CARBOHYDRATE COMPOSITION, IN VITRO DIGESTION, AND EFFECTS OF XYLANASE AND PHYTASE ON NUTRIENT AND ENERGY DIGESTIBILITY BY PIGS IN GRAINS AND GRAIN COPRODUCTS

BY

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ABSTRACT

Four experiments were conducted to determine carbohydrate composition, in vitro digestion, and effects of xylanase and phytase on ileal and total tract digestibility of nutrients and energy in grains and grain coproducts. The objective of Exp. 1 and Exp. 2 was to determine the efficacy of xylanase and phytase supplementation to growing swine diets based on corn, soybean meal, distillers dried grains with solubles (**DDGS**), and wheat bran. In Exp. 1, the apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of energy and nutrients in ileal cannulated growing pigs were determined. Four diets were formulated to meet the pig's AA requirements using a low amount of crystalline AA, whereas 3 additional diets were formulated to contain greater amounts of crystalline AA. The 4 low-AA diets included the control diet that contained no phytase or xylanase, the phytase diet that contained 500 phytase units (**FTU**)/kg of phytase, and 2 diets that contained 500 FTU/kg of phytase and 2,000 or 4,000 xylanase units/kg. The 3 high-AA diets contained 500 FTU/kg of phytase and 2,000, 4,000, or 8,000 xylanase units/kg. Results indicated phytase added to the low-AA diets improved ($P < 0.05$) the AID of CP, Ca, P, and most AA by 2.2, 13.9, 9.4, and approximately 2%, respectively, compared with the control diet. Phytase also improved ($P < 0.05$) the ATTD of Ca and P by 18.1 and 13.8%, respectively, compared with the control diet. Xylanase added to the low-AA diets improved ($P < 0.05$) the AID of DM, GE, and NDF by 2.3, 1.9, and 5.8%, respectively, compared with the phytase diet. In Exp. 2, a 42-d growth assay was used to determine the growth performance of 288 pigs fed the same diets used in Exp. 1 with the exception that the diet with 8,000 units of xylanase was not used. No improvements in growth performance due to supplementation of phytase and xylanase to the diets were observed, indicating that the increases in digestibility of nutrients and energy by using xylanase and phytase in Exp. 1 were not large enough to improve

growth performance. In Exp. 3, the carbohydrate composition of 12 U.S. swine feed ingredients was determined to classify the amount of available substrate for carbohydrase supplementation. The 12 ingredients included 3 grains (corn, sorghum, and wheat), 3 coproducts from the dry grind industry (corn DDGS and 2 sources of sorghum DDGS), 4 coproducts from the wet milling industry (corn gluten meal, corn gluten feed, corn germ meal, and corn bran), and 2 coproducts from the flour milling industry (wheat middlings and wheat bran). The parent grains (corn, sorghum, and wheat) contained greater amounts of starch and less non-starch polysaccharides (**NSP**) compared with the 9 coproducts, except corn gluten meal. The concentration of soluble NSP was minimal in all ingredients. Results indicated that there are substantial amounts of NSP in these ingredients, which indicates that NSP-degrading enzymes such as xylanase may be effective in improving the nutritional value of these ingredients. Exp. 4 was conducted to determine in vitro ileal and total tract digestibility of DM and NSP in the 12 feed ingredients used in Exp. 3. In vitro ileal digestibility of NSP was close to zero in all feed ingredients, indicating that pepsin and pancreatin enzymes have no effect on degradation of NSP. However, a strong correlation ($R^2 = 0.97$) between in vitro ileal digestibility of DM and NSP concentration in the feed ingredients was observed, which indicates that in vitro ileal digestibility of DM may be used to estimate NSP concentration in feed ingredients. In vitro total tract digestibility of NSP ranged from 6.5% in corn bran to 55.0% in corn germ meal, which indicates that NSP composition in each ingredient plays an important role in determining the extent of NSP fermentation, as well as exogenous enzyme degradation. As a consequence, the amount of enzyme supplementation and energy value of each ingredient is influenced by the composition of the NSP fraction.

Key words: coproducts, digestibility, in vitro, phytase, pigs, xylanase

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CHAPTER 1

INTRODUCTION

Inexpensive nutrients and energy in swine diets, supplied by carbohydrates in grains such as corn and wheat, is a thing of the past. It is not possible to economically feed pigs without reducing the amount of expensive grains in the diet. Therefore, less expensive coproducts, which are produced from both the wet-milling and dry-milling industries, are being used. However, coproducts are higher in fiber compared with the parent grain (Stein and Shurson, 2009). Fiber in swine diets makes pigs less efficient because fiber is not enzymatically digested, but fermented, and there are energetic losses in methane through fermentation (Bach Knudsen, 2001). Also, increasing fiber in swine diets decreases energy and protein digestibility, increasing the amount of manure excreted (Noblet and Le Goff, 2001; Bindelle et al., 2008). A possibility to increase the efficiency of feeding fiber is to add exogenous fiber-degrading carbohydrases (e.g., xylanase) to the diet.

Carbohydrases have gained popularity in recent years due to increases in the amount of fiber in pig diets and the proven effectiveness of phytase, another exogenous enzyme. When phytase is supplemented to pig diets, there is almost always a positive response which includes an increase in P digestibility and a reduction in P excretion (Almeida and Stein, 2010). The reasons for using phytase are similar to reasons for using fiber-degrading carbohydrases. Fiber-degrading carbohydrases enzymatically break down fiber in the small intestine, potentially rendering nutrients and energy within fiber and the fiber matrix to be absorbed in the small intestine rather than fermented in the hind gut (Bedford, 1995). However, the efficacy of fiber-degrading carbohydrases is inconclusive, especially when supplemented to U.S. swine diets containing corn and corn coproducts (Jones et al., 2010; Yáñez et al., 2011). Also, it must be

determined if phytase and fiber-degrading carbohydrases interact because of the proven effectiveness of phytase. Therefore, the first objective of this thesis is to determine the effects of xylanase and phytase supplementation on ileal and total tract digestibility of energy and nutrients and on growth performance of growing pigs fed a diet containing corn, soybean meal, distillers dried grains with solubles, and wheat bran.

Secondly, the carbohydrate composition, specifically the amount of carbohydrase substrate, of grains and grain coproducts from the United States has not been reported. Therefore, the second objective of this thesis is to determine the carbohydrate composition of grains and grain coproducts used as feed ingredients in U.S. swine diets.

Finally, development of an in vitro procedure that simulates the digestive processes of the pig by Boisen and Fernández (1997) has made it possible to determine in vitro ileal and total tract DM and OM digestibility values that are similar to values obtained in vivo. Therefore, the third objective of this thesis is to determine in vitro ileal and total tract digestibility of DM and non-starch polysaccharides in grains and grain coproducts.

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CHAPTER 2

XYLANASE AND PHYTASE: LITERATURE REVIEW

INTRODUCTION

The increasing price of corn and other cereal grains is making it more expensive to feed swine using conventional corn-soybean meal diets. Due to this increase in conventional feed costs, it is attractive to use unconventional, low-cost ingredients. One such ingredient is distillers dried grains with solubles (**DDGS**), which is a coproduct of the ethanol industry. Distillers dried grains with solubles may substitute for corn and soybean meal in the diet because DDGS has similar concentrations of DE and ME as corn, and contains highly digestible P (Stein and Shurson, 2009). Despite the advantages of using DDGS and other coproducts in swine diets, there are disadvantages as well.

One main disadvantage of DDGS and many other low-cost ingredients is that they often have high concentrations of fiber. Dietary fiber is composed of non-starch polysaccharides (**NSP**) and lignin. Pigs cannot digest NSP or lignin because the pig does not produce the appropriate enzymes (Anguita et al., 2006). However, pigs may obtain energy from NSP by microbial fermentation in the hindgut, which supplies the pig with VFA, which is used by the pig to synthesize energy or adipose tissue (Bach Knudsen, 2001). The energy contribution from VFA is not as efficient as the energy contribution obtained from enzymatic hydrolysis in the small intestine (Bach Knudsen, 2001). However, a diet that enables the pig to absorb more nutrients and energy from fiber will be attractive to pork producers.

The use of exogenous carbohydrase enzymes is a relatively new technology that has the potential to change the way the pig utilizes fiber. By supplementing the diet with fiber-degrading enzymes, it may be possible to shift some of the degradation of fiber from the hindgut to the

small intestine. This shift may enable the pig to utilize more energy and nutrients from fiber-containing feed ingredients. There is, however, a need for research to evaluate exogenous carbohydrase enzymes to determine their efficacy in swine diets.

PLANT CARBOHYDRATES

Because almost all pig feed ingredients are of plant origin, consisting mostly of carbohydrates, which supply the majority of energy in the pig diet, it is necessary to categorize the carbohydrate constituents of plants. The increased use of exogenous carbohydrases in swine diets also has made it necessary to describe the plant carbohydrates that these enzymes target. The plant can be divided into 2 components: the cell wall contents and non-cell wall contents. Each component contains different carbohydrates with different physical and chemical properties.

The plant non-cell wall carbohydrates include starch, disaccharides, oligosaccharides, fructan polysaccharides, and resistant starch. The plant cell wall carbohydrates include cellulose, hemicellulose, β-glucans, pectins and gums, and lignin (Cervantes-Pahm, 2011; NRC, 2012). Carbohydrates can also be divided into digestible and non-digestible carbohydrates (Bach Knudsen et al., 2012). Digestible carbohydrates are those that the pig can digest through the secretion of endogenous enzymes. Digestible carbohydrates are sugars (glucose, fructose, sucrose, lactose, and maltose), certain oligosaccharides, and starch (Bach Knudsen et al., 2012). Non-digestible carbohydrates are those that are not digested by the end of the small intestine by the pig's endogenous enzymes and must be fermented in the hind gut (Bach Knudsen et al., 2012). Non-digestible carbohydrates consist of non-digestible oligosaccharides, resistant starch, and NSP (Bach Knudsen et al., 2012). Most non-digestible carbohydrates, as well as plant cell wall carbohydrates, may also be included in the term dietary fiber (**DF**), which is commonly

defined as, “all plant polysaccharides and lignin that are resistant to hydrolysis by human digestive secretions” (Trowell, 1976).

Dietary Fiber

Dietary fiber is a term used to describe non-digestible components of carbohydrates. The components of DF are NSP and lignin. Lignin is present in the cell wall of plants and the concentration of lignin increases as the plant matures to provide rigidity and strength to the plant. Lignin is made up primarily of polymers of phenylpropenoids and is associated with cell wall cellulose and hemicelluloses (Liyama et al., 1994). Lignin is not enzymatically digested and fermentation of lignin is negligible. Non-starch polysaccharides include pectins, cellulose, hemicelluloses, β-glucans, arabinoxylans, fructans, oligosaccharides, and resistant starch (Bindelle et al., 2008). However, the main NSP in cereal grains are arabinoxylans, cellulose, and β-glucans (Bach Knudsen, 2001). The presence of lignin and NSP is the main reason for the disadvantages of feeding ingredients with high concentrations of DF to swine.

Swine are unable to fully utilize NSP because they lack enzymes necessary to cleave these large polysaccharides into monosaccharides that can be absorbed in the small intestine. This results in microbial fermentation of NSP in the hind gut of pigs, and the subsequent production of VFA, which can be absorbed and utilized for energy. In addition, soluble NSP in swine diets increases intestinal digesta viscosity, which is closely associated with a decrease in total tract digestibility of nutrients (Barrera et al., 2004), and increasing the concentration of NSP in the diet results in a linear decrease in the apparent ileal digestibility (**AID**) of energy and nutrients (Yin et al., 2000). Reduced digestibility limits the amount of DF that can be economically used by pigs. Dietary fiber also decreases the retention time in the small and large

intestines, which reduces the diets' exposure to the pig's digestive enzymes, as well as fermentation time (Bindelle et al., 2008). Due to the negative effects that DF, and specifically NSP, have on nutrient utilization in pigs, diets are usually formulated to contain as little DF as possible.

However, if DDGS is used in swine diets, it is not possible to minimize the amount of DF. Distillers dried grains with solubles contains approximately 31.5% total dietary fiber (**TDF**; Urriola et al., 2010). Less than 50% of TDF in DDGS is fermented in the intestinal tract, which means that more than 50% of the TDF in DDGS passes through the pig without being fermented (Urriola et al., 2010). In essence, 50% of the fiber in DDGS does not produce any energy for the pig to utilize, and the 50% that is utilized is fermented, which is a less efficient way of utilizing energy than if monosaccharides are absorbed in the small intestine. More research needs to be conducted to elucidate ways to improve the pig's utilization of TDF in DDGS and other fiber-containing feed ingredients.

Fiber Analysis

There are several methods for analyzing different feedstuffs for their fiber components. The first and oldest method is the crude fiber method, which is part of the Weende Proximate Analysis System (Henneberg and Stohmann, 1859). This is a gravimetric procedure that incompletely measures cellulose, insoluble hemicellulose, and lignin. Peter Van Soest developed the detergent fiber method in the 1960's. This procedure analyzes 3 portions of fiber: acid detergent fiber (**ADF**), neutral detergent fiber (**NDF**), and acid detergent lignin (**ADL**; Van Soest, 1963). The ADF portion contains cellulose and lignin, whereas the NDF fraction contains cellulose, acid insoluble hemicelluloses, and lignin. Acid detergent lignin contains only lignin.

The concentration of insoluble hemicelluloses can be calculated by subtracting ADF from NDF, and cellulose concentration can be determined by subtracting ADL from ADF. The detergent fiber procedure is still used, but it does not account for soluble hemicelluloses, which may be a very significant component of fiber. To determine the soluble fiber fraction, a method called the TDF procedure, is used. If fiber is analyzed as TDF, the fiber is divided into insoluble dietary fiber and soluble dietary fiber (Prosky et al., 1985). The insoluble fiber component contains lignin, cellulose, and insoluble hemicelluloses, whereas the soluble fiber component contains soluble hemicelluloses, which are composed of pectins, β-glucans, and to a lesser extent arabinoxylans. The last of the major fiber analysis procedures is the Englyst method of fiber analysis, which measures total soluble and total insoluble NSP and their constituent sugars (Englyst et al., 1994; Theander et al., 1994). The individual sugars include rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and uronic acids (measured separately using a colorimetric method; Scott, 1979) and the total concentration of these sugars is equal to the total NSP (Bach Knudsen, 1997).

