and South Beach contain dietary fiber below the recommended levels (Slavin, 2005).

Dietary fiber can reduce weight gain by several mechanisms. Soluble dietary fiber decreases gastric emptying, reducing the rate of glucose and nutrient absorption, and promotes a feeling of prolong fullness (Lunn and Buttriss, 2007). This may result in lower food intake within a meal and until the next meal (Anderson et al., 2009). Insoluble dietary fiber may also control food intake by its bulking property, and its continued presence in the gastrointestinal tract may hasten the feeling of fullness although this may be short term (Slavin, 2005). The feeling of satiety is contributed by the secretion of the gut hormone, cholecystokinin, the secretion of which is stimulated by the prolonged presence of dietary fiber in the small intestines (Higgins, 2004). The products of fermentation, particularly acetate and propionic acid, may also inhibit glycolysis and glycogenolysis (Higgins, 2004). Acetate and propionate may, therefore, shift the metabolic energy source from glucose to fat metabolism, promoting fat oxidation (Higgins, 2004).

Effects of Dietary Fiber on Colorectal Cancer. The incidence of colon cancer is increasing and the majority of the new incidences of colon cancer came from sporadic occurrence of adenomatous polyps that may eventually transform into cancer cells (Anderson et

al., 2009). Dietary involvement in the development of colorectal cancer is strong, although the influence of dietary fiber in reducing the risk of colorectal cancer is not definitive (Lunn and Buttriss, 2007). Although some cohort studies reported reduction in the incidence of colorectal cancer on high fiber intakes (Lunn and Buttriss, 2007), other studies were not able to observe such beneficial effects (Kendall et al., 2004; Lunn and Buttriss, 2007). The positive association between glycemic index and glycemic load with colorectal cancer however, gives credit to a potential protective role of dietary fiber against colorectal cancer (Kendall et al., 2004).

Dietary fiber could reduce the incidence of colorectal cancer through several mechanisms. The increase in fecal bulk dilutes fecal carcinogens or procarcinogens in the large bowel. Fecal bulk also stimulates faster rate of passage through the large bowel. The dilution effect and the faster rate of passage in the colon reduce the exposure of the colonic mucosa to carcinogenic compounds, potentially reducing colonic damage (Lunn and Buttriss, 2007). Butyrate, one of the short-chain fatty acids produced during fermentation, also possesses protective effects against the development of cancer by inducing apoptosis on colon cancer cells (Wong et al., 2006). Additionally, the reduction in colonic pH reduces the solubility of bile acids and may inhibit bacterial 7α-dehydroxylase, which degrades primary bile acids (cholic acid and chenodeoxycholic acid) to secondary bile acids (deoxycholic acid and lithocholic acid; Wong et al., 2006). High concentration of secondary bile acids in the colon and in the feces increases the risk of colorectal cancer (Chaplin, 1998).

Effects of Dietary Fiber on Pig nutrition and Gut Health

Gut health is broadly defined as having 3 components - diet, gastrointestinal mucosa, and intestinal microflora (Montagne et al., 2003). Dietary fiber is known to interact with the intestinal mucosa, especially with the mucus layer that protects the epithelium from physical and

enzymatic insults existing in the gut lumen (Lien et al., 2001). Dietary fiber may also influence the intestinal microflora by being a preferred substrate for some population of bacteria, but not of others (Mul and Perry, 1994). The interaction of dietary fiber with intestinal mucin secretion and the intestinal microflora may, therefore, influence the digestibility of dietary nutrients and gut health.

Effects of Dietary Fiber on Gut Health. The large intestine is composed of a diverse range of organisms (Mul and Perry, 1994; Nyachoti et al., 2006). The quantity and type of bacterial population in the large intestine differs depending on several factors including age, physiological state, and diet composition (Montagne et al., 2003). Although the microflora in the large intestine convert undigested proteins and carbohydrates to short-chain fatty acids that can be absorbed by the host and utilized for energy, some populations of bacteria, such as *E. coli* and *Clostridium* spp. may also produce toxins or harmful metabolites such as indoles and phenols that can cause gastrointestinal disease or intestinal damage (Montagne et al., 2003; Montagne et al., 2004). Reducing the population of these types of non-beneficial bacteria or increasing the population of beneficial bacteria such as *Bifidobacteria* spp. and *Lactobacillus* spp. may, therefore, improve gut health.

Dietary fiber, particularly nondigestible oligosaccharide such as fructo-oligosaccharide, has been reported to have prebiotic effects (Mul and Perry, 1994). Prebiotics are non-digestible food or feed ingredients that alter the microbial populations in the gut in favor of the growth of beneficial bacteria (i.e., *Bifidobacteria and Lactobacillus spp*.) that promote gut health (Martinez-Villaluenga et al., 2008). Fructo-oligosaccharides are the preferred substrate of *Bifidobacteria spp.* (Mul and Perry, 1994) and feeding fructo-oligosaccharide to dogs increased the population of bifidobacteria relative to controls (Grieshop et al., 2004). An increase in the

population of bifibobacteria was associated with reductions in the populations of *E. coli* and *C. perfringens* (Mul and Perry, 1994). Bifidin, the antibiotic produced by *Bifidobacterium bifidum*, is also effective against *Salmonella, Staphylococcus,* and other disease-causing bacteria (Martinez-Villaluenga et al., 2008). Therefore, including non-digestible oligosaccharides in the diets for pigs may improve gut heath and improve animal growth. However, the effects of feeding non-digestible oligosaccharide to pigs have not been as consistent as in human trials (Mountzouris et al., 2006). Some studies in pigs fed non-digestible oligosaccharides reported improved pig performance and reduced incidence of diarrhea, whereas others did not observed any beneficial effect of feeding non-digestible oligosaccharide (Mikkelsen et al., 2003). Rearing and feeding conditions, inclusion rate of the oligosaccharide, and age may influence response of pigs to dietary oligosaccharide inclusion (Mikkelsen et al., 2003).

Mannan-oligosaccharide was also reported to improve gut health by preventing the colonization of the gut with certain pathogenic organisms such as *E. coli* and S*almonella spp.* (Mul and Perry, 1994). Although, it is also a substrate for microbial fermentation, mannan-oligosaccharide present in the gut may serve as alternative binding sites for certain organisms (i.e., *E. coli* and *Salmonella spp.*) that use mannose as a way to bind to the gut mucosa (Mul and Perry, 1994). In the presence of mannan-oligosaccharide, *E. coli* and *Salmonella spp.* can bind to the mannose in the oligosaccharide instead of the mannose-containing glycoprotein in the gut mucosa. Attachment of *E. coli* and *Salmonella spp.* to the gut mucosa is, therefore, minimized or prevented, avoiding the colonization of the gut with pathogenic microorganisms, and maintaining gut health (Mul and Perry, 1994).

Effects of Dietary Fiber on Nutrient Digestibility. Increasing fiber concentration by increasing the inclusion of wheat bran (0 to 40%), maize bran, soybean hulls, and sugar beet

pulp in the diet progressively decreased total tract energy digestibility (Wilfart et al., 2007; Le Gall et al., 2009). The reduction in dietary energy digestibility was associated with a reduction in dry matter, organic matter, and carbohydrate digestibility (Wilfart et al., 2007; Le Gall et al., 2009). The degree of energy reduction was calculated to be 1% for each 1% increase in NDF concentration (Le Gall et al., 2009). The solubility of the fiber influences energy digestibility because total tract digestibility of beet pulp was higher than soybean hulls (Mroz et al., 2000). The presence of lignin in the dietary fiber also reduce energy digestibility (Wenk, 2001).

Effects of Fiber on Amino Acid Digestibility. The effect of adding fiber sources in pig diets have been observed to disturb CP and AA digestibility. The addition of 7.5% citrus pectin to a soybean meal-cornstarch based diet reduced apparent ileal digestibility (AID) of CP and AA by as much as 8.2 to 28.7 percentage units (Mosenthin et at., 1994). A reduction in the standardized ileal diegestibility (SID) of CP and AA was also observed when 4 or 8% apple pectin was added on a wheat-corn-SBM based diet (Buraczewska et al., 2007). A linear decrease in ileal N digestibility was observed when a purified neutral detergent fiber (NDF), processed from wheat bran, was added at increasing concentration in a soy isolate-cornstarch based diet fed to pigs (Schulze et al., 1995). Adding 15% of a similar purified wheat NDF also reduced AID of AA by 2 to 5.5 percentage units except for the AID of Cys, Ala, and Gly which were reduced by 18, 16, and 12 percentage units, respectively (Lenis et al., 1996). Similar results were reported by Dilger et al. (2004). Increasing the concentration of NDF from 2.72 to 4.16% by adding graded levels of soyhulls (3 to 9%) to soybean meal-cornstarch based diets also induced a linear or quadratic reduction in AID and SID of most AA. However, when 10% cellulose and barley straw were added to a SBM-CS based diets, the AID of AA except Leu and Gly was not reduced (Sauer et al., 1991). Such a reduction in AID of CP and AA was also not observed when graded

levels of Solkafloc (4.3 to13.3%) were added to a SBM-CS based diet fed to young pigs (Li et al., 1994). In contrast, when carboxymethylcellulose was added to the diets, SID of CP and AA increased (Larsen et al., 1994; Bartelt et al., 2002; Fledderus et al., 2007). Insoluble and poorly fermentable fibers such as cellulose impact CP digestibility through their water holding property, whereas soluble fibers may mediate their effect via effects on viscosity, as in the case of carboxymethylcellulose, or via viscosity and fermentability, as in the case of pectin.

Dietary fiber can reduce the efficiency of CP and AA utilization by impairing the digestion process, decreasing CP absorption, or increasing endogenous CP and AA loss (Mosenthin et al., 1994). Certain physico-chemical characteristics of fibers, particularly solubility, viscosity, water holding capacity, and fermentability were suggested to have an impact on CP and AA endogenous loss (Souffrant, 2001, Bartelt et al., 2002).

When a diet with 20% purified wheat bran NDF was fed to pigs, Schulze et al. (1995) observe an increased in ileal N flow with 59% of the N of endogenous origin. Adding graded levels of pea inner fibers to protein-free diets caused an exponential increase in ileal N flow and this was correlated with increasing water holding capacity of the diet (Leterme et al., 1998). The ileal flow of epithelial cells also increased exponentially with a corresponding linear increase in crude mucin and bacteria (Leterme et al., 1998). When a viscous and non fermentable fiber (carboxymethylcellulose) was added, mucin secretion and endogenous N loss also increased but without a change in the ileal bacterial population (Bartelt et al., 2002; Piel et al., 2005). However, an increase in certain ileal populations of bacteria was observed by Owusu-Asieda et al. (2006) when viscous and fermentable fibers such as guar gum was fed to pigs. In contrast, adding cellulose, an insoluble and poorly fermentable fiber, at 3.31 to 16.5% to the diet did not induce an increase in endogenous CP and AA loss which may be the reason for the absence of a

reduction in the AID of CP and AA of the diets when cellulose was added (Li et al., 1994). The level and the source of dietary fiber are two important factors that influence endogenous CP and amino acid loss (Sauer and Ozimek, 1986); therefore, the inclusion of cellulose may become important only when a certain threshold level is exceeded (Li et al., 1994). Furuya and Kaji (1992) did observe a tendency for most endogenous AA and nitrogen to increase with increasing cellulose level.

The effect of fibers on pancreatic secretions and enzyme activity may also be modulated by the physico-chemical properties of fiber. Barley- or wheat-based diets increased bile and pancreatic juice secretions compared with cornstarch-casein-cellulose based diets without affecting enzyme output (Low, 1989). However, when 400 g of wheat bran was added to an isonitrogeous and isocaloric diet, chymotrypsin and trypsin secretions were higher than diets without wheat bran added (Langlois et al., 1986). In contrast, the addition of pectin did not increase pancreatic secretions or affect secretions and enzyme activities of trypsin and chymotrypsin (Monsenthin et al., 1994), whereas the addition of carboxymethylcellulose reduced pepsin activity in the stomach without affecting trypsin and chymotrypsin enzyme activities (Larsen et al., 1993).

Effects of Fiber on Fat Digestibility. The addition of 20 and 40% wheat bran to a cereal-based diet lowered total tract digestibility of ether extract by 7 to 12 percentage units compared with control diets (Wilfart et al., 2007). The addition of beet pulp to a basal diet also reduced ileal and total tract digestibility of fat whereas, the addition of wheat bran did not (Graham et al., 1986). In contrast, adding a combination of triticale, wheat, and wheat bran as a source of non-starch polysaccharide on cereal-based diets improved ileal and total tract digestibility of fat compared with the control diet (Högberg and Lindberg, 2004). This suggests that the solubility

of diets containing different sources of non-starch polysaccharides influence fat digestibility because when a mixture of wheat bran, maize bran, soybean hulls, and sugar beet pulp was added at graded levels to a low fiber diet, total tract fat digestibility was not affected despite increasing levels of total dietary fiber concentration (Le Gall et al., 2009). The level of dietary fiber inclusion also influences lipid digestibility because decreasing fat digestibility was observed as coconut expeller, soybean hulls, or sugar beet pulp was added at graded levels in the diet (Canh et al., 1998).

Effects of Fiber on Mineral Digestibility. Dietary fiber is composed of polysaccharides that have the capability to bind minerals (Kritchevsky, 1988); however, the results of studies on the effect of dietary fiber on mineral digestibility are not consistent. The addition of 6% cellulose depressed the apparent absorption of calcium, phosphorus, magnesium, and potassium (Metzler and Mosenthin, 2008). Serum concentrations of calcium, phosphorus, copper, and zinc per unit of mineral ingested were also lower in sows fed high fiber diets containing a combination of corn cobs and wheat bran or oats and oat hulls compared with corn-soybean meal diets (Girard et al., 1995). In contrast, the addition of oat hulls, soybean hulls, and alfalfa meal did not affect total tract Ca, P, Zn, or Mn absorption or retention (Moore et al., 1988). A similar observation was reported by Vanhoof and De Schrijver (1996) where ileal and fecal absorption and percentage retention of calcium, phosphorus, magnesium, and zinc was not affected by the addition of 6% inulin to the control diet fed to pigs. Ileal ash digestibility was also not affected by the addition of 20 or 40% wheat bran to the low fiber diet but fecal ash digestibility was reduced at high concentration of wheat bran in the diet (Wilfart et al., 2007).

The presence of phytate, which is closely associated with and frequently a component of dietary fiber, was suggested to be a confounding factor in many of the studies that involved

dietary fiber and mineral interactions. Phytate has a greater effect on mineral digestibility than dietary fiber itself, making the studies less conclusive with regard to the effect of dietary fiber on mineral digestibility (Harland, 1989; Harland and Oberleas, 2001) . On the other hand, some studies have shown an improvement in calcium and magnesium absorption, and this was associated with the addition of soluble and fermentable fibers that are able to reduce hind gut pH sufficiently to solubilize minerals and facilitate absorption in the hind gut (Harland, 1989; Vaquero et al., 2000).

PIGS AS MODELS FOR HUMAN GASTROINTESTINAL FUNCTION AND DISEASE

Animal models have been extensively used to study the development of various diseases that occur in man. The underlying concept of using animal models in biomedical research is that the results obtained also apply to humans. Genetic, biological, physiological, and anatomical differences exist between humans and the different animal models available (Baker, 1998). Thus, the animal model of choice must have characteristics that are similar to humans with respect to the specific area of research.

The use of rodents (rats and mice) for biomedical research has dominated over the years because they are relatively easy to handle, inexpensive to maintain, they have a short life span, and their genome is sequenced which facilitates faster turnover of rats or mice that are genetically modified (Corpet and Pierre, 2005; Spurlock and Gabler, 2008). However, rodents and humans have major differences in terms of nutrient and energy digestion and the anatomy of the gastrointestinal tract that may hinder the translation of results from experiments with rats or mice, to humans. Other major differences between rodents and humans include differences in eating behaviors (especially coprophagy in rats), energy expenditure relative to body size, life

span and body proportion, as well as differences in intestinal morphology and gut microbial population (Greger, 1992; Corpet and Pierre, 2005). Rats are also cecal fermenters whereas humans are colonic fermenters (Graham and Aman, 1987). Most of the mechanisms that are known today that explain the development of disease in man have been obtained through the use of rodents, but the usefulness of rodent models to predict what happens in humans has its limitations. For example, whereas wheat bran protected rats and mice from colonic tumors, such a protection was not observed in man (Corpet and Pierre, 2005). Resistin, an adipokine released by fat cells in a strain of obese mice, which causes insulin resistance, is only secreted in small amounts in obese and diabetic humans (Nagaev and Smith, 2001; Arner, 2005). Patho-physiological changes in rats are seldom observed in human disease state (Doggrell and Brown, 1998).

The young pig (*Sus scrofa domestics)* has been used as a model for human infants since the late 1960s. Young pigs and infant humans are composed of 82% water, 13-14% CP, and 3% ash (CP and ash calculated at a fat free basis; Patterson et al., 2008). The concentration of some electrolytes in young pigs is also within the range observed in human infants at birth (Patterson et al., 2008). Because the digestive physiology and nutrient requirements of the young pig are similar to that of human babies, infant formulas have been developed and evaluated using young pigs with the assumption that formulas that are suitable for young pigs are also suitable for human infants (Pond and Houpt, 1978). Recently, the pig is also recognized as a more suitable animal model for studying the interaction between diet and disease in human adults than rats (Spurlock and Gabler, 2008). Pigs have been used to study mechanisms for diabetes, obesity, and cardiovascular disease (Boullion et al., 2003; Bellinger et al., 2006; Spurlock and Gabler, 2008). Both farm and miniature breeds have been used in biomedical research but for chronic

studies, the relatively slow growing miniature breeds (Yucatan, Hanford and Gottingen) are widely used (Swindle and Smith, 2000) because of easier handling than farm pigs (Pond and Houpt, 1978).

There are several reasons why pigs are good models for humans. Just like humans, pigs are omnivorous and have the tendency for sedentary behavior (Reeds and Odle, 1996). The morphology and physiology of the gastrointestinal tract in humans is similar to that in pigs (Miller and Ullrey, 1987). Size and cellular anatomy of tissues taken from the pigs' stomach, duodenum, jejenum, ileum, and colon closely resemble that of humans (Kurihara-Bergstrom et al., 1986). The type of circular folds (plica circularis) that project into the small intestinal lumen of pigs are also similar to that in humans, but is not present in rats. Further, the height and shape of the pig villus is not very different between humans and pigs (villus height in pigs 0.41 to 0.77 mm vs. villus height in humans 0.5 to 0.8 mm (Kurihara-Bergstrom et al., 1986). Although the length of the small intestines and the large intestines in pigs (15 to 22 m and 4 to 6 m, respectively) are longer than in humans (5.5 to 7 and 1.5 m, respectively), the ratio of the total length of the small and large intestines relative to BW is 0.1 m /kg BW in both pigs and humans (Patterson et al., 2008).

There are also similarities in carbohydrate and CP metabolism between pigs and humans (Fleming and Arce, 1986). Except for Thr, Tyr, Phe, and Cys, the true ileal digestibility of the other AA were not different between 25- kg pigs and human adults (Rowan et al., 1994). Ileal and total tract digestibility of DM, and energy digestibility were also not different between pigs and humans (Rowan et al., 1994).

Pigs may have a greater capacity to ferment dietary fiber than man because of the presence of a caecum, and a greater microbial population in pigs than in man (Graham and

Aman, 1987). Humans degrade dietary fiber less effectively than pigs and this is reflected in a reduced concentration of SCFA in humans than in pigs (Ehle et al., 1982). The ability for pigs to better degrade dietary fiber than humans may also be contributed to a longer digesta retention time in pigs (50 h) than in man (40 h; Graham and Aman, 1987; Van Soest et al., 1982). However, estimates of mean retention time of 52-54 h in both man and pigs have also been observed (Ehle et al., 1982). Ingesta transit time was also noted to be comparable between humans and pig (Miller and Ullrey, 1987).

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CHAPTER 3

Energy Digestibility of 4 Novel Fiber Sources Fed to Growing Pigs

ABSTRACT: An experiment was conducted to measure the apparent total tract digestibility (ATTD) of total carbohydrates and the DE and ME of maltodextrin (MD), 2 sources of resistant starch (RS 60 and RS 75), soluble corn fiber (SCF), and pullulan. A total of 72 castrated male pigs (initial BW: 22.0 ± 1.2 kg) were housed in metabolism cages that allowed for total but separate collection of feces and urine and assigned to 6 treatments with 12 replicate pigs per treatment. A basal diet based on corn, soybean meal, and casein was formulated. Five additional diets were prepared by replacing 10% of the basal diet with MD, RS 60, RS 75, SCF, or pullulan. The daily feed allowance was calculated as 2.5 times the estimated energy requirement for maintenance and pigs were fed 2 equal meals every day. Following a 7-d adaptation period, feces and urine from all pigs were collected quantitatively during a 5-d period using the marker to marker procedure. The DE and ME for each ingredient were calculated using the difference procedure. The DE and ME of RS 60 (1,779 and 1,903 kcal/kg, respectively), RS 75(1,784 and 1,677 kcal/kg, respectively), and SCF (1,936 and 1,712 kcal/kg, respectively), were less ($P < 0.05$) than of MD (3,465 and 3,344 kcal/kg, respectively) and pullulan (2,755 and 2,766 kcal/kg, respectively) and pullulan contained less ($P < 0.05$) DE and ME than MD. However, there was no difference in the DE and ME for RS 60, RS 75, and SCF. The present results indicate that resistant starch and soluble corn fiber can be used as low energy carbohydrate sources.

Key words: resistant starch, soluble corn fiber, pullulan, digestible energy, metabolizable energy, pig

INTRODUCTION

There is a positive association between the intake of dietary fiber and human health (Jenkins and Kendall, 2000). Although dietary fiber is believed not to contribute to nutrient absorption in the small intestine because of its resistance to digestion by mammalian enzymes, dietary fibers are fermented by the microbial population in the cecum and colon where energy in the form of short-chain fatty acids is produced and absorbed (Elia and Cummings, 2007).

The need for increasing dietary fiber intake in humans has led to the development of novel carbohydrates that have properties similar to dietary fiber. Resistant starch (**RS**), which is starch and starch derivatives that are not digested in the small intestine, are already commercially available for food application (Brown, 2004). Soluble corn fibers (**SCF**) are composed of sugars derived from corn starch hydrolysis and contain less than 20% simple sugars (Kendall et al., 2008). Gums (i.e., pullulan) are complex polysaccharides that have glucose units that are not linked with α – bonds (Southgate, 2001). Gums are, therefore, indigestible by mammalian enzymes and are considered dietary fiber.

Government regulations assign a specific caloric values to dietary fibers however, these assigned caloric values may not be suitable for novel carbohydrates (Livesey et al., 2000). Estimates of DE and ME in new sources of dietary fiber are, therefore, needed to obtain a reliable caloric value for these carbohydrate sources. The objective of this experiment was to measure the DE and ME and the apparent total tract digestibility (**ATTD**) of carbohydrates of 4 novel fiber sources (i.e., 2 sources of RS, 1 source of SCF, and 1 source of pullulan) that have been developed for the human food industry.

MATERIALS AND METHODS

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

Animals, Housing, and Experimental Design

A total of 72 growing barrows (BW: 22.0 ± 1.2 kg) that were the offspring of line 337 boars mated to C-22 females (Pig Improvement Company, Hendersonville, TN) were individually housed in metabolism cages that were equipped with a feeder and a nipple drinker. Cages were also equipped with screens and funnels to allow for total, but separate, collection of feed refusals, urine, and feces. Pigs were randomly allotted to a completely randomized design with 6 diets and 12 replications per diet.

Ingredients, Diets, and Feeding

Four novel sources of fiber were used in this experiment (Table 3.1). Two of these fibers were resistant starches containing 60% and 75% total dietary fiber, (RS 60 and RS 75, respectively). The other 2 sources were SCF and pullulan. Maltodextrin (**MD**) was also used. All the ingredients were supplied by Tate and Lyle (Decatur, IL).

