University of Alberta

Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing co-products fed to grower pigs

by

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Dedication

This thesis is dedicated to my mother Budha Laxmi Shrestha and father Ghana Narayan Shrestha

Abstract

Corn distillers dried grains with solubles (DDGS) and wheat millrun are co-products from the ethanol and dry milling industries characterized by high non-starch polysaccharides (NSP) content that limit nutrient digestibility of co-products, which could be improved by supplementation of NSP-degrading enzymes. Nutrient digestibility of diets containing co-products with supplemented NSP-degrading enzymes was evaluated in 3 experiments. Supplemental NSP-degrading enzymes did not increase the nutrient digestibility of corn DDGS- and wheat millrun-based diets ($P > 0.10$) however, feed enzymes increased ($P < 0.01$) the apparent ileal digestibility (AID), apparent total tract digestibility (ATTD) of energy, AID and ATTD of NSP and standardized ileal digestibility (SID) of crude protein in a wheat grain diet including 40% wheat millrun. Furthermore, these enzymes increased ($P < 0.05$) the SID of Arg, His, Trp, Cys, Glu, Gly and Ser. In conclusion, supplementing NSP-degrading enzymes increased the limited energy, protein, and AA digestibility in wheat millrun.

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List of Abbreviations

AA	Amino acids
ADF	Acid detergent fiber
AID	Apparent ileal digestibility
ANF	Anti nutritional factors
BW	Body weight
ATTD	Apparent total tract digestibility
CP	Crude protein
DDGS	Distillers dried grains with solubles
DE	Digestible energy
DF	Dietary fiber
DM	Dry matter
EE	Ether extract
GC	Gas chromatography
GE	Gross energy
HPLC	High performance liquid chromatography
ME	Metabolizable energy
MT	Metric tonne
N	Nitrogen
NDF	Neutral detergent fiber
NRC	National Research Council
NSP	Non-starch polysaccharide
OM	Organic matter

SAS	Statistical analysis system
SEM	Standard error of the mean
SID	Standardized ileal digestibility

Chapter 1. Literature review

1.1 Introduction

Crop farms have historically produced cereal grains for human food and livestock feed consumption. However, cereal grains are also now used to produce ethanol for automotive gasoline. Corn and wheat are the major cereal grains used for ethanol production in the U.S. and eastern Canada and in western Canada, respectively. The rapid expansion of the ethanol industry in North America requires a large tonnage of cereal grains. In the U.S., 1.3 billion metric tonnes (MT) of corn are fermented in ethanol plants (WASDE, 2010). More and more cereal grain is used each year for ethanol production. This scenario together with a limited yield increase suggests that cereal grain prices will continue to increase in the future, unless subsidies for ethanol production are reduced. Currently, the feed industry pays the same cereal grain price as offered by the ethanol industry. As a result, feed cost is higher than some years ago, therefore increasing the cost of swine production. Distillers dried grain with solubles (DDGS) and wheat millrun are co-products from the ethanol and wheat flour industries, respectively. The nutrient concentration of co-products is higher than that of the parent grain, except for starch (Slominski et al., 2004; Robinson et al., 2008; Ortin and Yu, 2009). Feed cost is the highest portion of variable cost in swine production (Payne et al., 2007). Therefore, co-products could be alternative feedstuffs in cost-effective swine diet formulation. But DDGS and wheat millrun contain more fiber than cereal grains. Fiber not only improves gut health (Montagne et al., 2003;

Stein, 2007a), but also high fiber content in diets reduces nutrient digestibility in pig (Wenk, 2001, Degen et al., 2007). High dietary fiber can therefore be considered an anti-nutritional factor for swine. Fiber increases digesta viscosity, endogenous nutrient losses, and reduces feed intake, limiting nutrient digestion and absorption (Owusu-Asiedu et al., 2006; Sauer et al., 1977; Hedemann et al., 2006).

Arabinoxylan is the main component of NSP found in corn DDGS and wheat millrun (Widyaratne and Zijlstra, 2007; Zijlstra et al., 1999; Sramkova et al., 2009). The NSP are not hydrolyzed by digestive enzymes (Barrera et al., 2004). Therefore, the fiber matrix and nutrients entrapped in fiber bypass digestion. Diets composed of co-products have more fiber content and reduced nutrient digestion in pigs. Negative effects of fiber on nutrient digestion can be reduced by supplementation of exogenous enzymes such as xylanase, β-glucanase and cellulase (Kass et al., 1980; Hedemann et al., 2006; Degen et al., 2007). These enzymes partially hydrolyze NSP and thereby increase nutrient digestibility of co-products (Barrera et al., 2004; Nortey et al., 2008; Widyaratne et al., 2009). Supplementation of exogenous enzymes in proper combination and proportion play important role in efficient and effective nutrient utilization of co-products in swine (Zijlstra et al., 2010). Many studies have been conducted to determine the effects of NSP-degrading enzymes on nutrient digestibility of feedstuffs fed to pigs. However, the results are inconsistent (Adeola and Cowieson, 2011), and little information is available on nutrient digestibility of diets containing co-products supplemented with NSP-degrading enzymes such as endo-β-xylanase

and endo-β-glucanase. The prefix "endo" refers to the function of β-xylanase and β-glucanase: to hydrolyze 1-4 and 1-3(4) bonds of NSP components. Therefore, studies were conducted to determine the effect of NSP-degrading enzymes on nutrient digestibility of diets containing co-products and cereal grain. If supplementation of NSP-degrading enzyme reduces the anti-nutritional character of NSP in co-products, nutritionists could use enzymes to mitigate the risk of including co-products in swine diets.

1.2 Co-products

Co-products are produced by the food and bio-processing industries during production of main products. In the ethanol industry, starch of cereal grains is fermented and converted into ethanol and CO_2 resulting in DDGS as a co-product. Similarly in the wheat milling process most of the starch in wheat grain is separated to produce flour for food and residual products are considered as wheat by-products (Holden and Zimmerman, 1991).

1.2.1 Co-products from milling process of wheat

Milling is a process by which wheat grain is ground for production of flour for human consumption (Weigel et al., 1997). Wheat grain contains 2-3% germ, 13-17% bran and 80-85% endosperm (Holden and Zimmerman, 1991; Belderok et al., 2000). In wheat flour production, most of the starch in wheat grain is extracted as flour and the remaining portions become wheat co-products (Figure 1.1). Wheat flour production process includes the following 3 steps:

Cleaning. Cleaning is the process of removing foreign materials, damaged and shrunken kernels and separation of kernels by shape and size. Magnetic separators are used to remove iron or steel particles from the grain mass. Stones, leaves, sand, and stems are removed using cleaning machines. The cleaned whole grain kernels are then passed into conditioning bins.

Conditioning. Conditioning takes place before milling to achieve uniform moisture content throughout the grain. The cleaned wheat grains are tempered by adding water. Moistening the grain helps to prevent the break-up of bran during milling and improves separation from the floury endosperm. Three types namely cold, hot and steam conditioning exist in wheat milling process.

Grinding and Separation. After conditioning, wheat grain is ground on a series of corrugated and reducing rolls. Subsequently, ground material passes through a sieving system where particles are sifted according to size. The clear fine flour is wheat flour that is destined for human food. The products collected after separation of wheat flour are bran, shorts, screenings, middlings and germ, which are examples of North American wheat co-products. These co-products once combined together with some offal from milling process are collectively named wheat millrun.

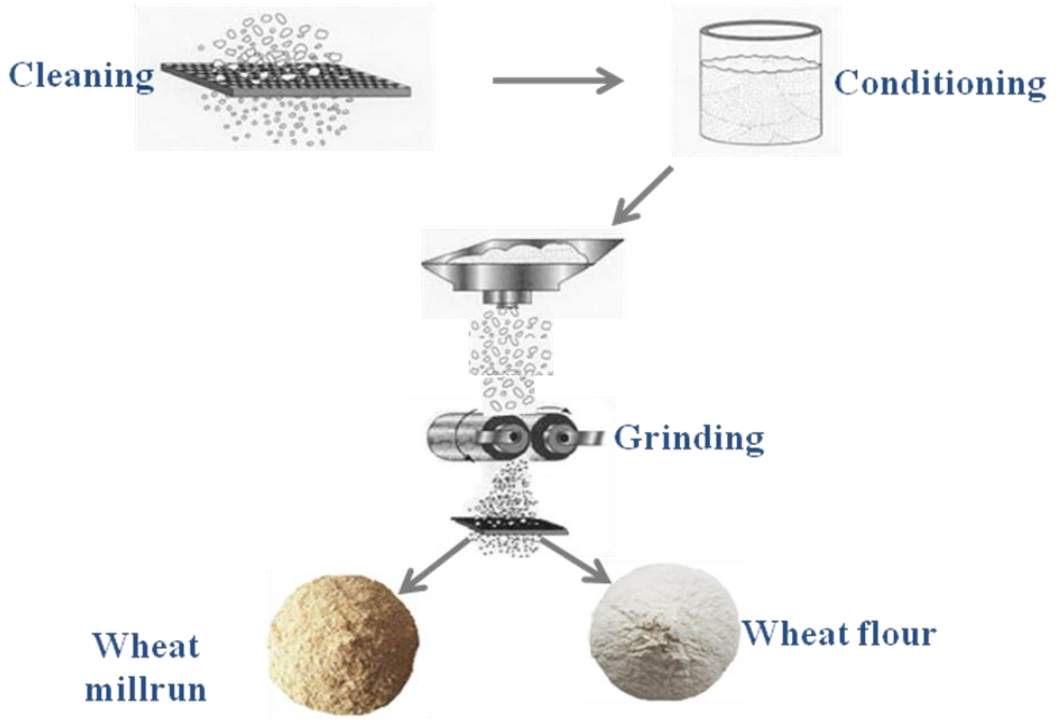

Figure 1.1 Simplified diagram of wheat milling process (www.madehow.com/Volume-3/Flour.html)

Wheat bran is rich in fiber, protein and minerals (Belderok et al., 2000). The main objective of the milling process is to extract the starch as much as possible from the endosperm of wheat grain (Skylas et al., 2000; Shewry et al., 2002). Wheat millrun therefore contains more fiber, protein, minerals and less starch than wheat grain (Holden and Zimmerman, 1991, Slominski et al., 2004). Fiber is not digested by endogenous enzymes, therefore, wheat millrun containing high fiber has lower nutrient digestibility than wheat grain (Nortey et al., 2007b).

1.2.2 Co-products from ethanol industry

Distillers dried grains with solubles is the main co-product of the ethanol industry (Stein et al., 2006) obtained after removal of ethyl alcohol from the wet grain mash after fermentation (Sauer et al., 1977). During the fermentation

process, starch is converted to alcohol and CO_2 released. The remaining nutrients (protein, fiber, minerals and vitamins) therefore concentrate 2 to 3 fold in DDGS (Newland and Mahan, 1990). Therefore, DDGS offers higher nutrient concentration than the parent grain and can be cost effective in diet formulations for livestock, poultry and fish.

Ethanol is commercially produced in two ways, using either wet or dry milling. In wet milling, the grain is soaked into diluted sulfuric acid and water for 24 to 48 hours in order to separate the grain into its many components like germ, fiber, gluten and starch prior to fermentation. Corn gluten is then dried to make corn gluten meal. Corn oil is another by-product of this process. Corn starch is then processed for fermentation to produce ethanol or dried to produce cornstarch and corn syrup. In dry milling, the grain is ground first and fermented without prior separation into its components.

In ethanol production (Figure 1.2), firstly grain is cleaned and coarsely ground. Then, the milled grain is mixed with water. The slurry pH is adjusted to about 5.8 and α-amylase enzyme is added to break down the starch into dextrin. The slurry is then heated to 82-87°C for 45 minutes to reduce its viscosity. The slurry is then pumped through a pressurized jet cooker at 105°C and held for 5 minutes (Batie et al., 2011). Then the mixture is cooled and stored in a liquefying tank for 1-2 hours at 82-87°C then α-amylase and glucoamylase are added. Then, the slurry is pumped into a fermentation tank where α-amylase breaks down the dextrin into glucose. Yeast is added to convert the sugar into ethanol and CO_2. The mash is fermented for 50–60 hours. During this step again some

saccharifying enzymes and protease are added into the slurry. Finally, the fermented mash is pumped into a multi-column distillation system where distillation of ethanol is performed. The 95% pure ethanol is named anhydrous ethanol (Hammond and Prevost, 2006).

The wet residue is named stillage that contains non-fermentable solids and water, and is pumped from the bottom of the distillation columns into the centrifuges for separation into thin stillage (a liquid with 5–10% solids) and wet distillers grain (WDG). Most of the water in thin stillage is then evaporated to produce condensed distiller soluble (CDS), a thick syrup containing 20-25% solids. The CDS is mixed back into WDG to produce WDG with solubles and dried together to produce dried distillers grain with soluble (DDGS). If WDG is dried separately the co-product is called dried distillers grain (DDG).

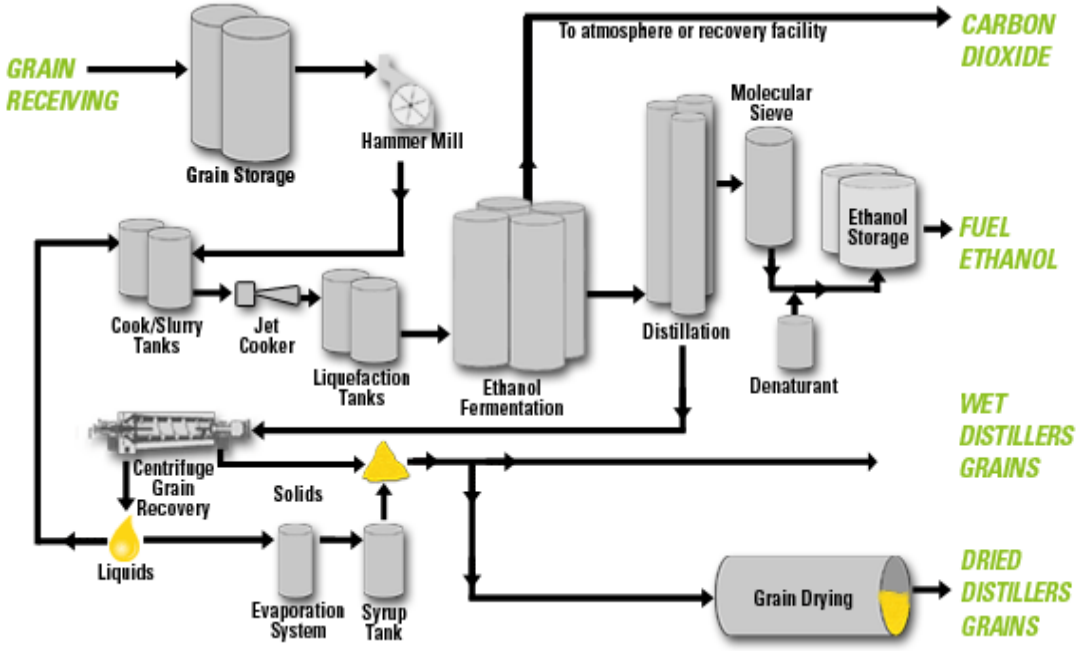

Figure 1.2 The ethanol production process

(www.icminc.com/ethanol/production_process)

The chemical composition of DDGS differs among sources and processing conditions (Table 1.1). Lysine is the most sensitive AA to heat damage (Cromwell et al., 1993, Pahm et al., 2009). Lysine in DDGS is damaged due to excessive heat or extended drying that reduces lysine bio-availablility (Hurrell and Carpenter, 1981. The Maillard reaction can occur during DDGS production yielding unavailable or unreactive lysine, because reducing sugars bind to the NH_2 group of lysine (Weigel et al., 1997; Martinez-Amezcua et al., 2007). The Maillard reaction changes the color of DDGS. The brown color of DDGS after drying wet distillers grain with soluble is a sign of scorching of the protein in DDGS (Ergul et al., 2003, Shurson, 2011).

Table 1.1 Nutrient composition of corn grain and corn DDGS (as is basis)

		Corn DDGS		
Item, %	Corn grain	Mean	Low	High
DM	86.7	87.6	86.2	89.7
CP	7.2	28.3	25.9	32.4
Ether extract	2.9	10.2	8.6	12.4
Starch	57.1	7.2	4.1	12.7
ADF	2.3	10.3	8.6	12.0
NDF	6.7	24.3	20.0	26.7
Ash	0.94	3.82	2.98	4.16
Ca	0.02	0.04	0.01	0.15
P	0.20	0.60	0.51	0.69
GE, Mcal/kg	3.90	4.76	4.57	4.85

(Pedersen et al., 2007)

1.3 Physical properties of co-products

The DDGS varies in physical and chemical properties. Physical characteristics of DDGS may be used as an indicator of the nutritive value of DDGS. Color of DDGS is one of the physical characteristics used to predict the protein quality of DDGS (Pahm et al., 2009). Color of DDGS varies from dark to golden yellow. Dark colored DDGS is an indicator of protein scorching. Therefore, light color DDGS is considered to have better quality than dark DDGS. The smell of the DDGS ranges from sweet fermented smell to burnt or smoky smell. Theoretically, golden-yellow, sweet-smelling DDGS is likely to have higher nutrient digestibility specifically lysine providing opportunity to include higher rates of DDGS into a swine diet.

1.4 Chemical properties of co-products

Processing methods influence the chemical and nutritional composition of DDGS. The nutrient composition of DDGS varies among grain sources, over time within plant and type of plant (Spiehs et al., 2002; Belyea et al., 2004; Cozannet et al., 2009; Ortin and Yu, 2009; Urriola et al., 2009).

1.4.1 Energy content

Starch, fat and fiber are major sources of dietary energy. Therefore, the energy value of feedstuffs depends on their starch, fat and fiber content. The gross energy (GE) of corn DDGS was determined to be greater than corn grain (4.8 vs. 3.9 Mcal/kg DM; Pedersen et al., 2007). The DE of corn DDGS for pigs (3.9 vs.

3.4 Mcal/kg DM) is higher than the value given by NRC (1998) (Spiehs et al., 2002; Hastad et al., 2004; Stein et al., 2005; Pedersen et al., 2007).

Wheat millrun and wheat grain contain 26 and 56% starch, respectively (Slominski et al., 2004). Wheat millrun has high fiber and lower energy digestibility, and thus has a lower DE value DE than wheat grain (3.10 vs. 3.50 Mcal/kg DM; Nortey et al., 2007a). Dietary inclusion of wheat millrun therefore reduces the AID and ATTD of energy by 10 and 9%-units, respectively, when 30% wheat millrun was included in a wheat grain diet (Nortey et al., 2007a). Because of the low digestibility of fiber relative to other nutrients such as protein, fat, and starch (40 to 60 vs. 80%; Noblet and Le Goff, 2001), the DE value is lower in wheat co-products compared to wheat grain (Stein et al., 2005; Stein and Shurson, 2009).

1.4.2 Fiber content

DDGS contains about 3 times more fiber than the parent grain (Spiehs et al., 2002). The main source of NSP in DDGS obtained from co-fermentation of wheat and corn grain is arabinoxylan, which represents 53% of total NSP (Yanez et al., 2011). Corn DDGS contains more insoluble fiber (30 to 35%) and less soluble fiber (1 to 6%) (Stein and Shurson, 2009; Urriola et al., 2010). Corn grain contains 4% ADF and 15% NDF. The fiber content of DDGS varies widely among DDGS samples. The ADF and NDF content of corn DDGS ranges from 11-18% and 29-50%, respectively (Cromwell et al., 1993; Ortin and Yu, 2009, Urriola et al., 2010) with a mean and standard deviation of 16 and 28% for ADF and 42 and 13% for NDF in corn DDGS, respectively (Spiehs et al., 2002).

The fiber content of wheat co-products varies among co-products and within a co-product. Similar to corn DDGS, the fiber content in wheat millrun is higher than in wheat grain. The ADF, NDF, and NSP (17, 39, and 26% as fed) content in wheat millrun are higher than that of wheat grain (3, 11, and 19% as fed), respectively (Slominski et al., 2004; Nortey et al., 2008). The main source of NSP in wheat millrun is also arabinoxylan, which represents 59% of NSP (Stanogias and Pearce, 1985; Twomey et al., 2003; Nortey et al., 2008). Wheat millrun contains more insoluble (25 vs. 1%) than soluble NSP (Nortey et al., 2008). Insoluble fiber increased peristaltic movement, digesta passage rate and thereby reduced nutrient digestibility (Jorgensen et al., 1996). So, both corn DDGS and wheat millrun contain more insoluble than soluble fiber. In addition, arabinoxylan induces the formation of a viscous digesta in non-ruminants that has negative correlation with nutrient digestibility (Zijlstra et al., 1999). Thus due to high fiber content, lower nutrient digestibility is determined in co-products compare to parent grains.

1.4.3 Crude protein content

Plant cell-walls contain small amounts of protein along with high amounts of non-starch polysaccharides (Englyst et al., 1992). Co-products containing high fiber also contained higher amounts of protein compared to parent grains. Corn grain contains about 8-9% CP, but it ranges from 22 to 42% in corn DDGS (Kleinschmit et al., 2007; Widmer et al., 2008; Saunders and Rosentrater, 2009; Schingoethe et al., 2009) with a CV of 6% (Spieh et al., 2002). Therefore, corn DDGS is considered a good source of CP. The corn DDGS from modern ethanol

plants contained higher CP (30% vs. 28%) than the DDGS that originated from older plants (Spiehs et al., 2002). The amino acids are highly concentrated in corn DDGS compared to corn grain (Widmer et al., 2008), but lysine content was the most variable (CV=17%) in DDGS followed by methionine (14%) (Spiehs et al., 2002). Thin stillage contains soluble protein. Therefore, crude protein content in the solubles is higher than in distillers grains, however, amino acid digestibility particularly lysine digestibility is reduced when condensed distillers solubles are mixed back to wet distillers grain and dried together (Yanez et al., 2011), most likely due to scorching of protein and reduced protein availability in solubles after prolonged drying.

Some protein is entrapped within the fiber matrix of cereal grain. Wheat co-products mainly consisting of the outer kernel coat layer contain high fiber that entraps significant amount of protein (Schulze et al., 1994; Lenis et al., 1996). So, wheat co-products containing high fiber contain higher amount of CP (17% vs. 13%) than wheat grain (NRC, 1998; Slominski et al., 2004). The CP content in wheat millrun ranges from 14 to 20% (Slominski et al., 2004; Nortey 2007). The protein fractions associated with fiber likely have a low digestibility, because digestive enzymes have limited access to digest nutrient entrapped in fiber (Bjergegaard et al., 1991). As a result, the CP entrapped within the fiber avoids digestion (Huang et al., 2001). Feeding high fiber diets increases endogenous loss because not only fiber stimulates greater intestinal mucosal secretion, but also its passage induces greater gut lumen wall sloughing (Montagne et al., 2003;

Brunsgaard, 1998). Therefore, AID of protein is greatly reduced when high fibrous diets are fed to pigs.

1.4.4 Mineral content

The DDGS is rich in minerals. For example, Ca and P concentrations are higher in corn DDGS than in corn grain (0.05 vs. 0.02% and 0.77 vs. 0.29%, respectively) (Ortin and Yu, 2009). In wheat millrun and wheat grain, the trend is similar for Ca and P (0.12 vs. 0.05% and. 1.09 vs. 0.37%, respectively; Nortey et al., 2008).

The digestibility of P in DDGS is greater than in the parent grain. The apparent total tract digestibility of P in corn DDGS is 59% vs. 19% in corn (Pedersen et al., 2007; Stein, 2007). Therefore, if the DDGS is included in swine diets a greater portion of the organic P would be digested and absorbed, thus reducing the need for adding inorganic P to the diets. In ethanol production, corn grain goes through the liquefaction and fermentation and some of the bonds that bind P in the phytate complex are hydrolyzed during these processes (Widmer et al., 2007). Therefore, P in DDGS is more digestible to the pig than P in parent grain.