NON-STARCH POLYSACCHARIDES

Non-starch polysaccharides, as the name indicates, include the non-α-glucan polysaccharides present in plant material excluding starch (Cummings and Stephen, 2007). Non-starch polysaccharides are composed of many monosaccharide units joined mostly by β linkages, making them resistant to enzymatic digestion in the pig. Non-starch polysaccharides may be grouped into 3 groups: cellulose, non-cellulosic polymers, and pectic polysaccharides (Bailey, 1973). Cellulose is a group of its own, non-cellulosic polymers contain arabinoxylans, β-glucans, mannans, galactans, and xyloglucans, and pectic polysaccharides include polygalacturonic acids, which may be substituted with arabinans, galactans, and arabinogalactans (Choct, 1997). Also,

NSP can be classified as either soluble or insoluble based on their reaction with water (Kumar et al., 2012). Soluble NSP may form dispersions in water and increase digesta viscosity, whereas insoluble NSP are bulking agents. The most abundant plant cell wall NSP includes cellulose and hemicelluloses (Kumar et al., 2012).

Cellulose

Cellulose is the most abundant organic substrate on earth and is the main structural component of plant cell walls. Cellulose is a linear polymer consisting of β1,4 linked glucose units that can be stacked upon each other by forming hydrogen bonds, therefore making them very inaccessible to water and subsequent degradation (Cummings and Stephen, 2007; Kumar et al., 2012). Because of the complex nature of cellulose and the fact that the pig does not produce cellulase, cellulose is not digested by the pig.

Arabinoxylans

Arabinoxylans are the main constituent of hemicelluloses and are predominantly present in wheat bran (23.8%), in corn coproducts like DDGS (15.6%) and, to a lesser extent, wheat (7.6%) and corn (5.2%; Bach Knudsen, 1997). Arabinoxylans have a high variability in plant cell walls. They consist of D-xylose joined by β1, 4 linkages and single residues of arabinose attached along the xylan chain (Bedford, 1995). However, arabinose is substituted randomly and other substitutes such as α-D-glucuronic acid and acetyl groups also are attached to the xylan backbone. The degree of substitution of arabinose to xylose within arabinoxylans can be an indication of interchain and intermolecular complexities and the potential of arabinoxylan degradation (Williams et al., 2011). The random substitution of arabinose makes it possible for arabinoxylans to cross-link and form gel networks which, along with the ability to absorb

approximately 10 times their weight in water, gives arabinoxylans the ability to increase digesta viscosity (Choct, 1997; Kumar et al., 2012). The aleurone layer contains cell walls that have a large amount of feruloylated arabinoxylans and whose contents contain lipids and proteins (Benamrouche et al., 2002). The encapsulation of lipids and proteins in arabinoxylans indicate that breakdown of arabinoxylans may render these trapped nutrients available for digestion and absorption. Due to their complex nature, arabinoxylans require multiple xylanase enzymes to degrade them. These enzymes include β-(1, 4)-endo-xylanase (EC 3.2.1.8), β-D-xylosidase (EC 3.2.1.37), α-L-arabinofuranosidase (EC 3.2.1.55), α-D-glucuronosidase (EC 3.2.1.139), acetyl-esterase (EC 3.1.1.6), and feruloyl-esterase (EC 3.1.1.2; Benamrouche et al., 2002). However, pigs do not synthesize xylanase or other arabinoxylan degrading enzymes, making digestion of arabinoxylans impossible, but certain microbes in the gastrointestinal tract of pigs synthesize xylanase, which is the reason arabinoxylans can be partly fermented in the GI tract of pigs (Gdala et al., 1997; Barrera et al., 2004).

β-glucans, Galacturonans, Mannans, Arabinans, Galactans, and Xyloglucans

There are several other NSP besides cellulose and arabinoxylans. β-glucans are predominantly present in oats and barley and are linear polymers of glucose joined by mixes of β1, 4 and β1, 3 linkages (Kumar et al., 2012). The mixture of β1, 3 bonds changes the structure of this NSP from cellulose and makes it more soluble and flexible (Choct, 1997).

Galacturonans are chains of α1, 4 galacturonans with units of rhamnose, galactose, arabinose, xylose, fucose, and glucuronic acid randomly substituted as side chains (Choct, 1997). They function as intracellular cement holding up the plant's network of cellulose (Kumar et al., 2012).

Mannans, which can be gluco- or galacto-, are rarely present in cereal grains, but are present in legumes (Choct, 1997). Mannans are comprised of a β1, 4 linked mannose backbone randomly substituted by glucose or galactose. If substituted with glucose monomers they are called glucomannans and if substituted by galactose monomers they are called galactomannans (Choct, 1997). Both mannans are water soluble and they greatly influence digesta viscosity and may slow glucose absorption from the small intestine (Ebihara et al., 1981; Kumar et al., 2012).

Arabinans are composed of arabinosyl residues and galactans are linear chains of galactose with arabinose randomly substituted along the chain. If galactans have arabinose substituted at carbon 3 and 6, they are referred to as arabinogalactans (Kumar et al., 2012). These NSP are neutral pectic polysaccharides also acting as intracellular cement (Kumar et al., 2012).

Xyloglucans have a linear glucose backbone with single xylose units substituted along the backbone (Choct, 1997). The physicochemical properties of xyloglucans are determined by the number and position of xylose residues (Nishinari et al., 2007).

As indicated above, there are many different NSP, which are composed of many different concentrations of the monosaccharides rhamnose, fucose, arabinose, xylose, glucose, galactose, mannose, and neutral acids like uronic acids. When NSP analysis is performed, it is not the NSP that is being analyzed. Instead, the constituent sugars are determined and NSP is calculated by adding the sugars (e. g., arabinoxylans = arabinose + xylose).

XYLOSE AND ARABINOSE METABOLISM

If arabinoxylans are completely hydrolyzed, they will yield L-arabinose and D-xylose, which are usually absorbed from the small intestine, although, the exact mechanism is uncertain (Schutte et al., 1991; 1992). One theory is that D-xylose and L-arabinose have the same

stereochemical structure of carbon atoms 1 to 3 as D-glucose, indicating that they may be metabolized in a similar way as D-glucose (Wyngaarden et al., 1957). Indeed, D-xylose and L-arabinose are cleared by tissues in response to insulin. Human subjects injected with D-xylose or L-arabinose have elevated blood levels of these pentoses (Wyngaarden et al., 1957). The blood concentrations of D-xylose and L-arabinose decreased from 0 to 60 min, and after an insulin injection, an acceleration in the rate of disappearance occurred, indicating that tissues that transport D-xylose and L-arabinose are responsive to insulin, but it is not known if these pentoses can stimulate the pancreas to secrete insulin (Wyngaarden et al., 1957).

However, the weight gain of chicks is severely depressed if more than 20% D-xylose or L-arabinose is substituted for D-glucose in the diet, indicating that these pentoses are not well utilized by chicks compared with D-glucose (Wagh and Waibel, 1967). By injecting chicks subcutaneously with pure 1-^{14}C-labeled D-glucose, L-arabinose, or D-xylose, the amount of radioactivity in expired carbon dioxide, liver glycogen, and excreta was determined. Only 35.7, 22.5, and 28.2% of the total radioactivity of D-glucose, L-arabinose, and D-xylose, respectively, were recovered in expired carbon dioxide, liver glycogen, and excreta combined, indicating considerable retention of these sugars. The retention of L-arabinose and D-xylose may occur in the tissues of the chick and when present in large amounts may cause death (Baker, 1977). However, the small amount of labeled C in expired carbon dioxide indicates that, in chicks, an isomerase may be present, which by isomerization and phosphorylation may enable D-xylose to enter the pentose phosphate pathway (Wagh and Waibel, 1967). But when D-xylose is fed in excess of 40% of the diet, the pentose phosphate pathway becomes overloaded and D-xylose accumulates in body tissues, primarily, the eyes causing cataracts in chicks (Baker, 1977).

Finally, when fed at 60% of the diet, D-xylose caused death in over half of chicks prior to the end of an 8 d experiment, suggesting a rapid toxicity in chicks (Baker, 1977).

In pigs, the AID of D-glucose, D-xylose, and L-arabinose is 99.3, 98.7, and 70.0% respectively, which demonstrates that almost all glucose and xylose and most arabinose disappear before the end of the small intestine (Schutte et al., 1991; 1992). However, it is not known if xylose and arabinose were absorbed or fermented. The pH decreased and total VFA increased in ileal digesta when D-xylose or L-arabinose was included in the diet, which indicates that some of the pentoses underwent microbial fermentation and that there is some microbial activity in the distal ileum (Schutte et al., 1991; 1992). No D-glucose was excreted in the urine, but 48.5% of the ingested D-xylose and 13.0% of the ingested L-arabinose were excreted in the urine. It is likely that the xylose and arabinose that disappeared in the small intestine, but was not excreted in the urine, was fermented in the small intestine. This hypothesis is supported by the decrease in digesta pH that was observed in pigs fed xylose or arabinose compared with pigs fed D-glucose. It is, however, also possible that some of the xylose and arabinose may have been absorbed and metabolized by the pigs, but further research is needed to demonstrate the exact fate of D-xylose and L-arabinose in the pig.

EFFECTS OF XYLANASE AND PHYTASE

Xylanase

The xylanase enzyme was developed in Europe where pork producers use more high fiber feed ingredients than in the United States. Therefore, most experiments conducted with xylanase use wheat-based diets because wheat is the major cereal grain used in pig diets in Europe, and because wheat contains greater amounts of arabinoxylans than corn.

Xylanase is an exogenous enzyme that may hydrolyze arabinoxylans. The enzyme first cleaves the arabinoxylan backbone randomly, which is composed of D-xylose molecules joined by β1, 4 bonds. "Accessory" enzymes cleave the small chains to monomers, D-xylose and L-arabinose, and potentially release other nutrients such as AA, Ca, and P that are associated with arabinoxylans located in the grain endosperm and aleurone layers (Paloheimo et al., 2011). Effects of xylanase are increased if the amount of arabinoxylans increases in the diet. The primary function of xylanase is to hydrolyze arabinoxylans to liberate xylose and arabinose before they reach the distal small intestine to promote absorption and possible utilization rather than microbial fermentation of these pentoses, therefore, possibly increasing the energy value of feed ingredients containing arabinoxylans. The secondary function of xylanase is to decrease the anti-nutritional factors associated with arabinoxylans (Bedford, 1995).

Xylanase supplemented to wheat-based diets fed to pigs did not affect the viscosity of ileal digesta (Mavromichalis et al., 2000). However, supplementing a wheat diet with 5,500 or 11,000 xylanase units/kg may improve the AID of CP and most dispensable and indispensable AA in growing pigs, indicating that xylanase has the capability to release AA that are bound to arabinoxylans in wheat (Barrera et al., 2004). It is also possible that arabinoxylans increase the secretion of endogenous AA. The differences in AID of AA and CP between wheat diets without and with xylanase are small, and it may not be possible to observe an improvement in pig performance due to xylanase supplementation.

Addition of 5,600 xylanase units/kg to wheat-based diets fed to weanling pigs increased the AID of OM, CP, most AA, crude fiber, and NDF (Diebold et al., 2004). The reason for improvements in digestibility may be that the young pig has an immature gastrointestinal tract, with a limited capacity for fiber fermentation; therefore, xylanase supplementation reduces the

anti-nutritional factors associated with feeding fiber. Xylanase supplementation had a tendency to increase VFA production before the terminal ileum, indicating an increase in microbial fermentation (Diebold et al., 2004). There was also a tendency for reduced VFA in the feces of pigs fed xylanase compared with that of pigs fed no xylanase, indicating that xylanase may have shifted fermentation from the hindgut to the ileum or proximal colon (Diebold et al., 2004).

Addition of xylanase and phytase to diets containing 20 or 40% wheat millrun, a coproduct of the wheat milling industry, improved the AID of GE, DE, DM, Met, and Thr compared with the AID of these nutrients in diets without exogenous enzymes (Nortey et al., 2007). Improvements in the AID of most nutrients and energy also were observed when xylanase was added to a wheat-based diet that contained 30% wheat bran (Nortey et al., 2008). However, adding 4,000 units of xylanase to a wheat-based diet that included 30% wheat coproducts (millrun, middlings, shorts, or screening) did not increase energy or nutrient digestibility (Nortey et al., 2008). These results indicate that the greatest effects of adding xylanase to diets fed to pigs are obtained if arabinoxylans are present in high concentrations in the diet.

For xylanase to be successfully utilized in the United States, it must be demonstrated that it can improve digestibility when supplemented to swine diets containing corn and corn coproducts. Corn and DDGS contain approximately 5.2 and 15.6% arabinoxylans, respectively (Bach Knudsen, 1997). The presence of arabinoxylans in DDGS indicates that it may be possible to improve energy and nutrient digestibility by adding xylanase to diets containing DDGS. However, addition of xylanase to a diet containing DDGS that was produced by co-fermenting wheat and corn at a 1:1 ratio had no effect on the AID and apparent total tract digestibility (**ATTD**) of nutrients and energy (Yáñez et al., 2011). This observation is in contrast with the hypothesis that increasing arabinoxylans in the diet will increase the effects of xylanase. The

performance of weanling pigs fed a corn-soybean meal diet that contained 30% DDGS and was supplemented with 4,000 xylanase units was also not improved compared with pigs fed similar diets without xylanase (Jones et al., 2010). More research is, therefore, needed to elucidate why xylanase seems to increase energy and nutrient digestibility in diets containing arabinoxylans from wheat, but not from corn.

Most cereal grains contain endogenous enzymes, including xylanase. However, these endogenous enzymes may be inactivated if the feed ingredients have been heat treated (e.g. millrun and DDGS), which is expected to increase the effect of exogenous xylanase supplementation on heat-treated feed ingredients (Coweison et al., 2005). Further research is necessary to determine the amount and the physiological importance of endogenous xylanases in grains and grain coproducts used as swine feed ingredients.

Phytase and Xylanase

For xylanases to be profitable, they must work in conjunction with microbial phytases. Approximately 65% of all manufactured pig feeds contain microbial phytases (Barletta, 2011). Phytase hydrolyzes bonds connecting P to the inositol ring in phytic acid, which releases P and other nutrients, and thereby increases the amounts of nutrients available to the pig (Selle and Ravindran, 2008). Phytase supplementation reduces the amount of inorganic P needed in the diet and also reduces P excretion in the feces (Simons et al., 1990; Almeida and Stein, 2010). Phytase is, therefore, included in most pig diets. The phytic acid, which the phytase hydrolyzes, is stored in the aleurone layer of the grain, which is hydrolyzed by xylanase (Adeola and Cowieson, 2011). It is, therefore, expected that the combination of xylanase and phytase can liberate more nutrients than either enzyme alone. However, results of recent experiments have not verified this

hypothesis and there are no indications that an additive effect is attained by adding both enzymes to diets fed to growing pigs (Woyengo et al., 2008; Yañez et al., 2011). It is, therefore, clear that more research is needed to investigate conditions that may result in positive effects of adding xylanase along with phytase to pig diets.