Six diets were formulated (Tables 3.2 and 3.3). The basal diet was a corn-soybean meal diet that also contained casein, soybean oil, vitamins, and minerals. The remaining 5 diets were prepared by mixing each of the 5 carbohydrate sources and the basal diet in a 1:9 ratio.

The daily feed allowance was calculated as 2.5 times the estimated maintenance requirement for energy (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998). The daily feed was divided into 2 equal meals that were provided at 0800 and 1500. Water was available at all times. Pigs were fed their assigned diets during a 14-d period.

Data and Sample Collection

Pig BW were recorded at the beginning of the experiment and the amount of feed provided each day was also recorded. Pigs were allowed 7 d to adapt to the experimental diet followed by 5 d of total collection of unconsumed feed, feces, and urine using the marker to marker approach (Adeola, 2001). Two grams of chromic oxide were added to the diet in the morning meal on d 8 and 2 g of ferric oxide were added to the morning meal on d 13. Fecal collection started upon appearance of chromic oxide in the feces and ceased upon appearance of ferric oxide in the feces. Feces were collected as soon as voided and stored at -20°C. Urine collection started on d 8 at 0800 and ceased on d 13 at 0800. Pre-weighed urine buckets were placed under the metabolism cages to allow for total collection. Buckets were weighed and emptied every morning and a preservative of 50 mL of 6 *N* HCl was added to the buckets each time they were emptied. Twenty percent of the daily urine collections were stored at -20°C.

At the end of the experiment, urine samples were thawed and mixed within animal and a subsample was collected for lyophilization and subsequent analysis. Fecal samples were dried at 57°C in a forced air oven and finely ground before a subsample was collected for chemical analysis.

Sample Analysis

Diets, ingredients, and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2005), CP (method 990.03; AOAC Int., 2005), and ash (method 942.05; AOAC Int., 2005). Total fat was analyzed by hydrolyzing the samples with 3 *N* HCl followed by ether extraction as described by Sanderson (1986). Gross energy of feces and lyophilized urine samples were determined using bomb calorimetry (model 6300, Parr Instruments, Moline, IL).

Calculations and Statistical Analysis

The DE and ME of each diet was calculated by subtracting the energy excreted in the feces, and the energy excreted in the feces and urine, respectively, from GE intake (Adeola, 2001). Using the difference approach, the DE and the ME contribution of the basal diet was subtracted from the DE and ME of the treatment diets to calculate the contribution of DE and ME from the test ingredients. By dividing this value by 0.10, the DE and ME of the test ingredients were calculated. By further correcting the DE and ME of the test ingredients for their respective DM concentration, the DE and ME of each ingredient was calculated on a DM basis.

The concentration of total carbohydrate was calculated by subtracting the concentration of CP, acid hydrolyzed ether extract, and ash from the concentration of DM in the diet and fecal samples. Apparent total tract digestibility of total carbohydrates was calculated by dividing the difference between the concentration of total carbohydrate consumed and the concentration of total carbohydrate excreted in the feces by the concentration of total carbohydrate consumed and expressed as a percent (Adeola, 2001).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure was used to verify homogeneity of the variances. Outliers were determined as values that deviated from the treatment mean by more than 1.5 times the interquartile range (Devore and Peck, 1993). Nine outliers were identified and removed from the data set. An ANOVA was conducted, with diet as the main effect and pig and period as random effects. Differences among treatment means were separated using the LSMEANS statement and the PDIFF option of PROC MIXED. The pig was the experimental unit for all analyses and an

alpha value of 0.05 was used to assess significance among treatments. An alpha value of less than 0.10 was used to assess a tendency towards significance.

RESULTS

Composition of Ingredients and Diets

The concentration of CP, acid hydrolyzed ether extract, and ash were less than 1% in all the novel fiber sources and in MD (Table 3.1). As a result, the concentration of total carbohydrate ranged from 89 to 96% in all ingredients (as-fed basis). The GE of MD was 3,914 kcal/kg, and the GE of RS 60, RS 75, SCF, and pullulan ranged between 3,738 and 3,849 kcal/kg.

The inclusion of 10% MD, RS 60, RS 75, SCF, and pullulan to the basal diet reduced the concentration of CP, acid hydrolyzed ether extract, and ash (Table 3.3). Gross energy was also lower in the diets containing MD, RS 60, RS 75, SCF, or pullulan than in the basal diet.

Energy and Carbohydrates in Diets

Gross energy intake among pigs fed diets containing MD, RS 60, RS 75, SCF, and pullulan did not differ from each other, but these values were greater ($P < 0.01$) than the GE intake of pigs fed the basal diet (Table 3.4). Fecal excretion of GE in pigs fed diets containing RS 60 and RS 75 was greater ($P < 0.01$) compared with the fecal energy excretion of pigs fed the diet containing SCF, but the fecal excretion of GE by pigs fed diets containing MD and pullulan was lower ($P < 0.01$) than the fecal excretion of GE from pigs fed the diet containing SCF. Pigs fed diets containing MD and pullulan excreted the same amount of GE, which was not different from the fecal energy excretion of pigs fed the basal diet. Urinary energy excretion of pigs fed diets containing SCF (94 kcal/kg) tended to be greater ($P = 0.07$) than for pigs fed diets containing RS 60 (64 kcal/kg), but no differences among pigs fed the other diets were observed.

The ATTD of GE in diets containing pullulan (88.9%), MD (89.5%), and the basal diet (89.0%) were not different from each other, but were greater ($P < 0.01$) than the ATTD of GE in diets containing SCF (87.6%). The lowest ($P < 0.01$) ATTD of GE (85.8%) was calculated for the 2 diets containing RS 60 and RS 75.

The DE and ME of diets containing MD (3,635 and 3,537 kcal/kg) was not different from the DE and ME of the basal diet (3,656 and 3,562 kcal/kg), but greater ($P < 0.01$) than the DE and ME of the diets containing pullulan (3,564 and 3,479 kcal/kg) and all other diets. The DE and ME of diets containing RS 60 (3,466 and 3,393 kcal/kg), RS 75 (3,467 and 3,370 kcal/kg), and SCF (3,482 and 3,374 kcal/kg) were not different from each other, but these values were lower ($P < 0.01$) than the DE and ME of the basal diet and the diets containing pullulan and MD.

The daily carbohydrate intake of pigs fed diets containing MD, RS 60, RS 75, SCF, and pullulan (range: 664 to 683 g) were not different from each other, but were greater ($P < 0.01$) than the daily carbohydrate intake of pigs fed the basal diet (580 g). However, daily carbohydrate fecal excretion was greater ($P < 0.01$) by pigs fed diets containing RS 60 and RS 75 (68 g and 65 g, respectively) than for pigs fed the other diets. Daily fecal excretion of carbohydrate for pigs fed diets containing pullulan (41 g) was not different from the daily fecal carbohydrate excretion of pigs fed the basal diet (36 g) or diets containing MD (39 g), but these values were lower ($P < 0.01$) than the daily fecal carbohydrate excretion observed for pigs fed the diet containing SCF (51 g).

Apparent total tract digestibility of total carbohydrates by pigs fed the diet containing pullulan (94.9%) was not different from the ATTD of total carbohydrates by pigs fed the diet containing MD (93.7 %). The ATTD of total carbohydrates by pigs fed the diet containing SCF (92.3%) was lower ($P < 0.01$) than by pigs fed diets containing pullulan and MD, but greater (*P*

< 0.01) than the ATTD of total carbohydrates in diets containing RS 60 (90.1%) and RS 75 (90.2%).

Energy and Carbohydrates in Ingredients

The DE and ME of MD (3,465 and 3,344 kcal/kg, respectively) were greater ($P < 0.01$) than the DE and ME of all other ingredients (Table 3.5). The DE and ME of pullulan (2,755 and 2,766 kcal/kg) was also greater ($P < 0.01$) than the DE and ME of RS 60 (1,779 and 1,903 kcal/kg, respectively), RS 75 (1,784 and 1,677 kcal/kg, respectively), and SCF (1,936 and 1,712 kcal/kg, respectively). No differences were observed in the DE and ME values among RS 60, RS 75, and SCF. When calculated on a DM basis, the DE and ME of MD and the novel fiber sources followed a similar pattern.

Daily total carbohydrate intake was not different among ingredients. However, daily fecal excretion of carbohydrates was greater ($P < 0.01$) for RS 60 (346 g) and RS 75 (325 g) compared with the other sources of fiber. Daily fecal carbohydrate excretion for SCF (184 g) was greater ($P < 0.01$) than the value observed for pullulan (84 g) and MD (62 g).
No differences were observed in the ATTD of total carbohydrates between RS 60 (78%) and RS 75 (76.2%) but these values were lower than the ATTD of total carbohydrates in the other fiber sources. The ATTD of total carbohydrates in pullulan (96.0%) was not different from the ATTD of total carbohydrates in MD (96.0%), but these values were greater ($P < 0.01$) than the ATTD of total carbohydrates in SCF (87%).

DISCUSSION

Novel fibers, such as RS 60, RS 75, SCF, and pullulan, are chemically or physically processed carbohydrates that are manufactured to provide concentrated levels of dietary fiber (Brown, 1994). They meet the definition established by the American Association of Cereal

Chemists for dietary fiber in terms of carbohydrate structure, resistance to small intestinal digestion, and associated physiological benefits (De Vries, 2004; Kendall et al., 2008).

Implied in the definition of dietary fiber is that it should have a low caloric value. Currently, the caloric value assigned to dietary fiber is based on Atwater conversion factors (De Vries, 2004) and may range from 0 to 4,000 kcal/kg. In the U. S., insoluble dietary fiber is assigned a caloric value of 0 kcal/g whereas soluble dietary fiber is assigned a caloric value similar to that of digestible carbohydrate at 4,000 kcal/kg (de Vries, 2004). In Australia, dietary fibers are assigned a caloric value of 1,800 kcal/kg and in Japan, dietary fibers, including fermentable fibers, are assigned a caloric value of 2,000 kcal/kg (Goldring, 2004). Given the physico-chemical and physiological properties of dietary fiber, and specifically novel fibers, the use of the Atwater conversion factors may be misleading (Livesey et al., 2000). Therefore, it is important to provide estimates of DE and ME for each of the novel fiber sources, not only for labeling purposes, but as a guide for its correct usage in "low calorie" diets or preparations.

Maltodextrin is a product of enzymatic or acidic hydrolysis of starch that is produced from cereal grains (Wang and Wang, 2000). It has a dextrose equivalent of of 10, and is mainly composed of digestible carbohydrates (Stewart et al., 2010). Maltodextrin is therefore often used as a control in experiments that aim at studying the physiological characteristics of novel fiber sources (Spears et al., 2005; Goda et al., 2006; Knapp et al., 2008). The greater DE and ME of MD compared with the DE and ME of novel fiber sources is, therefore, expected. In poultry, the true metabolizable energy of MD ranged from 3,690 to 4,060 kcal/kg (as-fed basis; White et al., 1988; Knapp et al., 2008). No comparable value is available for swine and to our knowledge, this is the first reported value for DE and ME of MD by pigs.

Resistant starch is defined as starch and starch derivatives that are not digested in the small intestines and, therefore, do not release glucose for absorption in the small intestine (Brown, 2004). Resistant starch has 4 subtypes. Type 1 RS is the physically inaccessible form of starch common in whole or coarsely milled grain. Type 2 RS refers to native starch granules that resist digestion because of the structure or configuration of the starch granule. Type 3 RS are retrograded starch formed during the cooling of gelatinized starch and type 4 RS are chemically modified starch (Brown, 2004).

Resistant starch 60 and RS 75 contain mainly Type 3 RS. It is produced by heating moisture-treated- high-amylose corn starch (Stewart et al., 2010). A covering around the semi-crystalline particle developed during processing of RS 60 and the high degree of crystallinity in RS 75 makes these starches resistant to enzymatic digestion and they are both considered insoluble fibers (Kendall et al., 2008).

The ME values for RS 60 (1,903 kcal/kg) and RS 75 (1,677 kcal/kg) that were measured in the current experiment are lower than the ME values (3,010 and 3,360 kcal/kg) for type 2 and type 3 RS that have previously been measured in pigs (De Schrijver et al. 1999), but they agree with the true metabolizable energy for type 3 RS in roosters (1,890 kcal/kg; Knapp et al., 2008). In humans, the energy value of RS was calculated at 2,720 kcal/kg (Elia and Cummings, 2007). Species differences may play a role in the wide range of caloric values for RS (De Schrijver et al., 1999), but differences among RS products also exist. The various processing techniques used to produce RS, the amount of dietary fiber it contains, the structure and conformation of the starch granule, the source of starch, and the proportion of RS to digestible carbohydrates may determine the digestibility of GE in this type of fiber (Livesey, 1990; Goldring, 2004). A single

caloric value for all RS products may, therefore, not be applicable because different RS may have different physiological properties (Ferguson et al., 2000; Brown, 2004).

Only 78.0 and 75.9% of the carbohydrates in RS 60 and RS 75 contributed to the caloric value of these dietary fibers. The low digestibility of carbohydrates may explain the low DE and ME values in RS 60 and RS 75 compared with the DE and ME in MD. This observation supports results of other studies that reported lower post-prandial glucose and insulin concentration when RS was included in beverages consumed by humans than if glucose was consumed (Kendall et al., 2008). A similar observation was reported in dogs (Knapp et al., 2008).

Soluble corn fiber is a product of corn starch hydrolysis. It has an average degree of polymerization of 10 (Stewart et al., 2010) and its resistance to digestion may be attributed to the predominance of α-1,6 glycosidic linkages present in the carbohydrate moiety as well as glycosidic linkages in the carbon 2 and carbon 3 positions that are also resistant to digestion (Kendall et al., 2008). Pullulan is a polysaccharide that is a product of bacterial fermentation. It has a degree of polymerization of 3,000 and a molecular weight of 486,000 (Stewart et al., 2010). Both SCF and pullulan are soluble dietary fibers that also reduce post- prandial glucose and insulin concentration (Kendall et al., 2008; Knapp et al., 2008).

The caloric value of a dietary fiber is dependent on the proportion of carbohydrates that are susceptible to digestion in the small intestine, the amount of substrate that is not digested in the small intestine, and the degree of fermentability of the carbohydrates that enter the large bowel (Meyer, 2004). The fact that the DE and ME in SCF was not greater than the DE and ME in RS 60 and RS 75 despite a greater ATTD of carbohydrate in SCF than in RS 60 and RS 75 suggests that a greater proportion of the carbohydrate in SCF were fermented in the large bowel.

Likewise, the lower DE and ME of pullulan than of MD despite a similar ATTD of carbohydrates with MD suggests that more carbohydrates in pullulan were fermented in the large bowel.

Fermentation is associated with the production of short chain fatty acids (mainly acetate, propionate, and butyrate). These short-chain fatty acids can also provide energy to the pig, but the absorption and subsequent oxidation of short-chain fatty acids provides less energy than if energy is absorbed as monosaccharides in the small intestine (Davies et al., 1991). Therefore, both enzymatic digestion and large bowel fermentation may contribute to the DE and ME of all the novel fibers.

The ME of SCF (1,712 kcal/kg) obtained in this study is consistent with the ME of other corn-based soluble fibers that were measured in roosters (Fastinger et al., 2007). Likewise, the DE of pullulan (2,755 kcal/kg) obtained in this study is similar to the calculated DE of 2,800 kcal/g for guar gum (Livesey, 1990).

In conclusion, the DE and ME of RS 60, RS 75, SCF, and pullulan ranged from 1,779 to 2,755 kcal/kg, and from 1,677 to 2,766 kcal/kg, respectively. The varying degrees of small intestinal digestibility and differences in fermentability among these novel fibers may explain the differences in the DE and ME values.

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Table 3.1. Analyzed energy and nutrient composition of maltodextrin, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis[1]

	Ingredients				
Item	Maltodextrin	Resistant starch 60[2]	Resistant starch 75[2]	Soluble corn fiber	Pullulan
DM, %	97.41	90.07	92.99	93.92	94.80
CP, %	0.74	0.68	0.70	0.44	0.44
Ash, %	0.19	0.04	0.10	0.10	0.04
Total carbohydrates[3], %	96.48	89.35	92.19	93.38	94.32
TDF, %	1.20	62.90	75.60	10.00	85.40
GE, kcal/kg	3,914	3,738	3,834	3,760	3,849

[1] Samples were analyzed for acid hydrolyzed ether extract but none was detected.

[2] Resistant starch 60 and resistant starch 75 contain 60 and 75% total dietary fiber, respectively.

[3]Total carbohydrates = DM – CP – ash.

Table 3.2. Ingredient composition (%) of experimental diets containing maltodextrin, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis

	Diet					
Ingredient	Basal	Malto-dextrin	Resistant starch 60[1]	Resistant starch 75[1]	Soluble corn fiber	Pullulan
Corn	53.88	48.48	48.48	48.48	48.48	48.48
Soybean meal, 48%	21.50	19.35	19.35	19.35	19.35	19.35
Sucrose	15.00	13.50	13.50	13.50	13.50	13.50
Casein	5.00	4.50	4.50	4.50	4.50	4.50
Soybean oil	2.00	1.80	1.80	1.80	1.80	1.80
Vitamin-mineral premix[2]	0.33	0.30	0.30	0.30	0.30	0.30
Limestone	0.95	0.86	0.86	0.86	0.86	0.86
Dicalcium phosphate	0.90	0.81	0.81	0.81	0.81	0.81
Salt	0.44	0.40	0.40	0.40	0.40	0.40
Maltodextrin	-	10.00	-	-	-	-
Resistant starch 60	-	-	10.00	-	-	-
Resistant starch 75	-	-	-	10.00	-	-
Soluble corn fiber	-	-	-	-	10.00	-
Pullulan	-	-	-	-	-	10.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1] Resistant starch 60 and resistant starch 75 contain 60 and 75% total dietary fiber, respectively.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 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 3.3. Analyzed energy and nutrient composition of experimental diets containing maltodextrin, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis

	Diet					
Item	Basal	Malto-dextrin	Resistant starch 60[1]	Resistant starch 75[1]	Soluble corn fiber	Pullulan
DM, %	88.91	89.53	88.82	88.89	88.64	89.03
CP, %	19.60	17.18	17.53	17.53	17.75	16.37
Ash, %	4.28	3.79	3.68	3.65	3.72	3.83
Acid hydrolyzed EE[2], %	4.72	4.28	4.35	4.42	4.61	4.56
Total carbohydrates[3], %	60.30	64.28	63.26	63.28	62.57	64.27
GE, kcal/kg	4,106	4,061	4,038	4,039	3,974	4,010

[1] Resistant starch 60 and resistant starch 75 contain 60 and 75% total dietary fiber, respectively.

[2]Acid hydrolyzed ether extract .

[3]Total carbohydrates = DM – (CP + ash + acid hydrolyzed ether extract).

Table 3.4. Daily energy and carbohydrate balance of pigs fed diets containing maltodextrin, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as fed-basis[1]

	Diet							
Item	Basal	Malto-dextrin	Resistant starch 60[2]	Resistant starch 75[2]	Soluble corn fiber	Pullulan	SEM	*P*-value
GE intake, kcal	3,193 [b]	3,539 [a]	3,567 [a]	3,472 [a]	3,440 [a]	3,465 [a]	57.12	0.002
GE in feces, kcal	351 [c]	372 [c]	505 [a]	492 [a]	426 [b]	386 [c]	15.89	0.001
GE in urine, kcal	74	86	64	82	94	74	8.85	0.066
ATTD [3] of GE, %	89.0 [a]	89.5 [a]	85.8 [c]	85.8 [c]	87.6 [b]	88.9 [a]	0.45	0.001
DE of diet, kcal/kg	3,656 [a]	3,635 [a]	3,466 [c]	3,467 [c]	3,482 [c]	3,564 [b]	17.79	0.001
ME of diet, kcal/kg	3,562 [a]	3,537 [a]	3,393 [c]	3,370 [c]	3,374 [c]	3,479 [b]	23.77	0.001
Daily carbohydrate intake, g	580[b]	678 [a]	683 [a]	664 [a]	666 [a]	674 [a]	10.83	0.001
Daily carbohydrate in feces, g	36 [c]	39[c]	68 [a]	65 [a]	51 [b]	41 [c]	2.49	0.001
ATTD[3] of total carbohydrate, %	93.7 [a]	94.2 [a]	90.1 [c]	90.2 [c]	92.3 [b]	94.9 [a]	0.35	0.001

[a-c] Values within a row lacking a common superscript differ ($P \leq 0.05$).

[1]Data are least squares means of 12 observations per treatment.

[2]Resistant starch 60 and resistant starch 75 contain 60 and 75% total dietary fiber, respectively.

[3]ATTD = apparent total tract digestibility.

Table 3.5. Energy concentration and daily carbohydrate balance in maltodextrin, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan[1]

	Ingredient						
Item	Malto-dextrin	Resistant starch 60[2]	Resistant starch 75[2]	Soluble corn fiber	Pullulan	SEM	*P*-value
DE of ingredients, kcal/kg	3,465 [a]	1,779 [c]	1,784 [c]	1,936 [c]	2,755 [b]	183.9	0.001
DE of ingredients, kcal/kg DM	3,558 [a]	1,977 [c]	1,918 [c]	2,062 [c]	2,906 [b]	194.0	0.001
ME of ingredients, kcal/kg	3,344 [a]	1,903 [c]	1,677 [c]	1,712 [c]	2,766 [b]	241.0	0.001
ME of ingredients, kcal/kg DM	3,434 [a]	2,116 [c]	1,804 [c]	1,823 [c]	2,918 [b]	254.1	0.001
Daily carbohydrate intake, g	1,527	1,610	1,422	1,440	1,528	101.0	0.695
Daily carbohydrate in feces, g	62 [c]	346 [a]	325 [a]	184 [b]	84 [c]	25	0.001
ATTD[3] of carbohydrate, %	96.0 [a]	78.0 [c]	76.2 [c]	87.0 [b]	96.0 [a]	1.93	0.001

[a-c] Values within a row lacking a common superscript differ ($P \leq 0.05$).

[1]Data are least squares means of 12 observations per treatment.

[2]Resistant starch 60 and resistant starch 75 contain 60 and 75% total dietary fiber, respectively.

[3]ATTD = apparent total tract digestibility.

CHAPTER 4

Effect of Novel Fibers on Ileal and Total Tract Disappearance of Energy and Nutrients in Purified Diets Fed to Growing Pigs

ABSTRACT: The objective of the experiment was to determine the effect of novel fibers on the apparent ileal disappearance (AID), apparent total tract disappearance (ATTD), and hindgut disappearance (HGD) of GE, total dietary fiber (TDF), DM, CP, acid hydrolyzed ether extract (AEE), ash, and total carbohydrates in diets containing 4 sources of dietary fiber. A second objective was to measure the endogenous flow of TDF and calculate the standardized ileal (SID) and standardized total tract (STTD) disappearance of TDF in 4 novel fibers fed to pigs. The 4 sources of dietary fiber were 2 types of resistant starch (RS) containing 60% (RS 60) or 75% (RS 75) TDF, a soluble corn fiber (SCF), and pullulan. Cellulose was added as a negative control. A maltodextrin-casein based diet was formulated and 5 additional diets were prepared by replacing 10% maltodextrin in the control diet with 10% of each of the sources of dietary fiber. Twelve growing barrows (BW: 20.0 ± 2.8 kg) that were fitted with an ileal cannula were randomly allotted to a replicated 6 × 5 Youden square design with 6 pigs and 5 periods per square. Fecal samples were collected on d 6 and 7, and ileal samples were collected on d 8 and 9 of each period. Pigs were fed at 3.0 times the estimated requirement for maintenance and no pig was given the same diet twice. Results of the experiment indicated that the AID of GE in diets containing cellulose or the novel fibers were less ($P < 0.05$) than in the maltodextrin diet and the AID of GE in RS 75 was less ($P < 0.05$) than in RS 60, SCF, and pullulan, but the ATTD of GE was not different among diets. The AID of DM and total carbohydrates were also reduced ($P < 0.001$) when dietary fiber was added to the diets. The addition of RS 60, RS 75, and SCF did not affect the AID of AEE, CP, or ash, but the addition of cellulose and pullulan reduced ($P < 0.01$)

the AID of CP. The average ileal and total tract endogenous losses of TDF were calculated to be 25.25 and 42.87 g/kg DMI, respectively. The SID of TDF in diets containing RS 60, SCF, and pullulan were greater ($P < 0.01$) than the SID of TDF in the cellulose diet, but the STTD of the SCF diet was greater ($P < 0.05$) than in the cellulose and pullulan diets.