1.5 Non-Starch Polysaccharides

Non-starch polysaccharides (NSP) are not starch but long chains of repeated carbohydrate monomer units joined together by β-glycosidic bonds. The NSP combined with lignin is total dietary fiber (Slavin, 1987; Prosky, 2000). Dietary fiber mainly constitutes plant cell wall material (cellulose, hemicelluloses,

oligosaccharides, pectin, arabinose, β-glucans, inulin, and lignin) and is mostly resistant to hydrolysis by mammalian digestive enzymes (Trowell, 1976; Degen et al., 2007). The NSP contributes 90% to the total dietary fiber (Trowell, 1976; Selvendran and Robertson, 1994). Thus, dietary fiber is defined as NSP to provide the best description of plant cell wall polysaccharides (Englyst and Hudson, 1987; Englyst, 1989).

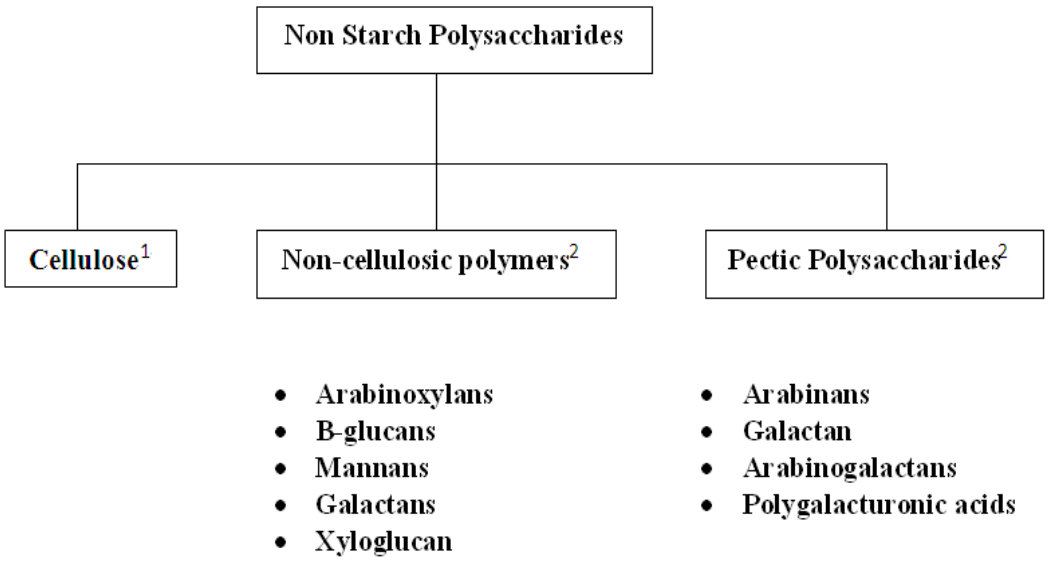

Figure 1.3. Components of NSP (Choct, 1997). [1]Insoluble and [2]partially soluble in water

The structure of NSP is complex. The NSP divide into three groups (Figure 1.3), namely cellulose, non-cellulosic polymers, and pectic polysaccharides (Choct, 1997). The NSP can be either soluble or insoluble (Englyst, 1989). Most plant foods contain both types of NSP although proportions vary. Wheat, barley, and rye are sources of soluble NSP. Solka floc, i.e., wood cellulose, is an example of insoluble NSP. Insoluble fiber reduces nutrient

digestibility more than soluble fiber (Souffrant, 2001; Renteria et al., 2008). Some starch is hydrolyzed by saliva but mostly hydrolyzed by pancreatic α-amylase in small intestine to release glucose. Instead, NSP are fermented by the intestinal microbiota in the large intestine of pigs (Bindelle et al., 2007) or partially hydrolyzed by supplemental NSP-degrading enzymes added to the feed (Diebold et al., 2004).

1.5.1 Estimation (or determination) of non-starch polysaccharides

Fiber can be presented as N-free extract, crude fiber, NDF, ADF, NSP, and total dietary fiber using a variety of methods (Souffrant, 2001, Degen et al., 2007). Due to non-specific analytical methods, these fiber measurements include a number of compounds (Figure 1.4, Souffrant, 2001). Development of an accurate method to quantify NSP was difficult due to the complexity and diversity of the polysaccharides. Analytical methods for dietary fiber can be divided into two types: measurement of total dietary fiber using a gravimetric method and measurement of the various components of dietary fiber, using analytical methods such as gas-liquid chromatography, high-performance liquid chromatography, or spectrophotometry (Lanza and Butrum, 1986; Englyst et al., 1992).

Determination of dietary fiber by gravimetric methods is laborious. Although crude fiber values are still reported, they do not include total NSP content accurately, because recovery of cellulose, hemicellulose, and lignin is less than 100% (Van Soest and McQueen, 1973). In gravimetric methods, parts that resist breakdown by starch-degrading enzymes are isolated as fiber (Asp et al., 1983; Prosky et al., 1984). For example, NDF measures cellulose, hemicellulose,

and lignin and ADF measures cellulose and lignin (Garcia et al., 1997). Thus, hemicellulose represents the difference between NDF and ADF. Although detergent methods were an improvement compared to crude fiber (Mendez et al., 1993), they underestimate total fiber due to the inability to recover soluble fiber, e.g., pectins, NSP. During digestion steps, 25% of soluble fiber fails to retrieve (Marlett et al., 1989). Consequently, the sum of total soluble and insoluble fiber did not add up to total fiber (Wolters et al., 1992).

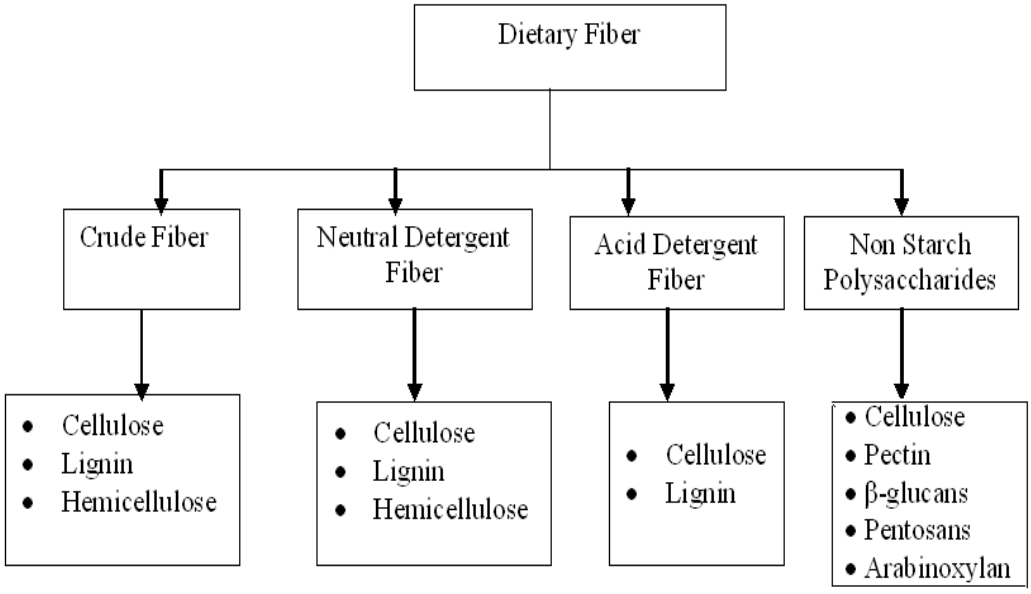

Figure 1.4 Fractions and components of dietary fiber (Souffrant, 2001)

Rapid technologies such as near infrared reflectance spectroscopy (NIRS) have been developed to predict dietary fiber content of feedstuffs. The NIRS analysis was developed to predict forage quality in the mid 1970s (Norris et al., 1976), and a method to predict ADF was developed a decade later (Barton and Windham, 1988). Now, NIRS is used with a reasonable degree of accuracy to predict total dietary fiber, ADF, and NDF (Archibald and Kays, 2000).

1.6 Effects of Non-Starch Polysaccharides

Dietary NSP have beneficial and detrimental effects for swine. Inclusion of fiber improved digestive enzymes secretion, gut health, and intestinal cell proliferation (Montagne et al., 2003). On the other hand, a high fiber content of swine diet reduced feed intake, growth performance, and nutrient digestion (Owusu-Asiedu et al., 2006).

1.6.1 Effects of NSP on the gastrointestinal tract

Gut morphology. Fiber content of the diet affects intestinal epithelial cell proliferation. Feeding high level of fiber increased villus height of mucosa in jejunum by 10% and in ileum by 16% (Schedle et al., 2008). Width of the intestinal villus and crypt depth increased in pigs fed the high-NSP content diet; however, high NSP did not affect villus height (Jin et al., 1994; Serena et al., 2008). Crypts are the principal sites for cell proliferation (Baserga and Ferrari, 1987). Therefore, growing pigs fed diets containing 10% wheat straw increased by 33 and 43% cell proliferation in the jejunum and colon with increased crypt depth respectively (Jin et al., 1994). High fiber content of diets increased sloughing of mucosal cell (Brunsgaard, 1998). Diets containing high fiber thus change intestinal morphology.

Endogenous secretion. Feeding dietary fiber increases mucosal enzymes activity in the digestive tract (Moharib, 2000; Hedemann et al., 2006). Pectin increased pancreatic enzymes (amylase and chymotrypsin) activity (Dunaif and

Schneeman, 1981). In contrast, starch replacement by pectin in diets fed to grower pigs reduced secretion of amylase (Mosenthin et al., 1994). Pigs fed 400 g wheat bran/kg diet increased pancreatic secretion by 115% (Langlois et al., 1987). Similarly, the activity of sucrase, lactase, maltase, and peptidase was increased in pigs fed high fiber diets (Hedemann et al., 2006). Dietary fiber increased endogenous secretion, thickness of mucus layer, and increased mucin-secreting goblet cells (Sakata, 1997). Doubling dietary fiber content also doubled secretion of saliva and gastric juice (Zebrowska et al., 1983). Insoluble fiber induced more endogenous secretion than soluble fiber in pigs (Hedemann et al., 2006). So, fiber is an important component in diet to influence endogenous losses and nutrient digestibility.

Viscera weight. Dietary fiber is positively correlated with the weight of empty viscera (Anguita et al., 2005; Serena et al., 2008); thus, dietary fiber reduces dressing percentage (Rijnen et al., 2001). Specifically, increased dietary NSP increases stomach, caecum, and colon weight (Jorgensen et al., 1996; Brunsgaard, 1998; Pluske et al., 1998; Nyachoti et al. 2000).

1.6.2 Effect of NSP on nutrient metabolism

Co-products generally contain more fiber than the parent grain. A diet containing high soluble fiber increased digesta viscosity and reduced digesta flow (Owusu-Asiedu et al., 2006). The amount of soluble fiber and digesta viscosity are correlated strongly (Bosscher et al., 2003). The NSP content of the diet increases the water-holding capacity which increases viscosity and gelling properties. High levels of soluble fiber increase digesta viscosity (Choct and

Annison, 1992). The viscosity and gelling properties of fiber hinder intestinal motility and nutrient digestion. The high viscosity impairs enzyme mixing rate, nutrient digestion and absorption (Vahouny and Cassidy, 1985). Digestive enzymes can not hydrolyze NSP (Wang et al., 2004). So, nutrients such as starch, AA, minerals, and vitamins entrapped within fiber are not available for digestion and absorption (Bosscher et al., 2003), and are released only after bacterial fermentation in hindgut (Lopez et al., 1999). But, efficiency of nutrient utilization is decreased in hindgut compare to upper gut (Ruppin et al., 1980).

Fiber digestion. The digestibility of nutrient components depends on the amount of fiber present in the diet, while digestibility of fiber components depends more on the types of the fiber (Stanogias and Pearce, 1987). Fiber is not digested by endogenous enzymes (Johansen et al., 1996; Wenk, 2001) but is fermented by microbes in the hindgut of pigs (Wang et al., 2004; Amguita et al., 2006; Bindelle et al., 2007). Short chain fatty acids, such as propionate, acetate and butyrate are produced by fiber fermentation in the large intestine (Bugaut and Bentejac, 1993; Macfarlane and Macfarlane, 2003). Butyrate stimulates intestinal cell proliferation (Velazquez et al., 1996; Kien et al., 2007), propionate is a glucogenic substrate (Overton et al., 1999) and acetate stimulates lipogenesis in liver (Fitch and Fleming, 1999). Fiber thus reduces nutrient digestibility but is also a source of VFA.

Digestibility of fiber depends on source, type, and age of pigs. Fiber digestibility of wheat bran ranges from 25 to 54% in grower pig and from 32 to 61% in adult sows (Noblet and Milgen, 2004). The ATTD of total dietary fiber in

corn DDGS ranges from 23 to 55% in grower-finisher pigs (Stein and Shurson, 2009). Soluble NSP is more fermentable, more digestible and provide higher contribution to energy utilization (Hogberg and Lindberg, 2004; Renteria et al., 2008). The variation in fiber digestibility exists due to complexity of physical and chemical structure of NSP (Karr et al., 2005). Ileal fiber digestion linearly decreased with increased level of dietary fiber (Jorgensen et al., 1996). Each 1 g NDF/kg dry matter (DM) reduces digestibility coefficients of gross energy by 0.1% (Degen et al., 2007). The efficiency of energy utilization was 50-60% for fiber and 80% for starch (Noblet and Le Goff, 2001). Thus, fiber reduces nutrient digestibility and efficiency of energy utilization when energy comes from fiber digestion.

Protein digestion. Plant cell walls contain protein in their matrix (Englyst et al., 1992); fiber-bound protein thus bypasses the small intestine. Protein digestibility depends on physical properties of fiber (Mosenthin et al., 1994). Soluble dietary fiber increased luminal viscosity (Johansen et al., 1996; Johansen et al., 1997) and high viscosity reduced diffusion rate of endogenous enzymes (Edwards and Johnson, 1988), thereby reducing digestion and absorption. High dietary fiber thus reduces protein digestion through several mechanisms. First, dietary fiber stimulates endogenous fluid secretion (Zebrowska et al., 1983; Langlois et al., 1987). Second, amino acids entrapped in fiber are not available for digestion and absorption because fiber is not hydrolyzed by digestive enzymes. Third, dietary fiber increased intestinal cell turnover (Jin et al., 1994). Combined,

each g NDF/kg diet reduced apparent ileal digestibility of CP by 0.03-0.08% (Degen et al., 2007).

Fat digestion. In practice, fiber components of diet dilute the nutrient concentration in feed. Therefore high-fiber diets are usually supplemented with fat or oil to compensate for the low energy density of diet. Dietary fiber also reduces the apparent total tract digestibility of fat (Noblet and Perez, 1993; Bakker et al., 1995; Le Goff and Noblet, 2001). Fibrous constituents bind bile acids in the digesta preventing fat emulsification (Kreuzer et al., 2002; Nakamura et al., 2004) and increase fat droplet size decreasing fat digestibility (Nakauma et al., 2008). Soluble fiber increases digesta viscosity and reduces enzyme diffusion that hampers liposysis (Pasquier et al., 1996). Soluble fiber binds with bile acid, prevents fat emulsification (Anderson et al., 1994) and increases bile excretion in feces.

Mineral digestion. Mineral utilization in swine varies according to the source and chemical composition of fiber. Generally, diets containing high fiber have reduced apparent absorption of minerals in pigs (Moore et al., 1986; Greger, 1999), lowered serum concentration of Ca, P, Cu, and Zn (Girard et al., 1995), and increased fecal Ca and P excretion (Baumgaertel et al., 2008). The apparent absorptions of Na and K were reduced with high dietary NDF; however, pigs fed diets with increasing NDF from hulls of soybean, field pea, oat, and maize did not reduce the apparent absorption of Ca, P, and Mg (Stanogias et al., 1994). Soluble dietary fiber reduced Ca, Fe, and Zn availability (Bosscher et al., 2003). Fiber and the amount of Ca trapped in fiber were correlated (James et al., 1978; Persson,

1991). Mineral entrapped in the fiber fraction is likely less available in the small intestine for digestion and absorption. However, minerals entrapped within fiber were available after fiber fermentation in the hindgut (Lopez et al., 1999). Fiber and mineral digestion were negatively correlated but P was highly digestible in co-products from the ethanol industry (Stein and Shurson, 2009).

Vitamin digestion. Pigs fed with high levels of dietary fiber had a reduced availability of vitamin B-complex, especially vitamin B_{12} (Girard et al., 1995). Soluble fiber reduced biotin availability (Baumgaertel et al., 2008).

1.7 Effect of NSP-degrading enzymes

A high amount of fiber in diets reduces nutrient digestion. Therefore, the growth performance of pigs was reduced when pigs were fed high fiber diets (Hedemann et al., 2006; Degen et al., 2007). The NSP can be partially hydrolyzed by supplementing NSP-degrading enzymes (Parkkonen et al., 1997; Nortey et al., 2007b). Therefore, nutrients entrapped by fiber can be released to some extent by hydrolysis of fiber with supplementation of exogenous enzymes (Tapingkae et al., 2008; Kumar and Wyman, 2009). The NSP-degrading enzymes partially degrade cell walls and increase cell wall permeability and release nutrient into digestive tract and increase nutrient digestibility.

1.7.1 Effect on growth performance and feed efficiency

Dietary fiber contributes to an improved gut health in pigs (Stein, 2007; Serena et. al., 2008). However, high fiber may also reduce diet nutrient digestibility and nutrient absorption in pigs (Stanogias and Pearce, 1985;

Jorgension et al., 1996). High fiber diet in growing period reduced growth performance of pigs (Len et al., 2008; Hedemann et al., 2006). The negative correlation of dietary fiber and growth performance of pig could be minimized by supplementation of exogenous enzymes (Annison and Choct, 1991; Wang et al., 2008). Weaned pigs fed with multienzyme supplemented diet improved average daily weight gain (ADG) and feed efficiency (Omogbenigun et al., 2004). Grower-finisher pigs fed with diets containing hull-less barley and supplemented with carbohydrase had a higher G:F (Grandhi, 2001). Similarly, growth performance and G:F was improved in grower pigs fed diet containing wheat and corn with xylanase supplementation (Van Lunen and Schulze, 1996).

The effect of supplemental exogenous enzymes on growth performance of pigs depends on their dose, combination of enzymes, age of pigs, and substrate availability present in feed ingredients. A combination of NSP-degrading enzymes improved feed conversion and live weight gain of piglets (Gill et al., 2000). Pigs fed a wheat DDGS diet containing high level of multicarbohydrase enzymes had a better growth performance (Emiola et al., 2009). Efficiency of carbohydrase enzymes is higher in young pigs and in diets containing low quality feedstuffs (Bedford et al., 1998; Cadogan et al., 2003; Omogbenigun et al., 2004), because young pigs have an immature gut and poor quality feed ingredients contain more substrates for the supplemental NSP degrading enzymes.

Results on feed efficiency and growth performance of pig fed with enzyme supplemented diet are not consistent. Average daily feed intake (ADFI), ADG, and G:F in weaned pigs fed wheat-soybean based diet supplemented with a

multi-enzyme product containing β-glucanase, hemicellulase, pentosanase and cellulase were not improved (Officer, 1995). Furthermore, ADFI, ADG, and G:F did not improve when growing-finishing pigs were fed with hullless barley diets supplemented with a multi-enzyme preparation containing β-glucanase, pentonase, cellulase, amylase and pectinase (Thacker et al., 1988). Similarly, supplementation of wheat and barley based diets with an endo-β-glucanase and endo-β xylanase cocktail did not improve G:F and growth performance of grower finisher pigs (Garry et al., 2007). In conclusion, efficiency of NSP-degrading enzymes depend on chemical complexity of NSP, amount of NSP present in feedstuffs, concentration of enzymes, age of animals and substrates specificity.

1.7.2 Effect on nutrient digestibility

For practical swine nutrition, supplementation of NSP-degrading enzymes to diets appears to be more effective in diets containing poorly digested feedstuffs (Cadogan et al., 2003). In weanling pigs, supplementation of xylanase to a wheat based diet increased organic matter digestibility (Diebold et al., 2004). NSP-degrading enzymes increased digestibility of GE, NDF, and NSP (Yin et al., 2001; Yin et al., 2001a; Kim et al., 2005; Woyengo et al., 2008). The NSP degrading enzymes randomly cut the NSP into small fragments and reduce their molecular weight (Tapingkae et al., 2008). Wheat millrun contains arabinoxylan as the main component of NSP that limits nutrient digestibility (Zijlstra et al., 1999; Nortey et al 2007b). Xylanase cleaves the long chain of arabinoxylan into small fragments, and increased energy digestibility (Nortey et al., 2007a; Kumar and Wyman, 2009).

The nutrients entrapped within fiber matrix (Englyst et al., 1992) can be released by NSP degrading enzymes supplementation and consequencetly ileal digestibility of protein and AA was increased (Haberer et al., 1997a; Woyengo et al., 2008). The NSP-degrading enzymes hydrolyzed the cell walls, increased permeability of the cell walls or cut the long chain polysaccharide (Parkkonen et al., 1997; Grandhi, 2001a; Tapingkae et al., 2008). Lysine is the first limiting amino acid; therefore, digestibility of essential AA such as lysine is more important rather than total protein digestibility for pig growth performance. In pigs, lysine digestibility was increased by supplementation of NSP degrading enzymes in diets (Yin et al., 2001; Diebold et al., 2004), therefore, supplementation of synthetic lysine can be minimized when NSP degrading enzymes are upplemented into the diets.

Furthermore, entrapped minerals are released from fiber by NSP-degrading enzymes, increased mineral absorption, and reduced mineral excretion in feces (Aulrich and Flachowsky, 1997). Pigs fed phytase-supplemented diets excreted 30% less P confirming that supplemental phytase improves P digestion and decrease P excretion (Htoo et al., 2007). Cocktails of enzymes are more effective for mineral digestion. Supplementation of xylanase alone did not improve P digestibility of DDGS (Yanez et al., 2011), but the combination of xylanase and phytase improved the ATTD of P and Ca of wheat based diet fed to grower pig (Kim et al., 2005; Woyengo et al., 2008). Efficiency of P digestibility was better when both xylanase and phytase were added (Nortey et al, 2007b).

Xylanase hydrolyzed bonds between fiber and phytate and released phytate to the phytase thereby increasing P digestibility.

Increased digesta viscosity reduced digesta passage rate (Owusu-Asiedu et al., 2006) and nutrient absorption (Mosenthin et al., 1994; Mosenthin, 1998). The NSP-degrading enzymes hydrolyze NSP, decrease digesta viscosity, and increase endogenous enzymes mixing rate with digesta components (Aulrich and Flachowsky, 1997; Diebold et al., 2004, Wang et al., 2008). The water holding capacity of fibrous feedstuffs was reduced after enzymatic fiber digestion, which reduced bulkiness of digesta, and improved feed intake of pigs. Combination of enzymes is more effective over single enzyme supplementation to improve nutrient digestibility. Cocktails of NSP-degrading enzymes improved digestibility of DM, GE, CP, AA, NDF, and NSP (Yin et al., 2001a; Hoare et al., 2003; Diebold et al., 2004).

The effects of supplementalenzymes on nutrient digestibility depend on concentration of enzymes in diet (Aulrich and Flachowsky, 1997). For example, a higher dose of NSP-degrading enzyme increased GE, OM and DM digestibility of diet more than a lower enzyme concentration (Omogbenigun et al., 2004; Emiola et al., 2009), the benefits of supplementing multi-enzymes decline with the age of pigs. Older pigs have a more developed hindgut and well-adjusted microbial population to digest dietary fiber (Brunsgaard, 1998). The NSP-degrading enzymes supplementation decreased hindgut fiber fermentation and probably improved ileal fiber digestion (Yin et al., 2001a). In conclusion, NSP degrading enzymes reduced detrimental effect of the fiber and improved nutrient

digestibility and digestible nutrient content. Furthermore, supplemental NSP-degrading enzymes efficiency depends on feed processing. Effectiveness of NSP-degrading enzymnes on nutrient digestibility is higher with a coarsely ground than with a finely ground diet (Mavromichalis et al., 2000; Amerah, 2008).

1.8 Summary

A critical issue in swine production is high feed cost. Opportunities to use co-products and thereby reduce feed cost are thus important. Consequently, pig producers have increased dietary inclusion of co-products resulting in diets with a higher NSP content than diets fed traditionally. The NSP are not digested by the pig's endogenous enzymes. High dietary NSP reduce: endogenous enzymes diffusion rate, digesta passage rate, nutrient digestion and absorption, and feed intake in pigs. Thus inclusion of high fiber co-products in swine diet reduces nutrient digestibility. Such detrimental effects of NSP on nutrition digestion could be minimized by supplementation of NSP degrading enzymes in diets containing co-products.