IN VITRO DIGESTIBILITY

In vitro techniques that simulate the digestion process in the pig may be beneficial to determine the efficacy of an exogenous enzyme like xylanase. The in vitro digestibility procedure described by Boisen and Fernández (1997) is a fairly inexpensive and reproducible procedure. However, when conducting in vitro digestibility experiments one must account for the following considerations: sequential use of digestive enzymes, an appropriate pH, concentration of enzymes, temperature for optimal enzyme activity, some degree of mixing, proper incubation time, and removal of end-products of digestion to separate the digested and undigested portions of feed (Longland, 1991). The procedure developed by Boisen and Fernández (1997) is a 3 step procedure that accounts for the previous considerations. First, the feed sample is weighed (0.500 g ± 1 mg) into 125-mL Erlenmeyer flasks. Next, 25 mL of phosphate buffer (0.1 *M*; pH 6.0) and 10 mL of 0.2 *M* HCl are added to each flask and flasks are stirred continuously using a magnetic stirrer. The pH of the solution is adjusted to 2 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH. One milliliter of freshly prepared pepsin solution (25 mg of pepsin/mL; P7000, Sigma Aldrich, St. Louis, MO) is added to each flask, and 0.5 mL of chloramphenicol solution (0.5 g chloramphenicol, Sigma No. C-0378, per 100 mL ethanol) is added to prevent bacterial growth and samples are placed in a shaking water bath that was heated at 39°C for 2 h. This first step simulates the gastric phase of digestion.

After 2 h, 10 mL of phosphate buffer (0.2 *M*; pH 6.8) and 5 mL of NaOH (0.6 *M*) is added to each flask and the pH is adjusted to 6.8 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH. One milliliter of freshly prepared pancreatin solution (100 mg of pancreatin/mL; P1750, Sigma Aldrich, St. Louis, MO) is added to each flask and samples are placed in a shaking water bath at 39°C for 4 h. This second step simulates the small intestinal phase of digestion. The pitfall of this procedure is this step in which a neutral phosphate buffer is used. This will solubilize the soluble fiber; thus, when the residue is filtered, the soluble fiber is removed, indicating complete digestion of soluble fiber by the end of the small intestine (Monro, 1992). However, this is not what happens in the pig. Soluble fiber is not enzymatically digested in the small intestine, but rather is easily fermented in the hind gut (Cho et al., 1997). To simulate in vivo conditions more accurately, 80% ethanol must be added to precipitate the soluble fiber (Monro, 1992). Once precipitated, the soluble fiber will not be removed through filtration and will be left in the undigested residue.

After 4 h, 10 mL of a 0.2 *M* EDTA solution is added to each flask and the pH is adjusted to 4.8 ± 0.01 with 30% acetic acid. Viscozyme (Viscozyme L V2010, Sigma-Aldrich, St. Louis, MO) is added to each flask in the amount of 0.5 mL. The flasks are placed in a shaking water bath at 39°C for 18 h. This third and final step simulates the fermentation processes in the large intestine.

To determine digestibility, undigested residues in the flasks are filtered in previously weighed Gooch crucibles containing celite 545 (0.400 g ± 5 mg; Sigma Aldrich, St. Louis, MO). The undigested material collected in the crucibles is washed twice with 10 mL of ethanol (96%) and twice with 10 mL of acetone (99.5%). The crucibles are dried in an oven for 2 h at 130°C, cooled in the desiccator, and weighed to measure total tract DM residues (Boisen and Fernandez,

1997). Two extra flasks, which contain no samples (blanks), but where all reagents and enzymes are added, are included to correct the final DM weight of the residues.

The in vitro digestibility of DM is calculated using the following equation (Cervantes-Pahm, 2011):

$$In\ vitro\ digestibility\ of\ DM, \% = \frac{[Sample\ DM - (Residue\ DM - Blank\ DM)]}{Sample\ DM} \times 100$$

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 samples respectively, and calculated as the difference between the combined dry weight of the crucible, celite, and residues, and the combined dry weight of crucible and celite (Cervantes-Pahm, 2011).

Using the procedure described above, Regmi et al. (2009) compared the in vitro digestibility of DM in wheat and the in vivo ATTD of energy and reported that the ATTD of energy is well correlated with in vitro digestibility of DM (R^2 = 0.82). Modifications to the original procedure by Boisen and Fernández (1997) have been proposed and longer incubation times (6, 18, 24 h) are sometimes used. Using this longer incubation procedure, it was observed that in vitro digestibility of GE in barley and in vitro digestibility of DM have a perfect relationship (R^2 = 1.00; Regmi et al., 2008). In vitro digestibility of OM and in vivo ATTD of OM in 79 mash diets for pigs that varied widely in ingredient and nutrient composition are also well correlated (R^2 = 0.82; Noblet and Jaguelin-Peyraud, 2007). Results of several experiments demonstrate that in vitro DM and OM digestibility procedures correlate well with energy, DM, and OM digestibility values obtained in vivo.

In the Boisen and Fernández (1997) procedure, an enzyme solution (viscozyme) that contains several carbohydrases (arabinase, cellulase, β-glucanase, hemicellulase, xylanase, and pectinase) is used to simulate fiber fermentation in the hind gut of pigs. This is not a perfect simulation because in pigs, these enzymes are synthesized by microbes in the cecum and large intestine. However, the extent, amount, and type of enzymes secreted by the microbial population are unknown and the type of diet also influences which enzymes are secreted by the microbial population.

Both Boisen and Fernández (1997) and Regmi et al. (2009) have tested the effects of using a cellulase enzyme instead of viscozyme to simulate hind gut fermentation. Boisen and Fernández (1997) observed an increase in in vitro digestibility of OM of multiple pig feed ingredients, especially high fiber ingredients, when using cellulase rather than viscozyme. Regmi et al. (2009) reported a poor relationship ($R^2 = 0.55$) between in vivo ATTD of energy and in vitro digestibility of DM in wheat using cellulase rather than viscozyme. Based on these observations it appears that cellulase overestimates fiber degradation in the hind gut of the pig, resulting in a poor correlation to in vivo ATTD of energy.

Another procedure developed by Urriola (2010) used a fecal inoculum in the third step of the in vitro procedure and compared the effectiveness of fecal inoculum and viscozyme to estimate DM and nutrient digestibility in 8 different samples of DDGS. In vitro digestibility of DM and NDF obtained with fecal inoculum increased by 20% compared with in vitro digestibility of DM and NDF obtained with viscozyme. This observation indicates that fecal inoculum had a greater amount of enzyme activity compared with the viscozyme solution. However, the fecal inoculum procedure, although greatly overestimating in vitro digestibility and in vivo digestibility of DM and NDF, is likely the most accurate way to mimic hind gut

fermentation because it uses the actual microbes that are present in the hind gut of the pig. Dilution procedures of feces and the incubation time need to be investigated to better simulate in vivo fiber fermentation.

In conclusion, the in vitro procedure developed by Boisen and Fernández (1997) is often used to simulate the digestibility of DM and OM in feed ingredients and complete pig diets. This procedure allows for alterations, allowing greater flexibility to investigate certain aspects of digestion. The flexibility of the procedure will provide a basis for an investigation into the use of xylanase.

CONCLUSION

The efficacy of xylanase to improve the digestibility of nutrients and energy in pig diets based on wheat and wheat coproducts has been demonstrated. Also, the combination of xylanase and phytase have no negative interactions, however; there are no reports on synergistic effects between xylanase and phytase. The efficacy of xylanase and phytase supplemented to swine diets based on corn, soybean meal, and DDGS has not been demonstrated. Also, the carbohydrate composition and in vitro digestion of NSP has not been reported for U.S. feed ingredients. Therefore, the scope of this thesis is to determine the carbohydrate composition, in vitro digestion, and effects of xylanase and phytase on ileal and total tract digestibility of nutrients and energy by swine in grains and grain coproducts produced in the United States.

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CHAPTER 3

EFFECTS OF PHYTASE AND XYLANASE ON ILEAL AND TOTAL TRACT DIGESTIBILITY OF ENERGY AND NUTRIENTS AND ON GROWTH PERFORMANCE OF GROWING PIGS FED DIETS CONTAINING CORN, SOYBEAN MEAL, DISTILLERS DRIED GRAINS WITH SOLUBLES, AND WHEAT BRAN

ABSTRACT

Two experiments were conducted to determine the efficacy of xylanase and phytase supplementation to swine diets based on corn, soybean meal, distillers dried grains with solubles, and wheat bran. In Exp. 1, the apparent ileal digestibility (**AID**) and the apparent total tract digestibility (**ATTD**) of energy and nutrients were determined. Eight pigs (initial BW: 24.1 ± 1.9 kg) were surgically fitted with a T-cannula in the distal ileum and randomly allotted to an 8 × 8 Latin square design with 8 diets and 8 periods. Four diets were formulated to meet the pig's AA requirements using a low amount of crystalline AA, whereas 3 additional diets were formulated to contain greater amounts of crystalline AA. The last diet was unrelated to this experiment. The 4 low-AA diets included the control diet that contained no phytase or xylanase, the phytase diet that contained 500 phytase units (**FTU**)/kg, and 2 diets that contained 500 FTU/kg of phytase and 2,000 or 4,000 xylanase units/kg. The 3 high-AA diets contained 500 FTU/kg of phytase and 2,000, 4,000, or 8,000 xylanase units/kg. Results indicated phytase improved ($P < 0.05$) the AID of CP, Ca, P, and most AA by 2.2, 13.9, 9.4, and approximately 2%, respectively, compared with the control diet. Phytase also improved ($P < 0.05$) the ATTD of Ca and P by 18.1 and 13.8%, respectively, compared with the control diet. Xylanase added to the low-AA diets improved ($P < 0.05$) the AID of DM, GE, and NDF by 2.3, 1.9, and 5.8%, respectively, compared with the

phytase diet. No negative interactions between xylanase and phytase were observed. In Exp. 2, all diets except the diet with 8,000 units of xylanase were fed to 288 pigs (initial BW: 28.9 ± 8.3 kg) in a 42-d growth assay. Results indicated that the high-AA diet with 2,000 xylanase units/kg and 500 FTU of microbial phytase/kg improved ($P < 0.05$) ADG, G:F, and final BW during the first 21 d of the experiment. However, this improvement was due to the addition of crystalline AA and no growth performance differences due to xylanase or phytase were detected. In conclusion, phytase and xylanase independently may result in small improvements in nutrient digestibility.

Key words: digestibility, phytase, pigs, xylanase

INTRODUCTION

The increase in ethanol production and the increased costs of traditional feed ingredients has made distillers dried grains with solubles (**DDGS**) an attractive feed ingredient for swine (Stein et al., 2006). Distillers dried grains with solubles can substitute for corn in swine diets because it has similar concentrations of DE and ME as corn and contains highly digestible P (Stein and Shurson, 2009). However, it is possible that more nutrients and energy may be obtained from DDGS if the large amounts of non-starch polysaccharides (**NSP**) in DDGS can be better utilized by the pig. Non-starch polysaccharides reduce the digestibility of DM and GE in DDGS because NSP are resistant to the pig's digestive enzymes (Stein and Shurson, 2009). One main NSP in DDGS is arabinoxylans (Chapter 4). Arabinoxylans are included in the hemicellulose fraction and consist of a D-xylose backbone joined by β1, 4 linkages and single residues of arabinose attached along the xylose backbone (Bedford, 1995). Arabinoxylans are

very difficult to hydrolyze due to their complex nature and the fact that the pig does not secrete xylanase, the enzyme needed to cleave the β1, 4 bonds linking the xylose units. Nutrient and energy digestibility is, therefore, reduced if concentrations of dietary NSP increase (Yin et al., 2000).

Supplementation of exogenous xylanase and phytase to diets containing wheat and wheat coproducts may reduce intestinal viscosity, improve ileal AA and total tract P digestibility, and improve G:F (Barrera et al., 2004; Nortey et al., 2007; 2008). However, limited research has been conducted on the effects of xylanase supplementation on nutrient digestibility and growth performance in diets based on corn, soybean meal (**SBM**), and DDGS. It has, however, been demonstrated that microbial phytase reduces P excretion and improves P digestibility in corn-SBM-DDGS diets (Almeida and Stein, 2010), but there is a lack of information about effects of combinations of phytase and xylanase in diets based on corn, SBM, and DDGS. The objective of this research, therefore, was to determine the ileal and total tract digestibility of nutrients and energy and growth performance responses of growing pigs fed phytase- and xylanase-supplemented diets containing corn, SBM, DDGS, and wheat bran.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for these experiments.

Exp. 1: Energy and Nutrient Digestibility

Animals, Diets, and Experimental Design. Eight barrows with an average initial BW of 24.1 ± 1.9 kg were used to determine effects of phytase and xylanase on ileal and total tract digestibility

of nutrients and energy in diets based on corn, SBM, DDGS, and wheat bran. Animals were randomly allotted to an 8 × 8 Latin square design with 8 diets and 8 periods (Kim and Stein, 2009). Pigs were surgically fitted with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). After the surgery, pigs were housed in individual pens. Each pen was equipped with a feeder and a nipple drinker and had a slatted tribar metal floor.

Corn, SBM, DDGS, and wheat bran were procured (Table 3.1) and the same batches of these ingredients were used in all diets. Eight experimental diets were prepared (Tables 3.2 and 3.3). Four diets were formulated to meet the pig's AA requirements using a low amount of crystalline AA, whereas 3 additional diets were formulated above the pig's AA requirements and thus contained greater amounts of crystalline AA. The last diet was unrelated to this experiment. The 4 low-AA diets included the control diet that contained no microbial phytase or xylanase, the phytase diet that contained 500 phytase units (**FTU**)/kg, and 2 diets that contained 500 FTU/kg of microbial phytase and 2,000 or 4,000 xylanase units/kg. The 3 high-AA diets contained 500 FTU/kg of microbial phytase and 2,000, 4,000, or 8,000 xylanase units/kg. The concentration of AA was increased in the final 3 diets to support a possible increase in energy release from the breakdown of arabinoxylans by xylanase. All 7 diets contained corn (36.5 or 38.0%), SBM (20.0%), DDGS (35.0%) and wheat bran (5.0%) as the fiber sources. Chromic oxide was added at 0.4% to each diet as an indigestible marker.

Feeding and Sample Collection. Pigs were fed once daily at 3 times the estimated requirement for maintenance energy (i.e., 106 kcal of ME/kg$^{0.75}$; NRC, 1998). Water was available at all times. The BW of each pig was recorded at the beginning of the experiment and at the end of each period. Each diet was fed during one 7-day period. The initial 4 d was considered an adaptation period to the diet. On d 5, fecal samples were collected and stored at – 20°C

immediately after collection. Ileal digesta were collected for 8 h on d 6 and 7. A 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 min or whenever full and replaced with a new bag. Digesta were stored at – 20°C immediately after collection.