Key words: digestibility, energy, maltodextrin, novel fibers, TDF, pigs

INTRODUCTION

The lack of dietary fiber in highly processed foods contributes to some of the metabolic diseases in the Western world (Eastwood and Kritchevsky, 2005). Intake of dietary fiber is recommended at 20-30 g/d, but the recommended intake is seldom met (Jones, 2001). Novel carbohydrates that act as dietary fiber such as resistant starch (**RS),** soluble corn fiber (**SCF**), and pullulan are commercially available and can be mixed into most food preparations to increase the concentration of dietary fiber (Brown, 2004). The physiological behavior that defines the health benefits associated with dietary fiber is influenced by the physico-chemical characteristics of the carbohydrates in the fiber, but because these characteristics of dietary fiber are diverse, the physiological behavior of dietary fiber is expected to differ among different sources of fiber (Dreher, 2001). The health benefits that novel fiber sources provide depend on the disappearance of carbohydrates in the small intestine and the fermentability of the carbohydrates that enter the large intestine. The low small intestinal disappearance of carbohydrates in novel fiber sources reduce post- prandial glucose and insulin responses in humans and dogs, which may be beneficial in diabetic management (Kendall et al., 2008; Knapp et al., 2008). Fermentation of carbohydrates in the large bowel may also increase the absorption of short-chain fatty acids, which may have benefits in controlling colonic diseases (Slavin, 2001). The reduced absorption of energy as glucose in the small intestine may also reduce the

caloric value of novel fiber sources compared with foods rich in starch, which may improve weight management (Elia and Cummings, 2007). The site of carbohydrate disappearance, therefore, plays an important role in providing health benefits associated with the consumption of dietary fiber.

The objective of this experiment was to determine the effect of novel fibers on the apparent ileal disappearance **(AID)** and the apparent total tract disappearance **(ATTD)** of GE, total dietary fiber **(TDF)**, DM, CP, acid hydrolyzed ether extract **(AEE)**, ash, and total carbohydrates in diets containing 4 sources of dietary fiber, and to calculate hindgut disappearance **(HGD)** of GE, TDF, DM, CP, AEE, and total carbohydrates in these diets when fed to growing pigs. A second objective was to measure the endogenous flow of TDF to be able to calculate the standardized ileal disappearance **(SID)** and the standardized total tract disappearance **(STTD)** of TDF in the 4 novel fibers fed to pigs.

MATERIALS AND METHODS

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

Animals, Housing, and Experimental Design

Twelve growing barrows (initial BW: 20.0 ± 2.8 kg) that were the offspring of line 337 boars mated to C-22 females (Pig Improvement Company, Hendersonville, TN) were surgically fitted with a T-cannula in the distal ileum using the procedure of Stein et al. (1998). Pigs were randomly allotted to a replicated 6 × 5 Youden square design with 6 pigs and 5 periods per square. After surgery, pigs were housed in individual pens (0.9 × 1.8m) that were equipped with a feeder and a nipple drinker. Pigs were allowed 10 d to recover from surgery and pigs were fed a corn-soybean meal grower diet (20% CP) during this time. Prior to the start of the experiment,

pigs were transferred to metabolism cages were equipped with individual feeders and nipple drinkers, as well as screens to allow for separate collection of feed refusals and feces.

Ingredients, Diets, and Feeding

Four sources of novel dietary fiber were used in this experiment (Table 4.1). Two of these carbohydrates were resistant starches containing 60 and 75% TDF (RS 60 and RS 75, respectively). The 2 other sources of carbohydrates were SCF and pullulan. The 4 novel fiber sources were supplied by Tate and Lyle (Decatur, IL). Synthetic cellulose (Solka floc, International Fiber Corp., Urbana, OH) was included as a negative control.

A maltodextrin-casein based control diet was formulated (Table 4.2). Five additional diets were prepared by replacing 10% maltodextrin in the control diet with 10% of each of the 4 novel dietary fiber sources or with 10% cellulose. Sucrose was added at 20% in all diets to improve diet palatability. Soybean oil, minerals, and vitamins were also added to the diets. Chromic oxide, an indigestible marker, was included at 0.50% in all diets.

The daily feed allowance was calculated as 3.0 times the estimated maintenance requirement for energy (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998). The daily feed ration was divided into 2 equal meals that were provided at 0800 and 1600 except on ileal collection days when feed was provided before and after ileal collection at 0600 and 1800, respectively. Water was available at all times.

Data and Sample Collection

Pig BW were recorded at the beginning of each period and the amount of feed provided each day was recorded. Pigs were allowed 5 d to adapt to the experimental diets. Fecal samples were collected on d 6 and 7. On d 8 and 9, a 225-mL plastic bag was attached to the opened cannula barrel using a cable tie and digesta that flowed into the bag were collected from 0600 to

1800. Bags were removed and replaced hourly. Collected digesta were immediately stored at -20°C.

At the conclusion of the experiment, fecal and ileal samples obtained over the 2-d collection periods were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. A sample of each diet and of each ingredient was also collected. Digesta samples were lyophilized and ground before chemical analysis.

Sample Analysis

Diets, ingredients, and ileal and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007) and TDF (method 991.43, AOAC Int., 2007). Acid hydrolyzed ether extract was also analyzed in these samples by acid hydrolysis (Sanderson, 1986) followed by ether extraction (method 2003.06; AOAC Int., 2007). Gross energy of diets, ingredients, ileal samples, and fecal samples were determined using bomb calorimetry (model 6300, Parr Instruments, Moline, IL). Chromium concentration of diets and ileal and fecal samples was determined using the Inductive Coupled Plasma Atomic Emission Spectrometric Method (method 990.08; AOAC Int., 2007). Samples were prepared for chromium analysis using nitric acid-perchloric acid (method 968.08D; AOAC Int., 2007). Water binding capacity of the diets was measured using the procedure described by Robertson et al. (2000). Briefly, 1000 ± 5 mg of sample was weighed in pre-dried centrifuged tubes and the sample was hydrated with 30 mL of distilled water for 18 h. After centrifugation, the supernatant was separated from the sample by inverting the tube and letting water drain from the pellet for 1 h. The fresh and dried weights of the pellet were recorded.

Calculations and Statistical Analysis

The concentration of total carbohydrates in the samples was calculated by subtracting the concentration of CP, AEE, and ash from the concentration of DM in the samples. The AID of GE was calculated in all diets using Eq.[1] (Stein et al., 2007):

$$AID_{GE} = \{1-[(GE_{digesta}/GE_{feed}) \times (Cr_{feed}/Cr_{digesta})]\} \times 100 \quad [1]$$

in which AID is the apparent ileal disappearance of GE (kcal/kg), $GE_{digesta}$ is the concentration of GE in the ileal digesta DM, GE_{feed} is the concentration of GE in the feed DM, Cr_{feed} is the concentration of chromium in the feed DM, and $Cr_{digesta}$ is the concentration of chromium in the ileal digesta DM. The AID for TDF, DM, CP, AEE, ash, and total carbohydrates was calculated using the same equation.

The ATTD of GE, TDF, DM, CP, AEE, ash, and total carbohydrates was also computed using Eq. [1] except that the concentration of GE, TDF, DM, CP, AEE, ash, total carbohydrates, and chromium in the feces was used rather than the concentration in the ileal digesta. Hindgut disappearance of GE was calculated by subtracting the concentration of GE in the ileal digesta from the concentration of GE in the feces (Urriola and Stein, 2010). Hindgut disappearance of TDF, DM, CP, AEE, ash, and total carbohydrates was calculated using the same equation.

The ileal endogenous loss **(IEL)** of TDF was calculated based on the flow of TDF obtained from feeding the maltodextrin diet using Eq. [2] (Stein et al., 2007):

$$IEL_{TDF} = [TDF_{digesta} \times (Cr_{feed}/Cr_{digesta})] \quad [2]$$

in which IEL_{TDF} is the basal ileal endogenous loss of TDF (g/kg DMI) and $TDF_{digesta}$ is the concentration of TDF in the ileal digesta. The total tract endogenous loss **(TTEL)** of TDF was also calculated using Eq. [2], but the concentration of TDF in the feces was used instead of the concentration of TDF in the ileal digesta.

Standardized ileal disappearance of TDF (SID_{TDF}) was calculated by correcting the AID of TDF for the IEL_{TDF} for each diet using Eq. [3] (Stein et al., 2007):

$$SID_{TDF} = [AID + (IEL_{TDF}/TDF_{feed}) \quad [3]$$

The STTD of TDF was calculated using the same equation except that the ATTD of TDF was used instead of AID, and the total tract endogenous loss of TDF was used instead of IEL of TDF. Water binding capacity was calculated as the difference between the fresh and dry weight of the pellet (g) divided by the dry weight of the pellet (Robertson et al., 2000).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure was used to verify that the variances were homogenous. Outliers were defined as pigs having at least 3 observations that deviated from the treatment mean by more than 1.5 times the interquantile range (Devore and Peck, 1993). Three pigs were considered outliers and were removed from the data set. Six pigs voided no fecal samples at the time of collection and were also removed from the data set.

An ANOVA was conducted, with diet as the main effect and pig and period as random effects. Differences among treatment means were separated using the LSMEANS statement and the PDIFF option of PROC MIXED. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among treatment means. An alpha value between 0.05 and 0.10 was considered a tendency towards significance.

RESULTS

The concentration of CP in maltodextrin, cellulose, and in the 4 novel fibers was below 1% (Table 4.1). Maltodextrin contained the least amount of TDF (1.2%) whereas cellulose was 100% TDF. This value is consistent with the observation of Cho et al. (1997). Except for SCF which contained 10% TDF, the concentration of TDF in the novel fiber sources ranged from 62.9

to 85.4% TDF. The novel fibers contained less GE (3,738 to 3,849 kcal/kg), DM (90.07 to 94.80%), ash (0.04 to 0.10%), and total carbohydrates (89.35 to 94.32%) than maltodextrin and cellulose. Because of the low concentration of TDF in maltodextrin and SCF, the TDF concentration of diets containing these ingredients was also low (2.19 and 2.81%, respectively; Table 4.3).

The AID values for GE in diets containing the novel fibers were less ($P < 0.05$) than that of diets containing maltodextrin, and the AID of GE in RS 75 was also less ($P < 0.05$) than that of RS 60, SCF, or pullulan (Table 4.4). However, the AID of GE in diets containing cellulose was the least ($P < 0.05$) among all the diets. The ATTD of GE was not different among the diets, but the HGD of GE in diets containing cellulose, RS 60, RS 75, or SCF was greater ($P < 0.05$) than the HGD of GE in diets containing maltodextrin or pullulan.

The AID of TDF in diets containing RS 60 and pullulan was greater ($P < 0.05$) than the AID of TDF in the diets containing cellulose, whereas the AID of TDF in diets containing RS 75 or SCF was not different from that of the cellulose-containing diet. The ATTD of TDF and the HGD of TDF in the diet containing SCF was less ($P < 0.05$) than the values obtained in the cellulose diet, but greater ($P < 0.01$) than the ATTD and HGD of TDF in the diet containing maltodextrin. However, no differences were obtained among the remaining diets.

Diets containing maltodextrin had the greatest ($P < 0.01$) AID of DM among all diets. Diets containing cellulose and RS 75, however, had the least ($P < 0.01$) AID of DM compared with the AID of DM in the other diets. The ATTD of DM in diets containing novel fibers was not different from each other, and was also not different from the AID in the maltodextrin diet. However, the ATTD of DM in the cellulose diet was less ($P < 0.01$) than in all other diets except in the RS 75 and the pullulan diet. The HGD of DM in diets containing cellulose, RS 60, RS 75,

or SCF was greater ($P < 0.05$) than in the maltodextrin diet, but the HGD of DM in the diet containing pullulan was not different from values obtained for the maltodextrin diet.

The addition of cellulose and pullulan, but not RS 60, RS 75, or SCF to a maltodextrin-based diet, reduced ($P < 0.01$) the AID of CP. The ATTD of CP was not affected by the addition of fiber ingredients to the maltodextrin based diet, but the HGD of CP in the cellulose diet was greater ($P < 0.05$) than in all other diets except for the SCF diet.

The AID of AEE in the diet containing cellulose tended to be less ($P < 0.10$) than in diets containing RS 75 and pullulan, but not different from the AID of AEE in the other diets. However, the ATTD and HGD of AEE in diets containing RS 60, RS 75, or SCF were not different from the ATTD and HGD in the diet containing cellulose, but the ATTD and HGD of AEE in the diets containing cellulose, RS 60, or SCF were greater ($P < 0.01$) than the ATTD and HGD of AEE in the diet containing maltodextrin. The ATTD and the HGD of AEE in the pullulan diet was less ($P < 0.05$) than in the cellulose diet, but not different from the other diets.

The AID of ash in diets containing RS 60, RS 75, SCF, or pullulan was not different from the values observed in the maltodextrin diet, but the AID of ash in the diet containing cellulose is less ($P < 0.01$) than that of the maltodextrin diet. No differences were observed in the ATTD of ash among diets, but the HGD of ash in the cellulose diet was greater ($P < 0.01$) than the HGD of ash in all other diets except for the pullulan diet.

The AID of total carbohydrate in all the fiber containing diets was less ($P < 0.01$) than in the maltodextrin diet, but the AID of total carbohydrate in diets containing cellulose or RS 75 were less ($P < 0.01$) compared with diets containing RS 60, SCF, or pullulan, but the AID of total carbohydrates in the diets containing SCF or pullulan was greater ($P < 0.01$) than in the diet containing RS 60. The ATTD of total carbohydrates in the diet containing cellulose was also

less ($P < 0.01$) than the ATTD of total carbohydrates in all other diets, and the ATTD of total carbohydrates in the RS 75 diet was also less ($P < 0.01$) than in the maltodextrin and the RS 60 diet. The HGD of total carbohydrates in diets containing RS 60 or RS 75 was not different from that of the cellulose diet and these values were greater ($P < 0.01$) than the HGD for all other diets. The HGD of total carbohydrates in the SCF diet was also greater ($P < 0.01$) than in the maltodextrin and the pullulan diets.

Standardized ileal disappearance of TDF in diets containing RS 60, SCF, or pullulan were not different from each other, but was greater ($P < 0.01$) than the SID of TDF in the cellulose diet (Table 4.5). The SID and STTD of TDF in the RS 60 and RS 75 diets were not different from each other, but the STTD of TDF in the SCF diet was greater ($P < 0.05$) than in the cellulose and pullulan diets. Disappearance of TDF in the hindgut was not different among the fiber containing diets.

DISCUSSION

The effect of dietary fibers obtained from traditional food ingredients such as cereal brans and vegetables have been studied (Gallaher and Schneeman, 2001;Wolever and Jenkins, 2001). However, with the update of the dietary fiber definition, novel carbohydrates that act as dietary fiber have been incorporated in food and beverages to increase dietary fiber intake (Brown, 2004). In this experiment, a semi-purified diet was used to study the effects of different novel fibers on energy and nutrient disappearance. The use of semi-purified ingredients avoids confounding factors (i.e., different components of a feedstuff) and isolates the effects of the addition of novel fibers. Maltodextrin was used as the basal carbohydrate for all the diets because it is a very digestible carbohydrate. The maltodextrin control diet also served as a control to which the effects of the addition of different types of fiber sources could be compared. No source

of dietary fiber was added to the maltodextrin diet; however, a small proportion of the diet was TDF. The maltodextrin ingredient also contained a small amount of TDF, which suggests that the TDF method may have limitations in analyzing very digestible carbohydrates such as maltodextrin. The low concentration of TDF in SCF ingredient and SCF diet also demonstrates the limitations of AOAC method 991.43 to measure dietary fiber fractions that are of low molecular weight. The fiber components in SCF are low molecular weight carbohydrates (Knapp, 2010) that are soluble in ethanol. Therefore, these low molecular weight dietary fiber fractions are poorly recovered in the analysis for TDF. This indicates that AOAC method 991.43 is not the suitable procedure for TDF determination in SCF.

The maltodextrin diet is very digestible and it is expected that none or very little dietary DM would be present in the ileal digesta of pigs fed this diet. However, TDF was analyzed in the ileal digesta and feces in pigs fed the maltodextrin diet and this is the reason negative values for AID and ATTD of TDF were calculated. The ileal digesta is composed of undigested DM from the diet, as well as endogenous enzymes, sloughed epithelial cells, bacterial cells, and mucin (Leterme et al., 1998, Stein et al., 2007). In the feces, the concentration of mucin is low because most of the mucin is fermented in the hind gut (Lien et al., 2001); however, the concentration of microbial matter in the feces is high (De Lange et al., 1989). The negative AID and ATTD of TDF in the maltodextrin diet strongly indicates that certain compounds in the endogenous losses and in the microbial matter may be analyzed as TDF. A similar observation was reported by Jørgensen et al. (1996) and by Wilfart et al. (2007).

The AID and ATTD of TDF in the diet containing SCF were also negative, but to a lesser extent than the values observed for the maltodextrin diet. The low recovery of TDF in the SCF diet may contribute to this negative value, but the possible presence of non-dietary sources of

TDF in the ileal digesta and in the feces from pigs fed this diet makes the interpretation of this result difficult. However, relative to the HGD of TDF in the maltodextrin diet, the lesser negative value for the HGD of TDF in the SCF diet indicates that the TDF in the SCF diet was not fermented because more TDF was excreted in the feces of pigs fed the SCF diet compared with pigs fed the maltodextrin diet. The interpretation of this result is not consistent with results of other experiments that indicated that TDF from SCF is fermented in the hindgut (Knapp et al., 2007; Stewart and Slavin, 2007). Therefore, the IEL and TTEL of TDF were calculated to remove the influence of endogenously synthesized metabolites and microbial matter that are analyzed as TDF. The STTD of TDF in the SCF diet is more than 100%. This indicates that the fiber in SCF is fermentable and confirms the results of Knapp et al. (2007) and Stewart and Slavin (2007). Therefore, a better estimate of TDF disappearance is obtained by correcting the AID of TDF for the basal endogenous loss of TDF.

The assumptions used for calculating basal endogenous losses of TDF are based on the concept used to calculate the SID of CP and AA digestibility (Stein et al., 2007). The IEL and TTEL of TDF obtained from a fiber-free diet (maltodextrin diet) is assumed to be associated with the normal physiologic and metabolic functions of the gut and is not affected by the components of the diet (Stein et al., 2007). Therefore, this is referred to as basal endogenous loss. The average IEL and TTEL of TDF obtained from this experiment is 25.25 and 42.87 g/kg DMI, respectively. To our knowledge, this if the first estimate of IEL and TTEL of TDF in pigs.

Cellulose or each of the novel fibers added to the diets were the only source of TDF in each of the diets. By definition, dietary fiber is resistant to digestion by mammalian enzymes, but the AID and SID of TDF of the fiber containing diets indicate that some of the TDF disappeared in the small intestines. The presence of a microbial population in the ileum has been

recognized (Wang et al., 2004), and the disappearance of TDF in the small intestines is likely due to the fermentative capability of the microbial population in the ileum.

The greater SID of TDF in diets containing SCF and pullulan than in the RS 75 and cellulose diets is consistent with the observation that soluble fiber is more fermentable than insoluble fiber (Cho et al., 1997, Urriola and Stein, 2010). The greater STTD of TDF in the RS 60 and RS 75 diets than in diets containing cellulose and pullulan confirms results from other studies indicating that the fiber in resistant starch is fermentable (Maathuis et al., 2009), whereas, the fiber in cellulose is poorly fermentable (Middelbos et al., 2007).

The reduced AID of GE in the diets that contained cellulose or the novel fibers was also reflected in a low AID of DM and total carbohydrates in these diets. Addition of dietary fibers to a starch containing diet increases the recovery of starch at the end of the ileum (Rideout et al., 2008) and depresses the AID of starch in pigs (De Schrijver et al., 1999). The reduced AID of DM and total carbohydrates in diets containing novel fibers indicates that the amount of glucose that was digested and absorbed in the small intestine of pigs fed these diets was also reduced compared with pigs fed the maltodextrin diet. This result supports the results of experiments that indicated that the addition of novel fibers to a diet may be beneficial for diabetic management because it can reduce post prandial blood glucose concentrations (Kendall et al., 2008; Knapp et al., 2008). However, the ATTD of GE in diets containing different novel fibers was not reduced. The reason for this observation is that nutrients that were not digested in the small intestine may be fermented in the hindgut and the products of carbohydrate fermentation can also be a source of energy for the pig (Anguita et al., 2006). However, this energy is absorbed in the form of short-chain fatty acids and will, therefore, not contribute to an increase in blood glucose concentration. The energetic efficiency of absorbed short-chain fatty acids is also less than that

of glucose (Black, 1995), which contributes to a reduced caloric utilization of dietary fiber compared with starch. Therefore, although the concentration of GE in all the diets in this experiment is similar and the ATTD of GE in diets containing cellulose or novel fibers are not different from that of the maltodextrin diet, the caloric value of the fiber-containing diets are expected to be less than the caloric value of the maltodextrin diet.

The addition of dietary fiber may reduce the AID of CP (Wang et al., 2002) although this is not always the case (Middelbos et al., 2007; Rideout et al., 2007). In the present experiment, the addition of cellulose or pullulan, but not RS 60, RS 75, or SCF, to a maltodextrin-based diet reduced the AID of CP. Pullulan also reduced the AID of CP when added to diets for dogs (Spears et al., 2005). These observations indicate that the effect of dietary fiber on CP disappearance depends on the type and, possibly, the concentration of fiber in the diet. Soluble corn fiber and pullulan are both soluble fibers, but the pullulan diet had a greater concentration of TDF and also a greater capacity to bind water than the SCF diet. Increasing the concentration of TDF in the diet increases endogenous CP flow by increasing ileal flow of epithelial cells, bacterial cells, and crude mucin (Leterme et al., 1998), and increased endogenous N flow is associated with reduced AID of N (Schulze et al., 1994). Increased water binding capacity also increases the endogenous loss of N that is induced by dietary fiber (Leterme et al., 1998). Therefore, the combined effects of the level of TDF and the WBC of the diet are likely the main reasons for the reduced AID of CP in the diet containing Pullulan compared with the diets containing maltodextrin or the other novel fibers.

Cellulose, RS 60, and RS 75 are insoluble fibers. Cellulose is a poorly fermentable fiber whereas RS 60 and RS 75 are easily fermentable fibers. The absence of a reduction in the AID of CP in diets containing RS 60 and RS 75 is consistent with data from other experiments (De

Schrijver et al., 1999; Rideout et al., 2008). However, the reduction in the AID of CP in the diet containing cellulose is not consistent with data indicating that the addition of cellulose has no effect on the AID of CP (Li et al., 1994; Middelbos et al., 2007). The cellulose (Solka floc 100) used in the present experiment has a fiber length of approximately 40 μ and the water binding capacity of cellulose increases with increasing fiber length up to 100 μ (Ang, 1991). The fiber length of the cellulose used by Li et al. (1994) and Middelbos et al. (2007) was not reported. However, the cellulose diet used in this experiment had a water binding capacity that was greater than that of the other diets. Water binding capacity is directly proportional to the swelling capacity (Auffret et al., 1994) or the ability of the diet to form "bulk" (Kyriazakis and Emmans, 1995). Pea fibers with a high water binding capacity increase ileal endogenous flow of N although the mechanism is unclear (Leterme et al., 1998). Therefore, the reduction in the AID of CP in diets containing cellulose may be a result of the capacity of cellulose to bind water.