Therefore, we hypothesized that NSP contained in co-products limit nutrient digestibility of diets containing co-products and digestibility can be improved by supplementation of NSP-degrading enzymes. Previous studies indicated that supplementation of NSP-degrading enzymes improved nutrient digestion, growth performance and feed efficiency in pigs; however, results are inconsistent. Still, limited information is available on nutrient digestibility of co-products (corn DDGS and wheat millrun) supplemented with multi NSP-

degrading enzymes fed to grower pigs. Therefore, three experiments were conducted to determine:

1. The effects of NSP-degrading enzymes on energy and protein digestibility of diets containing either corn DDGS or wheat millrun or wheat grain;
2. The digestible nutrient profile of co-products and wheat grain used in the studies.

Experiment 1 and 2 were conducted with corn DDGS and wheat millrun based diets. Experiment 3 was conducted with a diet containing wheat grain and wheat millrun with and without supplementation of NSP-degrading enzyme. Xylanase and β-glucanase activity were provided to the experimental diets by adding enzyme preparations Roxazyme G2G, Ronozyme WX (CT), and Ronozyme VP (CT) and tested in ileal-cannulated pigs.

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Chapter 2. Effects of NSP-degrading enzymes on nutrient digestibility of corn DDGS and wheat millrun diets fed to grower pigs

2.1 Abstract

High feed cost is a perduring concern in swine production. Feed cost could be reduced by feeding co-products like corn DDGS and wheat millrun. Co-products contain more non-starch polysaccharides (NSP) and less starch than parent grain. High dietary NSP in swine diet reduce digestibility of nutrients, but NSP could yield dietary energy if they could be hydrolyzed even to some extend by NSP-degrading enzymes. The effects of NSP-degrading enzymes on nutrient digestibility of diets containing corn DDGS (Exp. 1) and wheat millrun (Exp. 2) were investigated in a 7 × 7 Latin-square design using 7 ileal-cannulated barrows. The 7 diets were, a N-free diet, and 6 diets contained 60% either corn DDGS or wheat millrun as sole source of protein and fiber in diets. Five diets contained combinations of 3 commercial enzyme products [Roxazyme G2G, Ronozyme WX (CT) and Ronozyme VP (CT); DSM Nutritional Products, Basel, Switzerland] at regular, commercial doses to provide glucanase and xylanase activity in the diet. A N-free diet was fed to determine basal endogenous N and amino acid losses. Pigs were fed twice daily at 2.8 × maintenance. Each 9-d period consisted sequentially of a 5 d diet adaptation, and a 2-d feces and 2-d digesta collection. The apparent ileal (AID) and apparent total tract digestibility (ATTD) of energy and standard ileal digestibility (SID) of CP did not differ (P > 0.10) among corn DDGS diets (Exp. 1) and wheat millrun diets (Exp. 2).

However, combined Ronozyme WX (CT) and VP (CT) increased ($P < 0.05$) AID of energy in wheat millrun. In conclusion, supplementation of NSP-degrading enzyme at commercial dose did not increase energy and protein digestibility in corn DDGS and inconsistently in wheat millrun.

2.2 Introduction

Feed cost is the highest variable cost in pig production (Payne and Zijlstra, 2007). This cost has increased recently, partly due to high demands by the ethanol industry for feed grains (Hill et al., 2006). Feed cost can be reduced by inclusion of corn distillers dried grain with solubles (DDGS) and wheat millrun, however inclusion of co-products in swine diets may reduce nutrient digestibility and growth performance (Whitney et al., 2006, Nortey et al., 2007).

Arabinoxylan is the major NSP in corn DDGS and wheat millrun (Widyaratne and Zijlstra, 2007, Nortey et al., 2008). The NSP are not hydrolyzed by endogenous enzymes (Barrera et al., 2004), and can be considered an anti-nutritional factor (Englyst, 1989; Owusu-Asiedu et al., 2006). The NSP-degrading enzymes may reduce detrimental effects of NSP and increase nutrient digestibility (Barrera et al., 2004; Woyengo et al., 2008). Previously, digestibility studies determined the effects of NSP-degrading enzymes on diets containing cereal grain or cereal grain plus co-products (Nortey et al., 2007; Ji et al., 2008);, however, results were inconsistent (Olukosi et al., 2007; Lyberg et al., 2008). Furthermore, limited information exists for effects of glucanase and xylanase on nutrient digestibility of corn DDGS and wheat millrun.

We hypothesized that NSP limits energy, protein, and AA digestibility of corn DDGS and wheat millrun that could be increased by supplemental NSP-degrading enzymes. Thus, two studies were conducted to determine effects of such enzymes on nutrient digestibility of corn DDGS and wheat millrun fed to grower pigs. If the studies would confirm beneficial effects of NSP-degrading enzymes on nutrient digestibility of corn DDGS and wheat millrun, nutritionists might thus use NSP-degrading enzymes to maximize opportunity use of corn DDGS and wheat millrun inclusion in swine diets to reduce feed cost.

2.3 Materials and methods

Experimental procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee for Livestock. Pigs were handled in accordance with guidelines established by the Canadian Council on Animal Care (CCAC, 1993). Two experiments were conducted at Swine Research Technology Centre of the University of Alberta.

2.3.1 Experimental Diets and Design

Each experiment had 7 diets: 6 diets containing 60% of the feedstuff corn DDGS in Exp. 1 and wheat millrun in Exp. 2. These feedstuffs were the sole source of NSP and CP in these diets (Table 2.1). One of the 6 diets was the control and NSP-degrading enzymes were added to 5 diets. The seventh diet was cornstarch-based and was used to determine basal endogenous N and AA losses and to calculate energy digestibility of corn DDGS and wheat millrun. Chromic

oxide was added to the diets as an indigestible marker to calculate nutrient digestibility.

The 5 enzyme-supplemented diets contained either a single enzyme preparation or a combination of Roxazyme G2G, Ronozyme WX (CT), and Ronozyme VP (CT) obtained from DSM Nutritional Products (Basel, Switzerland). The doses of supplemental enzyme products were added as recommended by the manufacturer. The units of each enzyme activity were defined differently for each product, and these can thus not be directly compared. The origin and enzyme activities present in the enzyme products are described in Appendix 1.

Pigs were weighed at the start of each period to calculate their daily feed allowance. The daily feed allowance was set at 2.8 × maintenance energy requirement (110 kcal DE × $BW^{0.75}$; NRC, 1998) divided in two daily meals (08:00, 15:00) of approximately equal size. Diets were fed as a dry mash and pigs had free access to water throughout the experiment.

The study design was a 7 × 7 Latin-square using ileal-cannulated grower finisher pigs. The 7 experimental diets were fed to 7 pigs in each period, so that each pig consumes each diet over 7 experimental periods to provide 7 observations per diet. Each 9-d period contained sequentially a 5-d diet adaptation, 2-d feces collection, and 2-d digesta collection.

2.3.2 Experimental procedure

Seven cross-breed barrows (Duroc × Large White) with initial BW of 30 ± 1.3 kg (Exp. 1) or 27 ± 1.4 kg (Exp. 2) were surgically fitted with a T-cannula at

the distal ileum (Sauer et al., 1983; De Lange et al., 1989). Upon recovery from surgery, average BW of pigs was 39 kg in Exp. 1 and 32 kg in Exp. 2 when pigs started to eat the diet for period 1. Pigs were housed individually in metabolic pens measuring 1.2 × 1.2 × 0.9 m (width, length, height). Pen sides were made of solid plastic boarding with plexiglass windows. Flooring was made from plastic-coated metal. Pens were raised 0.4 m off the floor. Each pen was equipped with a stainless steel, single space dry self-feeder, and a bowl drinker. The room temperature was maintained at 22 ± 2.5ºC and lights were turned on from 08:00 to 20:00 throughout the experiment.

Feces were collected continuously using plastic bags attached to the skin around the anus for 2 d (Van Kleef et al., 1994). Bags were replaced whenever pigs defecated. Digesta was collected using PVC plastic bags containing approximately 15 mL of 5% formic acid attached to the opened cannula barrel for 10 hr over 2 days. Bags were removed whenever filled with digesta. Collected feces and digesta were separately pooled by pig and frozen at –20°C. Prior to analyses, feces, and digesta were thawed, homogenized, and sub-sampled. Samples were freeze dried and subsequently ground in a centrifugal mill (model ZM 200, Retsch, Newtown, PA) using a 1-mm screen for energy and CP analyses and 0.5-mm screen for AA and NSP analyses.

2.3.3 Chemical analyses

Samples were analyzed for GE using an adiabatic bomb calorimeter (model 5003, IKA-Werke GMBH and Co KG, Staufen, Germany) and DM by drying at 135°C in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Benzoic acid was used as the standard in bomb calorimeter. Chromic oxide content of samples were analyzed by spectrophotometer (model 80-2097-62, LKB-Ultraspec III, Pharmacia, Cambridge, UK) at 440 nm wave length after ashing at 450°C overnight (Fenton and Fenton, 1979). Particle size of corn DDGS and wheat millrun was measured using 13 sieves (4.00, 2.26, 1.70, 1.18, 0.85, 0.60, 0.43, 0.30, 0.21, 0.15, 0.11, 0.08, and 0.05 mm) and a pan on a sieve shaker (W. S. Tyler, Mentor, OH) using method S319.4 (ASAE, 2001). Ingredients, diets, feces and digesta were analyzed for DM (method 934.01; AOAC, 2006), CP (method 990.03; AOAC, 2006), complete AA profile (method 982.30E; AOAC, 2006), ether extract (method 920.39A; AOAC, 2006), crude fiber (method 978.10; AOAC, 2006), ADF (method 973.18, A-D; AOAC, 2006), NDF (Van Soest et al., 1991), total dietary fiber (method 985.29; AOAC, 2006), ash (method 942.05; AOAC, 2006), Ca (method 968.08; AOAC, 2006), P (method 946.06; AOAC, 2006), and available lysine (method 975.44; AOAC, 2006), and starch (method 76-13, Amer. Assoc. Cereal Chemists) at the University of Missouri, Columbia, MO. Following grinding over a 0.5-mm screen in Retsch mill, Ingredients, diets, feces and digesta were analyzed for total, soluble, insoluble NSP and constituent sugars by gas chromatography (Englyst and Hudson, 1987; Englyst, 1989) at the University of Alberta.

2.3.4 Calculations

Nutrient digestibility of diets was calculated using chromic oxide concentrations in feces and digesta in relation to feed. The apparent ileal and total tract nutrient digestibility was calculated using the following equation (Stein et al., 2007).

Digestibility, % (DE_C) = 1-($C_D \times N_E$) / ($C_E \times N_D$)

Where C_D is dietary concentration of Cr_2O_3, N_E is nutrient concentration in excreta, C_E is Cr_2O_3 concentration in excreta, and N_D is dietary concentration of nutrient. Energy digestibility of diet is also referred as coefficient of digestible energy (DE_C) and DE (Mcal/g) of diets was calculated as the product of DE_C and the gross energy concentration of the diet. Similarly, the DE value of corn DDGS and wheat millrun was calculated by multiplying energy digestibility and GE content of corn DDGS and wheat millrun, respectively. Pigs were fed the N free diet to calculate basal endogenous CP and AA losses. The cornstarch diet containing cornstarch, sugar and canola was considered the basal diet to determine energy digestibility of corn DDGS and wheat millrun using the indirect method (Adeola, 2001; Kim et al., 2009).

Digestibility of test feedstuffs = $[(T \times T_p) - (B \times B_p)]/A_p$

Where, T is the digestibility of diet (basal ingredient plus test feedstuffs); T_p is amount of component in the diet contributed by the basal diet plus test feedstuffs; B is the digestibility of the component in basal diet; B_p is the proportion of the component in the total diet contributed by the basal diet; A_p is

the proportion of the component in the total diet contributed by the test feedstuff; Tp = Bp + Ap = 100%

Therefore, digestibility of corn DDGS or wheat millrun = {Digestibility of test diet – (Digestibility of N-free diet × proportion of cornstarch in test diet)}/proportion contributed by test ingredient in test diet.

Test diet contained 33% cornstarch and 60% either corn DDGS or wheat millrun that contributed 36% and 64% to the test diet, respectively.

Therefore, digestibility of corn DDGS or wheat millrun = {Digestibility of test diet – (Digestibility of N-free diet × 0.36)}/0.64.

The basal ileal endogenous (Iend) loss of AA and crude protein was calculated by using following equation for the N-free diet (Stein et al., 2007).

Iend = {AA or CP in digesta × (Cr_2O_3 in feed/Cr_2O_3 in digesta)}

Standardized ileal digestibility (SID) value for each AA was calculated by correcting the AID of AA with basal endogenous losses (Stein et al., 2007).

SID = {AID + (I_{end}/AA in feed) × 100}

The test feedstuffs were the sole dietary source of AA in Exp. 1 and Exp. 2. Therefore, digestibility of AA in the test diet itself was considered AA digestibility of corn DDGS and wheat millrun. Thus, SID AA content of corn DDGS and wheat millrun was determined by multiplying SID of diet with the total AA content of corn DDGS and wheat millrun, respectively.

2.3.5 Statistical analyses

The cornstarch diet was used to determine basal endogenous N losses and calculate the nutrient digestibility of corn DDGS and wheat millrun, and was thus

excluded from statistical analyses. Data were analyzed using mixed model procedure of SAS 9.2 software (SAS Inst. Inc., Cary, NC) in a 7 × 7 Latin-square. The main effect of enzyme supplementation on nutrient digestibility of diets was analyzed using a model that included diet as a fixed effect, and period and pig as random effects. The individual pig was considered the experimental unit for the model. To test the hypotheses, $P < 0.05$ was considered significant. If $0.05 \leq P < 0.10$, these were considered trends.

2.4 Results

Pigs consumed the offered diet in both Exp. Pigs remained in good health in Exp. 1; however, digesta collection was completed with difficulty due to frequent blockage of cannulas by digesta in Exp. 2. Hence, fewer observations per diet were completed in Exp. 2.

2.4.1 Composition of ingredients and diets

The corn DDGS contained (as is basis) 29.0% total dietary fiber, 19.7% NSP, 10.1% ether extract, 29.8% CP, and 3.95% starch (Tables 2.2 and 2.3). Similarly, wheat millrun contained (as is basis) 33.3% total dietary fiber, 22.9% NSP, 3.5% ether extract, 16.7% CP, and 28% starch. The lysine was 92.4 and 93.0% available and the lysine to CP ratio was 3.25 and 3.41 in corn DDGS and wheat millrun, respectively. Corn DDGS and wheat millrun contained arabinoxylan as the main NSP (Table 2.3). The amount of insoluble NSP was higher than soluble NSP in corn DDGS (19 vs. 1%) and wheat millrun (18 vs. 5%).

Three enzymes products Roxazyme G2G, Ronozyme WX (CT), and Ronozyme VP (CT) were supplemented to diets. Analyzed enzyme activity in feed was similar to expected, indicating that enzymes were active and mixed properly into diets (Table 2.4). Results of in-feed analytical determinations of added enzyme products are provided in terms of product equivalents. The unit of each enzyme activity was defined differently for each product (Appendix 1).

Nutrient content was consistent among the 6 corn DDGS diets (Table 2.5) and 6 wheat millrun diets (Table 2.6). Particle size ranged from 729 to 773 µm for corn DDGS diets (Table 2.7) and from 396 to 538 µm for wheat millrun diets (Table 2.8), and did not differ among diets.

2.4.2 Nutrient digestibility of ingredients and diets

Corn DDGS. In Exp. 1, individual supplemental NSP-degrading enzyme treatments did not increase ($P > 0.10$) the AID and ATTD of energy and DE value of corn DDGS diets (Table 2.9) and thus corn DDGS (Table 2.10). Similarly, supplementation of NSP-degrading enzymes did not increase ($P > 0.10$) the AID and SID of CP and digestible protein content in the corn DDGS diet (Table 2.9) and thus corn DDGS (Table 2.10).

Wheat millrun. In Exp. 2, the AID and ATTD of energy and DE value did not differ ($P > 0.10$) among diets (Table 2.11) or for wheat millrun (Table 2.12). However, combined Ronozyme WX (CT) and VP (CT) increased ($P < 0.05$) AID of energy by 5.4%-units and ileal digested energy by 0.22 Mcal/kg for the diets (Table 2.11) or increased ($P < 0.05$) AID of energy by 9%-units and ileal digested energy by 0.37 Mcal/kg for wheat millrun (Table 2.12). In addition, supplemental

NSP-degrading enzymes did not increase ($P > 0.10$) AID and SID of CP and digestible protein content in the wheat millrun diet (Table 2.11) and wheat millrun (Table 2.12).

2.5 Discussion

2.5.1 Nutrient concentration in corn DDGS and wheat millrun

One purpose of the present study was to characterize the corn DDGS and wheat millrun in detail for digestible nutrient content and potential substrates for enzymes. The nutrient content and nutrient digestibility of corn DDGS and wheat millrun varies widely among their sources (Martinez-Amezcua et al., 2007; Nortey et al., 2008; Urriola et al., 2009). During fermentation, most grain starch is converted into ethanol and CO_2. Consequently, corn DDGS contains little starch and fiber and protein in corn DDGS is higher than in the parent grain (Young, 2008; Urriola et al., 2010). In flour milling, starchy endosperm of wheat grain is separated into wheat flour and wheat millrun that contains bran, aleurone, germ, and offal. Thus, dietary fiber, minerals, and protein are higher in wheat millrun than wheat grain (Belderok et al., 2000).

The corn DDGS in Exp. 1 contained more CP and fiber (ADF and NDF) than some reports (Martinez-Amezcua et al., 2007; Young, 2008; Stein and Shurson, 2009). The reactive lysine availability and lysine to CP ratio (3.25) in corn DDGS was higher than the mean ratio (2.98) among corn DDGS sources (Stein et al., 2006; Urriola et al., 2009). Combined, these data indicate that lysine quality of corn DDGS in the present study was excellent.

The wheat millrun in Exp. 2 contained less ADF, NDF, and NSP (12, 32, and 23% vs. 17, 39, and 26%, respectively) than before (Nortey et al., 2008). Arabinoxylan was the main NSP in wheat millrun, because millrun contains mostly bran (Joyce et al., 2005) and xylose and arabinose are two major pentosan sugars in wheat NSP (Manisseri and Gudipati, 2010). The xylose to arabinose ratio was 2.0:1 compared to 1.8:1 previously (Nortey et al., 2008).

2.5.2 Energy and protein digestibility of corn DDGS and wheat millrun

Arabinoxylan was the main NSP in corn DDGS (54%) and wheat millrun (61%). A high content of fiber has been correlated with reduced nutrient digestibility in pigs (McClean, 1993; Zijlstra et al., 1999; Yin et al., 2000; Degen et al., 2007; Widyaratne and Zijlstra, 2007). A high amount of dietary fiber may increase water-holding capacity and gelling properties of digesta thereby reducing mixing of digesta with digestive enzymes (Vahouny and Cassidy, 1985; Wenk, 2001; Johnston et al., 2003). Insoluble dietary fiber increases peristalsis of the gut (Jorgensen et al., 1996); thus, insoluble fiber reduces digesta retention in the small intestine (Wilfart et al., 2007) ultimately reducing nutrient digestibility (Yin et al., 2000).

The AID and ATTD of energy in corn DDGS were 10% lower than measured previously (Fastinger and Mahan, 2006; Widyaratne and Zijlstra, 2007). In the present study, corn DDGS contained less starch (4 vs. 7%) and more fiber (mostly insoluble) than mean values (Pedersen et al., 2007; Stein and Shurson, 2009). The ATTD of insoluble dietary fiber in corn DDGS is lower (40 vs. 90%) than of soluble dietary fiber (Urriolla et al., 2010). Energy digestibility of DDGS

was higher when DDGS was fed in combination with cereal grain instead of cornstarch (Kandel et al., 2011). Therefore, diet formulation, lower starch content, and higher insoluble fiber content may explain the lower energy digestibility of corn DDGS in the present study.

Digestibility of protein and AA in diets represented their digestibility in the feedstuffs (corn DDGS and wheat millrun), because these feedstuffs were the sole protein sources in the diets. In the present study, AID of CP in corn DDGS was higher than the mean AID of CP (70 vs. 63%) of 5 samples (Fastinger and Mahan, 2006). Furthermore, SID of CP in corn DDGS was higher than the mean SID of CP (78 vs. 73%) of 8 samples (Urriola et al., 2009). Combined with lysine availability and lysine to CP ratio data, the CP and AA data indicate that protein was not damaged during processing; and that the corn DDGS in Exp. 1 had excellent protein quality.

The AID and ATTD of energy in wheat millrun was 7 and 11% higher than the 51 and 56% reported previously in grower pigs (Nortey et al., 2008), likely because wheat millrun in Exp. 2 contained more soluble NSP than previously (5 vs. 1%; Nortey et al., 2008) that has a higher digestibility than insoluble NSP (Urriolla et al., 2010). Moreover, wheat millrun used in present study contained more starch (28 vs. 26%) than mean starch (Slominski et al., 2004). Higher soluble NSP and starch content may thus explain the higher energy digestibility in wheat millrun.

2.5.3 Effects of NSP-degrading enzymes

Corn DDGS and wheat millrun had a high content of NSP that are a physical barrier for nutrient digestion. The diets contained 60% of the test feedstuffs that should provide sufficient NSP to reduce nutrient digestibility in grower pigs. Supplementing NSP-degrading enzyme to high NSP diets may minimize detrimental effects of NSP and improve nutritional value of feedstuffs (Zijlstra et al., 2010). These enzymes hydrolyze NSP bonds, degrade cell wall, release fiber-entrapped nutrients, and increase mixing of digesta components with digestive enzymes (Kumar and Wyman, 2009), including for corn DDGS and wheat millrun.

Energy and protein digestibility did not differ among diets containing corn DDGS (Exp. 1) and wheat millrun (Exp. 2) at the used inclusion of NSP-degrading enzymes, although, combined Ronozyme WX (CT) and VP (CT) increased AID of energy in wheat millrun. Similarly, supplemental xylanase and β-glucanase increased AID of energy in cereal grain (Li et al., 1996; Yin et al., 2000, Yin et al. 2001), although increased AID of energy by xylanase supplementation does not necessarily increase ATTD of energy in corn DDGS (Widyaratne et al., 2009). The NSP-degrading enzymes may increase trypsin and α-amylase activity (Wang et al., 2008), and bacterial activity in the upper digestive tract (Hirsch et al., 2006), explaining the greater influence on AID than ATTD of energy for wheat millrun. Furthermore, effects on ATTD of energy might be masked by microbial fermentation in the lower digestive tract.

The present study is similar to other pig studies that did not detect beneficial effects of enzyme supplementation on nutrient digestibility of DDGS-diets (Sigfridson and Harldsson, 2007, Widyaratne et al., 2009; Jacela et al., 2010; Jones et al., 2010; Yanez et al., 2011) or wheat- and corn-based diets (Ji et al., 2008; Lyberg et al., 2008; Owusu-Asiedu et al., 2010; Susenbeth et al., 2011) including nursery pigs (Olukosi et al., 2007; Diebold et al., 2004, 2005). Enzyme supplementation did not increase growth performance of pigs fed diets containing corn, soybean meal, and corn DDGS (Jones, 2010) or containing wheat or hull-less barley and soybean meal (Officer, 1995; Thacker et al., 1988). In contrast, a multi-enzyme complex increased nutrient digestibility in pig diets with 30% wheat DDGS (Emiola et al., 2009). Likewise, supplementation of β-glucanase and protease to a corn soybean based diet fed to grower pigs increased ATTD of energy, CP, and fiber (Ji et al., 2008). Similarly, supplementation of a multi-enzyme to a corn soybean diet increased growth performance of weaned pigs (Omogbenigun et al., 2004). The results of Exp. 2 contrast that xylanase increased nutrient digestibility of wheat (Barrera et al., 2004; Diebold et al., 2004; Woyengo et al., 2008) or wheat co-products from flour milling (Yin et al., 2000; Nortey et al., 2007, 2007a, 2008). Effects of NSP-degrading enzymes in pigs are thus inconsistent (Mavromichalis et al., 2000; Adeola and Cowieson, 2011). Many factors may affect efficacy of NSP-degrading enzymes such as type of NSP, age of pigs, digesta pH and digesta retention time, enzyme activity in digesta, quality of feedstuffs, concentration of substrate, and particle size (Cadogan et al., 2003; Olukosi et al., 2007).