Chemical Analysis. Diets, ingredients, and the freeze-dried samples of digesta and feces were ground through a 1.0 mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). All samples were analyzed for DM (Method 930.15; AOAC Int., 2007), ash (Method 942.05; AOAC Int., 2007), crude fiber (Method 978.10; AOAC Int., 2007), ADF (Method 973.18; AOAC Int., 2007), and NDF (Holst, 1973). The concentration of GE in all diets and ingredients and in ileal and fecal samples was determined using an adiabatic bomb calorimeter (model 6300, Parr Instruments, Moline, IL). Benzoic acid was the standard for calibration. Calcium and total P were measured in diets and ingredients and in ileal and fecal samples by inductively coupled plasma (**ICP**) spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. All diets, ingredients, and ileal digesta samples were analyzed for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. All diets, ingredients, and ileal samples also were analyzed for AA on a Hitachi AA Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc, Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [Method 982.30 E(a); AOAC Int., 2007]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [Method 982.30 E(b); AOAC Int., 2007]. Tryptophan was

determined after NaOH hydrolysis for 22 h at 110°C [Method 982.30 E(c); AOAC Int., 2007]. The chromium concentration in all diets and ileal and fecal samples were determined using an ICP procedure (Method 990.08; AOAC Int., 2007). Samples were prepared using nitric acid-perchloric acid [Method 968.08 D(b); AOAC Int., 2007].

Calculations and Statistical Analysis. Values for apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) were calculated according to Stein et al. (2007). Data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC). Homogeneity of the variance among treatments was confirmed using the UNIVARIATE procedure of SAS. Outliers were determined as values that deviated from the treatment mean by more than 3 times the interquartile range (Devore and Peck, 1993). Three outliers were identified and removed from the data set. Means were calculated using the LSMEANS statement in SAS. Differences were evaluated using preplanned contrasts that included 1) control vs. the phytase diet, 2) phytase vs. xylanase, 3) low-AA vs. high-AA, and 4) linear effects of xylanase. There were no quadratic effects of xylanase. The pig was the experimental unit for all analyses and an α-value of 0.05 was used to assess significance among means. A *P*-value between 0.05 and 0.10 was considered a trend.

Exp. 2: Growth Performance

Animals, Diets, and Experimental Design. A total of 288 pigs with an average initial BW of 28.9 ± 8.3 kg were used in a 42-d growth assay. Pigs were individually weighed and randomly allotted using the Experimental Animal Allotment Program (Kim and Lindemann, 2007) to 1 of 6 dietary treatments in a randomized complete block design based on BW and sex. There were 4 pigs per pen, 6 dietary treatments, and 12 replicate pens per treatment. Each pen was equipped

with a single feeder and a nipple drinker to allow ad libitum feed and water consumption. Pigs were weighed individually at the beginning of the experiment, on d 21, and at the conclusion of the experiment. Daily allotments of feed were recorded and feed left in the feeders was recorded on d 21 and at the conclusion of the experiment. Data for ADG, ADFI, and G:F were calculated and summarized for each diet at the conclusion of the experiment.

Experimental diets containing corn, SBM, DDGS, and wheat bran were prepared. Ingredient composition of the diets was similar to the diets used in Exp.1 with the exception that the diet containing 8,000 units of xylanase was not used in this experiment and chromic oxide in the diets was replaced with cornstarch. Diets were analyzed as described for Exp. 1 (Table 3.4).

Statistical Analysis. Data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC). Homogeneity of the variance among treatments was confirmed using the UNIVARIATE procedure of SAS. The LSMEANS statement was used to calculate mean values and differences were evaluated using preplanned contrasts that included 1) control vs. the phytase diet, 2) phytase vs. xylanase, 3) low-AA vs. high-AA, and 4) linear effect of xylanase. There were no quadratic effects of xylanase. The pen was the experimental unit and an α-level of 0.05 was used to determine significance among means. A *P*-value between 0.05 and 0.10 was considered a trend.

RESULTS AND DISCUSSION

Exp. 1: Ileal and Total Tract Digestibility

All pigs consumed their daily allotments of feed, and feces and ileal digesta were successfully collected from all pigs. The analyzed concentration of most nutrients in the control diet was similar to the concentration of nutrients in other diets. However, as expected, the

concentrations of Lys, Met, Thr, and Trp were greater in the high-AA diets compared with the low-AA diets.

The AID of DM was greatest ($P < 0.05$) in the low-AA diet containing 4,000 xylanase units/kg (Table 3.5). An increase in the AID of DM also was observed when pigs were fed a diet containing wheat and wheat coproducts supplemented with 4,000 xylanase units/kg (Nortey et al., 2008). Xylanase supplementation increased ($P < 0.05$) the AID of DM compared with phytase supplementation, and the AID of DM in the low-AA diets was greater ($P < 0.05$) than the AID of DM in the high-AA diets. There was also a tendency ($P < 0.10$) for the AID of DM to increase linearly as the concentration of xylanase increased. The AID of GE was greater ($P < 0.05$) when xylanase and phytase were both used compared with no supplementation or phytase supplementation alone. Xylanase also tended ($P < 0.10$) to linearly increase the AID of GE, which is in contrast to data indicating that there is no effect on the AID of GE when growing pigs are fed a diet containing DDGS co-fermented from wheat and corn and supplemented with 4,000 xylanase units/kg (Yáñez et al., 2011). The contradicting results may be due to differences in ingredient composition between the experiments, which may have resulted in differences in arabinoxylan concentration. In the current experiment, the experimental diets contained arabinoxylans from corn DDGS and wheat bran, as well as from corn and SBM, whereas in the experiment by Yáñez et al. (2011), the sole source of arabinoxylans was the co-fermented DDGS. Arabinoxylans differ in their structure and solubility among ingredients. Corn, SBM, and wheat bran contain approximately 0.3, 1.1, and 1.7% soluble arabinoxylans and 7.8, 3.4, and 22.1% insoluble arabinoxylans, respectively (Bach Knudsen, 1997). Distillers dried grains with solubles is believed to contain approximately 3 times as many arabinoxylans as their parent grains because starch is removed from the grain during ethanol production.

Phytase supplementation decreased ($P < 0.05$) the AID of crude fiber and xylanase supplementation increased ($P < 0.05$) the AID of crude fiber compared with the diet containing phytase only, but the AID of crude fiber for the xylanase diet was not different from the AID of crude fiber in the control diet. The AID of ADF was greater ($P < 0.05$) in diets containing xylanase than in the diet containing only phytase, but was not different from the control diet, with the exception of the low-AA diet supplemented with 2,000 xylanase units/kg. Xylanase added to the low-AA diets improved ($P < 0.05$) the AID of NDF, but the AID of NDF in the high-AA diets was not different from the control or phytase only diets. When the AID of hemicellulose was calculated, however, there were no differences among diets. The hypothesis was that xylanase hydrolyzes arabinoxylans, which are included in the hemicellulose fraction, in the small intestine; therefore, an increase in the AID of NDF is expected if xylanase is included in the diet and this was only observed in the low-AA diets. However, the NDF procedure only determines insoluble hemicelluloses and not soluble hemicelluloses (Bach Knudsen, 2001). This may be the reason for the results obtained in this experiment because xylanase may only have an effect on soluble arabinoxylans, which are not included in NDF.

Addition of phytase to the diets increased ($P < 0.05$) the AID of Ca and P, but supplementation with both phytase and xylanase did not improve the AID of Ca and P compared with phytase supplementation only, which indicates that phytase, but not xylanase, is effective in degrading the phytate complex. These results are consistent with data indicating an increase in the AID of Ca and P with phytase supplementation and no interactions between phytase and xylanase (Yáñez et al., 2011).

The AID of CP increased ($P < 0.05$) with the addition of phytase to the low-AA diets, but xylanase had no effect on the AID of CP. The low-AA diets had a greater ($P < 0.05$) AID of CP

compared with the high-AA diets. This was potentially caused by an increase in endogenous N losses in the high-AA diets, which may be due to an interaction between xylanase or phytase and crystalline AA. However, greater AA intake is expected to reduce the effect of endogenous N on the calculation of AID of AA (Mariscal-Landin and Reis De Souza, 2006). Furthermore, there is no effect on AID of CP, Lys, Thr, and Met of increasing dietary CP level from 20 to 24%, which also increased the AA concentration of the diet (Fan et al., 1994). Excess AA were supplemented to test the hypothesis that if xylanase supplementation increases the digestibility of nutrients and energy, then the pig will have excess nutrients and energy for growth, which requires more AA to support maximum protein synthesis. However, results demonstrated that there were minimal increases in available nutrients and energy and, thus, excess AA do not need to be added to compensate for the potential release of excess energy and nutrients by xylanase and phytase. The high-AA diets had a greater ($P < 0.05$) AID of Lys, Met, Thr, and Trp compared with the low-AA diets, which was expected because of the addition of crystalline sources of Lys, Met, Thr, and Trp to these diets and because crystalline AA are believed to be 100% digestible. However, the AID of all other AA was greater ($P < 0.05$) in the low-AA diets compared with the high-AA diets. Some endogenous AA may be released in response to phytate and fiber in the diets, but all diets contained similar amounts of phytate and fiber; therefore, the difference in the AID of most AA between the low-AA diets compared with the high-AA diets cannot be explained (Adeola and Cowieson, 2011). Phytase supplementation increased ($P < 0.05$) the AID of most AA in the low-AA diets compared with the control diet. Phytic acid has a high capacity for chelation with free amino groups of Arg and Lys, and the terminal amino group of proteins (Maenz, 2001). Thus, phytase supplementation breaks the phytate bonds, releasing the chelated AA or proteins, and subsequently increases AA digestibility. However, Adeola and Sands (2003) concluded that

there is a lack of research supporting the hypothesis that AID of AA is improved if phytase is included in the diet. Therefore, it is difficult to assess the efficacy of phytase on the improvement of AID of most AA in the current study. Supplementation of low-AA diets with 4,000 xylanase units/kg improved the AID of Lys and Met compared with the control diet and the diet containing only phytase. However, the AID of Lys and Met in the low-AA diet supplemented with 4,000 xylanase units/kg was less ($P < 0.05$) than the AID of Lys and Met in the high-AA diets. Addition of 4,375 xylanase units/kg to diets containing wheat millrun improved ($P < 0.05$) the AID of Ile and Phe and also increased ($P < 0.05$) the AID of Arg, Ile, Leu, Lys, Phe, Ser, Thr, Tyr, and Val (Nortey et al., 2007; 2008). This observation indicates that supplementation of 500 FTU/kg and 4,000 xylanase units/kg to diets containing coproducts, such as DDGS, may improve the AID of most AA and, especially Lys and Met, which are typically the first and fourth limiting AA respectively in pig diets based on corn and SBM. There are 2 mechanisms by which xylanase may increase AA digestibility: 1) xylanase may decrease intestinal viscosity caused by soluble arabinoxylans, which increases the capacity for proteins to be hydrolyzed and absorbed, and 2) as arabinoxylans are hydrolyzed by xylanase, the AA that are randomly associated with arabinoxylans may be rendered more available to digestion and absorption (Campbell and Bedford, 1992; Simon, 1998).

The ATTD of DM was greater ($P < 0.05$) in the low-AA diets compared with the high-AA diets (Table 3.6). Supplementation of 2,000 or 4,000 xylanase units/kg improved ($P < 0.05$) the ATTD of DM compared with the control diet. No differences due to xylanase and phytase were observed for the ATTD of GE, crude fiber, ADF, and NDF. The ATTD of Ca and P increased ($P < 0.05$) when phytase was added to the diet, but xylanase had no effect on the ATTD of Ca. However, when 4,000 xylanase units/kg were added to the diet, the ATTD of P

tended to improve ($P < 0.10$) compared with phytase supplementation alone. An increase in the ATTD of P also was observed when 23-kg pigs were fed a diet containing wheat, wheat middlings, and SBM supplemented with 500 FTU/kg and 4,000 xylanase units/kg (Olukosi et al., 2007). Woyengo et al. (2008) observed that supplementing 4,000 xylanase units/kg to wheat-based diets fed to growing pigs improved ($P < 0.05$) the AID of Ca, concluding that Ca in wheat is highly associated with arabinoxylans and, thus, supplementing xylanase can increase available Ca through hydrolyzing arabinoxylans. Applying this hypothesis to corn, SBM, DDGS, and wheat bran with regards to P, it can be hypothesized that P in these ingredients may be associated with arabinoxylans and through hydrolysis of arabinoxylans by xylanase, more P is available for absorption, thus increasing the ATTD of P. However, more research needs to be conducted to verify this hypothesis.

Exp. 2: Growth Performance

Xylanase and or phytase supplementation did not affect the overall growth performance of pigs (Table 3.7). The only performance increases observed were during the first 21 d and were due to the supplementation of crystalline AA in the high-AA diets compared with the low-AA diets, which indicates that AA concentrations in the low-AA diets may have limited growth performance of pigs. The high-AA diet supplemented with 2,000 xylanase units/kg improved ($P < 0.05$) ADG, G:F, and final BW for d 0 to 21 compared with the low-AA diets. When xylanase was added to corn-SBM-based diets containing 30% DDGS and fed to weanling pigs, no differences in pig growth performance were observed (Jones et al., 2010). However, G:F was improved when diets containing 20 or 40% wheat millrun was supplemented with 4,375 xylanase units/kg (Nortey et al., 2007). Thus, variations in growth performance responses to addition of xylanase to diets of pigs have been reported. These variations may be caused by several factors.

The biggest factor seems to be the origin of arabinoxylans in the diet. If they are from wheat or wheat coproducts, there seems to be a response, but if they are from corn or corn coproducts, no responses have been observed. This difference is likely a result of differences in the arabinoxylan complex within the grains. Arabinoxylans in wheat may have more AA and other nutrients associated with them, and when xylanase hydrolyzes arabinoxylans, these nutrients are released. However, corn and wheat both have similar concentrations of arabinoxylans, but the ratio of arabinose to xylose is much different. Corn is much more substituted with a ratio of 0.81 compared with wheat, which has a ratio of 0.66 (Chapter 4). The smaller degree of substitution in wheat may make arabinoxylans more accessible to xylanase compared with corn, enabling a greater effect of xylanase on wheat than on corn. However, more research is necessary to elucidate the structure of the arabinoxylan complex within the fiber matrix to better understand how xylanase interacts with arabinoxylans.

Conclusion

The AID of DM, GE, most AA, and the ATTD of DM, Ca, and P were improved by addition of 4,000 xylanase units/kg and 500 FTU/kg of phytase to a diet based on corn, SBM, DDGS, and wheat bran. No negative interactions between xylanase and phytase were observed. However, the digestibility increases were not great enough to elicit a growth performance response. In conclusion, to successfully utilize xylanase and phytase they must be supplemented to diets formulated to contain lower amounts of GE, AA, Ca, and P, therefore, reducing diet costs while maintaining growth performance, subsequently increasing producer margins. However, more research is necessary to determine a consistent amount of nutrients and energy released by xylanase and phytase and if digestibility increases are additive among ingredients when both xylanase and phytase are added to growing pig diets.