The AID of AEE was not reduced when cellulose and novel fibers were added to the diet. This is consistent with data from Kil et al. (2010) that indicated that the AID of AEE is not affected by the level of synthetic cellulose in the diet. The AID of AEE is also not affected by the inclusion of different sources of dietary fiber (De Schrijver et al., 1999; Middelbos et al., 2007) although the addition of beet pulp reduced the AID of fat compared with diets containing increasing levels of cellulose (Muir et al., 1996). Soluble and fermentable fiber may increase digesta viscosity and reduce rate of lipolysis in vitro (Pasquier et al., 1996). In the present experiment, soluble and insoluble fiber did not reduce AID of AEE, but the ATTD of AEE was less than the AID of AEE. This observation is consistent with previous results (Shi and Noblet, 1993; Bakker, 1996; Kil et al., 2010). The presence of dietary fiber in the hindgut stimulates microbial growth and consequently, increase endogenous losses of fat (Kil et al., 2010). The

values for the HGD of AEE in all the diets were negative, suggesting a large contribution of microbial fat in the hind gut and the absence of lipid absorption post- ileum. Recent data from Kil et al. (2010) also indicate that fat is not absorbed in the hindgut. However, the more negative HGD of AEE in the maltodextrin diet than in the other diets indicates that more endogenous fat was present in the feces of pigs fed the maltodextrin diet. In addition to microbial synthesis of fat in the hindgut, additional endogenous fat may be contributed by the lipid components of mucin and intestinal epithelial cells (Larhed et al., 1998; Waheed et al., 1998) that may be present in the hind gut. However, the contribution of these non-microbial sources of endogenous fat is likely low in a low fiber diet such as the maltodextrin diet used in this experiment.

Pullulan is a polysaccharide produced by *Aureobasidium pullulans*. It was described to be a slowly digestible carbohydrate (Wolf et al., 2003). In this experiment, about 72% of the fiber in pullulan was fermented in the small intestine, which indicates that the fiber in pullulan is rapidly fermentable. This result is consistent with the data reported by Maathuis et al. (2009).

The negative AID of ash in the cellulose-containing diet indicates that the concentration of ash in the ileum of pigs fed the cellulose diet was greater than what was provided in the diet. Dietary fiber increases pancreatic juice and mineral secretions in pigs (Zebrowska and Low, 1987), but dietary and endogenous minerals are usually absorbed prior to the ileum (Wilfart et al., 2007). The negative AID of ash in the cellulose diet indicates that cellulose may have stimulated more endogenous secretion of minerals (Zebrowska and Low, 1987) or reduced the absorption of minerals in the small intestine by binding the minerals (Low, 1989). However, the ATTD of ash in all diets was similar, indicating that the absorption of minerals in the hind gut of pigs fed the cellulose diet compensated for the reduced small intestinal absorption of ash in pigs fed this diet. Dietary fiber may shift mineral absorption to the hindgut (Demigné et al., 1989),

and mineral absorption in the hindgut may be mediated by the solubilization of the minerals as a consequence of a low pH in the hind gut resulting from the production of short-chain fatty acids by microbial fermentation (Bongers and van den Heauvel, 2003). This mechanism is usually true for fermentable fibers, but the fiber in cellulose is poorly fermentable. An additional mechanism for enhanced absorption of minerals in the hindgut is that the "bulk" of cellulose may increase the surface area for absorption in the hindgut (Bongers and van den Heauvel, 2003). This mechanism is supported by an increase in size and weight of the cecum and colon of pigs fed dietary fiber (Pond et al., 1988). The absence of a reduction in the AID of ash in diets containing SCF, RS 60, and RS 75 is consistent with the results of Weaver et al. (2010).

In conclusion, addition of cellulose or novel fibers to a maltodextrin-based diet reduced the AID of DM and total carbohydrates with a concomitant reduction in the AID of GE, but the ATTD of GE was not reduced in the fiber-containing diets. Resistant starch 60, RS 75, and SCF did not affect the AID of AEE, CP, or ash, but the addition of cellulose and pullulan reduced the AID of CP. A proportion of the TDF in RS 60, RS 75, and pullulan disappeared in the small intestine. The negative AID and ATTD of TDF in diets containing maltodextrin and SCF indicates that small amounts of endogenous compounds that are analyzed as TDF are secreted into the intestinal tract. Therefore, measurement of basal endogenous losses of TDF and calculation of the SID and STTD of TDF is a better indicator of TDF fermentability. The addition of 10% novel fibers to the diet may help in diabetic and weight management without compromising CP, fat, or mineral disappearance.

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Table 4.1. Analyzed concentration of GE, TDF, DM, CP, ash, and calculated concentration of total carbohydrates in maltodextrin, cellulose, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis[1]

	Ingredient					
Item	Malto-dextrin	Cellulose	Resistant starch 60[2]	Resistant starch 75[2]	Soluble corn fiber	Pullulan
GE, kcal/kg	3,914	3,957	3,738	3,834	3,760	3,849
TDF, %	1.20	100.00	62.90	75.60	10.00	85.40
DM, %	97.41	96.21	90.07	92.99	93.92	94.80
CP, %	0.74	0.67	0.68	0.70	0.44	0.44
Ash, %	0.19	0.19	0.04	0.10	0.10	0.04
Total carbohydrates[3], %	96.48	95.35	89.35	92.19	93.38	94.32

[1] All samples were analyzed for AEE but AEE was not detected in any samples.

[2] Resistant starch 60 and resistant starch 75 were expected to contain 60 and 75% TDF, respectively.

[3]Total carbohydrates = DM – (CP + ash + AEE).

Table 4.2. Ingredient composition of experimental diets containing maltodextrin, cellulose, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis

Treatment	Malto-dextrin	Cellulose	Resistant starch 60[1]	Resistant starch 75[1]	Soluble corn fiber	Pullulan
Maltodextrin	59.00	49.00	49.00	49.00	49.00	49.00
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00
Casein	14.00	14.00	14.00	14.00	14.00	14.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00
Ground limestone	0.95	0.95	0.95	0.95	0.95	0.95
Dicalcium phosphate	0.70	0.70	0.70	0.70	0.70	0.70
NaCl	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Cellulose	-	10.00	-	-	-	-
Resistant starch 60	-	-	10.00	-	-	-
Resistant starch 75	-	-	-	10.00	-	-
Soluble corn fiber	-	-	-	-	10.00	-
Pullulan	-	-	-	-	-	10.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1] Resistant starch 60 and resistant starch 75 were expected to contain 60 and 75% TDF, respectively.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 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 4.3. Analyzed concentrations of GE, TDF, DM, CP, AEE, ash, and total carbohydrates in experimental diets containing maltodextrin, cellulose, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis

	Diet					
Item	Malto-dextrin	Cellulose	Resistant starch 60[1]	Resistant starch 75[1]	Soluble corn fiber	Pullulan
GE, kcal/kg	4,207	4,249	4,205	4,210	4,234	4,248
TDF, %	2.19	12.16	10.92	12.40	2.81	10.98
DM, %	96.35	96.45	95.94	96.15	96.45	96.64
CP, %	13.15	13.54	13.63	13.55	13.30	13.27
AEE, %	0.72	0.95	0.78	0.70	0.75	0.81
Ash, %	2.70	2.53	2.95	2.56	3.29	2.66
Total carbohydrates[2], %	79.78	79.43	78.59	79.35	79.11	79.89

[1] Resistant starch 60 and resistant starch 75 were expected to contain 60 and 75% TDF, respectively.

[2]Total carbohydrates = DM – (CP + ash +AEE).

Table 4.4. Water binding capacity, apparent ileal disappearance (AID), apparent total tract disappearance (ATTD), and hindgut disappearance (HGD) of GE, TDF, CP, AEE, ash, and total carbohydrates in pigs fed diets containing maltodextrin, cellulose, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan, as-fed basis[1]

		Diet							
Item		Malto-dextrin	Cellulose	Resistant starch 60	Resistant starch 75	Soluble corn fiber	Pullulan	SEM	*P*-value
Water binding capacity, g/g dry wt.		0.38 [d]	0.67 [a]	0.43 [c]	0.41 [cd]	0.40 [cd]	0.48 [b]	0.01	0.001
GE, kcal/kg									
	AID	96.8[a]	85.7 [d]	90.5 [b]	88.6 [c]	92.0 [b]	92.2 [b]	0.66	0.001
	ATTD	94.4	91.9	94.4	93.6	95.5	92.6	0.98	0.111
	HGD	-2.5 [b]	6.0 [a]	3.8 [a]	5.1 [a]	3.8 [a]	0.3 [b]	1.04	0.001
TDF, %									
	AID	-10.9[d]	15.7 [bc]	44.4 [a]	28.2 [ab]	-4.3[cd]	49.5 [a]	7.68	0.001
	ATTD	-88.2[c]	35.1[a]	56.2[a]	59.5 [a]	-36.9 [b]	48.6 [a]	12.34	0.001
	HGD	-77.4[c]	25.7[a]	11.7[a]	31.3[a]	-32.6[b]	-0.9 a[b]	13.28	0.001

Table 4.4. (Cont.)

DM, %									
	AID	95.4 [a]	84.84 [c]	89.55 [b]	86.58 [c]	91.10 [b]	90.67 [b]	0.69	0.001
	ATTD	94.2 [a]	90.58 [b]	93.43 [a]	92.68 [ab]	94.68 [a]	92.36 [ab]	0.90	0.027
	HGD	-1.1 [c]	5.78 [a]	3.96 [ab]	6.05 [a]	3.56 [ab]	1.72 [bc]	1.01	0.001
CP, %									
	AID	90.7 [a]	82.4 [c]	90.4 [a]	90.5 [a]	90.3 [ab]	87.1 [b]	1.09	0.001
	ATTD	91.3	92.8	92.9	92.9	94.4	89.8	1.14	0.119
	HGD	0.6 [b]	10.4 [a]	-2.8 [b]	2.3 [b]	4.2 [ab]	2.6 [b]	2.52	0.014
AEE, %									
	AID	77.9 [ab]	67.8 [b]	76.0[ab]	82.1[a]	75.8 [ab]	81.4 [a]	3.47	0.086
	ATTD	-6.2[c]	66.8 [a]	45.4 [ab]	37.5 [ab]	50.9 [ab]	9.9 [bc]	16.41	0.009
	HGD	-80.5 [c]	-1.9 [a]	-31.8 [ab]	-40.8 [abc]	-32.6 [ab]	-70.6 [bc]	18.77	0.011
Ash, %									
	AID	28.0 [abc]	-3.5 [d]	51.0 [ab]	24.7 [bc]	53.1 [a]	6.9 [cd]	11.18	0.001
	ATTD	43.9	46.2	50.9	44.3	61.6	51.6	5.37	0.143

Table 4.4. (Cont.)

HGD	14.7 [bc]	53.3 [a]	0.6 [c]	19.3 [bc]	5.0[c]	42.5 [ab]	12.41	0.009
Total carbohydrates[2], %								
AID	98.6 [a]	87.7 [d]	90.7 [c]	88.1 [d]	93.2 [b]	94.2 [b]	0.63	0.001
ATTD	97.4 [a]	92.0 [c]	97.2 [a]	94.9 [b]	96.6 [ab]	95.1 [ab]	0.87	0.001
HGD	-1.0 [c]	4.3 [ab]	6.7 [a]	6.6 [a]	3.3 [b]	0.8 [c]	0.89	0.001

[a-d] Values within a row lacking a common superscript differ ($P \leq 0.05$).

[1]Data are least squares means of 9 observations per treatment except for diets containing SCF and pullulan that have 8 and 7 observations, respectively.

[2]Total carbohydrates = DM – (CP + ash + AEE).

Table 4.5. Standardized ileal disappearance (SID), standardized total tract disappearance (STTD), and hindgut disappearance (HGD) of TDF in pigs fed diets containing cellulose, resistant starch 60, resistant starch 75, soluble corn fiber, and pullulan

Item		Diet						
		Cellulose	Resistant starch 60	Resistant starch 75	Soluble corn fiber	Pullulan	SEM	*P*-value
TDF, %	SID	35.8 [c]	66.6 [ab]	47.8 [bc]	85.7 [a]	71.7 [a]	7.55	0.001
	STTD	68.8 [b]	93.4 [ab]	93.0 [ab]	114.9 [a]	83.6 [b]	10.49	0.045
	HGD	31.7	27.9	44.9	29.0	10.7	9.76	0.129

[a-d] Values within a row lacking a common superscript differ ($P \leq 0.05$).

[1]Data are least squares means of 9 observations per treatment except for diets containing SCF and pullulan that have 8 and 7 observations, respectively.

CHAPTER 5

Digestibility of Starch, Fiber, Energy and Other Nutrients in Cereal Grains Fed to Growing Pigs

ABSTRACT: An experiment was conducted to determine the DE and ME, the apparent ileal **(AID)** and apparent total tract **(ATTD)** digestibility of GE, and to calculate hindgut disappearance **(HGD)** of nutrients, starch, and total dietary fiber **(TDF)** in 8 cereal grains fed to pigs. The 8 cereal grains were yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, polished rice, rye, sorghum, and wheat. Twenty-four growing barrows (BW: 30.7 kg) were fitted with a T-cannula in the distal ileum and housed in metabolism cages that allowed for the total, but separate, collection of feeds, feces, and urine. The cereal grains were ground and 8 diets were prepared with each of the cereal grains included at 97.4% of the diet. Titanium dioxide was added at 0.40% as an inert marker. The pigs were randomly allotted to the 8 cereal diets in a completely randomized design. The experiment consisted of three 14-d periods. The first 5 d of each period was the adaptation period. Chromic oxide was added to the diet on d 6 and ferric oxide was added on d 11. Quantitative collection of feces was initiated upon the appearance of chromic oxide and ceased upon the appearance of ferric oxide in the feces. Quantitative collection of urine was also initiated at d 6 and ended at d 11. For each period, each diet was fed to 3 pigs, and at the end of the experiment, each diet was fed to a total of 9 pigs and no pig was fed the same diet twice. Results indicated that the AID of GE, OM, and total carbohydrates was greater ($P < 0.001$) in rice than in all other cereal grains. The AID of starch was also greater ($P < 0.001$) in rice than in yellow dent corn, dehulled barley, rye, and wheat. The ATTD of GE was greater ($P < 0.001$) in rice than in yellow dent corn, rye, sorghum, and wheat. With few exceptions, the AID and ATTD of GE and nutrients in Nutridense corn was not different from the

values for dehulled oats. Likewise, with few exceptions, the AID, ATTD, and HGD of GE, OM, total carbohydrates, and TDF in yellow corn, sorghum, and wheat were not different from each other. The AID of GE and AEE in dehulled barley was greater ($P < 0.001$) than in rye. The AID of starch in dehulled barley, rye, and sorghum was less ($P < 0.001$) than in all other cereal grains. The ATTD of GE and most nutrients was greater ($P < 0.001$) in dehulled barley than in rye. Dehulled oats had the greatest ($P < 0.001$) ME (kcal/kg DM) and rye the least ME (kcal/kg DM) among the cereal grains. In conclusion, dehulled oats, dehulled barley, and rice have the greatest concentration of ME, but sorghum and rye may be more suitable to control diabetes and manage BW in humans.

Key words: cereal grains, digestibility, energy, Nutridense corn, starch, TDF

INTRODUCTION

Cereal grains are the major source of energy in most human and animal diets. The cereal grains that are commonly used for human consumption are corn, barley, oats, rice, rye, sorghum, and wheat. In developed countries, cereal grains are mostly consumed as processed and refined products. However, the interest in whole grains has increased because of recent studies indicating that consumption of whole grains is beneficial in preventing and managing diabetes (Venn and Mann, 2004), and BW (Williams et al., 2008).

In the U.S., corn is the major source of energy in most diets fed to pigs and poultry. However, the demand for corn has increased because of its use for ethanol production. Alternative sources of energy for swine diets, therefore, become increasingly important. Barley, oats, sorghum, and wheat, may also be used as sources of energy in swine diets (NRC, 1998). Aside from contributing energy, barley, oats, and wheat have the advantage of containing greater

concentration of CP than corn (NRC, 1998; Pedersen et al., 2007). Rye is also a good source of energy and it also contain more CP and AA than corn (NRC, 1998), but the use of rye in swine diets is not common (Thacker et al., 2002), because rye may reduce feed intake and growth performance in pigs (Bazylo, 1990). However, recent research has indicated that diets with relatively high inclusions of rye may be fed to pigs without reducing performance (Thacker et al., 1991; 2002). Rice is not included in traditional swine diets, but several experiments have shown that addition of rice to the diet benefits young pigs (Hopwood et al., 2004; Vicente et al., 2008).

The protective effect of whole cereal grains against metabolic diseases was suggested to be a result of the dietary fiber, i.e., non-starch polysaccharides and resistant starch, in the cereal grain. However, the presence of dietary fiber reduces energy and nutrient digestibility (Wenk, 2001; Högberg et al., 2004) and the greater concentration of total dietary fiber may negatively affect energy and nutrient digestibility in these cereal grains. Therefore, the objective of this experiment was to determine the DE and ME in yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, polished rice, rye, sorghum, and wheat fed to growing pigs, and to determine the apparent ileal **(AID)** and apparent total tract **(ATTD)** digestibility of GE, OM, CP, acid hydrolyzed ether extract **(AEE)**, starch, total carbohdyrates, total dietary fiber **(TDF)** in these cereal grains when fed to pigs.

MATERIALS AND METHODS

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

Animals, Housing, and Experimental Design

Twenty four growing barrows (initial BW: 30.7 ± 3.2 kg) that were the offspring of G-performance boars mated to F-25 females (Genetiporc, Alexandria, MN) were fitted with a T-cannula in the distal ileum as described by Stein et al. (1998). Pigs were allowed to recover after surgery for 10 d and they were provided with ad libitum access to water and a corn-soybean meal diet during this time. Pigs were housed in metabolism cages inside an environmentally controlled room. Each metabolism cage has a feeder and a nipple drinker, and is equipped with screens to allow for total, but separate, collection of feces and feed refusals, and funnels to allow for total collection of urine. Pigs were randomly allotted to 8 diets that were fed during each of 3 periods in a completely randomized design. During each period, each diet was fed to 3 pigs. Therefore, each diet was fed to a total of 9 pigs during the experiment and no pig was given the same diet more than once.

Ingredients, Diets, and Feeding

Eight diets were formulated based on each of 8 cereal grains (Tables 5.1 and 5.3). The 8 cereal grains were yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, polished white rice, rye, sorghum, and wheat obtained from a commercial source. All grains were ground in a hammer mill using a 1.6-mm screen. Each cereal grain was the only source of total carbohydrate, CP, acid hydrolyzed ether extract **(AEE)**, starch, GE, and TDF in the diet. Vitamins and minerals were added to all diets to meet or exceed the current requirements for growing pigs (NRC, 1998). All diets also contained 0.4% titanium dioxide (Kronos Titanox, Houston, TX) as an indigestible marker.

Pig BW were recorded at the start of each period and the pigs were fed 2 times their estimated maintenance requirement for energy (i. e., 106 kcal ME/kg$^{0.75}$). The daily feed

allowance was supplied in 2 equal meals that were provided at 0800 and 1700 except on digesta collection days when pigs were fed at 600 and 1800. Water was available at all times.

Sample Collection

Each period lasted 14 d. The initial 5 d of each period was considered the adaptation period to the diet. During these days, in addition to their daily feed ration, pigs were given 50 g of an AA mixture per feeding to reduce the impact of feeding pigs a diet that is deficient in CP (Pedersen et al., 2007). Chromic oxide was added in the morning meal of d 6 and ferric oxide was added in the morning meal of d 11. Quantitative collection of feces was initiated upon the appearance of chromic oxide and ceased upon the appearance of ferric oxide in the feces as described by Pedersen et al. (2007). Quantitative collection of urine was also initiated in the morning of d 6 and ceased in the morning of d 11 as described by Pedersen et al., (2007). Freshly voided feces were collected in the morning of d 6 and pH of the fecal samples was immediately measured using a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA). Fecal samples (20 g) were mixed with 2 *N* HCl in a 1:1 ratio and the samples were stored at -20ºC until the concentration of short-chain fatty acid (**SCFA**) from these fecal samples was analyzed. Ileal digesta were collected on d 13 and 14 for 10 h each day as described by Pedersen et al. (2007). The pH of the ileal digesta from each pig was also measured from ileal samples collected at 900, 1100, 1300, 1500, and 1700 on each collection day. The pH of the ileal digesta was measured in the same way as described for fecal samples.

At the conclusion of the experiment, ileal samples and urine samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. Samples of each diet and each cereal grain were also collected. Digesta and urine samples were lyophilized and

ileal digesta were ground prior to chemical analysis. All fecal samples were dried in a forced air oven at 60°C and also ground prior to chemical analysis.

Chemical Analyses

Cereal grains, diets, and ileal and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007), and TDF (method 985.29; AOAC Int., 2007). Samples were also analyzed for AEE by boiling the samples in 3*N* HCl (Sanderson, 1986) followed by ether extraction (method 2003.06; AOAC Int., 2007). Gross energy of diets, ingredients, ileal samples, and fecal samples were determined using bomb calorimetry (model 6300, Parr Instruments, Moline, IL). The concentration of titanium in the diets and ileal and fecal samples was analyzed based on the procedure of Myers et al. (2004).

Diets and cereal grains were analyzed for total starch (Thivend et al., 1972) and resistant starch (**RS**; Muir and O'Dea, 1992, 1993). Yellow dent corn and Nutridense corn were also analyzed for rapidly digestible starch **(RDS)** and slowly digestible starch **(SDS**; Englyst et al., 1992). Water binding capacity of the cereals was measured based on the procedure described by Robertson et al. (2000). Briefly, 1,000 ± 5 mg of sample was weighed in pre-dried centrifuged tubes and the sample was hydrated with 30 mL of distilled water for 48 h. After centrifugation (2,850g × 20°C), the supernatant was separated from the sample by inverting the tube and letting water drain from the pellet. The fresh and dried weights of the pellets were recorded.

Particle size distribution was determined by pouring 50 g of each diet on top of a stack of pre-weighed sieves (US Standard Testing Sieves, Fisher Scientific Co., Pittsburgh, PA). The pore sizes of the sieves were 300, 600, 850, 1,200, and 1,700 μm. The cereal grains and diets were separated into different particle size fractions by shifting the set of sieves mechanically for

15 min using a portable sieve shaker (Model RX-24, Tyler Industrial Products, Mentor, OH). After 15 min, the weight of each sieve was recorded.

Viscosity of the ileal digesta was measured using a Brookfield LV-DV-II+ Viscometer (Brookfield Eng. Lab. Inc., Middleboro, MA). Containers containing frozen ileal digesta were placed in a water bath until sample temperature reached 39°C. The sample was then stirred and 30 mL of sample was placed in a 100 mL glass beaker and viscosity was measured as described by Dikeman et al. (2007). The viscosity of each ileal digesta sample was measured within a range of shear rates (2, 4, 6, and 8) and within a range of spindle revolutions (1 to 4 rpm).

The concentration of SCFA in the feces was measured using the fecal sample that was preserved in HCl. Preparation of the fecal samples for SCFA analysis was as described by Urriola and Stein (2010) except that 2 mL of the feces:HCl mixture was mixed with 8 mL of distilled water. The procedure for SCFA analysis was as described by Erwin et al. (1961) and by Urriola and Stein (2010).