Types of NSP. In the present study, corn DDGS and wheat millrun contained mostly insoluble NSP (97 and 78%, respectively) that may explain part of lack of positive effect of enzymes. The NSP-degrading enzymes are more effective to increase digestibility of soluble than insoluble NSP (Yin et al. 2004; Adeola and Cowieson, 2011). Thus, high insoluble NSP contained in the feedstuffs may limit efficacy of NSP-degrading enzyme in the present study. Insoluble fiber reduced retention time (Wilfart et al., 2007). An increased digesta passage rate could also explain the lack of increased nutrient digestibility of feedstuffs, due to less contact between enzymes and substrate at optimum pH.

Age of pigs. Age of the pig may influence responses to supplemental enzymes (Li et al., 1996a; Diebold et al., 2004; Olukosi et al., 2007). Young pigs respond more to feed enzyme supplementation than mature pigs (Yin et al., 2001a), because digestibility increases with age due development of the digestive tract, including increased microbial activity. For example, supplemental phytase and a cocktail of xylanase, amylase, and protease increased nutrient digestibility in 10- but not in 23-kg pigs (Olukosi et al., 2007). Similarly, supplemental enzymes increased nutrient digestibility and growth performance of weaned pigs (Omonbenigun et al., 2004; Vahjen et al., 2007; He et al., 2010) but not growth performance of grower-finisher pigs fed diets containing wheat, wheat middlings, and corn DDGS (Olukosi et al., 2007; Woyengo et al., 2008; Jacela et al., 2010). In the present study, mean BW of pigs was 32 to 39 kg at the start; thus, our pigs had a more mature digestive tract that may have reduced enzyme efficacy.

Digesta pH. Enzyme stability is important to have effects of enzymes on nutrient digestibility. The NSP-degrading enzymes must survive the pig stomach to have effects. Otherwise, enzyme activity would be restricted to the pre-gastric and gastric regions. In the stomach, HCl lowers gastric pH down to 2-3 (Canibe et al., 2005, DeRouchey et al., 2009). At low pH, enzyme activity declined quickly and less sugar moieties were released from NSP (Thacker and Baas, 1996, 1996a). Enzyme activity declines over time in the digestive tract. For example, 52 and 26% of initial β-glucanase activity was detected 60 and 240 min after feeding pigs and did not improve pig performance (Thacker and Baas, 1996a). Retention time in the pig stomach and small intestine is 6 to 9 h for cereal diets (Latymer et al., 1990). Therefore, gastric inactivation might explain that supplemental glucanase and xylanase did not increase nutrient digestibility of corn DDGS and wheat millrun.

Substrate availability and quality of ingredient. Effects of NSP-degrading enzymes depend on their substrates. For example, supplemented xylanase increased nutrient digestibility greatly for a diet containing wheat co-product but less for a wheat grain diet (Nortey et al., 2008), indicating that enzyme effects depends on substrate availability. Ingredient quality is the most important factor, affecting efficacy of NSP-degrading enzymes: Feed enzymes are more effective in low quality feedstuffs (Cadogan et al., 2003). In Exp. 1, protein digestibility of corn DDGS was higher than average value (Fastinger and Mahan, 2006; Urriola et al., 2009), and the data indicated that the corn DDGS had excellent protein quality. The energy digestibility in wheat millrun was 7-11% higher than

previously for wheat millrun in grower pigs (Nortey et al., 2008). Combined, corn DDGS and wheat millrun used for the present study were of good quality providing an explanation for lack of substantially improved protein and energy digestibility.

Particle size. Reduction of particle size increased nutrient digestibility and growth performance of pigs (Wondra et al., 1995; Yanez, et al., 2011). Supplemental fiber-degrading enzymes increased feed efficiency and nutrient digestibility for coarse (1300 µm) but not fine (400-600 µm) particles (Mavromichalis et al., 2000). Mean particle size of corn DDGS and wheat millrun diets ranged from 729-773 µm and 397-538 µm, respectively, in the present study; thus, finer particle size may partially explain the low efficacy of NSP-degrading enzymes.

Concentration of feed enzyme. Effects of NSP-degrading enzymes depend on their dose (Fang et al., 2007). Xylanase supplementation from 400 to 32,000 units/kg feed linearly increased DM digestibility in corn-based diets (Olukosi et al., 2007a). Similarly, supplemental xylanase from 2000 to 4000 U/kg linearly increased ATTD of DM in grower pigs fed a wheat-based diet (Woyengo et al., 2008). The dose of supplemental feed enzyme might not been sufficient to increase nutrient digestibility in the present study.

To increase ethanol yield, the grain can be treated (Parveen et al., 2009). Physicochemical and enzymatic pre-treatments of grain may hydrolyze fiber and release fiber-bound nutrients. Fiber may also undergo structural, chemical changes during fermentation. During ethanol production, sulfuric acid is used to

adjust pH (Nuez-Ortin and Yu, 2009) that may hydrolyze fiber (Englyst, 1989). Grain is treated with α-amylase and protease in the liquefaction tank and pumped to cooker jet at 105 to 125°C (Batie et al., 2011) to release starch and protein. At high temperatures, cellulose changes its physical and chemical structure (Graham et al., 1989; Sun and Cheng, 2002). Enzyme responses are affected by substrate characteristics and enzyme activity (Campbell and Bedford, 1992). Thus, pre-treatments and possible changes in physicochemical structure in fiber in corn DDGS during processing could explain the lacking effects of supplemental NSP-degrading enzymes on nutrient digestibility of corn DDGS.

In Exp. 2, digesta collection was difficult because the barrel was blocked by digesta and required frequent cleaning; 4 pigs were removed from the study due to health problems. Thus, fewer observations were obtained per diet and the SEM increased. Using statistical power analyses, we concluded that the number of observations per diet was not enough to detect effects of NSP-degrading enzymes at $P < 0.05$. However, we detected that glucanase and xylanase in combined Ronozyme WX (CT) and VP (CT) increased AID of energy in the wheat millrun with 4 observations. Some diets only reached 3 observations. Thus, reduced statistical power might have prevented detection of treatment differences for the wheat millrun diets.

In summary, NSP-degrading enzymes did not increase nutrient digestibility of corn DDGS. Four of 5 combinations of NSP-degrading enzymes did not increase nutrient digestibility of wheat millrun, although combined Ronozyme WX (CT) and VP (CT) increased AID of energy. In conclusion,

enzyme and substrate did not match for corn DDGS and most treatments for wheat millrun. The NSP thus limit energy digestibility of wheat millrun, but enzyme dose was likely not sufficient to increase nutrient digestibility. The enzyme substrates were not the most important factor limiting nutrient digestibility of corn DDGS.

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Table 2.1 Ingredient composition of experimental diets (Exp. 1 and Exp. 2)

Ingredient, %	Treatment diets[1]						
	CON	Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C	N-free
Cornstarch	33.87	33.77	33.72	33.57	33.62	33.72	85.30
Test ingredient[2]	60.00	60.00	60.00	60.00	60.00	60.00	-
Sugar	1.95	1.95	1.95	1.95	1.95	1.95	5.00
Solka-Floc	-	-	-	-	-	-	3.00
Canola oil	0.78	0.78	0.78	0.78	0.78	0.78	2.00
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.00
Dicalcium phosphate	-	-	-	-	-	-	1.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
KCO_3, 56% K	-	-	-	-	-	-	0.50
MgO, 58% Mg			-	-	-	-	0.10
Cr_2O_3	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[1]CON = Control, without enzyme; Roxazyme contained 0.1 g Roxazyme G2G; Ronozyme 1 contained 0.15 g Ronozyme WX (CT), Ronozyme 2 contained 0.3 g Ronozyme VP (CT), B + C contained 0.1 g Ronozyme WX (CT) and 0.15 g Ronozyme VP (CT), and A + C contained 0.075 g Roxazyme (G2G) and 0.15 g Ronozyme VP (CT) products/kg feed.

[2]Exp. 1 used corn DDGS and Exp. 2 used wheat millrun as a test ingredient.

[3]Vitamin premix provided per kilogram of feed: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg.

[4]Mineral premix provided per kilogram of feed: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO.

Table 2.2 Analyzed chemical composition of corn DDGS and wheat millrun (as is basis)

Item, %	Corn DDGS (Exp. 1)	Wheat millrun (Exp. 2)
Moisture	11.05	11.6
Ether extract	10.11	3.46
Fiber		
Crude fiber	6.44	8.94
ADF	10.26	12.08
NDF	26.89	31.74
Total dietary fiber	29.00	33.33
Starch	3.95	28.32
Gross energy, Mcal/kg	4.79	4.09
Digestible energy, Mcal/kg	3.24	2.38
Crude protein, 6.25 × N	29.8	16.70
Dispensable AA		
Aspartic acid	1.83	0.91
Serine	1.23	0.50
Glutamic acid	3.69	2.80
Proline	1.97	0.97
Glycine	1.16	0.70
Alanine	1.89	0.63
Cysteine	0.49	0.27

Tyrosine	1.02	0.36
Indispensable AA		
Methionine	0.54	0.22
Isoleucine	1.11	0.49
Leucine	3.19	0.93
Threonine	1.06	0.42
Phenylalanine	1.24	0.60
Valine	1.44	0.68
Lysine	1.05	0.57
Histidine	0.75	0.35
Arginine	1.33	0.85
Tryptophan	0.22	0.16
Available lysine	0.97	0.53
Lysine to CP, %	3.25	3.41
Lysine availability, %	92.4	93.0
Ash	4.11	4.52
Ca	0.21	0.06
P	0.81	0.86

Table 2.3 Analyzed contents of non-starch polysaccharides and its components in corn DDGS and wheat millrun (%, as is basis)

Item		Corn DDGS (Exp. 1)	Wheat millrun (Exp. 2)
Arabinose			
	Insoluble	4.22	3.66
	Soluble*	0.14	0.93
	Total	4.36	4.58
Xylose			
	Insoluble	6.3	7.31
	Soluble	-0.07	2.1
	Total	6.23	9.41
Mannose			
	Insoluble	0.73	0.16
	Soluble	0.36	0.02
	Total	1.1	0.18
Glucose			
	Insoluble	6.54	6.32
	Soluble	0.12	1.73
	Total	6.66	8.05
Galactose			
	Insoluble	1.23	0.48
	Soluble	0.14	0.24

Total	1.37	0.72
Non-starch polysaccharides		
Insoluble	19.02	17.92
Soluble	0.69	5.02
Total	19.71	22.94

*Water soluble NSP is the difference between total NSP and insoluble NSP.

Table 2.4 Enzyme addition and in-feed analyze enzyme equivalent in the diets (as fed basis, Exp. 1 and Exp. 2)

	Treatment diet					
Item,	CON	Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C
Added enzyme ,g/kg diet						
Roxazyme G2G	-	0.100	-	-	-	0.075
Ronozyme WX (CT)	-	-	0.150	-	0.100	-
Ronozyme VP (CT)	-	-	-	0.300	0.150	0.150
Analyzed enzyme equivalent, g/kg diet						
Exp. 1						
Roxazyme G2G	-	0.116	-	-	-	0.070
Ronozyme WX (CT)	-	-	0.147	-	0.79	-
Ronozyme VP (CT)	-	-	-	0.316	0.125	N/D*
Exp. 2						
Roxazyme G2G	-	0.077	-	-	-	0.079
Ronozyme WX (CT)	-	-	0.150	-	0.104	-
Ronozyme VP (CT)	-	-	-	0.315	0.110	N/D

N/D = not determined due to technological complications of feed analytics of mixed enzyme combinations.

Table 2.5 Analyzed chemical composition of corn DDGS diets (%, as fed basis, Exp. 1)

Item	Treatment diets					
	CON	Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C
Moisture	7.83	7.74	7.67	8.08	8.22	7.80
Ether extract	6.92	7.16	7.00	6.76	6.78	6.59
Crude fiber	3.46	3.52	3.61	3.33	3.35	3.47
ADF	5.73	5.81	5.70	5.28	5.70	5.09
NDF	20.13	20.47	20.94	20.30	20.40	22.55
Total dietary fiber	17.93	18.72	19.00	18.02	18.47	17.90
Starch	33.52	36.31	34.78	37.07	34.22	35.51
Gross energy, Mcal/kg	4.23	4.27	4.26	4.20	4.26	4.26
Calculated NSP	11.83	11.83	11.83	11.83	11.83	11.83
Crude protein, 6.25 × N	17.46	17.63	17.71	17.48	17.35	17.25
Dispensable AA						
Aspartic acid	1.19	1.24	1.30	1.16	1.23	1.29
Serine	0.79	0.86	0.85	0.76	0.76	0.78

Glutamic acid	2.71	2.79	2.93	2.57	2.79	2.89
Proline	1.37	1.43	1.48	1.31	1.41	1.44
Glycine	0.73	0.76	0.79	0.71	0.75	0.79
Alanine	1.29	1.36	1.41	1.25	1.33	1.37
Cysteine	0.31	0.33	0.34	0.31	0.32	0.34
Tyrosine	0.66	0.68	0.68	0.61	0.61	0.65
Indispensable AA						
Methionine	0.33	0.35	0.36	0.33	0.34	0.35
Isoleucine	0.70	0.71	0.77	0.69	0.75	0.77
Leucine	2.11	2.21	2.31	2.04	2.18	2.23
Threonine	0.69	0.73	0.75	0.67	0.69	0.71
Phenylalanine	0.90	0.93	0.98	0.87	0.93	0.96
Valine	0.92	0.93	1.01	0.90	0.97	1.01
Lysine	0.66	0.67	0.72	0.65	0.69	0.72
Histidine	0.48	0.50	0.53	0.47	0.51	0.52
Arginine	0.82	0.84	0.88	0.80	0.83	0.87

Tryptophan	0.12	0.15	0.16	0.15	0.16	0.15
Available lysine	0.63	0.64	0.69	0.62	0.66	0.69
Lysine to CP, %	3.78	3.80	4.07	3.72	3.98	4.17
Lysine availability, %	95.5	95.5	95.8	95.4	95.6	95.8
Ash	4.57	4.59	4.75	4.83	4.85	4.86
Ca	0.95	0.89	0.88	0.87	0.80	0.88
P	0.76	0.72	0.74	0.73	0.71	0.69

Table 2.6 Analyzed chemical composition of wheat millrun diets (%, as is, Exp. 2)

Chemical composition	CON	Treatment diet				
		Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C
Moisture	9.87	10.01	9.72	10.17	9.86	9.48
Ether extract	2.68	2.6	2.86	2.59	2.7	2.87
Fiber						
ADF	8.68	8.13	9.14	8.30	8.54	8.43
NDF	21.35	21.86	23.21	22.38	22.24	22.64
Total dietary fiber	20.29	20.96	21.74	20.90	21.13	21.45
Starch	49.67	47.65	46.45	47.92	48.58	49.91
Gross energy, Mcal/kg	3.91	3.88	3.93	3.89	3.87	3.87
Crude protein, 6.25 × N	10.13	9.91	9.72	9.85	10.14	9.90
Dispensable AA						
Aspartic acid	0.57	0.64	0.67	0.62	0.59	0.67
Serine	0.34	0.39	0.39	0.37	0.35	0.42
Glutamic acid	1.91	2.08	2.21	2.07	1.93	2.23

Proline	0.64	0.70	0.74	0.69	0.64	0.74
Glycine	0.44	0.48	0.51	0.48	0.45	0.51
Alanine	0.40	0.44	0.47	0.43	0.41	0.47
Cysteine	0.16	0.18	0.20	0.18	0.17	0.19
Tyrosine	0.22	0.25	0.26	0.24	0.22	0.26
Indispensable AA	0.00	0.00	0.00	0.00	0.00	0.00
Methionine	0.14	0.15	0.15	0.15	0.14	0.15
Isoleucine	0.30	0.32	0.35	0.32	0.30	0.34
Leucine	0.58	0.64	0.68	0.63	0.59	0.67
Threonine	0.27	0.31	0.32	0.30	0.28	0.33
Phenylalanine	0.38	0.42	0.44	0.41	0.39	0.44
Valine	0.41	0.44	0.48	0.44	0.42	0.46
Lysine	0.34	0.37	0.40	0.37	0.35	0.39
Histidine	0.22	0.24	0.25	0.24	0.22	0.25
Arginine	0.53	0.59	0.62	0.58	0.54	0.62
Tryptophan	0.10	0.10	0.10	0.10	0.10	0.10

Available lysine	0.32	0.35	0.38	0.34	0.33	0.37
Lysine to CP, %	3.72	4.03	4.29	3.96	3.78	4.15
Lysine availability, %	94.12	94.59	95.00	91.89	94.29	94.87
Ash	4.93	5.02	5.15	5.39	5.21	5.32
Ca	1.04	1.02	1.03	1.04	1.00	1.10
P	0.67	0.68	0.69	0.68	0.68	0.67

Table 2.7 Characteristic of particle size in corn DDGS diets (Exp. 1)

	Treatment diet					
	CON	Roxazyme	Ronozyme 1	Ronozyme 2	B + C	A + C
Grain characteristics		(A)	(B)	(C)		
Geometric mean particle size, µm	733	731	729	759	773	756
Geometric STDEV of particle size	1.83	1.80	1.83	1.77	1.80	1.76
Particles per gram	130.3	122.2	131.4	97.8	103.2	98.5
Surface area, cm^2/g	0.98	0.98	0.99	0.93	0.92	0.93
Distribution of particles*, %						
Sieve opening, µm						
4000	0.85	0.71	0.96	0.15	0.80	0.40
2360	1.36	1.26	1.41	1.26	1.86	1.21
1700	3.02	2.82	2.97	3.38	3.42	3.23
1180	13.62	13.72	13.02	16.41	15.54	15.10
850	23.93	23.65	23.43	24.38	23.98	24.70
600	24.59	25.26	25.14	24.63	24.94	25.76

425	14.58	14.98	14.88	13.88	13.98	13.89
300	10.36	10.29	10.61	9.54	9.30	9.39
212	4.88	4.74	4.93	4.19	4.12	4.14
150	2.06	1.87	2.01	1.72	1.66	1.77
106	0.65	0.66	0.55	0.45	0.40	0.40
75	0.10	0.05	0.10	0.00	0.00	0.00
53	0.00	0.00	0.00	0.00	0.00	0.00
Pan	0.00	0.00	0.00	0.00	0.00	0.00

*Values are the grams of a 100-g sample retained on top of sieves after 10 min of shaking on a Ro-Tap shaker (W. S. Tyler, Mentor, OH).

Table 2.8 Characteristic of particle size in wheat millrun diets (Exp. 2)

		Treatment diet				
	CON	Roxazyme	Ronozyme 1	Ronozyme 2	B + C	A + C
Grain characteristics		(A)	(B)	(C)		
Geometric mean particle size, µm	504	538	398	396	414	415
Geometric STDEV of particle size	2.04	1.97	2.23	2.28	2.26	2.44
Particles per gram	779	513	2875	3379	2790	5020
Surface area, cm^2/g	1.54	1.4	2.08	2.12	2.02	2.15
Distribution of particles*, %						
Sieve opening, µm						
4000	0.46	0.15	0.81	1.58	1.20	2.94
2360	0.30	0.76	0.61	1.17	0.80	1.11
1700	0.46	0.66	0.56	0.61	0.50	0.61
1180	5.73	6.47	2.99	2.04	3.31	2.63
850	16.84	17.33	11.17	7.85	11.75	9.52
600	24.05	25.42	17.97	16.45	18.47	17.53

425	17.10	18.90	15.99	20.94	15.16	20.16
300	13.75	11.93	16.55	18.80	16.97	13.17
212	7.76	6.87	11.42	8.91	10.34	8.81
150	5.68	5.46	8.78	7.69	9.64	7.29
106	4.92	4.40	6.19	6.27	5.32	9.32
75	2.64	1.67	5.03	5.25	4.72	4.56
53	0.30	0.00	1.73	2.34	1.71	2.23
Pan	0.00	0.00	0.20	0.10	0.10	0.10

[*]Values are the grams of a 100-g sample retained on top of sieves after 10 min of shaking on a Ro-Tap shaker (W. S. Tyler, Mentor, OH).

Table 2.9 Nutrient digestibility and digestible nutrient content of the corn DDGS diets (%; as fed basis, Exp. 1)

Item	CON	Treatment diets					Pooled	*P*-value	
		Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C	SEM	Diet	CON vs. Enzyme
Energy digestibility, %									
AID	67.3	68.4	68.7	70.6	68.2	70.3	1.94	0.76	0.33
ATTD	76.7	77.4	77.6	77.2	77.9	77.4	0.59	0.50	0.09
DE value, Mcal/kg diet									
Ileal digested energy	2.87	2.92	2.93	3.01	2.91	3.00	0.08	0.76	0.32
Total digested energy	3.27	3.30	3.31	3.30	3.32	3.30	0.03	0.43	0.08
Protein digestibility, %									
AID	70.0	71.8	70.1	72.3	71.0	73.0	1.90	0.70	0.38
SID	78.0	79.8	78.1	80.3	78.5	81.0	1.89	0.65	0.38
Protein content, %									
App. ileal digested	13.0	13.4	13.1	13.5	13.3	13.7	0.35	0.70	0.37
Stand. ileal digested	14.6	15.0	14.7	15.1	14.7	15.1	0.35	0.65	0.38

Table 2.10 Nutrient digestibility and digestible nutrient content of corn DDGS [%; as is basis, Exp. 1)

Item	CON	Treatment					Pooled	*P*-value	
		Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C	SEM	Diet	CON vs. Enzyme
Energy digestibility, %									
AID	59.5	61.2	62.8	64.6	60.7	64.1	3.89	0.74	0.29
ATTD	67.7	68.7	69.0	68.5	69.5	68.8	0.90	0.49	0.09
DE value, Mcal/kg									
Ileal digested energy	2.85	2.93	3.01	3.09	2.91	3.07	0.18	0.74	0.29
Total digested energy	3.24	3.29	3.31	3.28	3.33	3.29	0.04	0.42	0.08
Protein digestibility, %									
AID	70.0	71.8	70.1	72.3	71.0	73.0	1.90	0.70	0.38
SID	78.0	79.8	78.1	80.3	78.5	81.0	1.89	0.65	0.38
Protein content, %									
App. ileal digested	20.9	21.4	21.2	21.6	21.2	21.8	0.54	0.81	0.29
Stand. ileal digested	23.3	23.8	23.3	24.0	23.4	24.2	0.56	0.65	0.37

Table 2.11 Nutrient digestibility and digestible nutrient content of wheat millrun diets (as fed basis, Exp. 2)

Item	CON	Treatment diets					Pooled SEM	*P*-value	
		Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C		Diet	CON vs. Enzyme[1]
Ileal digestibility									
Energy, %	70.1[b]	71.9[ab]	71.3[ab]	71.5[ab]	75.5[a]	74.7[ab]	1.62	0.27	0.09
DE, Mcal/kg as fed	2.72[b]	2.79[ab]	2.79[ab]	2.80[ab]	2.94[a]	2.90[ab]	0.06	0.26	0.09
Number of observation	6	6	6	4	4	3			
Total tract digestibility,									
Energy, %	76.8	78.0	78.4	77.6	77.2	78.3	0.85	0.73	0.25
DE, Mcal/kg, as fed	2.99	3.04	3.05	3.02	3.00	3.05	0.03	0.73	0.25
Number of observation	6	6	7	5	4	4			
Protein digestibility, %									
AID	67.1	67.0	66.2	67.6	73.0	71.7	2.16	0.20	0.32
SID	79.5	79.4	78.6	80.0	85.4	84.1	2.15	0.20	0.32
Digestible protein content, %									
AID	6.7	6.7	6.6	6.7	7.2	7.1	0.64	0.20	0.32
SID	7.9	7.9	7.8	7.9	8.5	8.4	0.73	0.20	0.32

[abc]Means within a row without a common superscript differ ($P < 0.05$); [1]Contrast between control vs 5 treatment diets

Table 2.12 Nutrient digestibility and digestible nutrient content of wheat millrun (as is basis, Exp. 2)

Item	CON	Treatment diets[1]					Pooled SEM	P-value	
		Roxazyme (A)	Ronozyme 1 (B)	Ronozyme 2 (C)	B + C	A + C		Diet	CON vs. Enzyme[1]
Ileal digestibility									
Energy,%	58.3[b]	61.2[ab]	60.3[ab]	61.2[ab]	67.3[a]	65.9[ab]	3.02	0.25	0.09
DE, Mcal/kg as fed	2.38[b]	2.50 [ab]	2.47[ab]	2.50[ab]	2.75[a]	2.70[ab]	0.12	0.23	0.09
Total tract digestibility									
Energy,%	67.4	69.4	70.1	68.7	68.0	69.9	1.16	0.73	0.24
DE, Mcal/kg as fed	2.76	2.84	2.86	2.80	2.78	2.86	0.059	0.74	0.25
Protein digestibility, %									
AID	67.1	67.0	66.2	67.6	73.0	71.7	2.16	0.20	0.32
SID	79.5	79.4	78.6	80.0	85.4	84.1	2.15	0.20	0.32
Digestible protein content, %									
AID	11.2	11.2	11.0	11.3	12.2	12.0	0.36	0.20	0.32
SID	13.3	13.3	12.2	13.2	14.1	13.9	0.36	0.24	0.96

[abc]Means within a row without a common superscript differ ($P < 0.05$); [1]Contrast between control vs. 5 treatment diets.