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Table 3.1. Analyzed composition of ingredients (as-fed basis), Exp. 1

Composition	Ingredient			
	Corn	Soybean meal	Distillers dried grains with solubles	Wheat bran
DM, %	86.15	87.66	87.22	86.78
GE, kcal/kg	3,871	4,215	4,704	3,964
CP, %	7.04	49.57	24.88	15.04
Crude fiber, %	1.50	2.98	6.38	8.92
ADF, %	2.06	4.94	7.58	11.06
NDF, %	9.41	8.24	29.97	42.22
Ca, %	0.01	0.33	0.03	0.09
P, %	0.24	0.62	0.78	1.36
Indispensable AA, %				
Arg	0.38	3.73	1.23	1.16
His	0.22	1.37	0.76	0.48
Ile	0.26	2.34	1.05	0.54
Leu	0.80	3.97	2.93	1.06
Lys	0.26	3.24	0.92	0.69
Met	0.16	0.71	0.55	0.27
Phe	0.33	2.58	1.25	0.68
Thr	0.26	2.01	1.03	0.54
Trp	0.12	0.32	0.27	0.18
Val	0.36	2.43	1.35	0.80

Table 3.1. (Cont.)

Dispensable AA, %				
Ala	0.51	2.21	1.81	0.79
Asp	0.49	5.85	1.71	1.21
Cys	0.16	0.75	0.54	0.38
Glu	1.23	8.90	3.61	3.14
Gly	0.31	2.20	1.09	0.90
Pro	0.61	2.55	2.04	1.06
Ser	0.32	2.33	1.19	0.62
Tyr	0.22	1.87	0.95	0.44

Table 3.2. Composition of experimental diets (as-fed basis), Exp. 1 and 2[1]

	Low-AA				High-AA		
Xylanase	0	0	2,000	4,000	2,000	4,000	8,000
Phytase	0	500	500	500	500	500	500
Ingredient, %							
Corn	38.00	38.00	38.00	38.00	36.50	36.50	36.50
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Distillers dried grains with solubles	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Wheat bran	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Cornstarch	0.31	0.285	0.285	0.285	0.535	0.535	0.535
L-Lys HCl	0.03	0.03	0.03	0.03	0.54	0.54	0.515
DL-Met	-	-	-	-	0.32	0.32	0.32
L-Thr	-	-	-	-	0.33	0.33	0.33
L-Trp	-	-	-	-	0.09	0.09	0.09
Chromic oxide[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]The diet containing 8,000 xylanase units/kg was used only in Exp. 1.

[2]In Exp. 2, chromic oxide was replaced with cornstarch.

[3]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 $_{DL}$-α tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamine 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 composition of diets (as-fed basis), Exp. 1

	Low-AA				High-AA		
Xylanase	0	0	2,000	4,000	2,000	4,000	8,000
Phytase	0	500	500	500	500	500	500
DM, %	88.48	88.55	88.15	88.33	88.24	88.18	87.90
GE, kcal/kg	4,212	4,200	4,184	4,220	4,235	4,248	4,211
CP, %	19.88	21.23	20.41	19.38	20.54	20.56	19.51
Crude fiber, %	3.53	3.41	3.47	3.63	3.50	3.42	3.56
ADF, %	4.94	4.91	5.07	4.76	4.97	4.94	5.00
NDF, %	16.79	17.18	17.15	16.74	17.41	16.90	16.92
Ca, %	0.46	0.50	0.48	0.39	0.47	0.42	0.50
P, %	0.63	0.62	0.63	0.63	0.62	0.63	0.63
Indispensable AA, %							
Arg	1.24	1.33	1.28	1.21	1.23	1.23	1.18
His	0.56	0.60	0.58	0.54	0.55	0.55	0.52
Ile	0.84	0.92	0.88	0.84	0.84	0.82	0.78
Leu	1.94	2.08	1.99	1.88	1.92	1.91	1.82
Lys	1.04	1.11	1.07	1.00	1.35	1.44	1.32
Met	0.38	0.41	0.38	0.38	0.62	0.66	0.60
Phe	1.01	1.08	1.04	0.98	1.00	0.99	0.94
Thr	0.79	0.83	0.79	0.76	1.02	1.08	1.04
Trp	0.24	0.22	0.23	0.22	0.30	0.30	0.31
Val	1.02	1.09	1.06	0.98	0.99	0.99	0.93

Table 3.3. (Cont.)

Dispensable AA, %							
Ala	1.21	1.28	1.23	1.16	1.19	1.19	1.14
Asp	1.80	1.91	1.85	1.75	1.76	1.77	1.68
Cys	0.35	0.38	0.36	0.35	0.35	0.35	0.32
Glu	3.47	3.76	3.60	3.37	3.41	3.43	3.25
Gly	0.89	0.95	0.91	0.86	0.87	0.87	0.84
Pro	1.33	1.41	1.36	1.28	1.31	1.31	1.24
Ser	0.93	0.98	0.92	0.89	0.89	0.92	0.87
Tyr	0.72	0.76	0.74	0.70	0.71	0.71	0.69

Table 3.4. Analyzed composition of diets (as-fed basis), Exp. 2

	Low-AA				High-AA	
Xylanase	0	0	2,000	4,000	2,000	4,000
Phytase	0	500	500	500	500	500
CP, %	20.51	19.96	20.55	19.16	20.34	21.11
Crude fiber, %	3.74	3.86	3.84	4.09	3.89	3.88
ADF, %	4.75	4.91	4.99	5.01	5.02	5.04
NDF, %	18.00	18.35	16.14	17.49	16.93	17.03
Ca, %	0.48	0.49	0.56	0.42	0.47	0.41
P, %	0.57	0.57	0.56	0.56	0.56	0.56
Indispensable AA, %						
Arg	1.26	1.21	1.26	1.17	1.18	1.26
His	0.57	0.56	0.57	0.54	0.55	0.56
Ile	0.86	0.82	0.84	0.82	0.83	0.84
Leu	2.05	2.01	2.04	1.92	1.96	1.97
Lys	1.01	0.98	1.02	0.95	1.26	1.45
Met	0.38	0.37	0.39	0.35	0.59	0.62
Phe	1.08	1.05	1.08	1.01	1.02	1.05
Thr	0.81	0.79	0.82	0.75	0.99	1.06
Trp	0.25	0.24	0.25	0.24	0.33	0.31
Val	1.03	1.01	1.02	0.99	1.00	1.00
Dispensable AA, %						
Ala	1.25	1.24	1.25	1.17	1.21	1.20

Table 3.4. (Cont.)

Asp	1.83	1.74	1.83	1.69	1.70	1.82
Cys	0.37	0.35	0.38	0.34	0.34	0.37
Glu	3.60	3.47	3.61	3.34	3.40	3.53
Gly	0.91	0.88	0.90	0.85	0.86	0.89
Pro	1.42	1.40	1.42	1.33	1.36	1.36
Ser	0.95	0.93	0.97	0.86	0.89	0.93
Tyr	0.78	0.76	0.77	0.71	0.74	0.77

Table 3.5. Apparent ileal digestibility of DM, energy, and nutrients, Exp. 1

	Low-AA				High-AA							
Xylanase	0	0	2,000	4,000	2,000	4,000	8,000		Contrast *P*-Value			
Phytase	0	500	500	500	500	500	500	Pooled SEM	Control vs. phytase	Phytase vs. xylanase	Low-AA vs. high-AA	Linear[1]
DM, %	67.4	66.7	68.6	69.7	65.5	65.3	67.5	0.9	0.52	0.01	< 0.01	0.08
GE, %	71.7	71.0	72.5	73.6	70.2	70.4	72.0	0.8	0.47	0.02	< 0.01	0.06
Crude fiber, %	36.3	26.1	37.7	39.9	31.2	28.2	35.9	3.0	< 0.01	< 0.01	< 0.01	0.19
ADF, %	29.8	24.7	35.7	31.4	27.7	25.4	31.1	2.5	0.07	< 0.01	< 0.01	0.24
NDF, %	41.0	39.9	46.2	46.8	41.4	39.4	42.9	2.4	0.68	< 0.01	< 0.01	0.60
Ca, %	55.3	69.2	67.7	64.8	62.7	63.0	69.8	2.8	< 0.01	0.27	0.12	0.03
P, %	38.0	47.4	48.0	47.8	41.1	46.4	49.7	2.9	< 0.01	0.82	0.04	< 0.01
CP, %	75.3	77.5	77.1	77.8	74.0	74.7	75.5	0.9	0.02	0.94	< 0.01	0.10
Indispensable AA, %												

Table 3.5. (Cont.)

Arg	85.8	87.5	86.6	87.6	84.9	85.4	85.5	0.5	0.01	0.43	< 0.01	0.32
His	80.1	82.0	80.8	81.7	77.8	78.1	78.2	0.8	0.05	0.37	< 0.01	0.63
Ile	78.8	81.6	81.0	82.1	78.3	77.5	78.0	0.7	< 0.01	0.97	< 0.01	0.72
Leu	83.5	85.4	84.6	85.8	82.7	82.5	82.8	0.6	0.01	0.67	< 0.01	0.90
Lys	77.0	78.5	78.6	79.2	81.9	82.5	81.7	0.8	0.12	0.58	< 0.01	0.84
Met	84.8	86.0	85.1	86.9	90.1	90.5	90.2	0.5	0.05	0.89	< 0.01	0.80
Phe	82.2	84.4	83.5	84.7	81.4	81.2	81.3	0.6	0.01	0.67	< 0.01	0.84
Thr	70.0	72.6	70.2	71.7	75.6	77.2	77.0	0.9	0.02	0.10	< 0.01	0.24
Trp	78.5	77.0	78.0	79.4	81.7	82.4	83.2	0.9	0.14	0.06	< 0.01	0.12
Val	75.7	78.3	77.7	78.3	74.3	74.1	73.8	0.9	0.03	0.72	< 0.01	0.66
Dispensable AA, %												
Ala	79.4	81.0	80.7	81.9	78.5	78.2	78.4	0.9	0.11	0.75	< 0.01	0.97
Asp	73.0	76.0	75.0	76.1	71.5	72.4	72.0	1.0	0.01	0.66	< 0.01	0.71
Cys	69.5	72.4	69.1	72.0	65.1	67.3	64.3	1.5	0.08	0.19	< 0.01	0.60
Glu	82.3	84.7	83.6	84.8	79.8	81.1	81.2	1.0	0.03	0.64	< 0.01	0.20

Table 3.5. (Cont.)

Gly	64.4	68.4	66.4	66.8	61.0	62.5	62.9	1.8	0.03	0.24	< 0.01	0.27
Pro	75.1	76.8	76.5	77.9	74.7	75.8	75.4	2.0	0.21	0.73	0.04	0.61
Ser	78.5	81.0	78.7	80.4	76.9	77.8	77.4	0.7	< 0.01	0.07	< 0.01	0.57
Tyr	83.0	85.0	84.0	85.0	82.0	82.0	82.4	0.6	0.02	0.46	< 0.01	0.58

[1] Linear effect of xylanase added to low-AA diets.

Table 3.6. Apparent total tract digestibility of DM, energy, and nutrients, Exp. 1

	Low-AA				High-AA							
Xylanase	0	0	2,000	4,000	2,000	4,000	8,000		Contrast *P*-Value			
Phytase	0	500	500	500	500	500	500	Pooled SEM	Control vs. phytase	Phytase vs. xylanase	Low-AA vs. high-AA	Linear[1]
DM, %	81.8	82.6	83.4	83.2	81.9	81.7	82.4	0.4	0.16	0.15	< 0.01	0.35
GE, %	83.0	83.2	84.0	84.0	83.1	82.9	83.5	0.4	0.66	0.08	< 0.01	0.37
Crude fiber, %	52.3	51.0	55.0	53.8	50.5	49.5	52.4	1.7	0.57	0.08	< 0.01	0.38
ADF, %	57.3	56.7	58.5	55.1	56.1	54.5	55.3	1.5	0.76	0.94	0.30	0.67
NDF, %	60.5	62.6	63.0	59.9	61.3	59.3	59.3	1.3	0.18	0.39	0.30	0.20
Ca, %	50.8	68.9	72.7	71.0	66.3	66.2	72.9	3.1	< 0.01	0.36	0.04	0.08
P, %	47.7	61.5	63.9	65.9	60.4	62.4	63.6	2.1	< 0.01	0.10	0.04	0.18

[1] Linear effect of xylanase added to low-AA diets.

Table 3.7. Growth performance of pigs fed experimental diets, Exp. 2

	Low-AA				High-AA						
Xylanase	0	0	2,000	4,000	2,000	4,000		Contrast *P*-Value			
Phytase	0	500	500	500	500	500	SEM	Control vs. phytase	Phytase vs. xylanase	Low-AA vs. high-AA	Linear[1]
d 0 - 21											
Initial BW, kg	28.88	28.84	28.90	28.91	28.92	28.93	2.08	0.73	0.54	0.83	0.55
ADG, kg/d	0.70	0.73	0.71	0.73	0.81	0.74	0.06	0.28	0.91	< 0.01	0.72
ADFI, kg/d	1.59	1.62	1.59	1.60	1.64	1.58	0.14	0.44	0.56	0.58	0.74
G:F, kg/kg	0.44	0.45	0.45	0.46	0.50	0.47	0.01	0.50	0.76	< 0.01	0.45
Final BW, kg	43.63	44.07	43.87	44.30	46.01	44.54	3.17	0.34	0.97	< 0.01	0.62
d 21 - 42											
ADG, kg/d	0.90	0.94	0.94	0.90	0.89	0.90	0.06	0.41	0.59	0.37	0.35
ADFI, kg/d	2.10	2.15	2.12	2.13	2.15	2.08	0.19	0.38	0.57	0.77	0.72
G:F, kg/kg	0.43	0.44	0.44	0.42	0.41	0.44	0.02	0.70	0.83	0.36	0.30

Table 3.7. (Cont.)

Final BW, kg	62.63	63.71	63.53	63.22	64.64	63.49	4.24	0.23	0.66	0.28	0.58
d 0-42											
Cumulative											
ADG, kg/d	0.80	0.83	0.82	0.82	0.85	0.82	0.06	0.22	0.61	0.30	0.54
ADFI, kg/d	1.85	1.88	1.85	1.87	1.90	1.83	0.17	0.31	0.49	0.97	0.67
G:F, kg/kg	0.44	0.44	0.45	0.44	0.45	0.45	0.01	0.46	0.97	0.09	0.62

[1] Linear effect of xylanase added to low-AA diets.