Calculations and Statistical Analysis

The concentration of OM in the samples was calculated as the difference between the concentration of DM and the concentration of ash. The concentration of total carbohydrates in the samples was calculated by subtracting the concentration of CP, AEE, and ash from the concentration of DM in the samples. The concentration of SDS in yellow dent corn and Nutridense corn was calculated as the difference between the digestible starch and rapidly digestible starch obtained using the Englyst et al. (1992) procedure (Bauer et al., 2003). Resistant starch for the diets and ingredients was calculated as the difference between the total starch obtained from the method of Thivend et al. (1992) and the digestible starch obtained from the method of Muir and O'Dea (1992, 1993). Water binding capacity of the diets was calculated as

the difference between the fresh and dry weights of the pellet (g) divided by the dry weight of the pellet (Robertson et al., 2000).

The weight of the particle fractions on each screen (g) was calculated as the difference between the weight of the screen with the samples (g) and the weight of the empty screen (g). The weights of each of the particle fractions were expressed as a percentage of the total weight of the sample recovered after sieving. The cumulative weights (%) of the different fractions were then transformed to log 10 values and mean particle size was calculated as described by Waldo et al. (1971). The viscosity of the ileal digesta was calculated using the Wingather software (Brookfield Eng. Lab. Inc., Middleboro, MA). The NLREG statistical software (NLREG, Brentwood, TN) was used to report viscosity measurements in terms of the power law equation as described by Dikeman et al. (2007). The constant and the exponent values of each of the ileal digesta samples obtained from NLREG was analyzed by the MIXED procedure of SAS with pig and period as the random effect and diet as the fixed effect. The LSMEANS statement was used to detect differences in viscosity among cereal grains.

The AID of OM, CP, AEE, TDF, total carbohydrates, starch, and GE was calculated for each diet as described by Stein et al. (2007). The same equation was used to calculate the ATTD of OM, CP, AEE, TDF, total carbohydrates, starch, and GE in the diets. Hind gut disappearance was calculated as the difference between the concentration of nutrients in the ileal digesta and the concentration of nutrients in the feces (Urriola and Stein, 2010). The DE and ME of the diets and ingredients were calculated as described by Adeola (2001).

Data were tested for outliers, normal distribution, and homogeneity of variances using the UNIVARIATE procedure of SAS. Observations that were more than 3 SD away from the treatment mean were considered outliers; however, no outliers were identified. Analysis of

variance was conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with diet as the fixed effect, and pig and period as the random effects. Means were calculated using the LSMEANS statement of SAS and the PDIFF option of SAS was used to separate treatment means. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to denote statistical significance among treatment means.

RESULTS

The concentration of DM in the 8 cereal grains ranged from 86.42 to 88.21%. (Table 5.1). The concentration of CP in yellow dent corn, Nutridense corn, sorghum, and polished rice was between 7.54 and 9.76%, whereas the concentration of CP for the other cereal grains was between 11.75 and 13.11%. Polished rice had the lowest concentrations of AEE, ash, and TDF whereas dehulled oats contained the most AEE among the cereal grains, which is likely the reason that dehulled oats contained more GE than the other cereal grains. The concentration of total starch ranged between 56.84% (rye) and 75.10% (rice). The concentration of resistant starch was less than 2% in rice, rye, and wheat, whereas the concentration of resistant starch for the other cereal grains was between 6.24 and 18.55%. A large proportion of the digestible starch in yellow dent corn and Nutridense corn was SDS (Table 5.2).

The concentration of GE in the experimental diets ranged between 3,596 kcal/kg in the rice diet and 4,022 kcal/kg in the dehulled oats diet (Table 5.4). Because of the low concentration of CP in yellow dent corn, Nutridense corn, sorghum, and rice, the concentration of CP in diets containing these cereal grains were also low (7.4 to 9.6%). However, for diets containing dehulled barley, dehulled oats, rye, and wheat, the concentration of CP was between 11.3 and 12.7%. The low concentration of AEE, ash, and TDF in the rice diet was reflective of

the low concentration of these nutrients in the rice grain. Total carbohydrates in the diets ranged from 65.8% in the dehulled oats diet to 74.4% in the rice diet, but the concentration of starch ranged from 55.7% in the rye diet to 72.8% in the rice diet. No resistant starch was analyzed from the diet containing rice, but the concentration of resistant starch for the other diets were between 1.0% (rye diet) and 12.6% (sorghum diet). The concentration of TDF in the rice diet was the least among diets, whereas the concentration of TDF for the other diets ranged from 4.3 to 11.9%.

Dehulled barley had the greatest ($P < 0.001$) capacity to bind water whereas rice had the least ($P < 0.001$) capacity to bind water (Table 5.5). The water binding capacity of sorghum was not different from that of dehulled oats, wheat, rye, yellow dent corn, or Nutridense corn, but Nutridense corn had a greater ($P < 0.001$) capacity to bind water than yellow dent corn.

Mean particle size was greatest ($P < 0.001$) in dehulled barley and least ($P < 0.001$) in yellow dent corn. Mean particle size of dehulled oats and rye was greater ($P < 0.001$) than the mean particle size obtained for polished rice, sorghum, yellow dent corn, and Nutridense corn. Mean particle size of polished rice and sorghum was also greater ($P < 0.001$) than for Nutridense corn.

The AID of GE was greater ($P < 0.001$) in rice than in other cereal grains (Table 5.6). The high AID of GE in rice was also reflected in a greater ($P < 0.001$) AID of OM, and total carbohydrates in rice than in the other cereal grains. The AID of starch was also greater ($P < 0.001$) in rice than in yellow dent corn, dehulled barley, rye, and sorghum. Likewise, the ATTD of GE was greater ($P < 0.001$) in rice than in yellow dent corn, rye, sorghum, and wheat, but the ATTD of GE in Nutridense corn, dehulled barley, and dehulled oats were not different from that

of rice. The greater ($P < 0.001$) AID and ATTD of GE, OM, and total carbohydrates resulted in less ($P < 0.001$) HGD of GE, OM, and total carbohydrate in rice than in the other cereal grains.

Except for the AID of CP, which was less ($P < 0.001$) in Nutridense corn than in dehulled oats, and the AID and ATTD of TDF, which was greater ($P < 0.001$) in Nutridense corn than in dehulled oats, the AID and ATTD of GE and nutrients in Nutridense corn was not different from those of dehulled oats.

With a few exceptions, the AID, ATTD, and HGD of GE, OM, total carbohydrates, and TDF in yellow dent corn, sorghum, and wheat were not different from each other. The AID of CP and starch was greater ($P < 0.001$) in wheat than in yellow dent corn and sorghum, and the AID of starch in yellow dent corn was greater ($P < 0.001$) than in sorghum, but the AID of AEE in sorghum was greater ($P < 0.001$) than in yellow dent corn and wheat.

The AID of GE and AEE in dehulled barley was greater ($P < 0.001$) than in rye, but the AID of starch in dehulled barley was less than ($P < 0.001$) than in rye. Except for the ATTD of CP and TDF in dehulled barley, which was not different from that in rye, the ATTD of GE and other nutrients in dehulled barley was greater ($P < 0.001$) than in rye.

Gross energy intake of pigs fed the rye diet tended ($P = 0.08$) to be greater than GE intake of pigs fed diets based on yellow dent corn, Nutridense corn, dehulled barley, rice, and wheat (Table 5.7). However, the GE excretion in the feces was greater ($P < 0.001$) from pigs fed the rye diet than from pigs fed all the other cereals except sorghum. The GE excretion in the urine of pigs fed the rye diet was also greater ($P < 0.001$) than in pigs fed yellow dent corn. This resulted in values for DE and ME (kcal/kg DM) that were less ($P < 0.001$) in rye than in the other cereal grains. Dehulled oats had the greatest DE and ME (kcal/kg DM) among the cereal grains. The DE (kcal/kg DM) in Nutridense corn, dehulled barley, and rice were not different

from each other, but were greater than the DE (kcal/kg DM) in yellow dent corn, sorghum, and wheat. However, the ME (kcal/kg DM) in wheat was not different from that of Nutridense corn and dehulled barley, but was less ($P < 0.001$) than in rice. The ME (kcal/kg DM) in yellow dent corn and sorghum was greater ($P < 0.001$) than the ME in rye.

The viscosity constant for the ileal digesta of pigs fed the diet containing Nutridense corn was greater ($P < 0.001$) than that of the digesta of pigs fed the other grains except that pigs fed the rye diet had a viscosity constant that was not different from that of pigs fed the Nutridense corn diet (Table 5.8). The viscosity constant of ileal digesta in pigs fed the rye diet was also not different from the viscosity constant of the ileal digesta in pigs fed the sorghum diet, but the ileal digesta of pigs fed diets based on yellow dent corn, dehulled barley, dehulled oats, rice, and wheat were less ($P < 0.001$) viscous than the ileal digesta from pigs fed the rye diet.

Ileal pH in pigs fed wheat was not different from the pH in the ileal digesta from pigs fed Nutridense corn and dehulled oats, but was greater ($P < 0.001$) than the ileal pH of pigs fed the other cereal diets. The ileal pH of pigs fed dehulled barley and rye was less ($P < 0.001$) than that of pigs fed other cereal grains.

The fecal pH was greatest ($P < 0.001$) in pigs fed wheat and least ($P < 0.001$) in pigs fed yellow dent corn, Nutridense corn, and sorghum. The concentration of fecal acetate in pigs fed yellow dent corn and Nutridense corn was not different, but was greater ($P < 0.001$) than the concentration of fecal acetate in pigs fed all other cereal grains. The concentration of fecal propionate was greater ($P < 0.001$) in dehulled barley and sorghum than in all other grains except rye, and the concentration of fecal butyrate was greatest ($P < 0.001$) for sorghum and least ($P < 0.001$) for dehulled barley, dehulled oats, wheat, and rice. The concentration of total SCFA was

greater ($P < 0.001$) in feces from pigs fed yellow dent corn, Nutridense corn, dehulled barley, rye, or sorghum compared with pigs fed dehulled oats, rice, or wheat.

DISCUSSION

Although starch is the major carbohydrate in cereal grains, the non-starch carbohydrate component of cereal grains varies considerably and a proportion of cereal starch is usually resistant to digestion. The presence of non-starch polysaccharides and resistant starch in cereal grains may alter gastrointestinal functions (Solà-Oriol et al., 2010) and may reduce nutrient digestibility in these grains, which may be of benefit in promoting health of humans, but may cause reduction in feed conversion efficiency when fed to pigs.

The concentration of AEE in the rice used in this experiment was approximately 60% less than the concentration of ether extract in rice used in other experiments and this may be the reason for the low GE obtained in the rice used in this experiment compared with data from other experiments (Kim et al., 2007; Li et al., 2006). However, the concentrations of total starch, resistant starch, and TDF were within the ranges reported (Cho et al., 1997; Kim et al., 2007; Li et al., 2006). The high digestibility of GE, OM, starch, and total carbohydrates in rice supports the results of studies indicating that energy and nutrients from rice are better digested and absorbed than energy and nutrients from corn when fed to young pigs (Li et al., 2006; Vicente et al., 2008). The low concentration of TDF and resistant starch in rice may contribute to this effect, which results in less fermentable substrates in the hindgut of pigs fed rice than in pigs fed corn. This is likely the reason the concentration of total SCFA in the feces of pigs fed the rice diet was relatively low. A less fermentable substrate in the hindgut may, in turn, contribute to a reduction in the incidence of diarrhea in pigs fed rice-based diets compared with pigs fed corn-based diets (Pluske et al., 1996). However, the high AID of starch and total carbohydrates in rice

also indicates that white rice may be a high glycemic grain (Nanri et al., 2010), which may be a concern in diabetic management for humans. The high GE digestibility of rice results in the caloric value of rice being second only to dehulled oats, which further indicates that rice may not be the cereal of choice in weight loss management or glycemic control.

The concentration of TDF in dehulled oats used in this experiment is within the typical range for oat grouts (Mälkki, 2001). Approximately 50% of the TDF in oats is soluble fiber, of which β- glucans are the major component (Mälkki, 2001; Jha et al., 2010). The presence of soluble TDF and β- glucans in the diet increases digesta viscosity (Gallaher et al., 1999), and increased viscosity in the digesta can limit the interaction of nutrients and enzymes and reduce nutrient digestion and absorption (Ellis et al., 1996). In this experiment, total starch in dehulled oats was as digestible as the starch in rice, but there was also no difference in digesta viscosity between pigs fed dehulled oats and rice. However, a reduction in the AID of GE, OM, and total carbohydrates in dehulled oats was observed and this was probably because of the TDF component in dehulled oats. In this experiment, the AID of TDF was determined. Therefore, the contribution of endogenous components analyzed as TDF cannot be quantified, but the negative value for the AID of TDF is likely a result of endogenous secretions that are analyzed as TDF. However, because total carbohydrates were calculated and TDF is a component of total carbohydrates, and because there was no difference in the AID of starch between dehulled oats and rice, the reduced AID of total carbohydrates in dehulled oats is likely a result of the reduction in the digestibility of the non-starch components of total carbohydrates, namely TDF and resistant starch, in dehulled oats. Although the ATTD and HGD of TDF in dehulled oats and rice were not different, dehulled oats contained greater concentrations of TDF and resistant starch than rice and, therefore, the low concentration of SCFA in the feces of pigs fed dehulled

oats was unexpected. Although the AID and ATTD of GE in dehulled oats was less than in rice, the greater caloric value of dehulled oats compared with rice was a result of the greater concentration of AEE and the greater digestibility of AEE in dehulled oats than in rice.

The nutrient digestibility profile of Nutridense corn is relatively similar to that of dehulled oats, but the reduced caloric value of Nutridense corn compared with dehulled oats can be attributed to the reduced AID of CP and the reduced ATTD of total carbohydrates as well as the lower concentration of AEE in Nutridense corn than in dehulled oats. The potential use of Nutridense corn for human consumption has not been investigated, but results of this experiment support results of several studies with pigs and poultry that indicate that Nutridense corn is superior in GE and AA digestibility compared with yellow dent corn (Han et al., 1987; Hasted et al., 2005; Pedersen et al., 2007). The superiority of Nutridense corn compared with yellow dent corn is attributed to the greater AID of OM, CP, AEE, and starch, and total carbohydrates, as well as the greater concentrations of CP and AEE in Nutridense corn than in yellow dent corn. The viscous nature of the ileal digesta of pigs fed Nutridense corn is not a characteristic of conventional corn varieties because the concentration of soluble fiber in corn is low (Bach Knudsen, 1997). The reason for the high viscosity of digesta in pigs fed Nutridense corn is unknown, but indicators of fiber fermentation in pigs fed Nutridense corn indicate that the type of fiber in Nutridense corn was similar to that in yellow dent corn. The proportion of SDS that was determined for Nutridense corn and yellow dent corn was greater than the values reported by Murray et al. (1999). The method of analysis may account for this difference.

The nutrient digestibility profile for sorghum and wheat is relatively similar to that of corn, but in terms of grain structure and nutrient composition, sorghum is more related to corn (Taylor and Emmambux, 2010). However the nutritional value of sorghum is only 95% of that of

corn (Hancock, 2000) because CP digestibility and DE of sorghum is less than for yellow dent corn (NRC, 1998; Pedersen et al., 2007). The reduced CP digestibility of sorghum is attributed to the binding of tannins to the protein in sorghum, which makes the protein resistant to proteolysis (Duodu et al., 2003), whereas the reduced caloric value of sorghum is attributed to low starch digestibility resulting from the formation of disulfide crosslinks between the endosperm and the protein in sorghum (Taylor and Emmambux, 2010). However, in this experiment, the AID of starch, but not of CP, was less for sorghum than for yellow dent corn, but the reduction in starch digestibility did not reduce the caloric value of sorghum compared with yellow dent corn, which may have been a result of the greater AID of AEE in sorghum than in yellow dent corn. The lack of a difference in the nutritional value between yellow dent corn and sorghum in this experiment is consistent with the results of Lin et al. (1987) and Murray et al. (1999). Sorghum contains approximately 40% more resistant starch than corn. Therefore, the resistant starch in sorghum may be the reason for the reduced AID of starch, but the resistant starch appeared to be fermented in the hindgut because the total tract disappearance of starch was close to 100% for sorghum as it was for the other cereal grains. The greater concentration of butyrate in the feces of pigs fed sorghum is likely the result of fermentation of the resistant starch in sorghum. Nevertheless, compared with Nutridense corn, dehulled barley, dehulled oats, and rice, the ME of sorghum was reduced. The relatively low AID of starch in sorghum indicates that sorghum is a suitable grain to manage blood sugar levels (Carciofi et al., 2008).

Unlike corn, where more than 80% of the TDF is insoluble fiber (Bach Knudsen, 1997), soluble fibers, particularly β- glucans and arabinoxylans, are present in wheat, barley, and rye (Annison and Choct, 1991). These soluble fibers are believed to have health promoting effects in humans (Venn and Mann, 2004). In contrast, soluble fiber may reduce the nutritional value of

these grains to pigs. However, in this experiment, the AID of starch in wheat was greater than in corn, but the ME of wheat was not different from that of corn. This is likely a result of the low concentration of AEE in wheat and the relatively lower AID of AEE in wheat than in yellow dent corn. There are also differences in the nutritional value among varieties of wheat (Fuller et al., 1989; Zijlstra et al., 1999; Regmi et al., 2009). Results of this experiment indicate that at least some varieties of wheat have a nutritional value that is equal to the nutritional value of yellow dent corn.

The concentration of TDF in dehulled barley was below the range of 19 to 22% reported for hulled barley and below the range of 11 to 15.7% for hulless barley (Fastnaught, 2001). The low AID of OM in dehulled barley is a result of the low AID of starch and total carbohydrates in dehulled barley. The poor AID of starch and total carbohydrates may be a result of the greater particle size of the dehulled barley compared with the other cereal grains. Grains with large particle sizes have less surface area exposed for enzymatic degradation and, therefore, are more likely to have a reduced digestibility of starch (Al-Rabadi et al., 2009). However, the ATTD of GE in dehulled barley was greater than in yellow dent corn, which was the reason the DE in dehulled barley was greater than the DE in yellow dent corn.

The concentration of TDF in rye was less than the value of 17% reported (Vinkx and Delcour, 1996; Bach Knudesen et al., 1997). The concentration of arabinoxylans is greater than the concentration of β- glucans in rye (Bach Knudsen, 1997) and the arabinoxylan component of rye contributes to the desired properties in bread preparation (Vinkx and Delcour, 1996). The low caloric value of rye compared with other cereal grains may be caused by the digestibility of GE and nutrients in this grain. Therefore, the relatively low nutritional value of rye compared with other cereal grains that was observed in this experiment is consistent with previous values

(NRC, 1998). The relatively high viscosity of digesta from pigs fed the rye diet may also have contributed to the reduced digestibility.

Ileal pH, fecal pH, and the concentration of SCFA in the feces were measured as indicators of the degree of fermentation of TDF at the end of the terminal ileum and throguhout the total tract. For cereal grains that have a low concentration of resistant starch and TDF such as rice, ileal digesta pH and fecal pH was expected to be more basic than the ileal digesta pH of cereals with greater concentration of resistant starch and TDF. The concentration of SCFA is also expected to be less in feces of pigs fed digestible cereals than in the feces of pigs fed cereals with more resistant starch and TDF. However, this hypothesis was only confirmed for rice. For all other cereal grains, no consistent pattern between concentration of TDF and resistant starch and measures of ileal and fecal pH and concentration of fecal SCFA was observed. A possible reason for this observation is that because SCFA are continuously produced and efficiently absorbed along the small and large intestines, changes in digesta and fecal pH may not be reflective of the continuous flux of SCFA in the gut.

In conclusion, the GE, nutrient digestibility, and caloric value of rice were greater and the GE and nutrient digestibility of rye was less than that of the other cereal grains. The AID of starch in yellow dent corn, barley, rye, and sorghum, but not in Nutridense corn, dehulled oats, and wheat, was reduced compared with the AID of starch in rice, which may be a result of the presence of resistant starch and TDF in yellow dent corn, dehulled barley, rye, and sorghum. However, because of the relatively high digestibility of energy and all nutrients, all these cereal grains can be used as energy sources in diets fed to pigs with rice and dehulled oats being superior to yellow dent corn. It is also expected that these cereal grains are excellent sources of energy for humans. If the goal is to feed grains with a high caloric value and absorption of most

energy in the form of glucose, rice and dehulled oats are the preferred cereal grains. However, if the goal is to reduce the glycemic index and reduce weight gain, sorghum and rye may be the most ideal cereal grains.

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Table 5.1. Analyzed energy and nutrient composition of yellow dent corn, Nutridense corn, barley, oats, rice, rye, sorghum, and wheat (as-fed basis)

Item	Yellow dent corn	Nutridense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat
GE, kcal/kg	3,921	3,972	3,878	4,172	3,717	3,906	3,953	3,913
DM, %	87.5	87.0	86.4	87.6	86.5	88.2	87.4	87.3
CP, %	7.5	8.8	11.8	13.1	9.0	12.1	9.8	11.9
AEE[1], %	4.2	5.6	2.4	7.5	0.9	2.7	3.9	3.2
Ash, %	1.2	1.0	1.3	1.5	0.2	1.6	1.0	1.4
OM[2], %	86.3	86.0	85.1	86.1	86.3	86.6	86.4	86.0
Total starch, %	64.7	64.1	64.2	65.1	75.1	56.8	66.9	61.6
Total carbohydrate,[3] %	72.4	70.1	69.9	65.8	74.4	70.6	67.8	68.6
Resistant starch[4], %	10.0	10.9	6.4	6.2	1.7	1.4	18.5	1.1
TDF[5], %	10.2	9.4	7.0	6.4	1.1	11.7	9.0	9.9

[1]AEE = acid hydrolyzed ether extract.

[2] OM = DM - ash.

[3] Total carbohydrate = DM – (CP + AEE + ash).

[4] Resistant starch = difference between the concentration of total starch (method of Thivend et al., 1972) and the concentration of digestible starch (method of Muir and O'Dea, 1992, 1993).

[5] TDF = total dietary fiber using method 985.29 (AOAC Int., 2007).

Table 5.2. Starch fractions in yellow dent corn and Nutridense corn (% of digestible starch)

	Free glucose,%	Rapidly digestible starch,%	Slowly digestible starch,%
Yellow dent corn	1.7	20.3	78.0
Nutridense corn	1.8	23.0	75.2

Table 5.3. Ingredient composition (%) of experimental diets, (as-fed basis)

Ingredient	Diet
Cereal grain	97.4
Dicalcium phosphate	0.80
Limestone	0.70
Titanium dioxide	0.40
Salt	0.40
Vitamin-mineral premix[1]	0.30
Total	100.00

[1]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 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 5.4. Analyzed energy and nutrient composition of experimental diets (%, as-fed basis)

Item	Yellow dent corn	Nutridense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat
GE, kcal/kg	3,770	3,815	3,764	4,022	3,596	3,740	3,800	3,821
DM, %	87.0	86.4	87.5	88.1	86.7	88.4	86.8	87.8
CP, %	7.4	8.3	11.6	12.7	8.7	11.3	9.6	12.1
AEE[1], %	3.7	4.9	2.4	6.3	0.9	2.3	5.3	3.0
Ash, %	3.5	3.1	3.5	3.2	2.7	4.2	4.2	4.0
OM[2], %	83.6	83.3	84.0	84.9	84.0	84.2	82.6	83.8
Total starch, %	61.9	63.5	60.6	63.0	73.8	55.7	60.5	59.5
Total carbohydrates[3], %	72.4	70.1	69.9	65.8	74.4	70.6	67.8	68.6
Resistant starch[4], %	7.7	12.2	10.9	3.0	-0.6	1.0	12.6	5.3
TDF[5], %	9.07	8.16	6.90	4.31	0.98	11.69	10.60	10.16

[1]AEE = acid hydrolyzed ether extract.