Chapter 3. Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing wheat grain and wheat millrun fed to grower pigs.

3.1 Abstract

High feed cost of current swine production can be ameliorated by feeding co-products such as wheat millrun. However, using wheat millrun as a cereal grain substitute is limited due to its high content of non-starch polysaccharide (NSP) that hinders nutrient digestibility. Wheat millrun can be a potential dietary energy source in swine feed if NSP were hydrolyzed by supplementing NSP-degrading enzymes. Hence, our objective was to determine the effect of NSP-degrading enzymes on nutrient digestibility of diets containing wheat grain (wheat diet) or wheat grain plus wheat millrun (wheat millrun diet). Five ileal-cannulated barrows were fed 5 diets in a 5 × 5 Latin-square design. Nutrient digestibility of diets was evaluated in a 2 × 2 factorial arrangement with a N-free diet to determine the effects of supplemental feed enzymes. Arabinoxylan constituted 50 and 57% of total NSP in wheat grain and wheat millrun, respectively. Wheat grain contained more digestible energy (3.37 vs. 2.36 Mcal/kg) and starch (57 vs. 30%), and less NSP (9 vs. 23%) than wheat millrun. Supplementation of NSP-degrading enzymes increased ($P < 0.01$) the apparent ileal digestibility (AID) of energy (71-74 %), apparent total tract digestibility (ATTD) of energy (74-78%) and standardized ileal digestibility (SID) of CP (89-93%) of the wheat millrun diet but not of the wheat grain diet. Furthermore, SID of amino acids namely: Arg, His,

Trp, Cys, Glu, Gly, and Ser were increased ($P < 0.05$) and tended to be increased ($P < 0.10$) for Leu, Phe, Tyr, and Val by enzyme supplementation to the wheat millrun diet. Supplementation of NSP-degrading enzymes increased ($P < 0.05$) AID of total NSP (30-41%) and ATTD (34-50%) of total NSP in the wheat millrun diet but not the wheat grain diet. In conclusion, supplementing NSP-degrading enzymes improved dietary energy, protein, and AA digestibility in wheat millrun. Thus, the combination of wheat millrun and enzyme supplementation is a potential substituent for cereal grain in diets for grower pigs.

3.2 Introduction

Wheat grain is an energy-yielding feedstuff commonly used in swine diets in western Canada. However, wheat grain is used foremost for flour and ethanol production. Therefore, the price of wheat grain has risen substantially, resulting in higher feed cost that can be ameliorated using the co-product of flour milling, i.e., wheat millrun (Holden and Zimmerman, 1991) that includes the bran, germ, and aleurone. Wheat millrun contains more fiber, protein, mineral and less starch than wheat grain (Belderok et al., 2000, Slominski et al., 2004). Arabinoxylan is main non starch polysaccharide (NSP) in wheat grain and wheat millrun (Degen et al., 2007; Sramkova et al., 2009).

Pigs do not produce digestive enzymes to hydrolyze arabinoxylan present in wheat millrun (Barrera et al., 2004). Therefore, nutrient digestibility of diets containing wheat millrun is limited due to NSP (Nortey et al., 2007; Nortey et al., 2008). Supplementing NSP-degrading enzymes such as xylanase and β-glucanase

could reduce anti-nutritional effects of NSP and thereby increase nutrient digestibility (Diebold et al., 2004; Fang et al., 2007). These enzymes hydrolyze the chemical bonds of NSP and the increase availability of starch, protein, and mineral those are entrapped within cell walls (Kumar and Wyman, 2009; Parkkonen et al., 1997). Pigs could therefore utilize more nutrients from wheat millrun after supplementation of NSP-degrading enzymes. Therefore, we hypothesized that NSP present in wheat grain and wheat millrun limit nutrient digestibility that could be increased by NSP-degrading enzymes.

Previously, the effects of NSP-degrading enzymes on nutrient digestibility of diet containing cereal grain or cereal grain plus co-products were studied (Thacker et al., 1988; Yin et al., 2004; Nortey et al., 2007; Ji et al., 2008); however, the effects were not consistent (Dusel et al., 1998; Diebold et al., 2004; Olukosi et al., 2007; Lyberg et al., 2008). Furthermore, xylanase and β-glucanase did not increase nutrient digestibility of corn DDGS and wheat millrun (Chapters 2 and 3) when xylanase and β-glucanase used at regular, commercial inclusion levels. Thus, the present study determined the effect of a higher dose of NSP-degrading enzymes on nutrient digestibility of diets containing wheat grain and wheat millrun.

3.3 Materials and methods

Experimental procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee for Livestock. Pigs were handled in accordance with guidelines established by the Canadian Council on Animal Care

(CCAC, 1993). The experiment was conducted at Swine Research Technology Centre of the University of Alberta.

3.3.1 Experimental Diet and Design

The wheat millrun used in this study was sourced from Masterfeeds feed mill, Edmonton and was processed in Regina, Saskatchewan. The hard red spring wheat grain was grown in the St. Albert research farm and ground at the feed mill of the University of Alberta.

Five ileal-cannulated barrows were fed 5 diets in 5 × 5 Latin-square design. Nutrient digestibility of diets was evaluated in 2 × 2 factorial arrangement to determine the effect of wheat millrun inclusion and supplemental feed enzymes on nutrient digestibility of diets. The experimental design contained 2 control diets, 2 enzyme-supplemented diets, and 1 corn starch diet. The corn starch diet was used to determine the endogenous N and AA losses. The wheat grain diet contained 96.3% wheat grain and the wheat millrun diet was prepared by partially replacing wheat grain with 40% wheat millrun (Table 3.1). Wheat grain was the sole source of protein, energy, and fiber in the wheat diet. Therefore, the wheat grain diet was used as basal diet to calculate nutrient digestibility and digestible nutrient content of wheat millrun. Chromic oxide (0.4%) was added to the diets as an indigestible marker to calculate nutrient digestibility.

The 2 enzyme-supplemented diets contained a combination of 3 commercial enzyme products (Table 3.7) at increased dietary inclusion levels, namely 0.6 g Roxazyme G2G, 0.4 g Ronozyme WX (CT), and 0.25 g Ronozyme VP (CT) per kg feed, all obtained from DSM Nutritional Products (Basel,

Switzerland). More details about the origin and enzyme activity present in the used enzyme products are described in Appendix 1.The unit of each enzyme activity is defined differently, and these are thus not directly comparable.

The 5 experimental diets were fed to 5 pigs in each period, so that each pig consumed each diet over 5 experimental periods to provide 5 observations per diet. Each 9-d period consisted sequentially a 5-d diet adaptation, 2-d feces collection, and 2-d digesta collection. Pigs were weighed at the start of each period to calculate their daily feed requirement. The daily feed allowance was set at 2.8 × maintenance energy requirement (110 kcal DE × $BW^{0.75}$; NRC, 1998) divided in two daily meals (08:00, 15:00) of approximately equal size. Diets were fed as a dry mash and pigs had free access to water throughout the experiment.

3.3.2 Experimental procedure

Five cross-breed barrows (initial BW 25 ± 1.6 kg; Duroc × Large White) were surgically fitted with a T-cannula at the distal ileum (Sauer et al., 1983; De Lange et al., 1989). Pigs were 27 kg body weight when they started receiving period 1 diets. Pigs were housed individually in metabolic pens measuring 1.2 × 1.2 × 0.9 m (width, length, height). Pen sides were solid PVC plastic boarding with plexiglass windows. Flooring was made from plastic-coated metal. Pens were raised 0.4 m off the floor. Each pen was equipped with a stainless steel, single space dry self-feeder, and a bowl drinker. The room temperature was maintained at 22 ± 2.5ºC. Light was turned on from 08.00 to 20.00.

Feces were collected continuously using plastic bags attached to the skin around the anus for 2 d (Van Kleef et al., 1994). Bags were replaced whenever pig

defecated in the plastic bag. Digesta was collected for 10 hr over 2 d using plastic bags containing approximately 15 mL of 5% formic acid attached to the opened cannula barrel. Bags were removed whenever filled with digesta. Collected feces and digesta were pooled by pig and frozen at –20°C. Prior to analyses, feces and digesta were thawed, homogenized, and sub-sampled. Samples were freeze dried and subsequently ground in a centrifugal mill (model ZM 200, Retsch, Newtown, PA) using a 1-mm screen for energy and CP analyses and 0.5-mm screen for AA and NSP analyses.

3.3.3 Chemical analysis

Samples were analyzed for GE using an adiabatic bomb calorimeter (model 5003, IKA-Werke GMBH and Co KG, Staufen, Germany) and DM by drying at 135°C in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Benzoic acid was used as standard in bomb calorimeter. Chromic oxide content of samples were analyzed by spectrophotometer (model 80-2097-62, LKB-Ultraspec III, Pharmacia, Cambridge, UK) at 440 nm wave length after ashing at 450°C overnight (Fenton and Fenton, 1979). Particle size was measured using 13 sieves (4.00, 2.26, 1.70, 1.18, 0.85, 0.60, 0.43, 0.30, 0.21, 0.15, 0.11, 0.08, and 0.05 mm) and a pan on a sieve shaker (method S319.4; ASAE, 2001). Ingredients, diets, feces and digesta were sent to University of Missouri, Columbia, MO to analyze DM (method 934.01; AOAC, 2006), CP (method 990.03; AOAC, 2006), complete AA profile (method 982.30E; AOAC, 2006), ether extract (method 920.39A; AOAC, 2006), crude fiber (method 978.10; AOAC, 2006), ADF (method 973.18, A-D; AOAC, 2006), NDF (Van Soest et al., 1991), total dietary

fiber (method 985.29; AOAC, 2006), ash (method 942.05; AOAC, 2006), Ca (method 968.08; AOAC, 2006), P (method 946.06; AOAC, 2006), and available lysine (method 975.44; AOAC, 2006) and starch (method 76-13, Amer. Assoc. Cereal Chemists). Ingredients, diets, feces and digesta were analyzed at university of Alberta, Edmonton, AB for total, soluble, insoluble NSP and constituent sugars by gas chromatography (Englyst and Hudson, 1987; Englyst, 1989). Further, experimental diets were sent to DSM Biopract GmbH (Berlin, Germany) to analyze enzyme activity present after the completion of experiment.

3.3.4 Calculations

Nutrient digestibility was calculated using Cr_2O_3 concentrations in feces and digesta in relation to feed. The apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) was calculated using this equation (Stein et al., 2007).

Digestibility, % (DE_C) = $1-(C_D \times N_E) / (C_E \times N_D)$

Where C_D is dietary concentration of Cr_2O_3, N_E is nutrient concentration in excreta, C_E is Cr_2O_3 concentration in excreta, and N_D is dietary concentration of nutrient. Digestibility of diet is referred to as coefficient of digestible energy (DE_C). The DE value (Mcal/kg) of diets was calculated as the product of DE_C and the gross energy content of the diet.

The feedstuffs wheat grain and wheat millrun were considered the sole energy, protein, and NSP sources of the wheat grain and wheat millrun diets (Table 3.1). The energy and protein digestibility of wheat grain diet solely represented the digestibility of wheat grain. Energy and protein digestibility of wheat millrun was

calculated by indirect method using the wheat diet as basal diet (Adeola, 2001; Kim et al., 2005).

Digestibility of test feedstuffs = $[(T \times T_p)-(B \times B_p)]/A_p$

Where, T is digestibility of diet (basal diet plus test feedstuffs); T_p is amount of component in the diet (basal diet plus test feedstuffs); B is digestibility of the component in basal diet; B_p is the proportion of component in the total diet contributed by the basal diet; A_p is the proportion of component in the total diet contributed by the test feedstuff; Tp = Bp + Ap = 100%

Wheat millrun contained more NSP than wheat grain. Therefore, NSP content was higher in wheat millrun diet. Therefore, NSP digestibility of wheat millrun was calculated based on NSP contribution of wheat grain and wheat millrun. For example, 56.3 % of wheat grain and 40% of wheat millrun contribute 36.5 % and 63.5% NSP in wheat millrun diet respectively. Then, NSP digestibility of wheat millrun was calculated as follows

NSP digestibility of wheat millrun = [Digestibility of wheat grain plus wheat millrun diet – (Digestibility of wheat grain diet x 0.365)]/0.635

The basal ileal endogenous (Iend) loss of AA and CP was calculated by using following equation for the N-free diet (Stein et al., 2007).

Iend = {AA or CP in digesta × (Cr_2O_3 in feed/Cr_2O_3 in digesta)}

Standardized ileal digestibility (SID) value for each AA was calculated by correcting the AID of AA with basal endogenous losses (Stein et al., 2007).

SID = {AID + (I_{end}/AA in feed) × 100}

Wheat grain was the sole dietary source of AA in wheat grain diet.

Therefore, digestibility of AA of diet containing wheat gain itself was considered the AA digestibility of wheat grain. Thus, SID AA content of wheat was determined by multiplying SID of wheat diet with AA content of wheat.

3.3.5 Statistical Analyses

The N-free diet was used to determine endogenous N losses but was excluded from statistical analyses. Data were analyzed using the mixed model procedure of SAS 9.2 software (SAS, 2003). Effects of supplemented enzymes were analyzed using a model that included diet as a fixed effect and period and pig as random effects. The individual pig was considered the experimental unit. Statistical carryover test was performed to calculate carryover effect of diets on nutrient digestibility in pigs (SAS Inst. Inc., Cary, NC). To test the hypotheses, $P < 0.05$ was considered significant. If $0.05 \leq P < 0.10$, these were considered trends. In case $P < 0.10$ for the interaction term, the interaction was considered significant and means were separated using PDIFF.

3.4 Results

Pigs remained healthy during the experiment. Orts were not observed; all pigs consumed their daily feed allowance throughout the experiment.

3.4.1 Enzyme activity in diets

Three commercial enzymes products Roxazyme: G2G, Ronozyme WX (CT), and Ronozyme VP (CT) were supplemented to the diets; however, individual enzyme activity was not measured due to due to technological complications of feed analytics of cocktail enzymes. Therefore, total amount of

enzymes product was analyzed and presented as product equivalent per kg diet. Amount of analyzed enzyme product was similar to expected, indicating that enzymes were active and mixed properly into diets (Table 3.7).

3.4.2 Nutrient composition of ingredients and diets

Wheat grain contained more starch, similar CP and Ca, and less fiber and P than wheat millrun (Table 4.2). Mean particle size of ground wheat grain and wheat millrun was 1157 and 371 µm. Arabinoxylan constituted 50 and 57% of total NSP in wheat grain and wheat millrun, respectively (Table 4.3). The amount of insoluble NSP was higher in wheat grain (8 vs. 1%) and wheat millrun (21 vs. 1%).

Due to the difference in nutrient composition between wheat grain and wheat millrun (Table 3.3), nutrient composition differed between wheat grain and wheat millrun diets (Table 3.4); however, the difference was consistent with expectations. Especially NSP content was higher (7%-units) in wheat millrun diet than the wheat diet. The wheat millrun diet was finer than the wheat grain diet (Table 3.6). The difference in protein and gross energy content of diets was small; however, wheat millrun diets contained more NSP (8 vs. 15%; Table 3.5), more lysine (0.38 vs. 0.45%), and less starch (57 vs. 42%; Table 4.4). Lysine availability was 3%-units higher for wheat millrun than wheat.

3.4.3 Nutrient digestibility

Diet and enzyme effects and their interaction were analyzed for nutrient digestibility and digestible nutrient content of diets and the 2 ingredients wheat grain and wheat millrun.

Energy digestibility. Diet type and enzyme interacted ($P < 0.05$; Table 3.8) for ATTD of energy and DE value and tended to interact ($P < 0.10$) for AID of energy and ileal digested energy in the diet. Supplementation of NSP-degrading enzymes did not increase ($P > 0.10$) the AID and ATTD of energy and the DE value in the wheat grain diet. However, supplementation of enzyme increased ($P < 0.05$) the AID and ATTD of energy by 3.6%-units and the DE value by 0.14 Mcal/kg in the wheat millrun diet. Furthermore, between the control diets, the AID and ATTD of gross energy was decreased ($P < 0.05$) by 9% and 13%-units, respectively, when 40% wheat millrun was added to a wheat diet, consequently the ileal digested energy and total digested energy content was reduced ($P < 0.05$) by 0.26 and 0.42 Mcal/kg, respectively. A carry-over effect of diets was measured ($P < 0.05$) for AID of energy in pigs and adjusted by Mixed Procedure in SAS (SAS Inst. Inc., Cary, NC). This effect indicated that the 5-d diet adaptation should have been increased to avoid carryover effects for high-fiber diets.

Nutrient digestibility and digestible nutrient content of ingredients followed similar patterns to that of diets, including the ingredient × enzyme interaction ($P < 0.05$; Table 3.12). Enzyme supplementation increased ($P < 0.01$) the AID and ATTD of energy in wheat millrun by 7 and 8%-units, respectively. Consequently, supplementation of feed enzymes increased ($P < 0.05$) the ileal digested energy and total digested energy content by 0.28 and 0.34 Mcal/kg in wheat millrun.

NSP digestibility. Diet type did not affect AID of NSP ($P > 0.10$; Table 3.9). Diet type and enzyme interacted ($P < 0.05$; Table 3.9) for ATTD of total NSP. Between control diets, the ATTD of total NSP was reduced 23%-units ($P < 0.05$) when 40% wheat millrun was included. Supplementation of NSP-degrading enzymes improved ($P < 0.05$) the AID and ATTD of total NSP by 11 and 16%-units in the wheat millrun diet but did not increase ($P > 0.1$) in the wheat diet. Furthermore, supplementation of enzymes improved ($P < 0.01$; Table 3.12) the AID of total NSP in both feedstuffs, but improved ($P < 0.05$) the ATTD of total NSP only in wheat millrun.

Protein digestibility. Diet and enzyme did not interact for AID and SID of protein in the diet, but tended to interact ($P < 0.10$) for AID and SID digestible protein content of diets (Table 3.8). In the present study, basal endogenous loss for the N-free diet was 21.9 g CP/kg of DM intake, The SID of protein was 6%-units higher ($P < 0.001$) for the wheat diet than the wheat millrun diet. Enzyme supplementation increased ($P < 0.01$) AID and SID of protein by 4%-units in millrun diet but not for wheat diet. Consequently, supplementation of enzymes improved ($P < 0.01$) the SID protein content by 0.6% for the wheat millrun diet but did not increase it for the wheat grain diet. Ingredient and enzyme supplementation interacted ($P < 0.05$; Table 3.12) for SID of protein. The SID of CP was higher ($P < 0.05$) by 12%-units in wheat than wheat millrun. Supplementation of enzyme increased ($P < 0.05$) the SID of protein in wheat millrun by 7%-units but not in wheat. Consequently, supplementation increased SID protein by 1%-unit in wheat millrun.

Ileal digestibility of amino acids. Diet and enzyme did not interact for AID and SID of AA (Tables 3.10 and 3.11). The NSP-degrading enzymes improved ($P < 0.05$; Table 3.11) the SID of Arg, His, Trp, Cys, Glu, Gly, and Ser and tended to improve the SID ($P < 0.10$) of Leu, Phe, Val, and Tyr in the diets; however, supplementation of feed enzymes did not increase digestibility of limiting AA like Lys, Met, and Thr. The improvements ($P < 0.05$) of SID (Table 3.13) and SID content (Table 3.14) of AA in ingredients were similar to the diets.

3.5 Discussion

Feed cost represents more than 60% of variable cost in swine production (Noblet, 2011). Energy is the largest cost component in swine feed cost (Noblet and Milgen, 2004), accounting for 70% of feed cost (Payne and Zijlstra, 2007). Therefore, technologies such as supplementation of exogenous enzymes that improve nutrient digestibility of co-products from food or biofuel industries are important for cost-effective feed formulation. In the present study, supplemented enzymes (xylanase and β-glucanase) improved energy, protein, and AA digestibility in wheat millrun but not wheat grain.

Wheat millrun is a co-product from the flour industry. At milling, the starchy endosperm of wheat grain is removed; thus, wheat millrun comprises wheat bran, germ, and some offal of flour-mills (Holden and Zimmerman, 1991). Consequently, wheat millrun contained more fiber and less starch than wheat grain, consistent with previous studies (Zijlstra et al., 1999; Belderok et al., 2000; Slominski et al., 2004; Nortey et al., 2008). In the present study, CP content of

wheat grain and wheat millrun differed by less, which differed by 4% previously (Slominki et al., 2004), indicating that wheat millrun quality differs among samples. Higher lysine availability in wheat millrun than wheat grain indicated that the milling process like conditioning of wheat grain improved lysine availability. Arabinoxylan was the main NSP in wheat grain and wheat millrun and was mostly insoluble NSP. The NSP digestibility of wheat millrun in grower pig was less (26 vs. 54%) than NSP digestibility of wheat bran in adult sows (Noblet and Milgen, 2004), because sows have a more mature digestive system than grower pigs.

Fiber reduces nutrient digestibility (Noblet and Perez, 1993) and digestibility of fiber is lower than digestibility of starch (Noblet and Le Goff, 2001). High fiber content in diets plays an anti-nutritive role in pigs (Bedford and Schulze, 1998). Pigs do not produce endogenous enzymes to hydrolyze fiber; therefore, fiber digestion depends on bacterial fermentation (Bjergegaard et al., 1991; Choct et al., 2010). Lower energy digestibility in the wheat millrun diet than the wheat grain diet met expectations due to higher NSP and lower starch content in wheat millrun. That increasing fiber reduced energy digestibility was similar to previous research in grower pigs (Yin et al., 2000) indicating that AID of energy decreased by 32% with total NSP increasing from 83 to 193 g/kg. Furthermore, energy digestibility was reduced by 10%-units by substituting 30% wheat grain with wheat millrun in diets fed to grower pigs (Nortey et al., 2008). Fiber binds water and the water-holding capacity of fiber might be as high as to 9 times its weight (Xu et al., 2009). The branched structure of NSP present in wheat

millrun increase the water-holding capacity that stimulates gelling properties of digesta. The gelling properties decrease mixing of digesta components with digestive enzymes (Vahouny and Cassidy, 1985; Choct et al, 1996; Diebold et al., 2004) and increase the unstirred fluid layer creating a physical barrier at the absorption surface on microvilli (Johnson and Gee, 1981). The NSP present in wheat millrun was mostly insoluble. Insoluble dietary fiber increases peristaltic movement of the gut (Jorgensen et al., 1996) and decreases digesta retention time in the small intestine (Wilfart et al., 2007); this also reduces opportunities to mix digestive enzymes and nutrient absorption in small intestine. Therefore, increased gelling properties of digesta, reduced digestive enzymes diffusion rate, and reduced retention time might be reasons for decreased nutrient digestibility of diet when 40% wheat millrun was included in wheat grain diet. Apart from dietary energy, the high fiber content also reduced protein and AA digestibility in wheat millrun diet compared to the wheat diet, similar to grower pigs fed wheat co-products (Nortey et al., 2007a), due to reason explained above. Increasing dietary fiber also increases endogenous losses of N and AA (Schulze et al., 1994; Mariscal-Landín et al., 1995; Souffrant, 2001). Thus, fiber intake reduces apparent digestibility of protein and AA.