CHAPTER 4

CARBOHYDRATE COMPOSITION AND IN VITRO DIGESTION OF DRY MATTER AND NON-STARCH POLYSACCHARIDES IN GRAINS AND GRAIN COPRODUCTS

ABSTRACT

Two experiments were conducted to investigate aspects of fiber composition and digestibility. In Exp. 1, the carbohydrate composition of 12 feed ingredients was determined to quantify the available substrate for carbohydrase supplementation. The 12 ingredients included 3 grains (corn, sorghum, and wheat), 3 coproducts from the dry grind industry [corn distillers dried grains with solubles (**DDGS**) and 2 sources of sorghum DDGS], 4 coproducts from the wet milling industry (corn gluten meal, corn gluten feed, corn germ meal, and corn bran), and 2 coproducts from the flour milling industry (wheat middlings and wheat bran). The parent grains (corn, sorghum, and wheat) contained greater amounts of starch and less non-starch polysaccharides (**NSP**) compared with the 9 coproducts. The concentration of soluble NSP was minimal in all ingredients. Results indicated that there were substantial amounts of NSP in these ingredients, which indicates that NSP-degrading enzymes such as xylanase may be effective in improving the nutritional value of these ingredients. Experiment 2 was conducted to determine in vitro ileal and total tract digestibility of DM and NSP in the 12 feed ingredients used in Exp. 1. In vitro ileal digestibility of NSP was close to zero in all feed ingredients, indicating that pepsin and pancreatin enzymes have no effect on the degradation of NSP. However, a strong correlation (R^2 = 0.97) between in vitro ileal digestibility of DM and NSP concentration in feed ingredients was observed, which indicates that in vitro ileal digestibility of DM may be used to estimate NSP concentration in feed ingredients. In vitro total tract digestibility of NSP ranged from 6.5% in corn bran to 57.3% in corn gluten meal, which indicates that the fermentation of each ingredient

depends on the amount and type of NSP in a feed ingredient. Also, there was not as strong of a correlation (R^2 = 0.83) between in vitro total tract digestibility of DM and ingredient NSP concentration. In conclusion, there is a large range in carbohydrate composition of grains and grain coproducts, and in vitro ileal digestibility of DM has a strong correlation to ingredient NSP concentration.

Key words: coproducts, fiber, grain, in vitro digestibility, non-starch polysaccharides, starch

INTRODUCTION

It is not possible to economically feed pigs without reducing the amount of expensive grains in the diet. Therefore, less expensive coproducts from grains produced from both the wet-milling and dry-milling industries often are included in diets fed to pigs. However, coproducts often contain more fiber compared with the parent grain. Fiber in swine diets reduces energy and DM digestibility by pigs because fiber is not enzymatically digested, but fermented, and there are energetic losses in carbon dioxide and methane through fermentation (Bach Knudsen, 2001). One possibility for increasing the utilization of fiber fed to pigs is to include exogenous fiber-degrading carbohydrases in the diets. However, the efficacy of fiber-degrading carbohydrases is highly variable, especially when supplemented to U.S. swine diets containing corn and corn coproducts or sorghum and sorghum coproducts. The reason for the lack of a consistent response to carbohydrases may be that enzymes are substrate-specific. Therefore, the first objective of this experiment was to determine the carbohydrate composition of grains and grain coproducts used as feed ingredients in U.S. swine diets.

An in vitro procedure that simulates the digestive processes of the pig was developed by Boisen and Fernández (1997), and results using this procedure indicate that in vitro ileal and total tract DM and OM digestibility values are similar to values obtained when in vivo digestibility

methods are used (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007). However, in vitro ileal and total tract digestibility of non-starch polysaccharides (**NSP**) has not been reported. Therefore, the second objective of this experiment was to determine in vitro ileal and total tract digestibility of NSP in grains and grain coproducts.

MATERIALS AND METHODS

Feed Ingredients. Twelve samples of grains and grain coproducts produced in the United States and typically included in swine diets were used. Locally grown corn (yellow dent #2), soft wheat (Siemer Enterprises, Inc., Teutopolis, IL), and red sorghum (milo; Siemer Enterprises, Inc., Teutopolis, IL) were the grains used. Three sources of distillers dried grains with solubles (**DDGS**) from the dry grind process were used. One source of DDGS was from fermentation of corn (Poet Nutrition, North Manchester, IN) and 2 sources of DDGS were from fermentation of sorghum. One sorghum DDGS sample was sourced from Kansas (White Energy, Russell, KS) and the other was sourced from Texas (Levelland Holey Country Ethanol, Levelland, TX). The amount of solubles added to the dried grain was less for the sample from Texas than from the sample from Kansas. Four corn coproducts from the wet milling industry including corn gluten meal (ADM Alliance Nutrition, Quincy, IL), corn gluten feed (ADM Alliance Nutrition, Quincy, IL), corn germ meal (ADM Alliance Nutrition, Quincy, IL), and corn bran (National Corn-to-Ethanol Research Center, Edwardsville, IL) also were used. Corn gluten meal is a high protein feed ingredient that is produced when the germ and bran are removed from the corn grain and the gluten remains. Corn gluten feed is a combination of corn bran, screenings, distillers solubles, and other leftovers after the separation of corn. Corn germ meal is produced when germ is extracted from corn grain and oil is removed from the germ. Corn bran is left after separation and can be removed and used as a separate coproduct or added to gluten feed (Blasi et al., 2001).

Wheat middlings (soft wheat; Hal Roberts' Son, Inc., Postville, IA) and wheat bran (soft wheat bran; Siemer Enterprises, Inc., Teutopolis, IL) from the flour milling industry also were included. Wheat middlings consists of a combination of coproducts from flour milling such as wheat bran, wheat shorts, wheat germ, and wheat flour, whereas wheat bran consists only of the bran fraction, which contains the aleurone and pericarp portions of the outer layer of grain (AAFCO, 2011).

Non-Carbohydrate Analysis. All feed ingredients were analyzed in duplicate for DM (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007). The concentration of GE was determined using an adiabatic bomb calorimeter (model 6300, Parr Instruments, Moline, IL). Benzoic acid was the standard for calibration. Crude protein was determined using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. Acid hydrolyzed ether extract was determined by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN).

Starch Analysis. Starch was analyzed using an enzymatic colorimetric method as described by Bach Knudsen (1997). The 12 feed ingredients were weighed (150 mg) into 50 mL tubes and 30 mL of acetate buffer (0.1*M*; pH 5.0) and 100 μL thermostable α-amylase (EC 3.2.1.1 Megazyme Ltd. Cat. No. E-BLAAM, 53.7 units/mg) were added and incubated at 100°C for 1 h. Complete degradation of starch oligomers into glucose was accomplished by adding 200 μL amyloglucosidase (EC 3.2.1.3 Megazyme Int., Cat. No. E-AMGDF, 3,260 units/ mL) and incubating the samples for 2 h at 60°C. Samples then were centrifuged and the released amount

of glucose was quantitatively determined using glucose oxidase (EC 1.1.3.4, Megazyme Int., Cat No. K-GLUC).

NSP Analysis. Non-starch polysaccharides were measured based on their separate sugar constituents as alditol acetates using gas chromatography for neutral sugars and colorimetry for acidic sugars as described by Bach Knudsen (1997). The 12 feed ingredients were analyzed in 3 parallel runs for total NSP, insoluble NSP (**I-NSP**), and non-cellulosic polysaccharides (**NCP**). By completing these 3 procedures, it was also possible to calculate by difference concentrations of cellulose, soluble NCP (**S-NCP**), and insoluble NCP (**I-NCP**). Klason lignin was determined as the insoluble residue after treatment with 12 *M* H_2SO_4 (Theander and Åman, 1979; Theander and Westerlund, 1986; Bach Knudsen, 1997). Dietary fiber was calculated as the sum of total NSP and Klason lignin.

Total NSP Procedure. Before analysis, the 12 feed ingredients were ground through a 0.5 mm screen and weighed (250 ± 5 mg) into 50 mL tubes. After incubation with a thermostable α-amylase (EC 3.2.1.1 Megazyme Ltd. Cat. No. E-BLAAM, 53.7 units/mg) at 100°C for 1 h and amyloglucosidase (EC 3.2.1.3 Megazyme Int., Cat. No. E-AMGDF, 3,260 units/ mL) at 60°C for 2 h, starch was removed from ingredients. The soluble NSP (**S-NSP**) were precipitated by adding 99% ethanol, to give a final concentration of 80% ethanol, while samples were kept in an ice bath for 1 hr. Samples then were centrifuged, the supernatant was removed, and the residues were washed twice with 85% ethanol, once with acetone, and left to dry overnight. The non-starch residues were treated with 5.0 mL of 12 *M* H_2SO_4 for 1 h at 35°C to swell the cellulose and the NSP then were hydrolyzed to monosaccharides using 30.0 mL of 2 *M* H_2SO_4 for 1 h at 100°C. The acid hydrolysates were filtered through glass-fritted crucibles and the filtered hydrolysate was collected. The unfiltered residue was dried at 103°C for 20 h and then ashed at

520°C for 3.5 h and the difference in the two weights was used to calculate Klason lignin. An internal standard (allose, 2 mg L^{-1}) was added to an aliquot of the hydrolysate and the sugars were reduced to alcohols with potassium borohydride and acetylated to alditol acetates derivatives using 1-methylimidazole to catalyze the reaction (Connors and Pandit, 1978) and quantified using gas-liquid chromatography. Uronic acids were measured by a colorimetric method as described by Scott (1979).

I-NSP Procedure. The procedure described above was followed with the exception that S-NSP were extracted from the starch-free residue using 40 mL of a 0.2 *M* phosphate buffer.

NCP Procedure. The procedure described for total NSP was followed except the starch-free residue was directly hydrolyzed to monosaccharides with 2 *M* H_2SO_4 rather than swelling cellulose with 12 *M* H_2SO_4. Also, after acid hydrolysis, the hydrolysates were filtered through filter paper in a plastic funnel, thus Klason lignin was not determined. Also, uronic acids were not determined in this procedure.

Calculation of Carbohydrate Composition. NCP were calculated using Eq. [1]:

NCP = rhamnose + fucose + arabinose + xylose + mannose + galactose + glucose + uronic acids

Cellulose was calculated using Eq. [2]:

$$\text{Cellulose} = \text{Total NSP}_{\text{glucose (12 } M \text{ H2SO4)}} - \text{NSP}_{\text{glucose (2 } M \text{ H2SO4)}}$$

Soluble NCP were calculated using Eq. [3]:

S-NCP = Total NCP – I-NCP

Soluble dietary fiber (**S-DF**) was calculated using Eq. [4]:

S-DF = S-NCP

Insoluble dietary fiber (**I-DF**) was calculated using Eq. [5]:

I-DF = I-NSP + Klason lignin

Dietary fiber (**DF**) was calculated using Eq. [6]:

DF = Total NSP + Klason lignin

Arabinoxylans were calculated using Eq. [7]:

Arabinoxylans = arabinose + xylose

In Vitro Ileal Digestibility. Using a two-step in vitro procedure modified after Boisen and Fernández (1997), the in vitro ileal digestibility of DM of the 12 feed ingredients was determined. After determining in vitro DM digestibility, the remaining undigested residue was collected for NSP analysis, enabling the calculation of in vitro ileal digestibility of NSP.

Each feed ingredient was ground through a 0.5 mm screen and weighed (0.500 g ± 1 mg) into 300-mL Erlenmeyer flasks in triplicate. Twenty-five milliliters of phosphate buffer (0.1 *M*; pH 6.0) and 10 mL of 0.2 *M* HCl were added to each flask and the 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 milliliter of freshly prepared pepsin solution (25 mg of pepsin/mL; P7000, Sigma Aldrich, St. Louis, MO) was added to each flask, and 0.5 mL of chloramphenicol solution (0.5 g chloramphenicol, Sigma No. C-0378, per 100 mL ethanol) was added to prevent

bacterial growth and samples were incubated in a heating cabinet at 40°C for 75 min with constant stirring. This step of the procedure represented the digestion processes of the stomach.

After 75 min, 10 mL of phosphate buffer (0.2 *M*; pH 6.8) and 5 mL of 0.6 *M* NaOH were added to each flask and the pH was adjusted to 6.8 ± 0.01 by adding 1 *M* HCl or 1 *M* NaOH. One milliliter of freshly prepared pancreatin solution (100 mg of pancreatin/mL; P1750, Sigma Aldrich, St. Louis, MO) was added to each flask and samples were incubated in a heating cabinet at 40°C for 18 h with constant stirring. This step of the procedure mimics the digestion processes of the small intestine.

Following completion of step 2, 5 mL of 20% sulfosalicylic acid was added to each flask and the samples were left to stand at room temperature with constant stirring for 30 min. Sulfosalicylic acid was added to precipitate the undigested soluble proteins (Regmi et al., 2009). Next, 230 mL of ethanol (99%) were added to each flask to give a final ethanol concentration of 80% and were left in an ice bath for 1 h before filtration. Ethanol was added to precipitate the soluble fiber (Monro, 1992).

The undigested residues in the flasks were filtered in previously weighed Gooch crucibles containing sand (0.400 g ± 5 mg). 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 an oven overnight at 102°C, cooled in the desiccator, and weighed to measure ileal DM residues (Boisen and Fernández, 1997). Two extra flasks, which contained no samples (blanks), but where all reagents and enzymes were added, were included. The DM of the residue collected in these flasks after incubation was used to correct the final DM weight of the residues.

In Vitro Total Tract Digestibility. The in vitro total tract digestibility procedure was a 3 step incubation modified after Boisen and Fernández (1997). The first 2 steps of the procedure were similar to the in vitro ileal digestibility procedure described previously, but with 3 exceptions. First, there was no need to add ethanol to precipitate the soluble fiber because in the pig, most of the soluble fiber will be fermented and disappear by the end of the large intestine (Urriola et al., 2010). Second, in the second step, the incubation time was reduced to 3.5 h. Third, no sulfosalicylic acid was added following the small intestine incubation because the flasks went on to another incubation simulating the large intestine. In this step, 10 mL of a 0.2 *M* EDTA solution was added to each flask and the pH was adjusted to 4.8 ± 0.01 with 30% acetic acid. Viscozyme (Viscozyme L V2010, Sigma-Aldrich, St. Louis, MO) was added to each flask in the amount of 1 mL. The flasks were incubated in a heating cabinet at 40°C for 18 h. Following the 18 h incubation, the flasks were filtered and the in vitro total tract digestibility of DM and NSP were determined as previously described.

In Vitro Calculations. The in vitro digestibility of DM was calculated using Eq. [8]:

$$In\ vitro\ digestibility\ of\ DM, \% = \frac{[Sample\ DM - (Residue\ DM - Blank\ DM)]}{Sample\ DM} \times 100$$

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 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 sand (Boisen and Fernández, 1997).

In vitro digestibility of NSP was calculated using Eq. [9]:

$$In\ vitro\ digestibility\ of\ NSP, \% = 1 - \frac{NSP\ in\ residue\ (mg)}{NSP\ in\ ingredient\ (mg)} \times 100$$

NSP Analysis of In Vitro Undigested Residues. The undigested residues remaining after in vitro digestion were transferred quantitatively to 50 mL tubes for determination of total NSP as described previously. However, starch was not removed from undigested residues because it was assumed that all starch was removed in the in vitro incubations or otherwise would be considered resistant starch; thus, the NSP procedure began with acid hydrolysis using 12 *M* H_2SO_4 to swell the cellulose. There was no determination of NCP to make the calculation of cellulose possible; therefore, glucose in the undigested residues is from cellulose, β-glucans, and resistant starch; however, β-glucan and enzyme-resistant starch were negligible in the ingredients used in this experiment.