[2] OM = DM - ash.

[3] Total carbohydrate = DM – (CP + AEE + ash).

[4] Resistant starch = difference between the concentration of total starch (method of Thivend et al., 1972) and the concentration of digestible starch (method of Muir and O'Dea, 1992, 1993).

[5] TDF = total dietary fiber using method 985.29 (AOAC Int., 2007).

Table 5.5. Water binding capacity (WBC, g/g dry pellet wt.) and mean particle size (μm) of yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, rice, rye, sorghum, and wheat

Item	Diet									
	Yellow dent corn	Nutri-dense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat	SEM	*P*-value
WBC	1.06 [c]	1.20 [b]	1.36 [a]	1.25 [b]	0.86 [d]	1.19 [b]	1.17 [bc]	1.19 [b]	0.037	0.001
Mean particle size	510 [f]	589 [e]	1057 [a]	774 [bc]	684 [d]	830 [b]	700 [d]	767 [cd]	19.98	0.001

Table 5.6. Apparent ileal digestibility (AID), apparent total tract digestibility (ATTD), and hindgut disappearance (HGD) of OM, GE, CP, acid hydrolyzed ether extract (AEE), total starch, total carbohydrate, and total dietary fiber (TDF) in pigs fed diets based on yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, rice, rye, sorghum, or wheat[1]

		Diet									
Item		Yellow dent corn	Nutri-dense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat	SEM	*P*-value
GE, %											
	AID	73.0 ^{c}	79.3 ^{b}	66.7 ^{d}	80.8 ^{b}	91.4 ^{a}	62.3 ^{e}	72.0 ^{c}	71.2 ^{c}	1.34	0.001
	ATTD	91.3 ^{cd}	92.3 ^{b}	93.2 ^{b}	91.9 ^{bc}	97.9 ^{a}	89.6 ^{de}	89.3 ^{de}	91.9 ^{bc}	0.81	0.001
	HGD	16.4 ^{cd}	13.1 ^{de}	26.7 ^{a}	11.2 ^{e}	6.23 ^{f}	27.5 ^{a}	17.1 ^{bc}	20.8 ^{b}	1.23	0.001
OM, %											
	AID	76.7 ^{c}	82.7 ^{b}	69.8 ^{d}	83.5 ^{b}	93.1 ^{a}	66.9 ^{d}	73.9 ^{c}	75.4 ^{c}	1.16	0.001
	ATTD	93.5 ^{cd}	94.3 ^{bc}	95.1 ^{b}	94.5 ^{bc}	98.7 ^{a}	92.4 ^{d}	92.3 ^{d}	93.4 ^{cd}	0.60	0.001
	HGD	15.2 ^{bc}	12.4 ^{cd}	25.5 ^{a}	10.8 ^{d}	5.3 ^{e}	25.7 ^{a}	18.3 ^{b}	18.0 ^{b}	1.16	0.001

Table 5.6. (Cont.)

CP, %											
	AID	49.8 ^{d}	58.4 ^{b}	61.3 ^{b}	72.4 ^{a}	70.7 ^{a}	56.5 ^{bc}	50.0 ^{cd}	62.17 ^{b}	2.63	0.001
	ATTD	83.7 ^{c}	88.4 ^{b}	87.3 ^{bc}	88.9 ^{b}	94.5 ^{a}	85.0 ^{bc}	77.8 ^{d}	89.3 ^{b}	1.87	0.001
	HGD	29.8 ^{ab}	28.9 ^{ab}	28.4 ^{ab}	16.2 ^{d}	19.2 ^{cd}	27.9 ^{ab}	32.3 ^{a}	25.0 ^{bc}	2.30	0.001
AEE, %											
	AID	35.5 ^{c}	54.3 ^{ab}	27.7 ^{cd}	59.8 ^{a}	49.6 ^{b}	-13.3 ^{e}	60.0 ^{a}	24.2 ^{d}	3.34	0.001
	ATTD	58.2 ^{b}	69.8 ^{a}	56.0 ^{b}	66.3 ^{ab}	61.6 ^{ab}	31.8 ^{c}	69.9 ^{a}	64.7 ^{ab}	5.37	0.001
	HGD	17.3 ^{cd}	15.5 ^{d}	30.2 ^{bc}	6.4 ^{d}	12.4 ^{d}	45.8 ^{a}	9.8 ^{d}	39.4 ^{ab}	4.77	0.001
Total starch,%											
	AID	95.1 ^{b}	98.5 ^{a}	84.9 ^{e}	96.8 ^{ab}	98.6 ^{a}	92.3 ^{c}	89.0 ^{d}	98.0 ^{a}	0.70	0.001
	ATTD	99.7 ^{bcd}	99.9 ^{ab}	99.9 ^{ab}	99.8 ^{abc}	99.9 ^{a}	99.6 ^{cd}	99.4^{e}	99.6 ^{de}	0.08	0.001
	HGD	4.5^{d}	1.5 ^{e}	14.8 ^{a}	3.4 ^{de}	1.4 ^{e}	7.4 ^{c}	9.8 ^{b}	2.1 ^{e}	0.72	0.001
Total carbohydrates, %											
	AID	81.4 ^{c}	87.4 ^{b}	72.3 ^{d}	88.7 ^{b}	96.3 ^{a}	71.1 ^{d}	79.0 ^{c}	79.9 ^{c}	1.08	0.001
	ATTD	96.3 ^{c}	96.7 ^{c}	97.8 ^{b}	98.3 ^{b}	99.6 ^{a}	95.4 ^{d}	96.6 ^{c}	95.3 ^{d}	0.38	0.001

Table 5.6. (Cont.)

	HGD	15.0 [b]	9.3 [c]	25.6 [a]	9.4 [c]	3.3 [d]	24.2 [a]	17.3 [b]	15.3 [b]	1.13	0.001
TDF											
	AID	12.6 [b]	4.6 [bc]	-7.4 [cd]	-15.6 [d]	-29.8 [e]	-7.2 [cd]	40.6 [a]	0.9 [bc]	4.75	0.001
	ATTD	65.0 [ab]	66.2 [ab]	70.9 [ab]	53.2 [c]	60.0 [bc]	67.9 [ab]	76.0 [a]	62.0 [bc]	25.73	0.020
	HGD	50.6 [cd]	61.0 [bc]	79.6 [a]	68.3 [ab]	69.7 [ab]	70.5 [ab]	35.4 [d]	59.1 [bc]	7.92	0.001

[1] Data are means of 8 observations per treatment except for Nutridense corn, dehulled barley, and sorghum diets, where data are means of 9 observations per treatment.

Table 5.7. Daily energy balance in growing pigs fed diets based on yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, rice, rye, sorghum, and wheat and the ME and DE in each cereal grain [1]

Item	Diet									
	Yellow dent corn	Nutri-dense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat	SEM	*P*-value
GE intake, kcal	3296 [b]	3388 [b]	3302 [b]	3529 [ab]	3244 [b]	3722 [a]	3441 [ab]	3411 [b]	150.42	0.080
GE in feces, kcal	286 [c]	259 [c]	233 [c]	275 [c]	68 [d]	403 [a]	370 [ab]	299 [bc]	24.76	0.001
GE in urine, kcal	72 [e]	94 [bcd]	89 [cde]	113 [ab]	89 [cde]	106 [abc]	80 [de]	116 [a]	9.35	0.001
DE in diet, kcal/kg	3,441 [c]	3,521 [b]	3,507 [b]	3,695[a]	3,520 [b]	3,330 [d]	3,390 [cd]	3,509 [b]	29.19	0.001
ME in diet, kcal/kg	3,354 [bc]	3,416 [b]	3,413 [b]	3,566 [a]	3,421 [b]	3,241 [d]	3,300 [cd]	3,380 [b]	30.01	0.001
DE in ingredient, kcal/kg	3,533 [c]	3,616 [b]	3,601 [b]	3,792 [a]	3,613 [b]	3,418 [d]	3,481 [cd]	3,603 [b]	30.38	0.001
ME in ingredient, kcal/kg	3,443 [bc]	3,507 [b]	3,504 [b]	3,661 [a]	3,513 [b]	3,327 [d]	3,388 [cd]	3,471 [b]	30.82	0.001
DE in ingredient, kcal/kg DM	4036 [c]	4155 [b]	4167 [b]	4330 [a]	4188 [b]	3875 [d]	3985 [c]	4126 [c]	34.80	0.001
ME in ingredient, kcal/kg DM	3934 [de]	4030 [bc]	4055 [bc]	4180 [a]	4063 [b]	3772 [f]	3878 [e]	3975 [cd]	35.30	0.001

[1] Data are means of 8 observations per treatment except for yellow dent corn, Nutridense corn, and sorghum diets, where data are means of 9 observations per treatment.

Table 5.8. Ileal viscosity, ileal and fecal pH and concentrations of fecal volatile short-chain fatty acids (SCFA; μmol·g^{-1}, DM basis) in the feces of pigs fed diets based on yellow dent corn, Nutridense corn, dehulled barley, dehulled oats, rice, rye, sorghum, and wheat

	Diet									
Item	Yellow dent corn	Nutri-dense corn	Dehulled barley	Dehulled oats	Polished white rice	Rye	Sorghum	Wheat	SEM	*P*-value
Ileal viscosity										
Constant, cP	488 ^{c}	1429 ^{a}	416 ^{c}	545 ^{c}	652 ^{c}	1224 ^{ab}	664 ^{bc}	413 ^{c}	197.74	0.001
Exponent	-1.0	-1.2	-0.9	-0.9	-1.1	-0.9	-1.0	-1.0	0.08	0.084
R^2	0.99	0.99	0.98	0.99	0.98	0.98	0.99	0.99	-	-
Ileal pH	6.62 ^{b}	6.76 ^{ab}	6.40 ^{d}	6.71 ^{ab}	6.60 ^{b}	6.39 ^{d}	6.45 ^{cd}	6.83 ^{a}	0.068	0.001
Fecal pH	5.89 ^{c}	5.97 ^{c}	6.36 ^{b}	6.33 ^{b}	6.43 ^{b}	6.47 ^{b}	5.75 ^{c}	6.90 ^{a}	0.113	0.001
Fecal SCFA										
Acetate	104 ^{a}	103 ^{a}	88 ^{b}	63 ^{cd}	70 ^{cd}	85 ^{b}	76 ^{bc}	60 ^{d}	5.31	0.001
Propionate	22 ^{bc}	21 ^{bc}	34 ^{a}	16 ^{cd}	12 ^{d}	26 ^{ab}	34 ^{a}	16 ^{cd}	3.26	0.001
Butyrate	13 ^{bc}	10 ^{cde}	12 ^{bcd}	9 ^{de}	11 ^{bcde}	14 ^{b}	20 ^{a}	8 ^{e}	1.63	0.001
Total SCFA	143 ^{a}	137 ^{a}	134 ^{a}	88 ^{b}	92 ^{b}	128 ^{a}	128 ^{a}	83 ^{b}	9.18	0.001

CHAPTER 6

Evaluation of *in vitro* Procedures to Measure Disappearance of DM in Yellow Dent Corn

ABSTRACT: The objective of the experiment was to correlate DM or OM disappearance obtained using 3 in vitro procedures with apparent total tract digestibility **(ATTD)** of GE or with the concentration of DE in 50 corn samples that were fed to growing pigs. The second objective was to develop a regression model that would predict the ATTD of GE or the concentration of DE in corn. The third objective was to evaluate the suitability of using the Daisy^{II} incubator as an alternative to the traditional water bath when determining in vitro DM and OM disappearance. A total of 8 experiments was conducted to evaluate in vitro assays that could be used to predict the ATTD of GE or to predict the concentration of DE in corn. Sample size (0.5, 1.5, 2.5, or 3.5 g), type of filtration (Gooch crucible or filter paper), incubation length (24 or 48 h), type of incubation (water bath or Daisy ^{II} incubator), and 3 different procedures to estimate hindgut fermentation were evaluated for prediction of in vitro DM (IVDMD) and in vitro OM (IVOMD) disappearance in corn. The 3 procedures were the use of Viscozyme , the use of cellulase, and the use of fecal inoculum. Results of the experiment indicated that increasing samples size reduced IVDMD and in vitro GE disappearance in corn. Corn samples filtered using the filter paper has lower IVDMD than corn samples filtered using the Gooch crucible, but no differences were observed in IVDMD in corn samples incubated either in the Daisy^{II} incubator or in the water bath when the incubation period was for 48h. If the Daisy^{II} incubator was used and the samples were incubated for 48 h with Viscozyme, the ability of the procedure to detect small differences in the ATTD of GE or to detect small differences in the concentration of DE in corn, was improved ($P < 0.001$). Likewise, compared with using cellulose or fecal inoculum, the variability in the ATTD of GE and the variability in the DE in corn was better ($R^2 = 0.56$; $P =$

0.05 and R^2 = 0.53; *P* = 0.06, respectively) explained if Viscozyme was used than if cellulase (R^2 = 0.48; *P* = 0.09 and R^2 = 0.41; *P* = 0.12, respectively) or fecal inoculum (R^2 = 0.03; *P* = 0.70 and R^2 = 0.17; *P* = 0.36, respectively) was used. In conclusion, a validated regression model that predicted the DE in corn was developed using Viscozyme and with the corn samples incubated in the Daisy^{II} incubator for a long period.

INTRODUCTION

In the US, corn is the main source of energy in swine diets. Variation in the chemical composition of corn exists (Reynolds et al., 2005) and may contribute to differences in energy digestibility or caloric value of corn (Adeola and Bajjalieh, 1997; Sauber and Owens, 2001; Pedersen et al., 2007). Considering that energy is the most expensive component in swine diets (Noblet and Henry, 1993), an inaccurate estimation of the energy digestibility of corn will have an economic impact. To formulate diets on a least cost basis that also meet the energy requirement of the pig, it is necessary that a rapid and accurate procedure to estimate the energetic values of corn be identified.

A 3-step in vitro procedure that makes use of pepsin, pancreatin, and Viscozyme to simulate gastric digestion, small intestinal digestion, and hind gut fermentation, respectively, may be used to determine the energy value of feed ingredients and mixed diets (Boisen and Fernandez, 1997). Use of cellulase from *Trichoderma viridae* (Huang et al., 2003; Regmi et al., 2008) or the use of fecal inoculum (Lattimer et al., 2007) in the third step of the procedure instead of Viscozyme, has been suggested. These procedures provided results that correlate well with in vivo energy digestibility of diets and feed ingredients (Boisen and Fernandez, 1997; Noblet and Jaquelin-Peyraud, 2007) and specific feedstuffs such as barley and wheat (Regmi et

al., 2008; Regmi et al., 2009); however, these procedures have not been compared and they have not been used to predict energy digestibility in different hybrids of corn.

The Daisy^{II} incubator system has the advantage of having a large batch size capable of accommodating greater quantities of samples (Lattimer et al. 2007) than the traditional water bath, and this system may, therefore, be used for incubation when in vitro procedures are used to measure energy digestibility feed ingredients. Considering the potential of in vitro procedures to reduce the time and costs of energy measurements, there is a need to evaluate these procedures. Therefore, the objective of this experiment was to correlate DM or OM digestibility obtained from 3 in vitro procedures with the energy digestibility values obtained from in vivo measurements using corn as the test material. The second objective was to develop a regression model that can predict the apparent total tract digestibility **(ATTD)** of GE or that can predict the concentration of DE in corn. The third objective was to evaluate the suitability of using the Daisy^{II} incubator as an alternative to the traditional water bath.

MATERIALS AND METHODS

Exp. 1. In Vitro Disappearance of DM and OM Using Viscozyme

Collection of Samples and Incubation. The objective of Exp. 1 was to determine in vitro ATTD of DM and OM in 50 corn samples using the 3 step in vitro procedure described by Boisen and Fernandez (1997) and to correlate in vitro DM **(IVDMD)** or in vitro OM **(IVOMD)** disappearance with the in vivo ATTD of GE or DE that were previously measured in these corn samples. The corn samples were obtained from Pioneer Hi-Bred International, Inc. (Johnston, IA) and were chosen to provide variability in corn nutrient composition (Table 6.1). A second objective was to determine the repeatability of the in vitro procedure by including a standard

corn sample for each batch and compare the IVDMD and IVOMD of this corn sample obtained from the different batches.

The in vitro assay of Boisen and Fernandez (1997) is a 3- step procedure with each step representing the digestion processes in the stomach, small intestine, and large intestine, by using pepsin, pancreatin, and Viscozyme, respectively. All samples were ground to pass through a 1-mm screen using a Wiley Mill Model 4 (Thomas Scientific; Swedesboro, NJ). Each sample was weighed (0.500 g ± 1 mg) into 125-mL Erlenmeyer flasks in triplicate. Twenty-five mL of phosphate buffer (0.1 *M*; pH 6.0) and 10 mL of 0.2 *M* HCl were added to each flask and flasks were stirred continuously using a magnetic stirrer. The pH of the solution was adjusted to 2 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH. One mL of freshly prepared pepsin solution (25 mg of pepsin/mL; 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) that was previously heated at 39°C for 2 h.

After 2 h, 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 ± 0.01 using 1 *M* HCl or 1 *M* NaOH. One mL of freshly prepared pancreatin solution (100 mg of pancreatin/mL; P1750, Sigma Aldrich) was added to each flask that was then incubated in a shaking water bath at 39°C. After 4 h, 10 mL of 0.2 *M* EDTA solution was added to each flask and pH was adjusted to 4.8 ± 0.01 using 30% acetic acid. Viscozyme was added to each flask (0.5 mL; Viscozyme L V2010, Sigma-Aldrich) and the flasks were returned to the shaking water bath and incubated for 18 h at 39°C. At the end of the 18-h incubation, undigested residues in the flasks were filtered in previously weighed Gooch crucibles containing celite 545 (0.400 g ± 5 mg; Sigma Aldrich). The undigested material collected in the crucibles was washed twice with 10 mL of ethanol (96%) and twice with 10 mL of acetone (99.5%). The crucibles were dried in the oven overnight at 130ºC, cooled in the desiccator, and weighed to

measure DM concentration prior to ashing at 500°C for 4 h (Boisen and Fernandez, 1997). The weight of the cooled crucibles was recorded to determine the concentration of ash in the residues. Two extra flasks, which contained no samples (blanks), but where all reagents and enzymes were added, were included in each batch. The DM and OM of the residue collected in these flasks after incubation were used to correct the final DM and OM weight of the residues.

Calculations and Statistical Analyses. The IVDMD was calculated using Eq. [1]:

$$\text{IVDMD}, \% = \frac{[\text{Sample DM} - (\text{Residue DM} - \text{Blank DM})]}{\text{Sample DM}} \times 100 \quad [1]$$

where sample DM is the concentration of DM in the sample (g), residue DM and blank DM are the concentration of DM in the residues obtained from flasks with and without corn samples respectively, and calculated as the difference between the combined dried weight of the crucible, celite, and residues, and the combined dried weight of crucible and celite.

The IVOMD was also calculated using Eq. [1]. Normality of the data and presence of potential outliers were determined using the UNIVARIATE procedure of SAS. The PROC REG of SAS was used to determine the relationship between ATTD of GE and IVDMD or IVOMD, as well as the relationship between DE and IVDMD or IVOMD. Regression equations with a *P* value less than 0.05 were considered significant and $P > 0.051$ to $P < 0.10$ was considered to have a tendency for significance. The repeatability of the procedure was tested using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) after establishing normal distribution of data by the UNIVARIATE procedure. Differences among means of different batches were considered significant at $P < 0.05$.

Exp. 2. Evaluation of Filtration Method

Samples and Incubation. Weighing of celite into Gooch crucibles and ashing of the Gooch crucibles for 4 h is part of the preparatory step in the Boisen and Fernandez (1997) procedure. This preparatory step prolongs the assay by at least 8 h. An alternative in vitro procedure (Regmi et al., 2008) uses filter paper instead of the Gooch crucible. The objective of

Exp. 2 was to compare values for IVDMD and IVOMD in selected corn samples (n = 8) obtained using the Gooch crucibles to values obtained using filter paper in the filtration step of the Boisen and Fernandez (1997) procedure.

Each of the 8 samples was weighed (0.500 g ± 0.1 mg) into 4 flasks. The corn samples were divided into 2 batches with each batch containing 16 flasks from 4 corn samples. Four blanks were also included in each batch to correct for DM and OM contribution of the solutions and enzymes. All the corn samples were analyzed according to the procedure described in Exp. 1 except that in the last step, 8 of the flasks belonging to the 4 corn samples and 2 blanks were filtered using the Gooch crucible and celite, and the other 8 flasks with corn samples and 2 blanks were filtered using hardened quantitative ash-free filter paper (Grade 541, Whitman Inc. Piscataway, NJ). The crucibles were pre-dried at 130°C whereas the filter papers were pre-dried at 80°C overnight and their weights were recorded prior to the filtration process. Crucible and filter papers with residues were dried in the same manner. The second batch was also performed in the same manner as the first batch.

Calculations and Statistical Analyses. Values for IVDMD and IVOMD obtained from either crucibles or filter papers were calculated using Eq. [1]. Normality of data was determined as described for Exp. 1. Differences between the 2 filtration procedures were analyzed as described in Exp. 1.

Exp. 3. In Vitro DM Disappearance Using Cellulase

The in vitro procedure developed by Huang et al. (2003) has been reported to adequately predict in vivo ATTD of GE from IVDMD in wheat and barley (Regmi et al., 2008, 2009) using cellulase from *Trichoderma viridae* instead of Viscozyme in the third step of the Boisen and Fernandez (1997) procedure. This procedure, however, has not been tested for corn. The objective of this experiment, therefore, was to determine the IVDMD in 7 selected corn samples

using the procedure developed by Huang et al. (2003) and to correlate IVDMD with the in vivo ATTD of GE or the in vivo DE that was determined previously in these corn samples.

Corn samples were weighed as described for Exp. 1 except that 1 g ± 1 mg of each sample was used. The first step of the in vitro procedure was as described for Exp. 1 except that the incubation time was 6 h and pepsin was added at 10 mg/mL instead of 25 mg/mL. The second step of the procedure was also as described for Exp. 1 except that 3 mL of pancreatin solution (50 mg/mL) was added to the flasks after the pH of the solution was adjusted to 6.80 ± 0.01. After an 18 h incubation, cellulase solution (20 mL; 3 units of cellulase/mL) prepared from *Trichoderma viridae* (C-9422, Sigma Aldrich. St. Louis, MO) was added. The flasks were incubated for an additional 24 h at 39ºC. At the end of the incubation, 5 mL of 20% sulfosalicylic acid was added to each flask at room temperature for 30 min. The solution was separated from the residues by using a filtration manifold. Residues were collected into plastic funnels lined with pre-dried and pre-weighed ash-free filter paper (Grade 541, Whatman Inc., Piscataway, NJ). Precipitates remaining in the flasks were rinsed and filtered with 1% sulfosalicylic acid. The filters with the residues were dried overnight at 80°C and weighed.

Calculations and Statistical Analyses. The IVDMD and the IVOMD for each corn sample was calculated using Eq. [1]. The relationship between ATTD of GE and IVDMD or IVOMD, as well as the relationship between DE and IVDMD or IVOMD, was analyzed as described for Exp. 1.

Exp. 4. In Vitro Disappearance of DM using the Daisy^{II} Incubator

Samples and Incubation. The Daisy incubator^{II} (Ankom Technology, Macedon, NY) consists of 4 incubation jars with each jar having a capacity of 25 bags and 2 L of solution. The objective of this experiment was to compare the IVDMD of selected corn samples incubated in

each of the 4 incubation jars. The 3- step in vitro procedure by Boisen and Fernandez (1997) was modified to adapt to the use of the Daisy^{II} incubator.