In the present study, NSP-degrading enzymes improved energy, protein, and AA digestibility of the wheat millrun diet, but not of the wheat grain diet. The lack of response for wheat grain was similar to previous research (Nortey et al., 2007a; Lyberg et al., 2008), although enzymes previously improved the AID of energy in wheat grain (Widyaratne et al., 2009). The improvement of energy

digestibility in the wheat millrun diet was consistent with previous research (Nortey et al., 2008) that indicated that xylanase improved ATTD of energy by 7%-units in a diet with 30% wheat millrun. The response to NSP-degrading enzymes for wheat millrun but not wheat grain might be due to the greater NSP content and thus more substrate for enzymes in wheat millrun than wheat grain. The NSP-degrading enzymes hydrolyze β-bonds of NSP (Parkkonen et al., 1997; Tapingkae et al., 2008); increase availability of starch, protein and AA that are encapsulated within cell walls (Emiola et al., 2009; Kumar and Wyman, 2009), and increase contact between digestive enzymes and fiber-entrapped nutrient. Enzyme supplementation to NSP-rich diets decreases digesta viscosity (Dusel et al 1998; Vahjen et al., 2007), and low digesta viscosity increases digestive enzymes diffusion rate (Edwards and Johnson, 1988). Exogenous NSP enzymes increased mixing of digesta components with digestive enzymes that coincided with increased digestive enzymes activity in the upper digestive tract of pigs (Wang et al., 2008; Fan et al., 2009). Consequently, greater improvement was calculated in AID than ATTD of energy by NSP-degrading enzymes for wheat millrun. It may further explained by less energy substrate remaining and available for microbial fermentation in large intestine after greater enzymatic digestion in small intestine. The enzyme activity may also deteriorate with passing through the digestive tract (Thacker and Baas, 1996). Exogenous enzymes stimulation of bacterial proliferation is greater in the small intestine (Annison and Choct, 1991; Choct et al., 1996), explaining the greater influence of NSP degrading enzymes on ileal energy digestibility of wheat millrun.

The NSP-degrading enzymes improved protein digestibility of wheat millrun but not of the wheat grain diet. Data on effects of supplementation of exogenous NSP-degrading enzyme on protein digestibility of wheat diets fed to pigs are not consistent. Protein and AA digestibility were improved by supplementation glucanase and xylanase in some studies (Yin et al., 2000; Barrera et al., 2004; Diebold et al., 2004) but not in others (Li et al., 1996; Susenbeth et al., 2011). In our study, supplementation of NSP-degrading enzymes in the wheat millrun diet improved AID of AA, similar to xylanase supplementation improved CP and AA digestibility in grower pigs fed wheat co-products (Nortey et al., 2008). The improved AID of AA could be due to increased availability of AA that was associated with arabinoxylans in wheat millrun. Basal endogenous losses were higher than the average basal endogenous losses measured previously for N-free diet (Boisen and Moughan, 1996). After correction of AID, the SID of glycine and proline was increased more similar to AID of proline in grower pigs fed with wheat grain (Woyengo et al., 2008), because proline and glycine are main constituents of endogenous protein (Nyachoti et al. 1997). The improved AID and SID of AA could also be attributed to reduced secretion of endogenous AA due to hydrolysis of arabinoxylans by NSP degrading enzymes.

The improved energy digestibility of the diet was explained by improved NSP digestibility by NSP-degrading enzymes. Supplementation of exogenous enzymes hydrolyzes binding bonds within the long chain of polysaccharides and releases monosaccharides (Haberer et al., 1997; Parkkonen et al., 1997). Xylanase degrades endosperm cell walls structure (Bedford and Autio, 1996) and releases

soluble, fermentable oligomers (Choct et al., 1996). For example, the mosaccharide arabinose and xylose were released from arabinoxylan after supplementing xylanase (Kumar and Wyman, 2009; Bedford, 1995). The NSP-degrading enzymes thus improved digestibility of NSP and individual moieties (arabinose, xylose, etc.). The lowest AID was measured for galactose. In contrast, pigs fed a rye-wheat based diet had greater AID of galactose than arabinose and xylose (Bartelt et al., 2002). The low AID of galactose might be linked to endogenous secretion of carbohydrate-rich porcine mucin (Lien et al., 2001) that contains galactose and N-acetylgalactosamine (Choi et al., 1991). Because AID of galactose was not corrected for endogenous galactose losses and the resulting value was lower, provided the amount of galactose of endogenous origin present at terminal ileum was greater. This might be the case in our study as the dietary concentration of galactose was low and endogenous excretion of galactose might increase due to the enhanced digesta viscosity. The improved digestibility of all NSP-constituents in response to NSP degrading enzyme indicates that supplemental feed enzymes are able to degrade not only arabinoxylan but also other NSP components, although the response was greatest for arabinoxylan. A similar improvement was reported for arabinoxylan digestibility by Gdala et al. (1997), while xylanase and β-glucanase did not affect digestibility of arabinose and xylose in a wheat-barley based diet fed to pigs (Owusu-Asiedu et al., 2010). Furthermore, NSP-degrading enzymes did not improve AID of glucose and galactose even though the enzymes improved digestibility of other NSP-moieties similar to previous research (Bartelt et al., 2002). The increased digestibility of

NSP explains the improved energy digestibility by NSP-degrading enzymes for wheat millrun.

Reduction of particle size improved nutrient digestibility (Yanez, et al., 2011). However, feed efficiency and nutrient digestibility improved only with coarse particle size-diet supplemented with exogenous enzymes (Mavromichalis et al., 2000; Amerah, 2008). In contrast, in present study NSP-degrading enzyme improved nutrient digestibility in fine particle sized-diet indicating particle size should not limit efficiency of NSP-degrading enzymes on nutrient digestibility of diets although particle sized differed. Supplementation of NSP-degrading enzymes may improve nutritive value of feedstuffs in animal nutrition by this mechanism: breakdown of specific chemical bonds of NSP that are not digested by endogenous enzymes (Kumar and Wyman, 2009) and degradation and increased permeability of plant cell walls (Parkkonen et al., 1997). Combined, nutrient availability is increased of that are encapsulated within cell walls or bound in a chemical form that the animal is unable to digest. Furthermore, NSP-degrading enzymes increase mixing rate of digest components with digestive enzymes. In the present study, enzymes improved nutrient digestibility, indicating that enzymes worked by one or all of these mechanisms. The improved energy digestibility by NSP-degrading enzymes coincided with improved digestibility of NSP, clearly indicating that NSP limits nutrient digestibility in wheat millrun. Therefore, physical barrier of NSP on nutrient digestibility can be reduced by addition of NSP-degrading enzymes and wheat millrun can be used as a cereal grain substituent if NSP-degrading enzymes are supplemented in swine diets.

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Table 3.1 Composition of experimental diets (Exp. 3)

Ingredient, %	Wheat grain		Wheat millrun		N-free
	(-) Enzyme	(+) Enzyme[1]	(-) Enzyme	(+) Enzyme[1]	
Corn starch –Melojel	-	-	-	-	85.3
Wheat grain	96.3	96.3	56.3	56.3	-
Wheat millrun	-	-	40.0	40.0	-
Sugar	-	-	-	-	5.0
Solka floc	-	-	-	-	3.0
Canola oil	-	-	-	-	2.0
Limestone	1.1	1.1	1.1	1.1	1.0
Dicalcium phosphate	0.8	0.8	0.8	0.8	1.2
Salt	0.4	0.4	0.4	0.4	0.5
Vitamin premix[2]	0.5	0.5	0.5	0.5	0.5
Mineral premix[3]	0.5	0.5	0.5	0.5	0.5
KCO_3 56%K	-	-	-	-	0.5
MgO 58%Mg	-	-	-	-	0.1
Chromic oxide	0.4	0.4	0.4	0.4	0.4

[1]Enzyme supplemented diets contained 0.6 g Roxazyme , 0.4 g Ronozyme WX (CT) and 0.25 g Ronozyme VP (CT) per kg feed.

[2]Vitamin premix provided: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantithetic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine,1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg/kg of feed.

[3]Mineral premix provided: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO/kg of feed.

Table 3.2 Analyzed chemical composition of ingredients (as is basis, Exp. 3)

Item, %	Wheat grain	Wheat millrun
Moisture	13.5	11.0
Ether extract	1.77	4.07
Fiber		
Crude fiber	2.33	8.11
ADF	2.95	11.53
NDF	16.25	36.92
Total dietary fiber	11.05	32.40
Starch	57.30	30.31
Gross energy, Mcal/kg	3.80	4.10
Crude protein, N × 6.25	14.4	15.5
Dispensable AA		
Aspartic acid	0.65	0.92
Serine	0.58	0.57
Glutamic acid	3.38	2.81
Proline	1.20	1.00
Glycine	0.53	0.71
Alanine	0.46	0.65
Cysteine	0.27	0.27
Tyrosine	0.33	0.36
Indispensable AA		
Methionine	0.20	0.21

Isoleucine	0.43	0.49
Leucine	0.85	0.94
Threonine	0.38	0.44
Phenylalanine	0.57	0.60
Valine	0.53	0.68
Lysine	0.39	0.59
Histidine	0.28	0.36
Arginine	0.59	0.87
Tryptophan	0.17	0.18
Available lysine[1]	0.35	0.55
Lysine to crude protein, %	2.71	3.81
Lysine availability, %	89.74	93.22
Ash	1.65	4.19
Ca	0.1	0.13
P	0.32	0.75
Particle size, µm	1157	371

[1]Determined according to AOAC Official Method 975.44, Chp. 45.4.03, 2006

Table 3.3 Analyzed monosaccharides and NSP content in wheat grain and wheat millrun (as is basis, Exp. 3)

Item, %	Wheat grain	Wheat millrun
Arabinose		
Total	1.88	4.56
Insoluble	1.59	4.37
Soluble[1]	0.29	0.18
Xylose		
Total	2.72	8.18
Insoluble	2.5	8.21
Soluble	0.22	-0.02
Mannose		
Total	0.15	0.23
Insoluble	0.13	0.20
Soluble	0.02	0.02
Glucose		
Total	4.12	8.96
Insoluble	3.69	7.87
Soluble	0.44	1.09
Galactose		
Total	0.32	0.62
Insoluble	0.16	0.48
Soluble	0.16	0.14

Non-starch polysaccharides		
Total	9.20	22.54
Insoluble	8.07	21.13
Soluble	1.13	1.41

[1]Water soluble NSP is the difference between total and insoluble NSP.

Table 3.4 Analyzed chemical composition of wheat grain and wheat millrun diets (as fed basis, Exp. 3)

Items, %	Wheat grain		Wheat millrun	
	(-) Enzyme	(+) Enzyme	(-) Enzyme	(+) Enzyme
Moisture	12.3	12.2	10.8	11.3
Crude fat	2.12	2.05	3.28	2.9
Fiber				
ADF	2.90	2.73	7.97	6.59
NDF	10.85	11.29	22.38	19.83
Total dietary fiber	9.27	9.83	21.49	17.92
Crude fiber	2.13	2.28	5.82	4.73
Starch	56.8	57.1	40.3	43.8
Gross energy, Mcal/kg	3.73	3.78	3.89	3.83
Digestible energy, Mcal/kg				
Total NSP	7.99	8.21	15.50	14.66
Crude protein (6.25 × N)	14.15	14.11	14.90	14.71
Dispensable AA				
Aspartic acid	0.65	0.61	0.75	0.72
Serine	0.51	0.49	0.50	0.49
Glutamic acid	3.59	3.51	2.95	3.19
Proline	1.21	1.19	1.00	1.08
Glycine	0.52	0.49	0.56	0.55
Alanine	0.45	0.42	0.52	0.50

Cysteine	0.27	0.25	0.23	0.25
Tyrosine	0.37	0.38	0.37	0.37
Indispensable AA				
Methionine	0.19	0.17	0.18	0.19
Isoleucine	0.45	0.44	0.43	0.44
Leucine	0.84	0.83	0.82	0.84
Threonine	0.37	0.35	0.38	0.37
Phenylalanine	0.60	0.58	0.56	0.58
Valine	0.54	0.53	0.55	0.58
Lysine	0.38	0.37	0.46	0.43
Histidine	0.27	0.26	0.29	0.29
Arginine	0.59	0.56	0.70	0.67
Tryptophan	0.18	0.18	0.18	0.19
Available lysine[1]	0.36	0.34	0.42	0.40
Lysine to CP, %	2.66	2.61	3.03	2.93
Lysine availability, %	95.4	92.9	92.2	91.8
Particle size, µm	1,024	1,068	554	718
Ash	4.19	3.37	5.51	5.12
Ca	0.95	0.64	0.86	0.9
P	0.72	0.64	1.05	0.91

[1] Determined according to AOAC Official Method 975.44, Chp. 45.4.03, 2006

Table 3.5 Analyzed monosaccharides and non-starch polysaccharide content in experimental diets (as fed basis, Exp. 3)

	Wheat grain		Wheat millrun	
Item, %	(-) Enzyme	(+) Enzyme[1]	(-) Enzyme	(+) Enzyme[1]
Arabinose				
Total	1.93	1.96	3.23	3.14
Insoluble	1.45	1.45	3.00	2.40
Soluble	0.48	0.51	0.23	0.74
Xylose				
Total	2.71	2.79	5.53	5.26
Insoluble	2.16	2.21	5.55	4.38
Soluble	0.55	0.57	-0.03	0.89
Mannose				
Total	0.16	0.17	0.18	0.21
Insoluble	0.12	0.12	0.19	0.14
soluble	0.04	0.04	-0.01	0.06
Glucose				
Total	2.82	2.87	5.99	5.47
Insoluble	2.42	2.48	5.4	4.7
soluble	0.4	0.39	0.59	0.77
Galactose				
Total	0.33	0.34	0.46	0.47
Insoluble	0.16	0.16	0.34	0.29

Soluble	0.17	0.18	0.12	0.18
Non-starch polysaccharide				
Total	7.95	8.12	15.38	14.55
Insoluble	6.31	6.42	14.48	11.91
Soluble	1.64	1.70	0.90	2.64

Table 3.6 Particle size distribution of wheat grain, wheat millrun, and diets[1]

Item	Wheat grain diet		Wheat millrun diet		Wheat	
	CON	NSP-ase	CON	NSP-ase	Grain	Millrun
Grain characteristics						
Mean particle size, µm	1024	1068	554	718	1157	371
Standard deviation of particle size	2.25	2.18	2.49	2.44	2.11	1.91
Particles per gram	180	126	2473	960	80	1295
Surface area, cm^2/g	0.8	0.8	1.64	1.24	0.69	2.00
Distribution of particles[2], %						
Sieve opening, µm						
4000	0.00	0.00	0.00	0.00	0.00	0.00
2360	4.22	4.54	1.82	2.97	5.59	0.10
1700	27.89	29.19	11.99	18.60	32.45	0.10
1180	24.51	24.88	11.01	15.50	25.63	1.29
850	13.31	13.21	9.26	10.33	12.48	6.77
600	9.62	9.31	13.94	12.56	8.13	17.79

425	5.73	5.51	13.30	10.94	4.52	19.62
300	4.96	4.64	13.47	10.47	3.68	19.82
212	3.29	2.99	8.45	6.72	2.41	12.62
150	2.18	2.02	6.26	5.87	2.04	10.45
106	2.01	2.29	6.33	4.79	2.21	9.24
75	2.01	1.34	3.97	1.22	0.80	2.10
53	0.27	0.07	0.20	0.03	0.07	0.10
Pan	0.00	0.00	0.00	0.00	0.00	0.00

[1]Geometric mean particle size, log normal standard deviation of the particle size, and surface area were determined according to ASAE (1995) procedures.

[2]Values are the grams of a 100-g sample retained on top of sieves after 10 min of shaking on a Ro-Tap shaker (W. S. Tyler, Mentor, OH).

Table 3.7 Supplemented enzymes and results of in-feed analytical determination of enzymes in diets (as fed basis, Exp. 3)

	Wheat grain		Wheat millrun	
Variable, mg/kg	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]
Added enzyme, g/kg feed				
Roxazyme G2G	-	0.600	-	0.600
Ronozyme WX (CT)	-	0.400	-	0.400
Ronozyme VP (CT)	-	0.250	-	0.250
Total analyzed enzyme equivalents, g/kg diet	-	1.382	-	1.302

[1] CON = control diet, without enzyme.

[2] NSP-ase = diet contained combination of NSP-degrading enzymes products

Table 3.8 Nutrient digestibility and digestible nutrient content of wheat and wheat millrun diets (as fed basis, Exp. 3)

	Wheat grain		Wheat millrun		Pooled	*P*-value		
Variable	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Diet type	Enzyme	Diet type × Enzyme
Energy digestibility, %								
AID	79.4[a]	80.4[a]	70.6[c]	74.2[b]	0.90	<0.001	0.007	0.094
ATTD	87.7[a]	89.0[a]	74.4[c]	78.0[b]	0.53	<0.001	0.005	0.011
DE value, Mcal/kg								
Ileal digested energy	2.96[a]	2.99[a]	2.70[c]	2.84[b]	0.03	<0.001	0.009	0.079
Total digested energy	3.27[a]	3.28[a]	2.85[c]	2.99[b]	0.02	<0.001	0.004	0.010
Protein digestibility, %								
AID	80.3	81.3	75.8	79.4	0.7	<0.001	0.009	0.103
SID	94.5	95.5	89.3	92.9	0.7	<0.001	0.009	0.103
Digestible protein content[3], %								
AID	11.4[a]	11.5[ab]	11.2[b]	11.8[a]	0.1	0.54	0.009	0.088
SID	13.4[b]	13.5[ab]	13.2[b]	13.8[a]	0.1	0.54	0.009	0.088

[abc] Within a row, means without a common superscript differ ($P < 0.05$), [1] CON = control, without enzyme, [2] NSP-ase = combination of NSP-degrading enzymes products,[3]CP calculated by N × 6.25.

Table 3.9 NSP digestibility of wheat grain and wheat millrun diets (as fed basis, Exp. 3)

	Wheat grain		Wheat millrun		Pooled	*P*-value		
Variable	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Diet type	Enzyme	Diet type × Enzyme
Ileal digestibility, %								
Arabinose	28.1	33.5	24.1	33.4	4.40	0.508	0.037	0.524
Xylose	28.9	43.0	28.3	44.1	4.66	0.943	0.003	0.814
Mannose	43.4	52.8	36.5	42.0	3.95	0.004	0.009	0.398
Glucose	40.5	38.6	37.7	45.8	4.42	0.586	0.447	0.235
Galactose	13.0	9.2	10.3	8.8	6.33	0.813	0.685	0.860
Total NSP	31.3	37.2	30.0	40.7	3.97	0.713	0.020	0.416
Total tract digestibility, %								
Arabinose	51.4[a]	51.3[a]	30.8[b]	47.3[a]	3.10	0.004	0.029	0.028
Xylose	63.3[a]	63.2[a]	39.0[c]	54.1[b]	2.23	<0.001	0.007	0.007
Mannose	82.0	82.6	70.1	76.0	1.63	<0.001	0.056	0.111
Glucose	54.4[ab]	55.8[a]	32.3[c]	47.8[b]	2.63	<0.001	0.003	0.008
Galactose	63.9[a]	64.0[a]	36.6[c]	48.3[b]	2.06	<0.001	0.005	0.006
Total NSP	57.2[ab]	57.5[a]	34.4[c]	50.0[b]	2.42	<0.001	0.007	0.010

[abc] Within a row, means without a common superscript differ ($P < 0.05$), [1] CON = control, without enzyme.

[2] NSP-ase = combination of commercial NSP-degrading enzyme product

Table: 3.10 Apparent ileal digestibility of amino acids of wheat and wheat millrun diets (as fed basis, Exp. 3)

Variable	Wheat		Wheat millrun		Pooled	*P*-value		
	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Diet type	Enzyme	Diet type × enzyme
Indispensable AA, %								
Arginine	86.5	87.8	85.4	88.8	0.73	0.936	0.013	0.201
Histidine	85.5	86.4	83.1	86.5	1.09	0.263	0.058	0.241
Isoleucine	84.9	85.5	81.7	85.0	1.15	0.137	0.128	0.252
Leucine	86.6	87.2	83.0	86.3	1.00	0.039	0.055	0.179
Lysine	75.6	74.9	76.8	78.1	1.84	0.238	0.872	0.558
Methionine	88.1[a]	86.7[ab]	85.0[b]	87.2[ab]	0.83	0.117	0.596	0.065
Phenylalanine	88.6	89.1	84.6	88.0	0.96	0.021	0.05	0.126
Threonine	75.2	74.6	73.7	75.1	1.85	0.481	0.923	0.321
Tryptophan	90.1	91.1	85.5	89.3	1.11	0.01	0.034	0.166
Valine	82.1	82.2	78.4	82.1	1.26	0.132	0.137	0.161

Dispensable AA, %								
Alanine	74.4	72.8	73.0	76.0	1.7	0.613	0.668	0.179
Aspartic acid	75.8	74.1	75.5	76.7	1.56	0.454	0.874	0.331
Cysteine	87.5[a]	87.1[a]	79.5[b]	85.5[a]	1.5	0.005	0.053	0.033
Glutamic acid	93.4[a]	94.3[a]	89.8[b]	92.9[a]	0.52	0.001	0.004	0.067
Glycine	73.6	75.3	65.5	75.6	2.6	0.141	0.037	0.116
Proline	82.6	88.1	70.5	83.1	5.82	0.134	0.108	0.504
Serine	85.0	85.3	80.8	83.2	0.82	0.003	0.090	0.186
Tyrosine	86.9	87.6	84.2	87.0	0.95	0.081	0.067	0.258
Available Lys	78.3	77.9	78.0	80.2	1.84	0.586	0.631	0.465

[abc]Means within a row without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme.

[2]NSP-ase = combination of NSP-degrading enzymes.

Table: 3.11 Standardized ileal digestibility of amino acids of wheat and wheat millrun diets (as fed basis, Exp. 3)

Variable	Wheat		Wheat millrun		Pooled	*P*-value		
	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Diet type	Enzyme	Diet type × enzyme
Indispensable AA, %								
Arginine	96.7	98.5	94.2	97.8	1.27	0.081	0.013	0.201
Histidine	92.2	93.4	89.4	92.9	1.07	0.131	0.045	0.260
Isoleucine	91.5	92.2	88.6	91.7	1.2	0.222	0.151	0.347
Leucine	92.7	93.5	89.4	92.5	1.00	0.059	0.067	0.247
Lysine	85.9	85.5	85.4	87.3	1.7	0.845	0.667	0.506
Methionine	92.1	91.4	89.4	91.3	0.97	0.179	0.452	0.150
Phenylalanine	93.7	94.3	90.1[b]	93.4	1.1	0.042	0.061	0.182
Threonine	90.5	89.7	87.4	89.4	1.9	0.522	0.714	0.364
Tryptophan	96.9	97.9	92.2	95.7	1.2	0.014	0.045	0.220
Valine	91.6	92.3	87.7	91.2	1.2	0.098	0.117	0.263

Dispensable AA, %								
Alanine	87.3	86.7	84.1	87.8	1.7	0.409	0.360	0.214
Aspartic acid	87.2	86.2	85.3	87.2	1.6	0.775	0.760	0.361
Cysteine	93.6[a]	93.7[a]	86.6[b]	92.1[a]	1.5	0.011	0.048	0.062
Glutamic acid	95.8	96.9	92.8	95.7	0.56	0.004	0.005	0.124
Glycine	98.1	101.3	88.6	99.3	2.8	0.025	0.021	0.158
Proline	116.9	122.6	111.9	121.8	7.7	0.386	0.129	0.666
Serine	94.8	95.5	90.7	93.4	1.1	0.008	0.050	0.224
Tyrosine	93.22	93.87	90.6	93.3	0.9	0.100	0.086	0.262
Available Lys	87.73	87.86	86.1	88.9	1.7	0.704	0.402	0.447

[abc]Means within a row without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme;

[2]NSP-ase = combination of NSP-degrading enzymes.