RESULTS AND DISCUSSION

Non-Carbohydrate Analysis. Dry matter concentrations ranged from 87.5% in sorghum DDGS (KS) to 94.2% in corn gluten meal (Tables 4.1 and 4.2). Ash concentrations ranged from 1.4% in sorghum to 6.9% (DM basis) in wheat bran. The concentration of GE ranged from 4,320 kcal/kg in corn, 4,337 kcal/kg in sorghum, and 4,212 kcal/kg in wheat (DM basis), whereas GE in coproducts ranged from 4,708 kcal/kg in corn gluten feed to 5,667 kcal/kg in corn gluten meal (DM basis). Crude protein was 11.9, 9.0, and 12.2% (DM basis) in corn, sorghum, and wheat, but corn coproducts contained between 9.8 and 66.8% CP (DM basis). Sorghum DDGS contained 30.5 and 38.0% CP (DM basis) and wheat middlings and wheat bran contained 18.4 and 17.2% CP (DM basis), respectively. Acid hydrolyzed ether extract (**AEE**) ranged between 2.5 and 3.3% (DM basis) in the grains and between 2.3 and 10.9% (DM basis) in the coproducts. Non-carbohydrate values reported for corn and corn coproducts were similar to previously

reported values, except corn gluten meal in this experiment contained 8.0% AEE (DM basis), whereas previously reported values for AEE are much lower (Anderson et al., 2012; NRC, 2012). Also, non-carbohydrate values reported for sorghum, 2 sources of sorghum DDGS, wheat, and wheat coproducts were similar to previously reported values, except sorghum DDGS from Kansas had a lower concentration of CP and wheat middlings contained a greater concentration of AEE than previously reported values for CP and AEE (NRC, 2012).

Carbohydrate Analysis of Ingredients. Starch concentration in sorghum, corn, and wheat [69.0, 62.0, and 61.8% (DM basis), respectively] are in agreement with previously reported values (Bach Knudsen et al., 1988; Bach Knudsen, 1997; NRC, 2012). Starch concentration in corn DDGS was 8.6% (DM basis) and the 2 sources of sorghum DDGS contained only 3.1 and 2.1% starch (DM basis). The reason for the low concentration of starch in DDGS is that starch is removed through fermentation during the production of ethanol. Although the high starch concentration in corn DDGS obtained in this experiment is not out of the range reported by Stein and Shurson (2009), it is greater than most values reported (Urriola et al., 2010; Anderson et al., 2012). Differences in starch concentrations in DDGS samples demonstrate that the production of ethanol, through the fermentation of starch by yeast, is a biological process, making every batch of DDGS slightly different in composition. The other coproducts ranged from 11.1% starch (DM basis) in corn gluten meal to 22.6% starch (DM basis) in corn bran. Starch values for the corn coproducts are in agreement with Anderson et al., (2012), but, they are less than starch values reported by Bach Knudsen (1997), and the starch concentration in wheat middlings is much less than the value reported by Bach Knudsen (1997). However, Bach Knudsen (1997) analyzed feed ingredients produced in Europe, whereas the coproducts used in this work are from the U.S., and it is possible that production processes are different between Europe and the U.S.

Concentrations of S-NCP were very small in all samples, ranging from 0.4% in sorghum to 6.6% (DM basis) in corn germ meal. The S-NCP concentrations in corn, corn gluten feed, corn bran, wheat, and wheat bran were similar to reported values for European ingredients (Bach Knudsen, 1997; Gdala et al., 1997). However, the concentration of S-NCP in wheat middlings was less than reported values (Bach Knudsen, 1997; Nortey et al., 2008). The major components of S-NCP in most ingredients tended to be arabinose, xylose, and glucose. Xylose and arabinose mainly come from soluble arabinoxylans, whereas the majority of glucose in these types of ingredients stems from soluble β-glucans. Soluble NCP are important in pig diets because they are rapidly fermented in the cecum and almost always completely fermented by the end of the large intestine, resulting in increased VFA production and, subsequently, the VFA are used as an energy source for the pig (Urriola, 2010; Bach Knudsen, 2011). Due to this reason, S-NCP may provide a large energy contribution to the pig.

Insoluble-NCP concentrations in corn, sorghum, and wheat were between 3.8 and 6.2% (DM basis), whereas concentrations in coproducts ranged from 1.9% (DM basis) in corn gluten meal to 26.8% (DM basis) in corn bran. Insoluble NCP of grains and grain coproducts were mostly composed of xylose and arabinose. Xylose and arabinose concentrations ranged from 0.6 and 0.5% (DM basis), respectively, in corn gluten meal, to 14.2 and 7.8% (DM basis), respectively, in corn bran.

Cellulose concentrations in corn, sorghum, wheat, and corn gluten meal were between 0.9 and 1.7% (DM basis), whereas concentrations in all other ingredients ranged from 5.8 to 10.4% (DM basis). Also, it is interesting to note that cellulose concentrations were not as great as arabinoxylan concentrations in all ingredients.

Total NSP, which includes S-NCP, I-NCP, and cellulose, ranged from 3.6 to 9.5% (DM basis) in corn gluten meal, corn, sorghum, and wheat, whereas concentrations of total NSP ranged from 24.7 to 41.8% (DM basis) in coproducts, excluding corn gluten meal. Also, the concentrations of total NSP for the 3 DDGS samples used in this experiment were similar to the concentration of total NSP in a DDGS sample that was cofermented from wheat and corn (Yáñez et al., 2011).

Klason lignin ranged from 0.8% in corn to 11.5% in sorghum DDGS (TX), which had less solubles added to it, thus increasing the concentration of DM. When Klason lignin and total NSP are added, DF is calculated. Dietary fiber concentrations in corn, sorghum, and wheat ranged from 8.3 to 11.2% (DM basis), whereas DF concentrations ranged from 13.2 to 45.7% in coproducts.

In summary, pigs fed grains will receive diets higher in starch, whereas pigs fed coproducts will receive diets higher in dietary fiber, protein, and fat. Pigs receiving starchy diets will absorb most of their energy as glucose in the small intestine, whereas pigs fed diets higher in fiber will ferment it in the hind gut, producing VFA. The pig may use glucose for lipid synthesis, ATP production, synthesis of AA, and generating reducing equivalents through the pentose phosphate pathway, whereas VFA may be used for lipid synthesis, gluconeogenesis, or as an energy source for colonocytes (Bach Knudsen, 2011). Glucose absorption and use is more efficient than fermentation and subsequent VFA production because fermentation results in losses of methane (Bach Knudsen, 2001). Thus, pigs fed diets containing coproducts convert dietary energy to BW gain less efficiently than pigs fed diets based on grain.

In Vitro Ileal Digestibility. In vitro ileal digestibility of DM ranged from 47.7% in corn bran to 93.5% in corn gluten meal (Table 4.3). In vitro ileal digestibility of DM in corn and corn DDGS was much greater than previously reported (Urriola, 2010). The difference is due to the use of a longer incubation time in this experiment compared with the experiment by Urriola (2010). The 24 h incubation time was used to keep the incubation time the same for both in vitro ileal and total tract digestibility procedures. Amongst the corn coproducts, excluding corn gluten meal, in vitro ileal digestibility of DM ranged from 47.7 to 64.7%. Corn DDGS and sorghum DDGS (KS) had an in vitro ileal digestibility of DM of 64.7 and 65.6%, respectively, whereas sorghum DDGS (TX), which had less solubles added to it, had an in vitro ileal digestibility of DM of 57.3%. The difference in digestibility between these 3 samples is most likely due to NSP concentrations, which were 25.0% (DM basis) in corn DDGS, 24.7% (DM basis) in sorghum DDGS (KS), and 29.8% (DM basis) in sorghum DDGS (TX). Sorghum DDGS (TX) had less solubles added to it, resulting in a NSP concentration of 29.8% (DM basis) and a Klason lignin concentration of 11.5% and, because of these concentrations, the in vitro ileal digestibility of DM was 57.3%. Also, the grains had lower concentrations of NSP and for this reason had a high in vitro ileal digestibility of DM. This trend was similar for all ingredients - as NSP concentration increased in the ingredients, in vitro ileal digestibility of DM decreased. Yin et al. (2000) reported a decrease in ileal digestibility of DM when dietary NSP content increased in the pig's diet. The negative relationship between apparent ileal digestibility of DM and dietary NSP was linear (r = 0.99). In this experiment, a similar and strong correlation between in vitro ileal digestibility of DM and NSP concentration of ingredients was observed (R^2 = 0.97; Figure 4.1). The strong correlation between in vitro ileal digestibility of DM and NSP concentration of ingredients was expected because the in vitro ileal digestibility procedure removes almost all the

protein, starch, and lipid from the sample, whereas the fiber fraction, which is primarily NSP, is left.

Furthermore, in vitro ileal digestibility of NSP in all ingredients was slightly positive or negative, with corn gluten meal being the exception, with an in vitro ileal digestibility of NSP of 22.8%. This observation indicates that there is some error in the procedure because there should be no negative digestibility values in vitro because there are no endogenous losses in vitro. Also, there should be no in vitro ileal digestibility of NSP because no NSP degrading enzymes are added to the in vitro ileal digestibility procedure. The error is most likely due to ingredients, like corn gluten meal, that contain low amounts of NSP to begin with and in calculating in vitro digestibility of NSP, very small numbers are being used; thus, the digestibility of NSP can have large errors. However, for most ingredients, in vitro ileal digestibility of NSP was close to zero, which is in line with expectations. Further evidence that in vitro ileal digestibility of NSP was zero is that the mean of all ingredients for in vitro ileal digestibility of NSP was -2.7%.

In Vitro Total Tract Digestibility. Among corn and corn coproducts, in vitro total tract digestibility of DM ranged from 50.4% in corn bran to 94.3% in corn gluten meal (Table 4.4). Also, in vitro total tract digestibility of DM ranged from 59.3% in sorghum DDGS (TX) to 91.6% in sorghum, whereas sorghum DDGS (KS) had an in vitro total tract digestibility of DM of 71.4%. Finally, the range for in vitro total tract digestibility of DM for wheat and wheat coproducts was from 63.6% in wheat bran to 92.2% in wheat. In vitro total tract digestibility of DM for corn was 93.0%, which was greater than the average in vitro DM disappearance of 50 corn samples determined using a similar in vitro procedure (84.4%; Cervantes-Pahm, 2011). However, Cervantes-Pahm (2011) also reported an average in vitro DM disappearance of 93.1% for corn samples when incubated in a Daisy^{II} incubator, which is in line with in vitro total tract

digestibility of DM for corn reported in this experiment. Also, Urriola (2010) reported lower in vitro total tract digestibility values for 8 different corn DDGS samples and a sorghum DDGS sample compared to values obtained in this experiment. However, a 4 g sample was used in the experiment by Urriola (2010) and may explain the difference in digestibility values. A larger sample weight may need greater enzyme doses in order to get similar in vitro digestibility results to when a 0.5 g sample is used with the same enzyme dose. When fecal inoculum was used instead of viscozyme, in vitro total tract digestibility of DM in corn DDGS and sorghum DDGS were similar to values reported in this experiment (Urriola, 2010). Also, in vitro digestibility of OM for corn gluten feed reported by Anderson et al. (2012) was similar to in vitro total tract digestibility of DM for corn gluten feed used in this experiment. However, Anderson et al. (2012) reported lower in vitro digestibility of OM for corn germ meal, corn bran, and corn gluten meal compared to in vitro total tract digestibility of DM reported in this experiment. Finally, Regmi et al. (2009) determined in vitro total tract digestibility of DM in 20 wheat samples and found a range of 79.1 to 89.4%, which was less than in vitro total tract digestibility of DM in wheat in this experiment. However, Regmi et al. (2009) used 20 hard red spring-type wheat samples whereas a soft red wheat was used in this experiment, and this difference may account for the greater in vitro total tract digestibility of DM in this experiment. In summary, differences in ingredients and types of in vitro procedures account for most of the differences observed in in vitro total tract digestibility of DM. Also, in vitro total tract digestibility of DM had a wide range and was most likely due to differences in NSP concentration among ingredients. Finally, a weaker relationship ($R^2 = 0.83$) between in vitro total tract digestibility of DM and ingredient NSP concentration was found compared with the relationship between in vitro ileal digestibility of DM and ingredient NSP concentration (Figure 4.2).

In vitro total tract digestibility of NSP in corn gluten meal, corn germ meal, and wheat were 57.3, 55.0, and 44.9%, respectively, whereas in vitro total tract digestibility of NSP in corn bran and sorghum DDGS (TX) was only 6.5 and 7.1%, respectively (Table 4.4). In vitro total tract digestibility of NSP in corn DDGS was 32.7%, which was similar to in vitro total tract digestibility of total dietary fiber (**TDF**) in 8 corn DDGS samples (Urriola, 2010). The in vitro digestibility of TDF may be compared with in vitro digestibility of NSP because TDF is equal to NSP and lignin. Lignin is not enzymatically digested in vitro; therefore, in vitro digestibility of TDF should be similar to in vitro digestibility of NSP. Also, in vitro total tract digestibility of NSP for sorghum DDGS (KS) was 26.9% which was similar to an in vitro total tract digestibility of TDF of 29.8% in sorghum DDGS reported by Urriola (2010).

In vitro total tract digestibility of arabinoxylans in corn germ meal and wheat was 53.0 and 46.2%, respectively, indicating that arabinoxylans in these ingredients are most accessible to viscozyme. Wheat middlings had an in vitro total tract digestibility of arabinoxylans of 7.8% and wheat bran had an in vitro total tract digestibility of arabinoxylans of 22.3%. Corn germ meal and wheat middlings had the same concentration of arabinoxylans, but the difference in digestibility between the 2 ingredients indicates that the structure of arabinoxylans in the 2 ingredients is different.

Conclusion

The results of this experiment demonstrate the wide range of carbohydrate composition in feed ingredients used in U.S. swine diets. In grains, the predominant carbohydrate is starch, whereas the predominant carbohydrate in grain coproducts is NSP. In vitro ileal and total tract digestibility of DM had a strong correlation with the concentration of NSP in the ingredients.

However, in vitro NSP degradation varied among ingredients, which indicates that the composition of the NSP fraction in each ingredient plays an important role in determining the extent of fermentation of NSP. As a consequence, the composition of the NSP fraction is also expected to influence the energy value of the ingredient.