Each of the 11 selected corn samples were weighed into 8 pre-dried and pre-weighed polyester bags (5 cm × 10 cm bags; R510, Ankom Technology, Macedon, NY). The weight of the bag and the sample (0.500 g ± 1 mg) was recorded before the bags were heat-sealed to prevent spilling of the samples. Each jar was assigned 2 bags per sample. Three additional bags without samples were added to each jar to serve as blanks. Therefore, each jar contained a total of 25 bags. All the solutions for the 4 jars were prepared at the same time and originated from the same batch. The concentrations of the buffers, acids, bases, EDTA, and enzymes were added in proportion to the number of bags contained in each jar. Phosphate buffer (pH = 6.0, 625 mL), HCl (0.2 M, 250 mL), and chloramphenicol (12.5 mL) were mixed together in each jar and pre-heated inside the Daisy^{II} incubator until the internal temperature reached 39ºC. After adding the bags and shaking the jar to soak all the bags with the solution, the pH of the solution was adjusted to 2.0 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH. Pepsin was added (25 mL/jar; 25 mg pepsin/mL) and the jars were incubated for 2 h after a 20 min allowance to allow the solution temperature to reach 39ºC. A preheated mixture of phosphate buffer (pH = 6.8, 250 mL) and NaOH (0.6*M*, 125 mL) was added to the jar at the end of the 2-h incubation and the solution was adjusted to pH = 6.80 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH to the solution. Pancreatin was added (25 mL/jar; 100 mg/mL) and the jars were again incubated for 4 h. Preheated EDTA (0.2 *M*, 250 mL) was added in each jar and the pH of the solution was adjusted to 4.80 ± 0.01 by the addition of 30% acetic acid. Viscozyme was added (12.5 mL/jar) prior to the 18 h incubation. At the end of the incubation, the solution was drained from each jar and the bags inside the jar were rinsed twice with 400 mL distilled water. The bags were further rinsed with 95 % ethanol

(2 × 250 mL) and acetone (2 × 250 mL). The bags were dried overnight at 100ºC, cooled, and weighed.

Calculations and Statistical Analyses. The IVDMD for each corn sample was calculated using Eq. [1]. Normality of the data was determined as described in Exp. 1. Data were analyzed as described in Exp. 2.

Exp. 5. The Effect of Length of Incubation and Type of Incubation on In Vitro DM Disappearance of Selected Corn Samples

Samples and Incubation Length . The objective of this experiment was to determine the effect of method of incubation and time of incubation on IVDMD of selected corn samples using a 2 × 2 factorial design. The 2 methods of incubation were the water bath and the Daisy^{II} incubator. The 2 incubation times were 2, 4, and 18 h of incubation or 6, 18, and 24 h of incubation for pepsin, pancreatin, and Viscozyme steps, respectively.

Seven samples of corn were selected and all samples were weighed at the same time. Weighing of the samples and the in vitro analysis using the water bath were as described for Exp. 1 and weighing of the samples and the in vitro analysis using the Daisy^{II} incubator were as described for Exp. 4. The only difference between procedures used in this experiment and those used in Exp. 1 and 4 is the length of incubation. Because there was only one Daisy^{II} incubator, the long incubation time for both the water bath and Daisy^{II} incubator was performed first followed by the short incubation time. The position of the jar was established (Exp. 4) to have no effect on IVDMD; thus, the samples were only added to 1 jar, but the other jars were incubated in the same way except that distilled water was added.

Calculations and Statistical Analyses. The IVDMD for each corn sample was calculated using Eq. [1]. Normality of the data was determined as described in Exp. 1. Effect of type of incubation and length of incubation on IVDMD was tested as described in Exp. 1. The

relationship between ATTD of GE and IVDMD, as well as the relationship between DE and IVDMD was analyzed as described for Exp. 1.

Exp. 6. Effect of Sample Weight and Type of Incubation on In Vitro Disappearance of DM and GE in Corn

Sample Weight and Incubation Method. The amount of residue that was collected after the 3-step in vitro procedure as described by Boisen and Fernandez (1997) was not sufficient to conduct analyses other than DM and ash. Also, the procedure by Huang et al. (2003) used 1.0 g of sample instead of 0.5 g used by Boisen and Fernandez (1997). Therefore, the objective of this experiment was to determine if larger sample size can be used to determine vitro gross energy disappearance (**IVGED**) in corn. The sample weight groups that were evaluated were 0.5, 1.5, 2.5 and 3.5 g. The weighing procedure for samples used for the water bath was as described in Exp. 1 except that for 1.5 g ± 1 mg, the samples were weighed into 250-mL flasks, and the 2.5 and 3.5 g ± 1 mg samples were weighed into 500-mL Erlenmeyer flasks to accommodate the volume of solutions that would be added. The amount of solution, acids, bases, EDTA, and enzymes were 3, 5, and 7 times greater in the flasks containing 1.5 , 2.5, and 3.5g samples, respectively, than in flasks containing 0.5 g samples. For each weight group, 2 flasks containing no sample, but where all the reagents and enzymes were added, were also included. All other analyses were as described for Exp. 1 except that the residues were collected in pre-dried and pre-weighed filter papers as described for exp. 2.

The weighing procedure for samples used for the Daisy^{II} incubator was as described for Exp. 4 except that additional bags weighing 1.5, 2.5, and 3.5 g were prepared. Each jar contained duplicate samples of each weight group; therefore, each jar contained a total of 8 bags. Similar to the water bath, the concentration of solution, acids, bases, EDTA, and enzymes were

adjusted in proportion to the amount of samples inside each jar. All remaining procedures were as described for Exp. 4. The residues in the filter paper and the bags were analyzed for GE by bomb calorimetry (model 6300, Parr Instruments, Moline, IL).

Calculations and Statistical Analyses. The IVDMD and IVGED for the corn sample in each weight group was calculated using Eq. [1]. Differences among weight groups were analyzed as described in Exp. 1. The relationship between sample weight and IVDMD, and between sample weight and IVGED, was analyzed as described in Exp. 1.

Exp 7. Comparison of 3 In Vitro Procedures and the Effect of Length of Incubation in IVDMD Using the Daisy^{II} Incubator

The objective of this experiment was to compare 3 in vitro procedures and to determine the effect of length of incubation on IVDMD using the Daisy^{II} incubator in a 3 × 2 factorial design. The 3 in vitro procedures compared were the procedure by Boisen and Fernandez (1997) using Viscozyme, the procedure by Huang et al. (2003) using cellulase, and a modification of the Boisen and Fernandez (1997) procedure where fecal inoculum was used instead of Viscozyme in the third step of the procedure. The 2 incubation times were 2, 4, and 18 h or 6, 18, and 24 h for the first, second, and third steps, respectively, of the in vitro procedures. Weighing of the samples was as described in Exp. 4. All samples allocated to each in vitro procedure were placed in one jar. Therefore, jar 1 contained samples for the in vitro analysis using the Boisen and Fernandez (1997) procedure, jar 2 contained samples for the in vitro analysis using the Huang et al. (2003) procedure, and jar 3 contained samples for the modified Boisen and Fernandez (1997) procedure using fecal inoculum at the third step of the procedure. Jar 4 contained distilled water. The first 2 steps of the in vitro procedure were conducted as described in Exp. 4 except that the pepsin solution added to jar 2 contained 10 mg pepsin/mL of

solution instead of 25 mg pepsin/mL, and the pancreatin solution that was added was at 75 mL (50 mg pancreatin/mL) instead of 25 mL (100 mg pancreatin/mL solution). The third and the final steps of the procedure for Jar 1 were as described in Exp. 4. For jar 2, after the second incubation, 500 mL of cellulase solution (20 mL/g; 3 units cellulase/mL) was added to the jar. At the end of the third incubation, 125 mL of 20% sulfosalicylic acid was added and the jar was allowed to stand for 30 min before draining. The bags with residues were rinsed twice with 250 mL of 1% sulfosalicylic acid before oven-drying.

Fecal inoculum was prepared from freshly collected feces obtained from a pig fed a corn-soybean-DDGS-antibiotic free diet for at least 10 d. After collection, feces were placed in a bag that was sealed after most of the air was removed from the bag, and transported to the laboratory inside an insulated bag that was kept at approximately 39ºC. Feces were weighed and mixed with previously prepared anaerobic diluting solution (Bryant and Burkey, 1953) inside a blender (Waring Laboratory Products; Torrington, CT) at a 1:10 w/v ratio. The blender was continuously purged with copper dried carbon dioxide gas to keep the condition anaerobic. Under CO_2, the fecal mixture was then filtered with 4 layers of cheesecloth into a CO_2 purged flask that was subsequently sealed. The flask was kept in the water bath at 39ºC until inoculation. At the end of the second incubation, anaerobic media (650 mL; Bourquin et al., 1993) and fecal inoculum (100 mL) were added to jar 3 under a continuous stream of copper dried CO_2 prior to closing the jar. At the end of the third incubation, the jar was filled with 95% ethanol and allowed to stand for 1 h. The solution was drained and the bags were rinsed twice with 250 mL of 96% ethanol. The bags were further rinsed 2 times with 250 mL of acetone prior to oven-drying.

Calculations and Statistical Analyses. The IVDMD for each corn sample was calculated using Eq. [1]. Effect of in vitro procedure and length of incubation on IVDMD was analyzed as

described in Exp. 1. The relationship between ATTD of GE and IVDMD for each treatment group, as well as the relationship between DE and IVDMD, were analyzed as described in Exp. 1.

Exp 8. Development and Validation of Regression Models to Predict Apparent Total Tract Disappearance of GE or to Predict DE in Corn

The objective of this experiment was to develop a regression model that is able to predict the ATTD of GE or the concentration of DE in corn. The IVDMD of 38 corn samples were determined using Viscozyme, the Daisy [II] incubator, and a long incubation period as described in Exp. 7.

Calculations and Statistical Analyses. The IVDMD of each corn sample was calculated using Eq. 1. A total of 28 corn samples were used to develop the regression model. Ten corn samples, independent of the corn samples used for developing the regression model, were used to validate the model. Initially, a full model that included all the chemical and physical characteristics of the corn (Table 1) was explored. However, for model selection, the conceptual predictive statistic (Cp) and Akaike's information criterion (AIC) were used to choose the best model from each subset of candidate variables. The model that has a Cp value that approximates the number of variables in the model (i.e., the smallest difference between the Cp value and the no. of variables in the model), and that has the smallest AIC value, was the chosen model. The predictive capability of the chosen model was validated using the mean squared prediction error (MSPE) calculated using Eq. [2].

$$\text{MSPR} = \frac{\sum_{i=1}^{n*}(Yi-\hat{Y})^2}{n*} \qquad [2]$$

where Yi is the in vivo ATTD of GE or the concentration of DE in the corn sample, $\hat{Y}i$ is the predicted ATTD of GE or the concentration of DE in the corn samples, and n* is the number of

corn samples used for validation. The predictive capability of the final model is considered appropriate when the MSPR is not more than 3 times the MSE of the regression model.

RESULTS

Exp. 1. In Vitro Disappearance of DM and OM UsingViscozyme

In the 50 corn samples, the concentrations of ADF (CV = 55.16%) and ether extract (CV = 30.16%) were most variable, but the concentrations of GE and DE were the least variable (CV = 1.78 and 2.60%, respectively; Table 6.1). The average IVDMD and IVOMD of the 50 corn samples were 84.41% and 84.43%, respectively (Table 6.2). The IVDMD ($r^2 = 0.12$; $P = 0.02$) and IVOMD ($r^2 = 0.12$; $P = 0.01$) was correlated with DE, but the relationship was very weak (Table 6.3). No relationship was observed between IVDMD ($r^2 = 0.04$; $P = 0.15$) or IVOMD ($r^2 = 0.04$; $P = 0.14$) and ATTD of GE. No differences were observed in the IVDMD and IVOMD in the corn sample that was analyzed in 6 different batches (Table 6.4).

Exp. 2. Evaluation of Filtration Method

The IVDMD of corn samples filtered using the Gooch crucible was greater ($P < 0.01$) than the IVDMD of corn samples obtained using the filter paper (Table 6.5). However, the IVOMD that was measured using the Gooch crucibles was not different from the value that was observed when the residues were collected using filter paper.

Exp. 3. In Vitro DM Disappearance Using Cellulase

The average IVDMD or IVOMD for the selected corn samples were 72.50 and 72.85%, respectively (Table 6.6). No relationship was observed between IVDMD and ATTD of GE ($r^2 = 0.06$; $P = 0.38$) and between IVDMD and DE ($r^2 = 0.04$; $P = 0.49$). However, a relationship was observed between IVOMD and ATTD of GE ($r^2 = 0.64$; $P = 0.01$) and between IVOMD and DE

(r^2 = 0.60; *P* = 0.01; Table 6.7). The slopes of the regression lines were negative, indicating that IVOMD decreases as ATTD of GE or the concentration of DE increases.

Exp. 4. In Vitro Disappearance of DM Using the Daisy^{II} Incubator

The average IVDMD of the corn samples incubated from 4 different jars was between 92.35 and 93.87% (Table 6.8). No differences were observed in the IVDMD values for corn samples incubated in the 4 different incubation jars.

Exp. 5. The Effect of Length of Incubation and Type of Incubation on In Vitro DM Disappearance of Selected Corn samples

Regardless of type of incubation, the IVDMD of corn samples incubated for a short time were less (*P* < 0.01) than the IVDMD of corn samples incubated for a long time (Table 6.9). However, the difference in IVDMD values between corn samples that were incubated in the water bath for a short period and corn samples that were incubated for a long period, was 2-fold more than the difference in IVDMD values when corn samples were incubated in the Daisy^{II} incubator for a short and long period. As a consequence, corn samples that were incubated in the water bath for a short period had less (*P* < 0.01) IVDMD than corn samples that were incubated in the Daisy^{II} incubator for the same duration. Use of the water bath or the Daisy^{II} incubator had no effect on IVDMD of corn samples when the incubation period was long. The IVDMD of corn samples incubated for a long period in the Daisy^{II} had higher R^2 when regressed against ATTD of GE (Table 6.10) or against DE (Table 6.11) than the IVDMD of corn samples that had a short incubation period.

Exp. 6. Effect of Sample Weight and Type of Incubation on In Vitro Disappearance of DM and GE in Corn

Using the water bath, as sample size was increased from 0.5 g to 1.5, 2.5, or 3.5 g, a quadratic decrease was observed in IVDMD ($P < 0.01$) and IVGED ($P < 0.01$; Fig. 6.1). The IVDMD and IVGED in corn samples incubated using the Daisy^{II} incubator also decreased in a quadratic manner as sample size was increased ($P < 0.01$). However, beyond 1.5 g, no differences in IVGED was observed in corn samples assayed using either the water bath or the Daisy^{II} incubator.

Exp. 7. Comparison of 3 in Vitro Procedures and the Effect of Length of Incubation in IVDMD Using the Daisy^{II} Incubator

The average IVDMD in corn samples that was incubated with Viscozyme or cellulase for a long period was greater ($P < 0.01$) than using the same procedure but with a short incubation period (Table 6.12). But increasing incubation period resulted in a 2-fold incremental increase in IVDMD values when Viscozyme was used instead of cellulase. Increasing incubation period, however, had a negative effect on IVDMD when using the fecal inoculum. Thus, the IVDMD of corn samples incubated with Viscozyme for a short period was not different from the IVDMD of corn samples incubated with fecal inoculum for a long period. No difference in IVDMD was observed between corn samples that were incubated with cellulase for a long period and IVDMD of corn samples that was incubated with fecal inoculum for a short period. The use of either Viscozyme or cellulase, and a long incubation period, improved the R^2 between IVDMD and the ATTD of GE (Table 6.13) and between IVDMD and DE (Table 6.14) and was able to explain 48 to 56% of the variability in ATTD of GE and the variability in DE in the corn samples analyzed.

Exp 8. Development and Validation of Regression Models to Predict Apparent Total Tract Disappearance of GE or to Predict DE in Corn

The chemical characteristics that were consistently present in the models that were able to explain at least 47% of the variability in the ATTD of GE in corn were IVDMD, starch, and corn density (Table 6.15). Addition of new variables (NDF, DM, CP, Ash, GE, crude fat, and particle size) did not improve the ability of the model to explain the variations in the ATTD of corn beyond 54%. Therefore, the regression model that was chosen to predict ATTD of GE in corn only contained terms for IVDMD, starch, and corn density (Table 6.17). Validation of the chosen regression model for the ATTD of DE resulted in an MSPE of more than 3 times the MSE of the model. This indicates that the ability of the regression model to predict in-vivo ATTD of GE in corn is poor.

The chemical characteristics that were consistently present in the models that were able to explain 81% of the variability in the DE in corn were IVDMD, starch, corn density, GE and NDF (Table 6.16). Addition of more chemical characteristics (DM, ash, CP, crude fat, particle size) did not improve the ability of the model to explain the variations in the DE of corn beyond 82%. Therefore, the regression model that was chosen to predict DE in corn only contained these 5 variables (Table 6.17).

The relationship between the predicted DE and the observed DE has a slope that is not different from 1 (Fig. 6.2). Validation of the chosen regression model for the DE in corn resulted in an MSPE that is less than 3 times the MSE of the model. This indicates that the regression model has a good ability to predict the DE in corn. Because the regression model was validated by the 10 corn samples, a final model that included all 38 samples was developed to make the regression model more robust. Therefore, the final model to predict the DE in corn is:

$$\text{DE} = -2.02729 + 0.01395\ \text{IVDMD} - 0.01063\ \text{NDF} + 0.93343\ \text{GE} - 0.00774\ \text{starch} + 0.90637\ \text{corn density}\ .$$

DISCUSSION

Developing in vitro procedures as a tool to predict energy disappearance or DE of feeds or feedstuffs is often of interest because it is an inexpensive alternative to the use of animal models. In this experiment, attempts were made to evaluate in vitro assays that can be used to predict the ATTD of GE or the concentration of DE in corn. Despite the high variability in the concentration of ADF and EE among the corn samples, the CV for DE (2.60 Mcal/kg DM) and the ATTD of GE (1.68%) in corn were not as variable. The CV for DE and ATTD of GE in 20 wheat samples were 3.0 mcal/kg DM and 3.7%, respectively (Regmi et al., 2009) and the CV for DE and ATTD of GE in barley were 7.3 Mcal/kg DM and 7.5%, respectively (Regmi et al., 2008). This indicates that the energy digestibility and concentration of DE in corn is less variable compared with wheat and barley. The relative homogeneity of corn in terms of energy digestibility and in terms of DE necessitates that an in vitro procedure that is sufficiently sensitive to detect small differences in corn energy digestibility is identified. In this experiment, the procedure by Boisen and Fernandez (1997) was considered the standard procedure. The absence of a relationship between ATTD of GE and IVDMD or IVOMD, as well as the absence of a relationship between DE and IVDMD or IVOMD in the 50 corn samples analyzed using this procedure indicates that the procedure cannot be used to differentiate between corn samples that have a low or a high ATTD of GE or a high or low concentration of DE. The assay, however, produced consistent results from batch to batch. Good repeatability when using this procedure was also observed by Noblet and Jaquelin-Peyraud (2007).

Using the procedure of Huang et al. (2003), the negative slope of the regression line between IVOMD and ATTD of GE or between IVOMD and DE indicates that as the ATTD of GE or the concentration of DE in the corn samples increased, the IVOMD values decreased.

Using this procedure, a positive slope was obtained by Regmi et al. (2009) and 55% of the variability in the ATTD of GE in 20 wheat samples could be explained by the differences in IVDMD. Results of the current experiment indicate that the recovery of residues in corn samples that have high ATTD of GE or with high DE increases when this procedure is used. The bias may be a result of a low vacuum used during the separation of residues from the solution to avoid tearing of the filter paper in the process of filtration. The low vacuum may not be sufficient to remove precipitates that may normally be removed under high vacuum. Previously, no difference was observed between the IVOMD of corn samples that were filtered using the Gooch crucible or the filter paper when using the Boisen and Fernandez (1997) procedure (Exp. 2). The procedure by Huang et al. (2003) differed from the procedure of Boisen and Fernandez (1997) in several aspects, but the use of 20% sulfosalicylic acid to precipitate the soluble protein, and the absence of ethanol and acetone rinsing in the final steps of the procedure may increase the recovery of precipitated soluble protein and fat residues under low vacuum pressure resulting in low IVOMD values. This observation indicates that the filtration step needs to be improved if this procedure is used.

The absence of a difference between the IVDMD of corn samples that were incubated for a long period using either the Daisy^{II} incubator or the water bath indicates that the Daisy^{II} incubator is a suitable alternative to the water bath when the incubation period is long. The Daisy^{II} incubator can also replace the use of individual tubes to determine IVDMD of feeds in ruminants (Holden, 1999). Under the conditions of this experiment, a long incubation period using the water bath would result in the analysis of 7 corn samples in 48 h compared with 28 samples when the Daisy^{II} incubator is used for the same time period. An advantage of the Daisy^{II} incubator, therefore, is greater productivity compared with the water bath.

Regardless of incubation length, the use of fecal inoculum instead of Viscozyme in the third step of the Boisen and Fernandez (1997) procedure did not improve the sensitivity of the assay. Although the feces were obtained from a single pig fed a single diet and care was taken that the preparation of the fecal inoculum and the media was as uniform as possible between the short and long incubation periods, the population of microbes in the feces is dynamic. The viability of the microbes in the fecal inoculum depends on the amount of substrate available and the maintenance of the anaerobic environment that is necessary for microbial growth. The fecal inoculum for the long incubation was stored for about 1 ½ h longer than the fecal inoculum used in the short incubation period. Prolonged storage of ruminal fluid may reduce the number or activity of the microbial population because gas production was reduced as the ruminal fluid was stored in an anaerobic environment at 39ºC for up to 24 h (Mould et al., 2005). The storage time of the fecal inoculum used for the long incubation may have affected the population or the activity of the fecal inoculum such that the IVDMD of corn samples incubated for a long period was reduced.

Using Viscozyme (Boisen and Fernandez, 1997), increasing the length of incubation of the corn samples in the Daisy^{II} incubator from 24 h to 48 h resulted in IVDMD values that explain the variability in energy digestibility or in the concentration of DE in corn from 53 to 56%. Increasing the length of incubation, therefore, makes this assay more sensitive to small differences in ATTD of GE or to the small differences in the concentration of DE in a relatively homogenous feed ingredient such as corn. The procedure of choice was, therefore, the long incubation of the corn samples in the Daisy^{II} incubator using Viscozyme because it provided a higher R^2 than the procedure using cellulase. The blend of cellulase, β-glucanase, hemicellulase,

and xylanase in Viscozyme may have degraded more fibrous components in corn than cellulase alone.

Despite the addition of several chemical or physical components to the regression model, the capability of the chosen regression model to predict ATTD of GE or to predict DE in corn was not improved. The relative homogeneity of the corn in terms of energy digestibility may be a limiting factor to optimize the model, but other predictor variables may provide additional information that could help explain the variability in the concentration of DE in corn. Variations in corn chemical characteristics from year to year were attributed to the use of different corn hybrids in different regions in the US (Jones et al., 2011). Therefore, adding corn genetic information may improve the ability of the model to predict DE to more than 81%.

The predictive capability of regression models could only be ascertained if the regression model is validated by samples that were not included in the model development (Kutner et al., 2004). In this experiment, MSPE was used to validate the chosen regression model to predict DE, and the low MSPE relative to the MSE of the regression model indicates that the regression model could predict the DE in other corn samples with reasonable accuracy.

In conclusion, a sample weight of 0.5 g is optimal to conduct in vitro analysis of corn. The Daisy^{II} incubator can substitute for the traditional water bath in doing routine in vitro measurements as long as the incubation period is 48 h. Using the Daisy ^{II} incubator, among the 3 in vitro procedures evaluated, increasing the incubation length of the Boisen and Fernandez (1997) procedure and using Viscozyme in the third step improved the sensitivity of the assay and provided a better R^2 between IVDMD and ATTD of GE, and between IVDMD and DE, than the procedures using cellulase or fecal inoculum. A validated regression model that predicted the

DE of corn with reasonable accuracy was, therefore, developed using Viscozyme and with the corn sample incubated in the Daisy^{II} incubator for a long period.