Table 3.12 Nutrient digestibility and digestible nutrient content of wheat grain and wheat millrun (as is basis, Exp. 3)

	Wheat		Wheat millrun		Pooled	*P*-value		
Variable	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Ingredient	Enzyme	Ingredient × Enzyme
Energy digestibility,%								
AID	79.4[a]	80.4[a]	58.9[c]	65.5[b]	1.63	<0.001	0.016	0.044
ATTD	87.7[a]	87.9[a]	56.9[c]	65.2[b]	1.06	<0.001	0.004	0.005
DE value, Mcal/kg								
Ileal digested energy	3.05[a]	3.07[a]	2.44[b]	2.72[a]	0.06	<0.001	0.015	0.041
Total digested energy	3.37[a]	3.38[a]	2.36[c]	2.70[b]	0.04	<0.001	0.004	0.005
Protein digestibility, %								
AID	80.3[ab]	81.3[a]	70.1[c]	76.8[b]	1.18	<0.001	0.010	0.036
SID	94.5[a]	95.5[a]	82.7 [c]	89.5[b]	1.18	<0.001	0.010	0.037
Digestible protein content, %								
AID	11.4[a]	11.5[a]	10.8[b]	11.9[a]	0.19	0.013	0.009	0.033
SID	13.6[a]	13.7[a]	12.8[b]	13.9[a]	0.18	0.012	0.009	0.033
NSP digestibility, %								

AID	31.3	42.2	28.6	43.7	3.51	0.035	0.006	0.603
ATTD	57.4[a]	57.5[a]	26.1[c]	46.0[b]	2.75	0.001	0.018	0.019

[abc]Means within a row without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme;

[2]NSP-ase = combination of NSP-degrading enzymes.

Table 3.13 Standardized ileal digestibility of amino acid in wheat grain and wheat millrun (as is basis, Exp- 3)

	Wheat		Wheat millrun		Pooled	*P*- value		
Item	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Ingredient	Enzyme	Ingredient × enzyme
Indispensable AA,%								
Arginine	96.7	98.5	92.7	99.1	1.88	0.120	0.049	0.222
Histidine	92.2	93.6	85.3	93.1	2.23	0.097	0.076	0.189
Isoleucine	91.5	92.2	83.9	91.1	2.58	0.161	0.166	0.252
Leucine	92.6	93.7	83.9	91.3	2.12	0.042	0.081	0.171
Lysine	85.9	85.6	86.7	91.9	3.38	0.554	0.484	0.444
Methionine	92.1[a]	91.4[a]	85.0[b]	91.7[a]	1.85	0.047	0.104	0.057
Phenylalanine	93.6	94.5	84.2	92.0	2.01	0.022	0.057	0.114
Threonine	90.6	89.5	82.49	89.9	3.43	0.316	0.369	0.23
Tryptophan	96.9	88	85.3	93.2	2.31	0.009	0.058	0.133
Valine	91.6	92.3	82.1	90.8	2.52	0.66	0.099	0.153

Dispensable AA, %								
Alanine	87.4	86.8	81.1	92.0	3.15	0.196	0.140	0.109
Aspartic acid	87	86.4	84.4	90.8	3.12	0.554	0.385	0.283
Cysteine	93.7[a]	93.6[a]	75.1[b]	90.2[a]	3.38	0.009	0.044	0.043
Glutamic acid	95.8[a]	96.8[a]	87.8[b]	93.7[a]	1.07	0.001	0.014	0.06
Glycine	99[a]	101[a]	77.0[b]	99.7[a]	5.66	0.039	0.050	0.097
Proline	117.4	124.6	95.2	116.6ab	10.8	0.177	0.136	0.446
Serine	94.8	95.4	84.5	90.3a	1.7	0.006	0.091	0.163
Tyrosine	93.2	94.0	86.4	92.2ab	1.8	0.064	0.107	0.201
Available Lys	87.6	88.0	85.5	92.4	3.73	0.629	0.359	0.404

[abc]Means within a row without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme;

[2]NSP-ase = combination of NSP-degrading enzymes.

Table 3.14 Standardized ileal amino acids content in wheat grain and wheat millrun (DM basis, Exp-3)

Item	Wheat		Wheat millrun		Pooled	*P*- value		
	CON[1]	NSP-ase[2]	CON[1]	NSP-ase[2]	SEM	Ingredient	Enzyme	Ingredient × Enzyme
Indispensable AA								
Arginine	0.63	0.64	0.93	1.0	0.017	<0.001	0.047	0.145
Histidine	0.28	0.29	0.34	0.37	0.008	<0.001	0.080	0.232
Isoleucine	0.48	0.48	0.47	0.51	0.014	0.335	0.190	0.279
Leucine	0.89	0.90	0.89	0.97	0.022	0.109	0.086	0.169
Lysine	0.36	0.36	0.57	0.61	0.021	<0.001	0.477	0.426
Methionine	0.21[b]	0.21[b]	0.21[b]	0.23[a]	0.004	0.044	0.152	0.075
Phenylalanine	0.62	0.63	0.59	0.64	0.014	0.109	0.056	0.109
Threonine	0.36	0.36	0.40	0.43	0.015	0.015	0.392	0.216
Tryptophan	0.2	0.20	0.19	0.20	0.005	0.118	0.058	0.115
Valine	0.58	0.58	0.64	0.71	0.019	0.004	0.117	0.137

Dispensable AA								
Alanine	0.43[c]	0.43[c]	0.60[b]	0.68[a]	0.021	<0.001	0.122	0.092
Aspartic acid	0.63	0.63	0.90	0.97	0.031	<0.001	0.360	0.305
Cysteine	0.28[a]	0.28[a]	0.22[b]	0.27[a]	0.01	0.008	0.036	0.037
Glutamic acid	3.93[a]	3.97[a]	2.9[c]	3.1[b]	0.039	<0.001	0.011	0.070
Glycine	0.57[b]	0.57[b]	0.60[b]	0.78[a]	0.042	0.019	0.046	0.075
Proline	1.64	1.73	1.06	1.31	0.129	0.008	0.151	0.519
Serine	0.53	0.54	0.47	0.50	0.010	0.008	0.101	0.234
Tyrosine	0.36	0.37	0.37	0.40	0.007	0.061	0.107	0.158
Available Lys	0.35	0.35	0.52	0.57	0.022	<0.001	0.371	0.371

[abc]Means within a row without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme.

[2]NSP-ase = combination of NSP-degrading enzymes.

Chapter 4. General Discussion

4.1 Introduction

Feed is largest variable cost in pork production and dietary energy represents the greatest proportion of this cost (Noblet and Milgen, 2004; Payne and Zijlstra, 2007; Noblet, 2011). Cereal grains have historically been the main dietary energy source in commercial swine diets. Presently, cereal grains are increasingly used for ethanol production. Consequently, feed cost has recently increased drastically. As such, inclusion of co-products such as distillers dried grains with solubles (DDGS) and wheat millrun in swine diets might be economically attractive, but also poses challenges such as reduced nutrient digestibility. Co-products contain more dietary fiber, protein, and minerals than the parent grain (Slominski et al., 2004). Nutrients entrapped in fiber are not readily available to the pig, because pigs do not produce enzymes to digest fiber (Barrera et al., 2004). High dietary fiber reduces nutrient digestibility in pig and is thus considered as an anti-nutritional factor in pigs (Englyst, 1989; Zijlstra et al., 1999). The NSP-degrading enzymes such as β-glucanase and xylanase reduce the anti-nutritional effects of dietary fiber and thereby increase nutrient digestibility (Diebold et al., 2004; Fang et al., 2007). Previously, many studies were conducted to determine the effects of NSP-degrading enzymes on nutrient digestibility on cereal-based diet fed to grower pigs; however, limited research has been conducted to determine the effects of β-glucanase and β-xylanase on nutrient digestibility of co-products. Furthermore, effects of exogenous enzymes on

nutrient digestibility are inconsistent (Adeola and Cowieson, 2011). Nutritionists are increasingly interested in incorporating co-products with supplemental fiber-degrading enzymes in swine diet, because these enzymes have potential beneficial effects on growth performance, nutrient digestibility, gut health, and nutrient excretion in pigs (Chapter 1). With this background, this thesis project was conceptualized and implemented to determine the effect of NSP-degrading enzymes including endo β-xylanase, endo β-glucanase on nutrient digestibility of diets containing corn DDGS, wheat millrun, wheat grain, or wheat grain plus wheat millrun as fiber sources for growing pigs.

4.2 Effect of NSP-degrading enzymes on nutrient digestibility

Effects of NSP-degrading enzymes on nutrient digestibility were studied in 3 experiments (Chapters 2 and 3). The hypothesis was that feed enzymes would increase nutrient digestibility; however, the results summarized in Chapters 2 did not confirm the hypothesis and indicated that a low level of supplemental feed enzymes did not increase nutrient digestibility of corn DDGS and wheat millrun in grower pigs. However, an increased dose of supplemental NSP-degrading enzymes increased digestibility of energy, protein, NSP, and some amino acids in the wheat millrun diet (Chapter 3) indicating a likely dose response. Enzyme activity per kg feed was lower in Chapter 2 than in Chapter 3. A higher concentration of NSP-degrading enzymes may thus increase nutrient digestibility of feedstuffs (Chapter 3).

In Chapter 2, diets were formulated on a cornstarch base. Such starch is almost completely digested at the end of the small intestine with digestibility values above 99% (Bach Knudsen and Canibe, 1997). Thus, concentration of undigested co-products in digesta increased in the distal part of the intestine for pig fed with the diet containing co-products and cornstarch. Both corn DDGS and wheat millrun (Chapter 2) contained less starch and more insoluble fiber. A high amount of insoluble fiber increased peristaltic movement and reduced digesta transit time in small intestine of pigs (Jorgensen et al., 1996; Wilfart et al., 2007). Increased peristaltic movement and reduced digesta transit time reduced mixing of dietary components with digestive enzymes (Wenk, 2001; Johnston et al., 2003), thereby reduced nutrient digestibility. Therefore, lower concentration of NSP-degrading enzymes and increased co-products concentration in digesta towards the distal intestine could be reasons that supplemented NSP-degrading enzyme did not improve nutrient digestibility of corn DDGS and wheat millrun diets (Chapter 2). In addition, both corn DDGS and wheat millrun were finely ground. Supplementation of fiber- degrading enzyme was less effective to increase nutrient digestibility in pigs when diets contained finely ground feedstuffs (Mavromichalis et al., 2000; Amerah, 2008). This could be another reason that a lower dose of NSP-degrading enzymes did not increase nutrient digestibility of corn DDGS and wheat millrun (Chapter 2).

Feeding diets with more fiber increases VFA production and lowers pH in the digestive tract (Drochner, 1991; Yongxi et al., 2002; Nortey, 2007). Enzyme activity declines at low pH and with increased time in the digestive tract (Thacker

and Baas, 1996, 1996a). The NSP-degrading enzymes need a neutral pH environment for optimum performance (Yen, 2001). Thus, low digesta pH might be linked to NSP-degrading enzymes not improving nutrient digestibility (Chapter 2). However, in Chapter 2, combined xylanase and glucanase from enzyme products Ronozyme-WX(CT) and Ronozyme-VP(CT) increased AID of energy in wheat millrun diet compared to control, indicating that these enzyme activities were more effective than others to improve nutrient digestibility of wheat millrun. A similar improvement in AID of energy was measured for xylanase supplementation (Nortey, 2007). The improved AID of energy likely reduced hindgut fermentation, indicating that enzymes shifted the site of digestion from the lower to the upper digestive tract thereby enhancing nutrient utilization.

In Chapter 3, a higher dose of NSP-degrading enzymes improved nutrient digestibility of wheat grain plus wheat millrun diet but not the wheat grain diet, confirming that NSP-degrading enzymes improved nutrient digestibility of wheat millrun that contained more NSP than wheat grain. The NSP are long chains of repeated carbohydrate monomers and nutrient can be entrapped inside NSP or fiber (Bosscher et al., 2003). Exogenous enzymes hydrolyze the NSP and release fiber-trapped nutrients (Kumar and Wyman, 2009; Parkkonen et al., 1997), and increase contact between digestive enzymes and nutrient released from fiber. Fiber-degrading enzymes supplementation to NSP-rich diets decrease gelling properties of digesta (Dusel et al 1998; Vahjen et al., 2007). These, in turn, increases digestive enzymes diffusion rate (Edwards and Johnson, 1988). Exogenous NSP enzymes may increase digestive enzyme activity (Wang et al.,

2008; Fan et al., 2009) and increase Bifidobacteria spp. populations in wheat grain diets (Garry et al., 2007). Therefore, supplementation of NSP-degrading enzyme improved the nutrient digestibility in wheat millrun, whereas the NSP contained in wheat grain did not limit the nutrient digestibility sufficiently for the enzymes to show beneficial effects.

4.3 Limitations and future research

Wheat millrun contained more insoluble fiber. A high amount of dietary insoluble fiber increased water-holding capacity (Owusu-Asiedu et al., 2006) consequently increased bulkiness of digesta and reduced feed intake in pigs (Ogle, 2006). Therefore, adding fibrous wheat millrun to a cornstarch diet might cause difficulties with cannulas and digesta collection (Chapter 2). Consequently, fewer observations were made per diet, indicating that diet formulations on cornstarch base should be avoided in future studies with fibrous co-products.

In Chapter 3, pigs fed the wheat grain diet took more time to finish their daily feed allowance than pigs fed the wheat grain plus wheat millrun diet, indicating that taste or particle size might have been an issue. Particle size greatly influences nutrient digestibility and efficacy of fiber-degrading enzymes (Mavromichalis et al., 2000; Amerah, 2008; Yanez et al., 2011). Mean particle size was greater for ground wheat grain than for wheat millrun (1157 vs. 371 µm). However, a feed particle size of 600 to 800 microns is recommended for most commercial swine diets (Whitney, 2007). This variable was not standardized

(Chapter 3), and less of a difference in particle size is recommended for future digestibility studies.

Low NSP-degrading enzymes concentration did not improve nutrient digestibility of wheat millrun (Chapter 2) but energy, protein and AA digestibility was improved at higher concentration of NSP-degrading enzymes (Chapter 3) Therefore, further digestibility study is required with graded levels of NSP-degrading enzymes to determine if a dose-response exists.

Fiber degrading-enzyme supplementation adds cost and adds benefit in swine feeding. Their most important benefit is less quantity of costly feedstuffs in swine diets. The calculation of actual dietary costs associated with supplementing exogenous enzymes and substitution with co-products allows cost effective feed formulation. This thesis did not include growth performance study of pigs. Therefore, cost benefit and growth performance study is required in commercial farm condition to verify the advantage of supplementing NSP-degrading enzymes.

4.4 Conclusions and implications

The results confirmed that:

- A low dose of NSP-degrading enzymes did not improve nutrient digestibility of corn DDGS and wheat millrun, showing that NSP present in corn DDGS and wheat millrun did not match with enzyme activity, NSP contained in corn DDGS and wheat millrun was not the limiting factor for nutrient digestibility, or added NSP-degrading enzymes activity was not sufficient in diets (Chapter 2).

- The NSP-degrading enzymes improved nutrient digestibility of wheat millrun, indicating clearly that NSP content in wheat millrun limits nutrient digestibility that can be improved by supplementation of NSP-degrading enzymes (Chapter 3).

The improvement of nutrient digestibility of wheat millrun has broadened the opportunity of wheat millrun to substitute costly cereal grain in swine diets with the supplementation of NSP-degrading enzymes. In practice, NSP-degrading enzymes can increase energy and protein digestibility of diets rich in wheat fiber from flour milling; however, attention should pay on enzyme cost and benefits while adding to fiber-rich feedstuffs.

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Appendix 1. Description of enzymes

1. Roxazyme G2G

Roxazyme G2G is soluble in water and odorless granule. It contains an enzyme complex derived from *Trichoderma longibrachiatum*. The main activities of the enzyme complex are endo-1,4-β- glucanase, endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase. The prefix "endo" refers to the function of glucanase and xylanase that supposes to hydrolyze 1-4 and 1,3(4) bonds of NSP components.

Enzyme activity:

Endo-1,4-β-glucanase activity: min. 8 000 units per gram

Endo-1,3(4)-β-glucanase activity: min. 18 000 units per gram

Endo-1,4-β-xylanase activity: min. 26 000 units per gram

Recommended dosage range:

50–200 g per tonne of complete feed.

Stability and storage:

Roxazyme G2G is practically stable at room temperature and can be stored for 18 months at a temperature below 25 °C.

Unit definition:

The unit definition of enzyme activity is the amount of enzyme (10 units) that releases 1 μmol of reducing moieties from the substrate per minute at pH 5.8, incubation temperature 40°C, and incubation time 20 min.

2. Ronozyme WX (CT)

Ronozyme WX (CT) is a non-dusty, light-brown granulated heat-stable endo-xylanase from *Thermomyces lanuginosus*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. The main activity is endo-1,4-β-xylanase. The prefix "endo" refers to the function of β-xylanase that supposes to hydrolyze 1-4 bonds of arabinoxylan. The bulk density is approx. 1.1 g/mL, and the average particle size is approx. 600 μm.

Fungal xylanase activity: min. 1000 fungal xylanase units (FXU) (W) per gram

Dosage range:

150–300 g per tonne of feed, mixed into the feed.

Stability and storage

Ronozyme WX (CT) maintains its declared activity for at least 12 months from the date of manufacture at a temperature below 25°C, and for at least 24 months from the date of manufacture at a temperature below 5 °C, when stored in the unopened original container.

Unit definition:

One fungal xylanase unit (FXU) is the amount of enzyme that releases 7.8 μmoles of reducing sugars (xylose equivalents) from wheat arabinoxylan per minute under the following reaction conditions:

pH:	6.0
Incubation temperature:	50°C
Incubation time:	30 min
Substrate concentration:	0.45% AZO-wheat arabinoxylan

3. Ronozyme VP (CT)

Ronozyme VP (CT) is a non-dusty, light-brown granulated carbohydrase preparation produced by submerged fermentation of an *Aspergillus aculeatus* microorganism. It contains endo-1,3(4)-β-glucanase, pentosanase, hemicellulase and pectic-substance hydrolyzing activities. The bulk density is approximately 1.1 g/mL, and the average particle size is 500–600 μm.

Fungal β-glucanase activity: min. 50 fungal β-glucanase units (FBG) per gram

Dosage range:

200–1200 g per tonne of feed, mixed into the feed.

Stability and storage:

Ronozyme VP (CT) is sensitive to heat. The product maintains its declared activity for at least 24 months when is stored in the unopened original container and at a temperature below 25 °C.

Unit definition:

One fungal beta-glucanase unit (FBG) is the amount of enzyme that releases 1 μmol of reducing sugars (glucose equivalents) from ß-glucan per minute under the following reaction conditions.

pH:	5.0
Incubation temperature:	30°C
Incubation time:	30 min
Substrate concentration:	0.5% ß-glucan

Appendix 2. Abstract of oral presentation in ASAS/ASDA joint annual meetings 2011[1]

Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing wheat and wheat millrun fed to grower pigs.

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A critical issue of current swine production is high feed cost that might be ameliorated by co-products including wheat millrun. However, feedstuffs such as millrun have physicochemical limitations such as a high non-starch polysaccharide (NSP) content. The NSP hinder nutrient digestibility but are also a potential energy source if hydrolyzed by bacteria or NSP-degrading enzymes. The objective was to determine the effect of NSP-degrading enzymes on diets containing wheat or wheat and wheat millrun on nutrient digestibility. Effects of diet (96% wheat or 56% wheat plus 40% wheat millrun) and xylanase (0 or 16,000 units xylanase and 15,600 units β-glucanase/kg of feed) were investigated in a 2 × 2 factorial arrangement with a N-free control diet for a total of 5 diets. Five pigs were fed 5 diets in a 5 × 5 Latin square. Arabinoxylans constituted 50 and 57% of the total NSP in wheat and millrun, respectively. The wheat used in this study contained 3.80 Mcal DE/kg of DM, 16% CP, and 11% NSP, whereas the millrun contained 2.90 Mcal DE/kg of DM, 17% CP, and 25% NSP. Supplementation of NSP-degrading enzymes to the wheat diet did not alter the

apparent ileal digestibility (AID) of energy, AID of CP, or the apparent total tract digestibility (ATTD) of energy. Supplementation of NSP-degrading enzymes to the wheat millrun diet increased ($P < 0.05$) the AID of energy and CP by 5% and increased ($P < 0.05$) the ATTD of energy by 5%. The improved energy digestibility of the millrun diet was supported by an increase ($P < 0.05$) of the AID and ATTD of NSP by 36 and 47%, respectively. Supplementation of NSP-degrading enzymes increased ($P < 0.05$) the content of ileal digested energy and DE of millrun by 0.34 & 0.41 Mcal/kg of DM. In conclusion, exogenous NSP-degrading enzymes match with the NSP contained in wheat millrun and can improve energy and protein digestibility of diets containing wheat millrun for grower pigs.

KEYWORDS

Pig Wheat millrun Xylanase

[1]ASAS/ASDA Joint Annual Meeting, July 10-14, 2011, New Orleans, Lousiana, USA

Appendix 3. Poster presented in Western Nutrient Conference, 2011[2]

Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing wheat and wheat millrun fed to grower pigs

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Introduction

High feed cost of current swine production can be ameliorated using co-products such as wheat millrun. However, use of wheat millrun as a cereal grain substituent is limited due to its high non-starch polysaccharide (NSP) content that hinders nutrient digestibility (Zijlstra et al., 1999; Slominski et al., 2004, Nortey et al., 2008). Wheat millrun can be a potential energy source in swine feed if NSP is hydrolyzed by supplementing NSP-degrading enzymes.

Hypothesis

- We hypothesized that nutrient digestibility of wheat and wheat millrun is limited by its NSP content, and this limitation can be reduced by supplementation of NSP-degrading enzymes.

Objectives

- The objectives were to determine the effect of NSP-degrading enzymes on energy, protein and AA digestibility of diets containing wheat or wheat plus wheat millrun fed to growerpigs.

Results

Arabinoxylan a main component of NSP constituted 50 and 57% of total NSP in wheat and wheat millrun respectively. Compared to wheat millrun more digestible energy (3.24 vs. 2.53 Mcal/kg), more starch (57 vs. 30%) but low NSP (9 vs. 23%) were measured in wheat grain. Supplementation of NSP-degrading enzymes improved standardize ileal digestibility (SID) of CP (89-92%), apparent ileal digestibility (AID) of energy (70-74%) and apparent total tract digestibility (ATTD) of energy (74-78%) in wheat millrun diet ($P < 0.01$) but did not improve energy and protein digestibility in wheat grain based diet. Furthermore, SID of amino acids viz. Arg, His, Leu, Phe, Try, Cys, Glu, Gly, and Ser were improved by such supplementation in wheat millrun diet ($P < 0.05$). Improved energy, protein, and AA digestibility of wheat millrun diet was considered due to improved energy and NSP digestibility of wheat millrun after enzyme supplementation ($P < 0.001$).

Methodology

Effects of NSP degrading enzyme was investigated in a 2 2 factorial arrangement with a corn starch diet for a total of 5 diets. Treatment diets contained combination of 3 enzymes namely Roxazyme-G2G, Ronozyme-WX(CT) and Ronoyme-VP(CT) to supply fungal β-glucanase, fungal xylanase, endo β-glucanase and endo β-xylanase. Five barrows were fed with 5 diets in a 5 5 Latin square design. Each 9-d period contained sequentially a 5-d diet adaptation, 2-d feces collection, and 2-d digesta collection. Data were analyzed using Proc Mixed procedure in SAS (SAS Inst. Inc., Cary, NC). In the model, diet was fixed effect, period and pig were random term and pig was experimental unit. To test the hypotheses, $P < 0.05$ was considered significant.

Table 1: Chemical composition of ingredients and diets.

	Wheat grain		Wheat millrun		
	CON[1]	NSP-ase	CON	NSP-ase	N-free
Ingredient, %					
Corn starch	-		-	-	85.3
Wheat grain	96.3	96.1	56.3	56.1	-
Wheat millrun	-	-	40.0	40.0	-
Enzymes	-	0.2	-	0.2	-
Sugar	-	-	-	-	5.0
Solka floc	-	-	-	-	3.0
Canola oil	-	-	-	-	2.0
Others[2]	3.7	3.7	3.7	3.7	4.7
Chemical composition, % as is					
Crude fat	2.1	2.1	3.2	2.9	2.0
Ca	0.9	0.6	0.9	0.9	0.7
P	0.7	0.6	1.0	0.9	0.4
ADF	2.9	2.7	7.9	6.6	0.2
NDF	10.9	11.3	22.4	19.8	3.4
Starch	56.8	57.1	40.3	43.8	76.4
Total NSP	8.0	8.1	15.4	14.7	N/A
Crude protein	14.2	14.1	14.9	14.7	N/A

[1]CON = CONTROL, [2]Minerals, vitamins, Cr_2O_3, salt, limestone, dical. phos.