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Table 4.1. Analyzed composition of corn and corn coproducts, DM basis[1]

Item, %	Corn	Corn DDGS[2]	Corn gluten meal	Corn gluten feed	Corn germ meal	Corn bran
DM	88.3	90.7	94.2	88.2	92.6	91.6
Ash	1.9	4.8	1.8	6.3	4.3	2.2
GE, kcal/kg	4,320	5,185	5,667	4,708	4,432	4,589
CP	11.9	29.2	66.8	25.8	26.2	9.8
AEE[3]	2.7	10.9	8.0	3.7	2.3	4.9
Starch	62.0	8.6	13.0	11.1	18.7	22.6
S-NCP[4]	2.5	3.4	0.7	5.1	6.6	5.2
Arabinose	0.5	0.9	0.1	1.2	2.3	1.1
Xylose	0.4	0.9	0.1	1.2	1.6	1.5
Mannose	0.1	0.7	0.1	0.7	N.D.[10]	N.D.
Galactose	0.4	0.2	0.1	0.3	0.8	0.4
Glucose	0.6	0.3	0.3	1.1	0.7	1.5
Uronic acids	0.4	0.4	0.1	0.5	1.1	0.7
I-NCP[5]	3.8	15.8	1.9	16.3	19.7	26.8
Arabinose	1.2	4.3	0.5	4.7	7.1	7.8
Xylose	1.7	6.2	0.6	7.4	7.5	14.2
Mannose	0.1	1.2	0.2	0.5	0.4	0.4
Galactose	0.4	1.1	0.2	1.4	1.7	2.4
Glucose	0.0	1.8	0.2	0.9	0.8	N.D.

Table 4.1. (Cont.)

Uronic acids	0.4	1.2	0.2	1.4	2.2	2.0
Cellulose	1.7	5.8	0.9	7.1	8.1	10.1
Total NSP[6]	8.1	25.0	3.6	28.7	34.5	41.8
Klason lignin	0.8	3.9	9.6	2.9	2.2	3.9
Soluble dietary fiber[7]	2.5	3.4	0.7	5.1	6.6	5.2
Insoluble dietary fiber[8]	6.4	25.5	12.4	26.5	30.1	40.6
Dietary fiber[9]	8.9	28.9	13.2	31.6	36.7	45.7

[1] Total rhamnose and fucose in all ingredients ranged from N.D. to 0.1% and thus was excluded from the table.

[2] DDGS = distillers dried grains with solubles.

[3] AEE = acid hydrolyzed ether extract.

[4] Soluble non-cellulosic polysaccharides.

[5] Insoluble non-cellulosic polysaccharides.

[6] Total NSP = S-NCP + I-NCP + cellulose.

[7] Soluble dietary fiber = S-NCP.

[8] Insoluble dietary fiber = I-NCP + cellulose + Klason lignin.

[9] Dietary fiber = soluble dietary fiber + insoluble dietary fiber.

[10] N.D. = not detected.

Table 4.2. Analyzed composition of sorghum and sorghum coproducts and wheat and wheat coproducts, DM basis[1]

Item, %	Sorghum	Sorghum DDGS[2] (KS)	Sorghum DDGS (TX)	Wheat	Wheat middlings	Wheat bran
DM	89.5	87.5	91.1	92.9	91.6	92.7
Ash	1.4	5.2	3.5	2.2	6.1	6.9
GE, kcal/kg	4,337	5,249	5,208	4,212	4,446	4,312
CP	9.0	30.5	38.0	12.2	18.4	17.2
AEE[3]	3.3	10.0	9.8	2.5	4.9	3.7
Starch	69.0	3.1	2.5	61.8	16.8	16.9
S-NCP[4]	0.4	3.5	4.9	1.9	1.2	3.8
Arabinose	0.1	0.9	0.8	0.6	0.2	0.9
Xylose	N.D.[10]	1.0	0.9	0.7	0.2	1.8
Mannose	N.D.	0.5	0.3	0.1	N.D.	0.1
Galactose	0.1	0.3	0.2	0.2	0.2	0.2
Glucose	N.D.	0.4	2.2	0.2	0.2	0.6
Uronic acids	0.1	0.3	0.5	N.D.	0.3	0.2
I-NCP[5]	4.7	14.4	14.6	6.2	22.7	24.3
Arabinose	1.6	3.8	4.5	1.7	7.0	6.8
Xylose	1.3	4.4	5.7	2.9	11.4	12.6
Mannose	0.1	1.1	1.1	0.2	0.3	0.4
Galactose	0.2	0.5	0.6	0.2	0.5	0.6

Table 4.2. (Cont.)

Glucose	1.0	3.6	1.4	0.9	2.3	2.8
Uronic acids	0.3	1.0	1.3	0.3	1.2	1.1
Cellulose	1.5	6.7	10.2	1.3	6.7	6.4
Total NSP[6]	6.6	24.7	29.8	9.5	30.7	34.5
Klason lignin	1.6	8.2	11.5	1.8	7.3	6.9
Soluble dietary fiber[7]	0.4	3.5	4.9	1.9	1.2	3.8
Insoluble dietary fiber[8]	7.8	29.4	36.3	9.3	36.9	37.6
Dietary fiber[9]	8.3	32.9	41.3	11.2	38.1	41.4

[1] Total rhamnose and fucose in all ingredients ranged from N.D. to 0.1% and thus was excluded from the table.

[2] DDGS = distillers dried grains with solubles.

[3] AEE = acid hydrolyzed ether extract.

[4] Soluble non-cellulosic polysaccharides.

[5] Insoluble non-cellulosic polysaccharides.

[6] Total NSP = S-NCP + I-NCP + cellulose.

[7] Soluble dietary fiber = S-NCP.

[8] Insoluble dietary fiber = I-NCP + cellulose + Klason lignin.

[9] Dietary fiber = soluble dietary fiber + insoluble dietary fiber.

[10] N.D. = not detected.

Table 4.3. In vitro ileal digestibility (%) of DM and non-starch polysaccharides in grains and grain coproducts

Item, %	Corn	Corn DDGS[1]	Corn gluten meal	Corn gluten feed	Corn germ meal	Corn bran	Sorghum	Sorghum DDGS (KS)	Sorghum DDGS (TX)	Wheat	Wheat middlings	Wheat bran	SEM
DM	89.4	64.7	93.5	63.7	60.5	47.7	90.1	65.6	57.3	84.9	59.1	49.8	0.9
NSP	-18.7	-6.8	22.8	8.3	3.6	-3.5	-5.9	-5.9	-10.4	0.0	-11.3	-5.2	3.7

[1] DDGS = distillers dried grains with solubles.

Table 4.4. In vitro total tract digestibility (%) of DM and non-starch polysaccharides in grains and grain coproducts

Item, %	Corn	Corn DDGS[1]	Corn gluten meal	Corn gluten feed	Corn germ meal	Corn bran	Sorghum	Sorghum DDGS (KS)	Sorghum DDGS (TX)	Wheat	Wheat middlings	Wheat bran	SEM
DM	93.0	75.2	94.3	72.2	79.5	50.4	91.6	71.4	59.3	92.2	67.0	63.6	0.3
Arabinose	39.1	32.1	11.4	12.4	58.4	8.9	37.7	26.9	-4.1	54.7	0.1	25.0	7.4
Xylose	12.0	15.2	56.3	1.9	47.5	1.6	12.8	20.1	-10.7	40.6	12.6	20.9	1.8
Mannose	48.8	84.9	78.5	85.4	68.5	54.2	36.1	64.0	44.0	93.7	69.8	52.7	7.4
Galactose	65.9	31.7	65.8	22.8	72.7	11.6	41.1	50.7	27.2	77.9	41.1	37.5	2.6
Glucose	28.4	35.1	71.1	30.5	50.0	6.4	-28.0	23.6	13.3	33.3	21.6	16.9	5.5
Uronic acids	55.8	38.6	70.3	41.8	69.1	17.1	3.2	24.9	27.4	6.4	16.3	7.0	1.8
Arabinoxylans	24.2	22.2	35.6	6.2	53.0	4.2	27.4	23.2	-7.7	46.2	7.8	22.3	4.0
NSP	33.6	32.7	57.3	20.1	55.0	6.5	12.9	26.9	7.1	44.9	13.6	20.6	2.8

[1] DDGS = distillers dried grains with solubles.

Figure 4.1. In vitro ileal digestibility of DM versus non-starch polysaccharide concentration of grains and grain coproducts

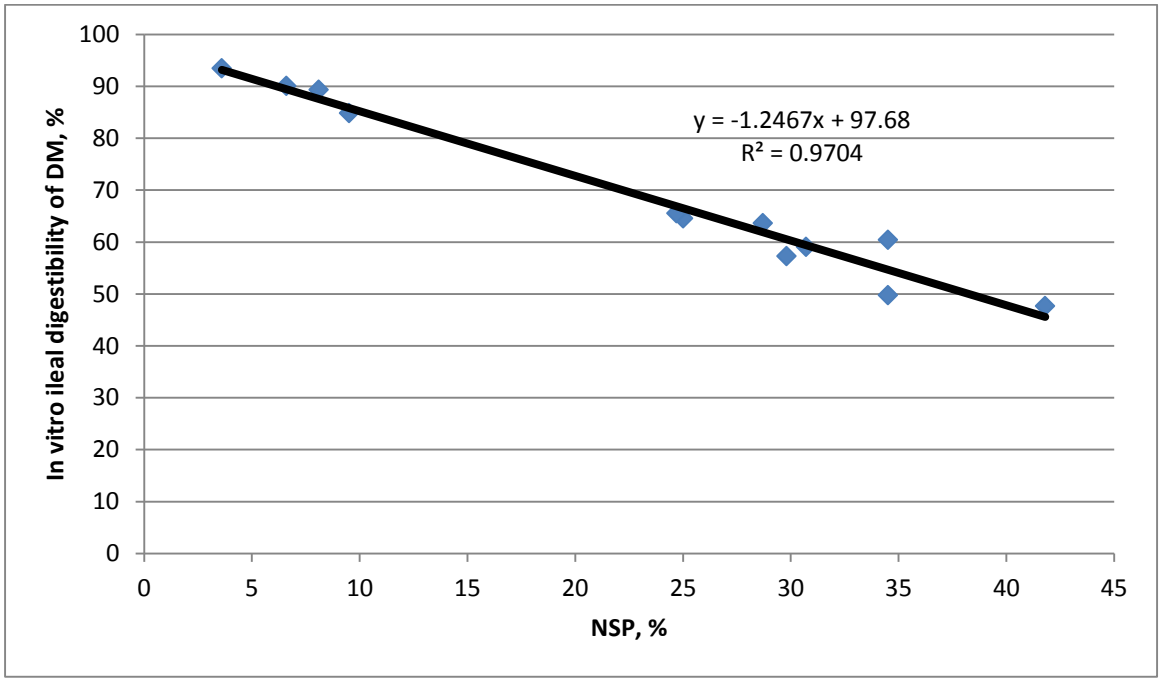
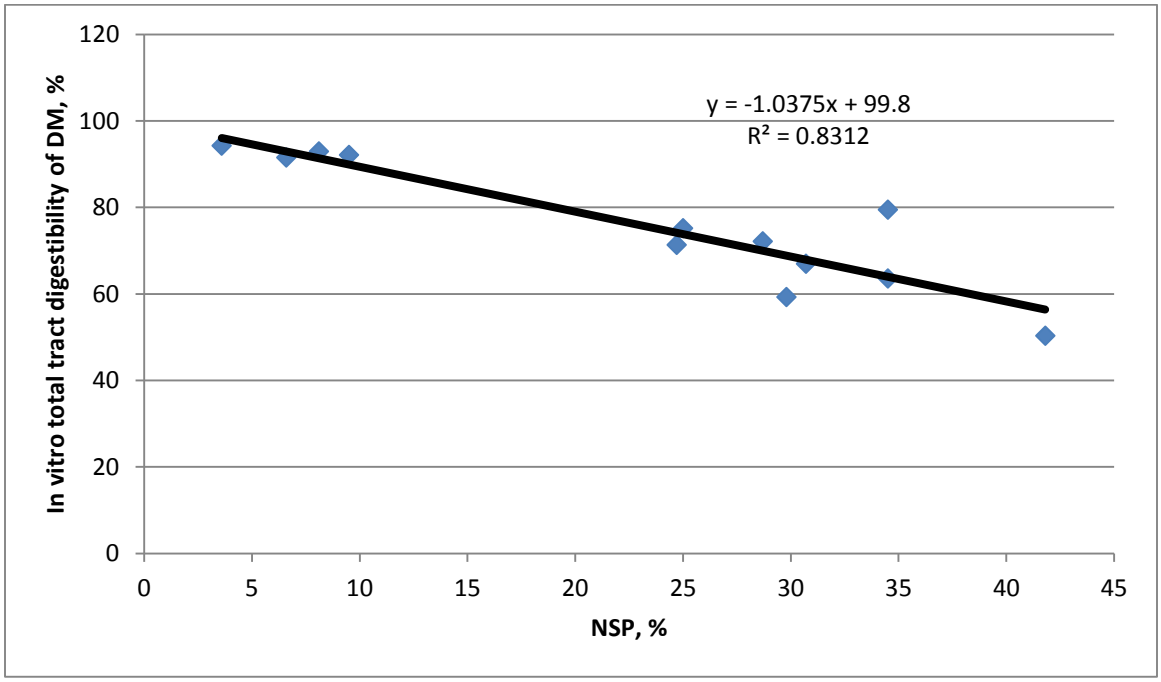


Figure 4.2. In vitro total tract digestibility of DM versus non-starch polysaccharide concentration of grains and grain coproducts



CHAPTER 5

CONCLUSIONS

Xylanase and phytase supplemented to swine diets containing corn, soybean meal, distillers dried grains with solubles, and wheat bran independently resulted in small improvements in apparent ileal and total tract digestibility of nutrients and energy in pigs. However, these improvements were not large enough to elicit a growth performance response. In practice, commercial pig diets supplemented with xylanase and phytase may be formulated to contain lower concentrations of DM, GE, some AA, Ca, and P due to the improved digestibility. However, more research is needed to determine consistent digestibility values using exogenous enzymes based on the amount of substrate in individual feed ingredients.

It was also concluded that there is non-starch polysaccharide (**NSP**) substrate in grains and even more NSP substrate in grain coproducts to favor the use of exogenous NSP-degrading enzymes. However, there was a difference in NSP composition between corn, wheat, and sorghum, and their subsequent coproducts. Therefore, it is concluded that the effectiveness of exogenous fiber-degrading enzymes depends on more than just the amount of NSP, but the type and structure of NSP, where further research is necessary.

Finally, it was concluded that in vitro ileal digestibility of DM had a strong correlation with NSP concentration in feed ingredients, enabling a method to estimate NSP concentration in feed ingredients. Also, in vitro total tract digestibility of NSP had a wide range among ingredients suggesting that the NSP composition of each ingredient plays an important role in determining the extent of NSP fermentation, and therefore, influences the energy value of the ingredients. Overall, more research is necessary to determine a correct method to get the most beneficial effects of fiber-degrading enzymes.