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Table 6.1. Physical, chemical, and energy characteristics in 50 corn samples, DM basis

Characteristics	Mean	Min	Max	SD	CV
Corn density, g/cc	1.31	1.18	1.40	0.04	2.94
Particle size, μm	509.9	423.0	624.0	40.4	7.84
CP,%	9.50	7.27	11.84	0.97	10.22
Crude fat[2], %	4.50	3.11	7.57	1.36	30.16
Ash %	1.31	1.03	1.65	0.14	10.65
NDF, %	9.03	6.85	12.92	1.43	15.83
ADF, %	2.66	1.09	7.56	1.47	55.16
Starch, %	70.75	59.99	77.03	3.09	4.37
GE, kcal/kg	4,513	4,410	4,719	80	1.78
DE, kcal/kg	4,040	3,777	4,266	105	2.60
In vivo ATTD of GE, %[1]	89.52	85.12	92.32	1.50	1.68

[1] ATTD = Apparent total tract digestibility.

[2] Crude fat by ether extraction.

Table 6.2. In vitro DM disappearance (IVDMD) and in vitro OM disappearance (IVOMD) of 50 corn samples assayed using Viscozyme, Exp. 1

Item	Mean	Min	Max	SD	CV
IVDMD, %	84.41	78.45	89.32	2.34	2.77
IVOMD, %	84.44	77.02	89.37	2.34	2.77

Table 6.3. Regression equations for the apparent total tract digestibility of GE (ATTD of GE) and for DE derived from in vitro DM disappearance (IVDMD, %) and in vitro OM disappearance (IVOMD, %) in 50 corn samples assayed using Viscozyme, Exp. 1

Regression equation	r^2	*P*-value
ATTD of GE = 113.0196 - 0.3196 IVDMD	0.04	0.15
ATTD of GE = 113.5597 - 0.3254 IVOMD	0.01	0.14
DE = 115.0252 - 7.5778 IVDMD	0.12	0.02
DE = 115.4026 -7.6641 IVOMD	0.12	0.01

Table 6.4. The effect of batch on in vitro DM disappearance (IVDMD) and in vitro OM disappearance (IVOMD) assayed using Viscozyme, Exp. 1

Batch	n	IVDMD, %	IVOMD, %
1	2	83.89	83.90
2	2	82.60	82.86
3	2	83.82	83.75
4	2	85.30	85.25
5	2	85.09	85.17
6	2	83.74	83.57
SEM		0.79	0.81
P - value		0.30	0.36

Table 6.5. The effect of method of filtration on in vitro DM disappearance (IVDMD) and in vitro OM disappearance (IVOMD) in corn samples assayed using Viscozyme, Exp. 2

Method of filtration	n	IVDMD,%	IVOMD,%
Gooch crucible	15	83.02	84.51
Filter paper	15	78.87	84.81
SEM		1.07	0.95
P- value		0.01	0.82

Table 6.6. In vitro DM disappearance (IVDMD) and in vitro OM disappearance (IVOMD) in corn samples assayed using cellulase, Exp. 3

Item	n	Mean	Min	Max	SD	CV
IVDMD, %	7	72.50	67.04	77.90	3.42	4.72
IVOMD, %	7	72.85	68.66	77.53	3.37	4.62

Table 6.7. Regression equations for the apparent total tract digestibility (ATTD, %) of GE and for DE (Mcal/kg DM) derived from in vitro DM disappearance (IVDMD, %) and in vitro OM disappearance (IVOMD, %) of corn samples assayed using cellulase, Exp. 3

Regression equation	r^2	*P*-value
ATTD of GE = 76.65 + 0.17 IVDMD	0.06	0.38
ATTD of GE = 137.19 - 0.53 IVOMD	0.64	0.01
DE = 3.30 + 0.01 IVDMD	0.04	0.49
DE = 7.13 - 0.01 IVOMD	0.60	0.01

Table 6.8. The effect of incubation jars on in vitro DM disappearance (IVDMD) of corn samples assayed using Viscozyme and incubated using the Daisy^{II} Incubator, Exp. 4

Incubation jar	n	IVDMD, %
1	8	93.46
2	8	93.87
3	8	93.10
4	8	92.35
SEM		0.48
P - value		0.16

Table 6.9. Effect of type of incubation and length of incubation on in vitro DM disappearance (IVDMD, %) of corn samples assayed using Viscozyme, Exp. 5

Type of incubation	Length of incubation[1]	IVDMD
Water bath	Short	87.79 [c]
	Long	92.04 [a]
Daisy[II]	Short	89.48 [b]
	Long	91.24[a]
SEM		0.32
P-value		
Type of incubation		0.19
Length of incubation		0.001
Type × Length		0.001

[a-c] Values within a column lacking a common superscript differ ($P \leq 0.05$).

[1]Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and Viscozyme steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and Viscozyme steps, respectively.

Table 6.10. Regression equations for the apparent total tract digestibility (ATTD, %) of GE derived from in vitro DM disappearance (IVDMD, %) of corn samples using Viscozyme, Exp. 5

Type of incubation	Length of incubation[1]	Regression equation	r^2	*P*-value
Water bath	Short	ATTD of GE = -13.59 + 1.17 IVDMD	0.09	0.57
	Long	ATTD of GE = -272.83 + 3.93 IVDMD	0.50	0.08
Daisy[II]	Short	ATTD of GE = 5.44 + 0.94 IVDMD	0.26	0.24
	Long	ATTD of GE = -349.14 + 4.80 IVDMD	0.83	0.01

[1]Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and Viscozyme steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and Viscozyme steps, respectively.

Table 6.11. Regression equations for DE (Mcal/kg DM) derived from in vitro DM disappearance (IVDMD) of corn samples using Viscozyme, Exp. 5.

Type of incubation	Length of incubation[1]	Regression equation	r^2	*P*-value
Water bath	Short	DE = -0.20 + 0.05 IVDMD	0.03	0.75
	Long	DE = -20.93 + 0.27 IVDMD	0.40	0.13
Daisy[II]	Short	DE = -0.02 + 0.05 IVDMD	0.11	0.47
	Long	DE = -27.38 + 0.34 IVDMD	0.72	0.02

[1]Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and Viscozyme steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and Viscozyme steps, respectively.

Table 6.12. Effect of 3 in vitro procedures and 2 incubation lengths on in vitro DM disappearance (IVDMD, %) in corn samples using the Daisy incubator, Exp. 7

In vitro procedure[1]	Length of incubation[2]	IVDMD, %
Use of Viscozyme	Short	88.84[d]
	Long	96.19[a]
Use of cellulase	Short	90.15[c]
	Long	94.11[b]
Use of fecal inoculum	Short	93.46[b]
	Long	87.81[d]
SEM		0.37
P-value		
Procedure		0.001
Length of incubation		0.001
Procedure × Length		0.001

[a-d] Values within a column lacking a common superscript differ ($P \leq 0.05$).

[1] The 3 in vitro procedures were Boisen and Fernandez (1997) using Viscozyme in the third step, Huang et al. (2003) using cellulase in the third step, and a modified Boisen and Fernandez (1997) using fecal inoculum in the third step.

[2] Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively.

Table 6.13. Regression equations for apparent total tract digestibility (ATTD) of GE (%) derived from vitro DM disappearance (IVDMD, %) obtained from 3 in vitro procedures and 2 incubation lengths in corn samples using the Daisy^{II} incubator, Exp. 7

Procedure[1]	Incubation time[2]	Regression equation	r^2	*P*-value
Use of Viscozyme	Short	ATTD of GE = 103.08 – 0.155 IVDMD	0.01	0.88
	Long	ATTD of GE = -257.55 + 3.60 IVDMD	0.56	0.05
Use of cellulase	Short	ATTD of GE = 89.92 - 0.01 IVDMD	0.01	0.99
	Long	ATTD of GE = -72.04 + 1.71 IVDMD	0.48	0.09
Use of fecal inoculum	Short	ATTD of GE = -61.15 + 1.61 IVDMD	0.09	0.52
	Long	ATTD of GE = 123.28 – 0.39 IVDMD	0.03	0.70

[1] The 3 in vitro procedures were Boisen and Fernandez (1997) using Viscozyme in the third step, Huang et al. (2003) using cellulase in the third step, and a modified Boisen and Fernandez (1997) using fecal inoculum in the third step.

[2] Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively.

Table 6.14. Regression equations for DE (Mcal/kg DM) derived from vitro DM disappearance (IVDMD, %) obtained from 3 in vitro procedures and 2 incubation lengths in corn samples using the Daisy[II] incubator, Exp. 7

Procedure[1]	Length of incubation[2]	Regression equation	r^2	*P*-value
Use of Viscozyme	Short	DE = 8.77 - 0.05 IVDMD	0.10	0.49
	Long	DE = -21.63 + 0.27 IVDMD	0.53	0.06
Use of cellulase	Short	DE = 7.45 - 0.04 IVDMD	0.06	0.60
	Long	DE = -7.33 + 0.12 IVDMD	0.41	0.12
Use of fecal inoculum	Short	DE = 1.96 + 0.02 IVDMD	0.01	0.90
	Long	DE = 9.98 - 0.07 IVDMD	0.17	0.36

[1] The 3 in vitro procedures were Boisen and Fernandez (1997) using Viscozyme in the third step, Huang et al. (2003) using cellulase in the third step, and a modified Boisen and Fernandez (1997) using fecal inoculum in the third step.

[2] Short incubation was 2, 4, and 18 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively. The long incubation was 6, 18, and 24 h for the pepsin, pancreatin, and either Viscozyme or cellulase or fecal inoculum steps, respectively.

Table 6.15. Candidate variables and models that predict apparent total tract digestibility (ATTD) of GE (%) using adjusted R^2, conceptual predictive statistic (Cp), and Akaike's information criterion (AIC)[1] , Exp. 8

No. of variables in model	Adjusted R^2	Cp	AIC	Variables in model
3	0.47	2.94	15.09	IVDMD starch density
4	0.49	3.34	15.07	IVDMD starch density NDF
5	0.52	3.28	14.23	IVDMD starch density NDF DM
6	0.52	4.19	14.59	IVDMD starch density NDF DM Ash
7	0.54	4.78	14.33	IVDMD starch density NDF DM CP particle size
8	0.52	6.37	15.64	IVDMD starch density NDF DM CP particle size Ash
9	0.51	8.01	17.02	IVDMD starch density NDF DM CP particle size Ash GE
10	0.48	10.00	19.00	IVDMD starch density NDF DM CP particle size Ash GE crude fat

[1]Adjusted R^2 is a model selection criterion that quantifies the amount of variation in the ATTD of GE in corn that can be explained by the model given the number of variables included in the model. Models with a large adjusted R^2 are candidate models. Cp is a model selection criterion that uses as few variables as possible to explain as much variation in the ATTD of GE in corn. Models where $Cp \leq p$ (p = number of variables + 1), are candidate models. AIC measures the goodness of fit. Smaller AIC value indicates a better fit of the model.

Table 6.16. Candidate variables and models that predict DE (Mcal/kg) using adjusted R^2, conceptual predictive statistic (Cp), and Akaike's information criterion (AIC) [1], Exp. 8

No. of variables in model	Adjusted R^2	Cp	AIC	Variables in model
5	0.81	4.76	-155.98	IVDMD NDF GE starch density
6	0.82	4.84	-156.71	IVDMD starch density GE ADF DM
7	0.82	5.37	-156.98	IVDMD starch density GE NDF DM Ash
8	0.82	6.68	-156.12	IVDMD starch density NDF DM GE CP particle size
9	0.82	8.04	-155.21	IVDMD starch density NDF DM GE CP particle size Ash
10	0.81	10.00	-153.27	IVDMD starch density NDF DM GE CP particle size Ash Crude fat

[1]Adjusted R^2 is a model selection criterion that quantifies the amount of variation in the ATTD of GE in corn that can be explained by the model given the number of variables included in the model. Models with a big adjusted R^2 are candidate models. Cp is a model selection criterion that uses as few variables as possible to explain as much variation in the ATTD of GE in corn. Models where $Cp \leq p$ ($p =$ number of variables + 1), are candidate models. AIC measures the goodness of fit. Smaller AIC value indicates a better fit of the model.

Table 6.17. Chosen regression models for the prediction of ATTD of GE (%) and for the prediction of DE (Mcal/kg) in corn, DM basis

Chosen regression model	Adjusted R^2	*P*-value	MSE	MSPE
ATTD of GE = 35.7804 + 0.4147 IVDMD – 0.1958 starch + 21.7608 corn density	0.47	0.01	1.50	6210
DE = -2.20367 + 0.01899 IVDMD – 0.00952 NDF + 0.89816 GE – 0.01051 starch + 0.94262 corn density	0.81	0.01	0.003	0.007

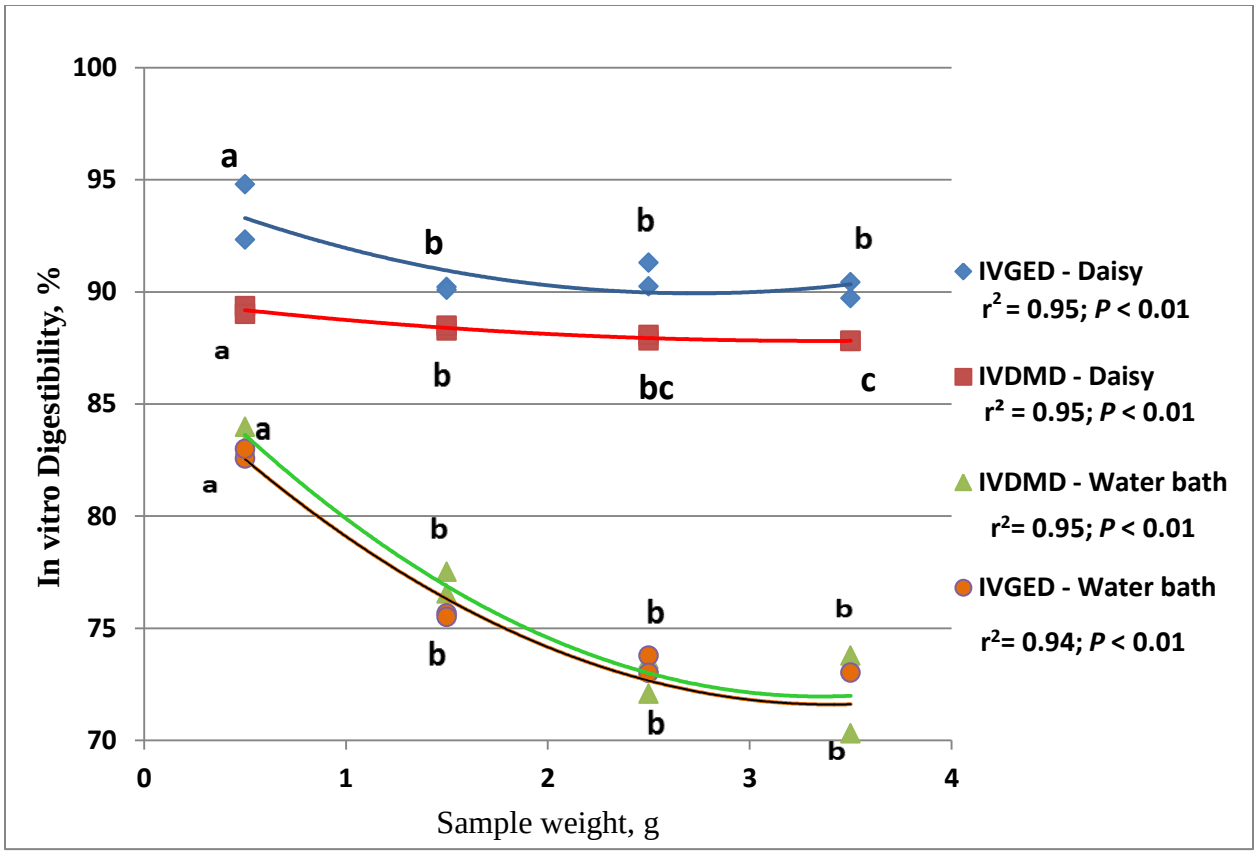

Figure 6.1. Relationship between in vitro DM disappearance (IVDMD,%) or in vitro GE disappearance (IVGED,%) and sample weight using the water bath or the Daisy^{II} incubator assayed using Viscozyme, Exp. 6.

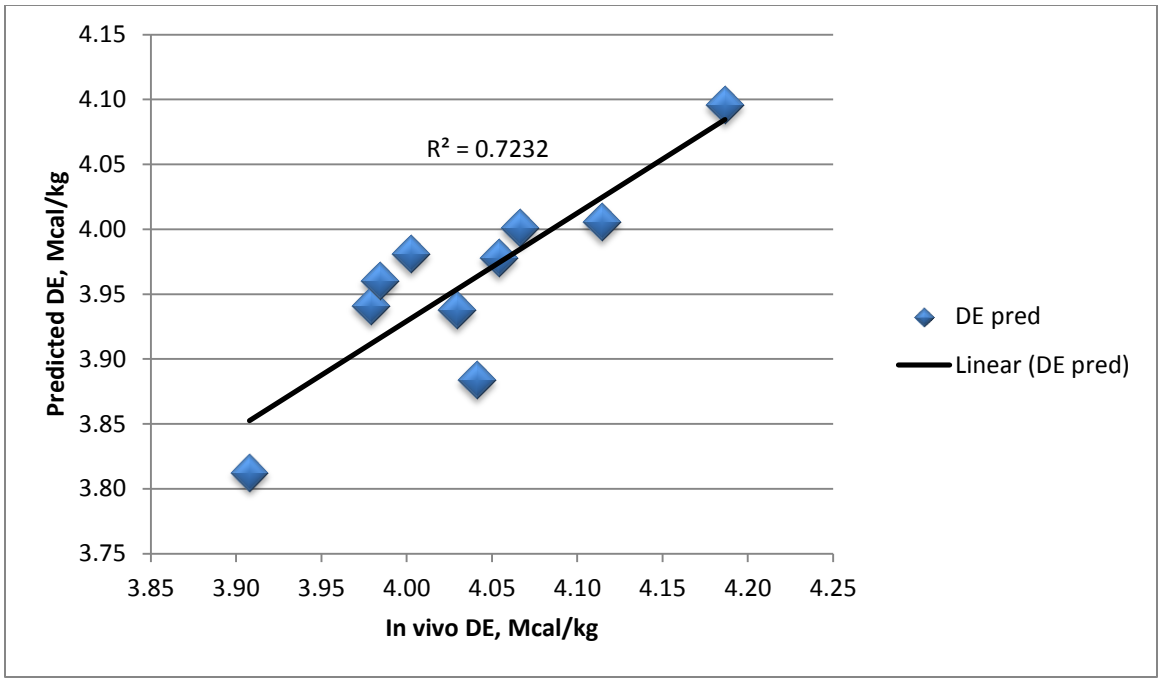

Figure 6.2. Relationship between in vivo DE and predicted DE derived from the chosen regression model DE = -2.20367 + 0.01899 IVDMD - 0.00952 NDF + 0.89816 GE - 0.01051 starch + 0.94262 corn density.

GENERAL CONCLUSIONS

Pigs can have dual functions. The meat of pigs can provide nutrients for humans, but pigs also may be used as models for human gastrointestinal function. Carbohydrates are consumed by both animals and man as a source of energy. For pigs, the more nutrients that are provided to them, the faster they grow and the better is the feed efficiency. Therefore, in feeding pigs, ingredients that have a high apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of GE and nutrients are preferred. However, for humans, food and food ingredients high in AID and ATTD of GE increase the risk of metabolic diseases such as diabetes and obesity. Therefore, for individuals who have a high risk for developing diabetes or obesity, food with a low AID and ATTD of GE are preferred.

In the western world, the increasing incidence of individuals that are obese or diabetic is of great concern. Thus the FDA recommended increasing dietary fiber intake to a concentration that is able to attenuate blood glucose concentration in humans (25-30 g/d). The food industry responded to this recommendation by producing novel carbohydrates that act as dietary fiber. Resistant starches, SCF, and pullulan are novel carbohydrates that may be incorporated in food and beverages to increase or supplement dietary fiber intake in humans. As expected, the ME of all the novel carbohydrates were low (between 1,804 to 2,918 kcal/kg DM) which is less than the ME of a very digestible carbohydrate such as MD. Inclusion of 10% of these novel carbohydrates into the diets for pigs also reduced the AID of energy of the diets. It is expected that the post prandial blood glucose concentration of individuals that consume diets containing these novel carbohydrates would be less than that of what can be observed in individuals that consume a diet without novel carbohydrates. Therefore, post prandial blood concentration is better managed when novel carbohydrates are included in the diet for humans.

Although inclusion of novel carbohydrates reduced the AID of GE, it should not reduce the AID of other critical nutrients in the diet. The inclusion of cellulose and pullulan, but not RS 60, RS 75 and SCF, reduced the AID of CP. This emphasizes the importance of evaluating novel carbohydrates, not only in their ability to attenuate blood glucose concentration, but also in their ability to positively or negatively influence the digestibility of other critical nutrients in the diet.

The negative values obtained for the AID of TDF indicate that endogenous compounds were analyzed as TDF. Using a fiber-free diet (maltodextrin diet), endogenous compounds that were analyzed as TDF were quantified. Calculation of SID and STTD of TDF indicated that the disappearance of TDF in the small intestines was substantial. Further studies are needed to identify and quantify endogenous compounds that are analyzed as TDF.

Aside from consuming food products that are fortified with novel carbohydrates, another way to increase dietary fiber intake is by the consumption of food that are inherently high in dietary fiber, such as cereal grains. The TDF in whole cereal grains of yellow dent corn, dehulled barley, rye, and sorghum contributed to a reduced AID of starch compared with the AID of starch in rice. However, not all cereal grains with low AID of starch have low DE and ME because the AEE and CP components of the cereal grains also contribute energy. Hindgut fermentability of TDF in the cereal grains also contribute to energy by the production and absorption of VFA. Therefore, among the 8 cereal grains evaluated, sorghum and rye were the recommended grains for diabetic and weight management because their ME and starch digestibility were relatively less compared with the other cereal grains. However, for undernourished individuals that need to increase caloric intake, dehulled oats and rice are recommended.

Although in vivo digestion studies are ideal, in vitro procedure are useful tools to determine caloric values for feed and food. By incubating the corn samples with Viscozyme for 48 h in a Daisy incubator, a validated regression equation was developed to predict the DE in corn.

AUTHOR'S BIOGRAPHY

Sarah Flordeliz Cervantes-Pahm is a native of Cebu, Philippines. She graduated with a BS in Agriculture major in Animal Science from the University of the Philippines at Los Banos. Before pursuing further studies, she worked as a poultry nutritionist for more than 10 years in one of the reputable poultry integrators in the Philippines. She started her Masters degree under the guidance of Dr. Hans H. Stein at South Dakota State University in the summer of 2005. After finishing 4 experiments, she accepted the invitation of Dr. Stein to join his new lab at the University of Illinois, Urbana-Champaign in 2006. Her work evaluated the amino acid digestibility of several soybean products including full fat soybeans, high –protein soybean meals, fermented soybean meals, and by-pass soybean meal proteins. Two of her thesis chapters have been published. She obtained her Master's degree in Animal Nutrition in May 2007. She decided to continue for her Ph. D. She studied energy and nutrient digestibility of novel carbohydrates and cereal grains using the pig as a model for human gastrointestinal function. After her graduation, she is going back to the Philippines.

She is married to Ameer Pahm who also obtained his Ph. D. from the University of Illinois, Urbana. They are blessed with a daughter, Emi Raquel, and a son, Ethan Gavril.