Table 2: Details of supplemented enzymes (g/kg) in diets.

Enzyme	U/kg feed	Enzyme activity	Enzyme efficiency
Roxazyme-(G2G)	31,200 U	Endo-β-glucanase & Endo-β-xylanase	10 U = 1 µmole
Ronozyme, WX (CT)3	400 FXU	Fungal xylanase	1 FXU= 7.8 µmole
Ronozyme, VP (CT)	12.5 FBG	Fungal β-glucanase	1 FBG = 1 µmole

Fig. 1: Eenergy digestibility of diets.

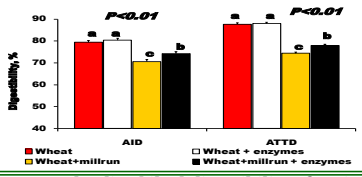


Fig. 2: Protein digestibility of diets

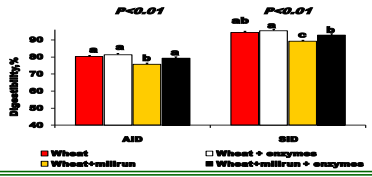


Fig.3: Standardized ileal digestibility of amino acid in diets.

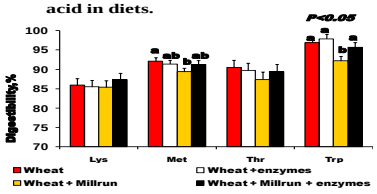


Fig. 4: Apparent ileal digestibility of NSP in diets

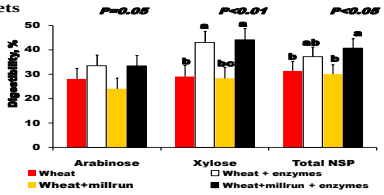
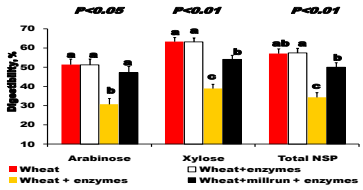


Fig.5: Apparent total tract digestibility of NSP in diets



Conclusion

- Energy, protein, and AA digestibility is limited by NSP in wheat millrun which can be improved by supplementation of NSP degrading enzymes
- Wheat millrun can be a potential alternative feed ingredient to substitute energy rich cereal gain

Implications

- NSP degrading enzymes can be used to increase energy and protein digestibility of diets rich in wheat fiber from flour milling
  - However, attention should be paid on cost and benefit of enzymes.

Acknowledgements

- DSM Nutritional Products (Basel, Switzerland)
- Agriculture and Food Council Alberta (Ed., Alberta)
- Alberta Livestock and Meat Agency (Ed., Alberta)
- Alberta Advanced Education and Technology (Ed., Alberta)

References

1. Slominski, B. A., D. Boros, L. D. Campbell, W. Guenter, and O. Jones. 2004. Wheat by–products in poultry nutrition. part I. chemical and nutritive composition of wheat screenings, bakery by–products and wheat mill run. Can. J. Anim. Sci. 84:421–428.
2. Nortey, T. N., J. F. Patience, J. S. Sands, N. L. Trottier, and R. T. Zijlstra. 2008. Effects of xylanase supplementation on the apparent digestibility and digestible content of energy, amino acids, phosphorus, and calcium in wheat and wheat by–products from dry milling fed to grower pigs. J. Anim. Sci. 86:3450–3464.
3. Zijlstra, R. T., C. F. M. de Lange, and J. F. Patience. 1999. Nutritional value of wheat for growing pigs: Chemical composition and digestible energy content. Can. J. Anim. Sci. 79:187–194.

[2]Western Nutrient Concerefe, September 14 – 15, 2011, Edmonton, Alberta, Ca

Appendix 4. Thesis presentation, 2011

Effect of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing co-products fed to grower pigs

Dharma Shrestha

MSc Thesis Defense Seminar

2011

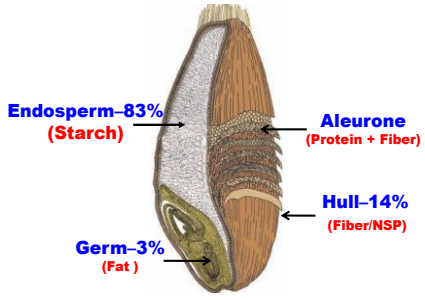
Introduction

- DDGS and Wheat millrun are available co-products
- Co-products are rich in fiber, CP, Min., Vit. *(Robinson et al., 2008)*
- High dietary fiber reduced nutrient digestibility *(Degen et al., 2007)*
- Fiber is source of energy *(Bach Knudsen, 2005)*
- Co-product a potential substituent of grain

Introduction

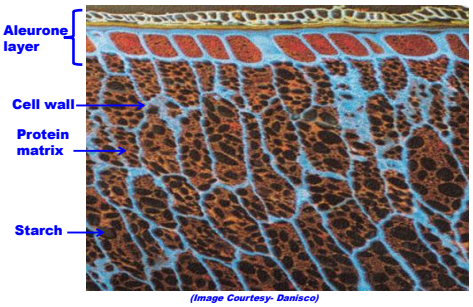
- High cost of pig production
  - Feed cost (60 - 70%) is the highest variable cost *(Payne and Zijlstra, 2007, Noblet, 2011)*
  - Energy represent greatest proportion of feed cost *(Noblet and Milgen, 2004)*
  - Wheat and corn are common source of energy in swine diet
  - Cereal grains are diverted to produce ethanol and wheat flour

Gross structure of grain



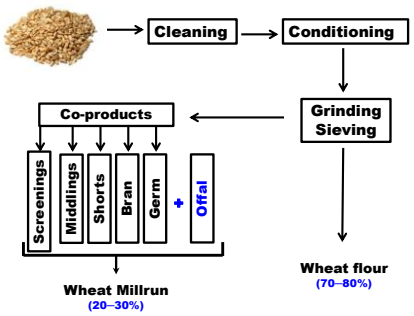
(Source: http://xim.hubpages.com/hub/Benefits-of-Whole-Wheat)

Microscopic structure of grain

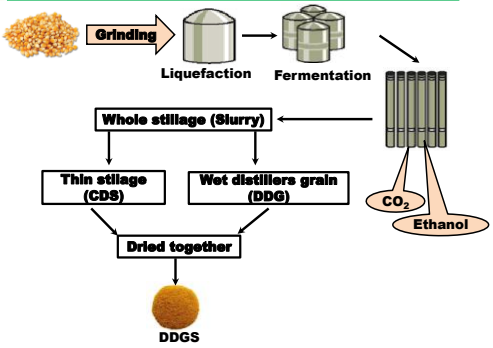


(Image Courtesy- Danisco)

Production of wheat millrun



Production of ethanol



Chemical composition

%, as is	Wheat grain	Wheat millrun	Corn DDGS
Starch	57.3	30.3	4.0
Total NSP	9.2	22.5	19.7
Insoluble NSP	8.1	21.1	19.0
Soluble NSP	1.1	1.4	0.7
Crude protein	14.4	15.5	29.8
Crude fat	1.8	4.1	10.1
Ash	1.7	4.2	4.1
Ca	0.1	0.2	0.2
P	0.3	0.8	0.8

Non starch polysaccharide

Advantages:

- **NSP improved gut health** *(Montague et al., 2003, Wellock et al., 2007)*
- **Influenced the release of hormones** *(Bach Knudsen, 2011; Hooda et al., 2010)*

Disadvantages:

- **Reduced nutrient digestibility**
  - Reduced digesta retention time
  - Increased endogenous loss
- **Endogenous enzymes do not digest NSP** *(Barrera et al., 2004)*

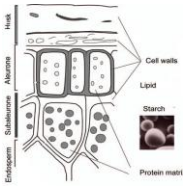
Previous studies

- **Studies focused on cereal grain based diet**
  - **Improved growth performance** *(Emiola et al., 2009)*
  - **Improved nutrient digestibility** *(Adeola and Cowieson, 2011)*
- **Results are less consistent**
- **Limited data available on nutrient digestibility of co-products**

Function of NSP-enzymes

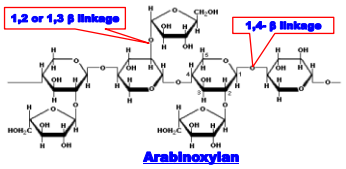
- **NSP-degrading enzymes**
  - **Improved growth performance** *(Emiola et al., 2009)*
  - **Improved nutrient digestibility** *(Adeola et al., 2011)*

- **Hydrolyze NSP bonds**
- **Degrade cell wall, increase permeability**
- **Release fiber entrapped nutrient**
- **Reduce endogenous loss**



Gap in knowledge

- **Limited information**
  - **Grower pigs**
    - Corn DDGS and wheat millrun
  - **NSP-degrading enzymes**
    - Endo-1,4-β-xylanase
    - Endo-1,3(4)-β-glucanase
    - Fungal xylanase *(FXYL)*
    - Fungal β-glucanase *(FBG)*



Arabinoxylan

Over view of study

- **Hypothesis:**
  - NSP reduces nutrient digestibility
  - NSP-enzymes could improve nutrient digestibility
- **Objective:**
  - Determine nutrient digestibility of diet with NSP-enzymes supplementation
  - Determine nutritive value of ingredients

Enzyme activity

Enzymes [1]	Enzyme activity
Roxazyme, (G2G)[2]	Endo-1,3(4)β-glucanase Endo-1,4-β-xylanase
Ronozyme, WX (CT)[3]	Fungal xylanase
Ronozyme, VP (CT)[4]	Fungal β-glucanase

[1]DSM Nutritional Products
[2]Roxazyme, (G2G); *Trichoderma longibrachiatum* *10 U = 1 µmole*
[3]Ronozyme, WX (CT); *Thermomyces lanuginosus* *1 FXU= 7.8 µmole*
[4]Ronozyme, VP(CT); *Aspergillus aculeatus* *1 FBG = 1 µmole*

Overview of study

3 Studies

Study- 1 and 2 | **Study- 3**

Corn DDGS | **Wheat millrun** | **Wheat grain or wheat grain + millrun**

- Ileal cannulated cross breed barrows

Study- 1 and 2

Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing corn DDGS or wheat millrun fed to grower pigs


Corn DDGS


Wheat millrun

Study- 1 and 2

Hypothesis:

- NSP content limits nutrient digestibility
  - Corn DDGS and Wheat millrun
- Nutrient digestibility could improve
  - β-xylanase, β-glucanase, FXYL, FβG

Objective:

- Determine nutrient digestibility of diet
  - Corn DDGS and Wheat millrun
- Determine nutritive value of corn DDGS and Wheat millrun

Data collection and analysis

- Design was 7 7 Latin square
  - Pig was experimental unit
  - Pig and period were random terms
  - Diet was fixed effect
- Experimental period:
  - 5 d adaptation, 4 d collection
- Digestibility calculation

$$\text{App. Dig.} = 1 - \left\{ \frac{Cr_2O_3 \text{ in feed}}{Cr_2O_3 \text{ in excreta}} \times \frac{\text{energy in excreta}}{\text{energy in feed}} \right\}$$

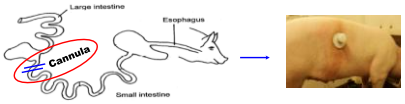
$$\text{SID} = [\text{AID} + (I_{end}/\text{AA in feed}) * 100]$$

(Stein, 2007)

- Data were analyzed using Mixed Procedure in SAS

Study- 1 and 2

- 7 Cross breed barrows
  - Body weight: 39/32 kg
  - T-cannula was fitted at distal ileum
  - Feed: 2.8 Maintenance (NRC,1998)
  - Free access to water
  - Pigs were weighed before each period



Experimental diets

(Study: 1 & 2)

Ingredients, %	Treatment diets CON	β-GX (A)	F-XL (B)	FβG (C)	B+C	A+C	N-free
Corn starch	33.8	33.7	33.7	33.7	33.7	33.7	85.3
Wheat Millrun	60.0	60.0	60.0	60.0	60.0	60.0	-
Sugar						2.0	5.0
Solka-Floc						-	3.0
Canola oil	0.8	0.8	0.8	0.8	0.8	0.8	2.0
Limestone							1.0
Others[1]	1.9	1.9	1.9				3.7

$$A, \% = \{(T \times T_p) - (B \times B_p) / A_p\}$$

$$I_{end} = \text{Digesta AA} \times \frac{Cr_2O_3 \text{ in feed}}{Cr_2O_3 \text{ in digesta}}$$

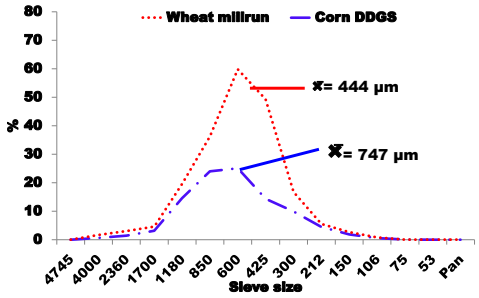
[1]Minerals. Vitamins, Cr_2O_3, Salt, limestone, Dical. Ph., KCO3, MgO

Chem. Composition of diet

Items, %	CON	β-GX (A)	F-XL (B)	FβG (C)	B+C	A+C
	Study- 1 (Corn DDGS diets)					
Ether extract	6.9	7.2	7.0	6.8	6.8	6.6
Total dietary fiber	17.9	18.7	19.0	18.0	18.5	17.9
Crude protein	17.5	17.6	17.7	17.5	17.4	17.3
DE Mcal/kg	3.27	3.30	3.31	3.30	3.32	3.24
Starch	33.5	36.3	34.8	37.1	34.2	35.5
	Study- 2 (Wheat millrun diets)					
Ether extract	2.7	2.6	2.9	2.6	2.7	2.9
Total dietary fiber	20.3	20.3	21.7	20.9	21.1	21.5
Crude protein	10.1	10.0	9.7	9.9	10.1	10.0
DE Mcal/kg	2.98	3.03	3.03	3.01	3.01	3.05
Starch	49.7	47.7	46.5	47.9	48.6	49.9

Particle size distribution of diet

(Study: 1 & 2)



Enzyme concentration

Enzyme activity, U/kg	CON	β-GX (A)	F-XL (B)	FβG (C)	B+C	A+C
	Study- 1					
Endo β-glucanase	-	3016	-	-	-	1820
Endo β-xylanase	-	3016	-	-	-	1820
Fungal Xylanase	-	-	147		79	-
Fungal β-glucanase	-	-	-	15.8	6.3	NA
Total	-	6032	147	15.8	75.3	3640
	Study- 2					
Endo β-glucanase	-	2704	-	-	-	2054
Endo β-xylanase	-	2704	-	-	-	2054
Fungal Xylanase	-	-	150	-	82	-
Fungal β-glucanase	-	-	-	22.3	12	NA
Total	-	5408	150	22.3	94	4108

Results

(Study: 2)

Digestibility, %	CON	β-GX (A)	F-XL (B)	FβG (C)	B+C	A+C	Pooled SEM	*P*-value
	Diets							
Energy, %								
AID	70.1	71.9	71.3	71.5	75.5	74.7	1.62	0.27
ATTD	76.8	78.0	78.4	77.6	77.2	78.3	0.85	0.26
Protein, %								
AID	67.1	67.0	66.2	67.6	73.0	71.7	2.16	0.20
SID	79.5	79.4	78.6	80.0	85.4	84.1	2.15	0.20

Results
(Study: 2)

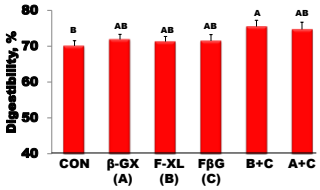
Digestibility, %	Diets CON	β-GX (A)	F-XL (B)	FβG (C)	B+C	A+C	Pooled SEM	*P*-value
Energy, %								
AID	70.1	71.9	71.3	71.5	75.5	74.7	1.62	0.27
ATTD	76.8	78.0	78.4	77.6	77.2	78.3	0.85	0.26
Protein, %								
AID	67.1	67.0	66.2	67.6	73.0	71.7	2.16	0.20
SID	79.5	79.4	78.6	80.0	85.4	84.1	2.15	0.20

Summary
(Study: 1 and 2)

- NSP-enzymes did not improve nutrient digestibility
  - Corn DDGS and wheat millrun diet
    - Instability of NSP-degrading enzymes *(Thacker and Haze, 1996)*
    - Finer and fibrous diet lower digesta pH *(Yongni et al., 2002; Horsley, 2007)*
    - Less effectiveness on fine particle size diets *(Mavromichalis et al., 2000; Amerah, 2008)*
    - Heat application may damage fiber structure *(Graham et al., 1989; Sun and Cheng, 2002)*
    - Possible enzymes use in ethanol plant

AID of energy in diet
(Setudy- 2)

P<0.05



- Shows higher efficiency of fungal enzymes
- Less number of observation per treatment

Conclusion

- NSP-enzymes did not improve nutrient digestibility
  - NSP concentration high in corn DDGS and wheat millrun
- The NSP may not have matched with supplemented enzyme activity
- NSP-degrading enzymes may not have been sufficient in diet

Study-3

Effect of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing wheat grain or wheat grain plus wheat millrun fed to grower pigs


Wheat grain


Wheat millrun

Materials & methods

- 5 Cross breed barrows
  - Body weight: 27 1.1 kg
  - T-cannula was fitted at distal ileum
  - Feed: 2.8 Maintenance (NRC,1998)
  - Pigs were weighed before each period
- 5 5 Latin square design and 2 2 factorial arrangement
  - Pig was experimental unit
  - Pig and period were random terms
  - Diet was fixed effect
- Experimental period:
  - 5 d adaptation, 4 d collection

Hypothesis and Objective

Hypothesis:

- NSP limits energy, protein and AA digestibility
  - Wheat grain diet
  - Wheat grain + wheat millrun diet
- Limited nutrient digestibility could improve
  - β-xylanase, β-glucanase, FXYL and FβG

Objective:

- Determine energy, protein, and amino acids digestibility of diet
- Determine nutritive value of wheat grain and wheat millrun

Experimental diets

	Wheat grain		Wheat millrun		
Ingredient, %	CON[1]	NSP-ase	CON	NSP-ase	N-free
Corn starch	-		-	-	85.3
Wheat grain	96.3	96.1	56.3	56.1	-
Wheat millrun	-	-	40.0	40.0	-
Enzymes					-
Sugar					5.0
Solka floc	-	-	-	-	3.0
Canola oil	-	-	-	-	2.0
Others[2]	3.7	3.7	3.7	3.7	4.7

$A, \% = \{(T \times T_p) - (B \times B_p) / A_p\}$

[1]CON = CONTROL
[2]Minerals. Vitamins, Cr_2O_3, Salt, limestone, Dical. Ph.

Chem. Composition diets

Items, %	Treatment diets			
	CON	NSP-ase	CON	NSP-ase
Starch	56.8	57.1	40.3	43.8
Total NSP	8.0	8.2	15.5	14.7
DE Mcal/kg	3.27	3.28	2.85	2.99
Ether extract	2.1	2.1	3.3	2.9
Crude protein	14.1	14.1	14.9	14.7
Available lysine	0.36	0.34	0.42	0.40
Calcium	0.95	0.64	0.86	0.90
Phosphorus	0.72	0.64	1.05	0.91

Data collection and analysis

- **Digestibility calculation**

$$\text{App. Dig.} = 1 - \left(\frac{Cr_2O_3 \text{ in feed}}{Cr_2O_3 \text{ in excreta}} \frac{\text{energy in excreta}}{\text{energy in feed}}\right)$$

$$I_{end} = \text{Digesta AA} \frac{Cr_2O_3 \text{ in feed}}{Cr_2O_3 \text{ in digesta}}$$

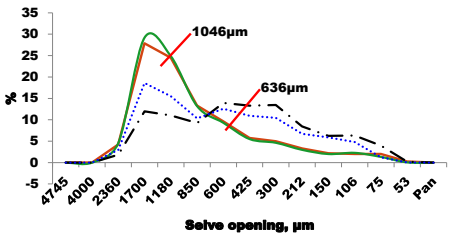
$$\text{SID} = [\text{AID} + (I_{end}/\text{AA in feed}) * 100]$$

(Stein, 2007)

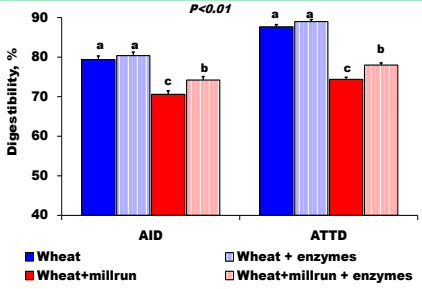
- **Data were analyzed using Proc mixed in SAS**

$$Y_{ijk} = \mu + D_i + E_j + D_i \quad E_j + \varepsilon_{ijk}$$

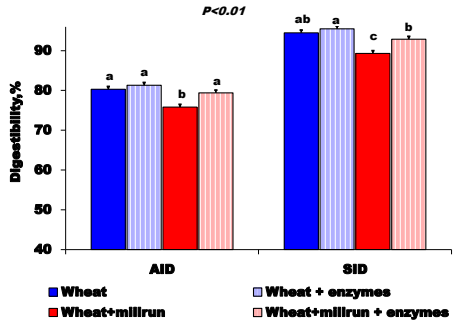
Distribution of particle size



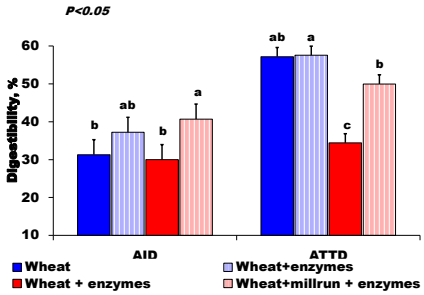
Energy digestibility of diet



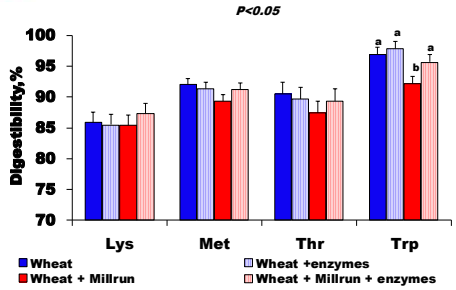
Protein digestibility of diet



NSP digestibility in diet



SID of amino acids in diets



Result and discussion

- **Improved nutrient digestibility by NSP-enzymes**
  - **Wheat grain + wheat millrun diet**
  - **Hydrolyze binding bonds of NSP** *(Kumar and Wyman, 2009)*
  - **Degrade cell wall & increase permeability** *(Parkkonen et al., 1997)*
  - **Reduce digesta viscosity** *(Vahjen et al., 2007).*
  - **Increase enzymes diffusion rate**
  - **Increase availability of fiber trapped nutrient**

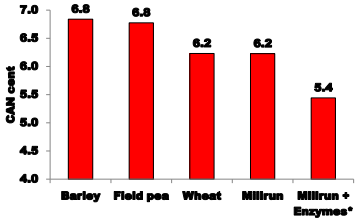
Summary

- NSP-enzymes improved
  - Energy , Protein, AA, NSP digestibility
- Improved nutrient digestibility of wheat millrun
  - Energy digestibility *(AID: 7%; ATTD: 6%)*
  - Protein digestibility *(SID: 7%)*
  - NSP digestibility *(AID: 15%; ATTD: 20%)*

Conclusion

- NSP present in wheat millrun limits
  - Energy, protein, and AA digestibility
  - NSP-enzymes improved nutrient digestibility

Unit cost/Mcal DE



	Barley	Field pea	Wheat	Millrun
Cost/MT	210	225	210	147

*Enzyme cost is not considerd

Implication

- NSP-degrading enzymes can be used
  - To increase energy digestibility
  - To increase protein/AA digestibility
    - Wheat millrun could be a potential substituent
- Should pay attention on cost and benefit while adding enzymes to fiber rich ingredients

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