ABSTRACT

MORAN RAMIREZ, KORY NATHALY. Enzyme Supplementation to Improve the Nutritional Value of Fibrous Feed Ingredients in Swine Diets Fed in Dry or Liquid Form. (Under the direction of Dr. Eric van Heugten).

The increasing industrial demand for feed grains, partly for biofuel production, has raised the price forecast for feed grains. Co-products are a short-term solution for commercial swine production to control feed cost, but these co-products have substantial amounts of fiber that decrease nutrient digestibility in pigs. Application of fiber degrading enzymes and liquid feeding methods may help to improve the nutritional value of fibrous feed ingredients. This study evaluated the effect of endo-1,4-β-xylanase supplementation (Xyl; with or without), feeding method (dry or liquid) and feedstuff (corn DDGS or wheat middlings) on apparent total tract digestibility (ATTD), apparent ileal digestibility (AID) of nutrients; intestinal morphology; caecal pH and VFA (volatile fatty acids) concentration in growing pigs. Sixty-four pigs (BW 25.9 ± 0.38 kg) were blocked by BW and sex, placed in individual pens and randomly assigned to 8 dietary treatments. Within each feedstuff, diets were fed either liquid or dry, without or with Xyl (24,000 BXU/kg feed). DDGS and wheat middlings-based diets contained 3.32 and 3.19 Mcal/kg ME and 1.03 and 1.07% SID (Standardize Ileal Digestible) lysine, respectively. Pigs were fed isocaloric amounts of ME per day and restricted at 3 times maintenance energy requirements (197 kcal ME/kg $BW^{0.60}$). The daily ration was fed in 2 equal meals. Liquid diets were prepared by steeping DDGS or wheat middlings with water (1:3 w:v) with or without Xyl for 24 h, followed by mixing with the respective basal diet and water to achieve a final ratio of 1:2.5 w:v. Diets were fed for 16 days and then pigs were euthanized. An interaction between feeding method, Xyl supplementation and feedstuff ($P < 0.10$) was observed for AID of GE and NDF. When Xyl was added in dry wheat middlings-

based diets an increase in AID of GE and NDF was observed as compared to dry wheat middlings-based diets without Xyl (64.50 vs. 54.67%; 52.88 vs. 31.69%, respectively). However, supplementation of Xyl did not impact AID of GE and NDF when liquid wheat middlings-based diets were offered. Supplementation of Xyl in liquid DDGS-based diets enhanced the AID of NDF as compared to liquid DDGS-based diets without Xyl, but Xyl did not affect AID of NDF in dry DDGS diets. Addition of Xyl to wheat middlings-based diets improved ATTD of GE and N as compared to wheat middlings diets without Xyl (80.37 vs. 78.07%; 80.23 vs. 77.94%, respectively); however, there was no effect of Xyl in DDGS diets (feedstuff by Xyl interaction, $P < 0.05$). DDGS diets in liquid form reduced ATTD of GE as compared to DDGS diets offered in dry form (81.10 vs. 82.97%); however, no effects on ATTD of GE were observed when wheat middlings diets were offered, regardless the feeding method (78.89 vs. 79.55%; feeding method by feedstuff interaction, $P = 0.010$).

Pigs fed DDGS diets had greater concentrations of butyrate in ceacum ($P = 0.001$) as compared to pigs fed wheat middlings diets (27.55 vs. 20.44 mmol/L). Pigs fed DDGS-based diets with Xyl had deeper crypt in the jejunum than pigs fed DDGS diets without Xyl (98.20 vs. 86.16 µm), however, there was no effect of Xyl in pigs fed wheat middlings-based diets.

Under the conditions of this experiment, the liquid feeding method and the application of Xyl demonstrated a limited potential to enhance nutrient digestibility in pigs fed corn DDGS-based diets. However, the supplementation of Xyl in wheat middlings-based diets improved the AID of NDF and ATTD of GE and N; but the liquid feeding as a pretreatment did not enhance further the nutritional value of wheat middling based diets.

Enzyme Supplementation to Improve the Nutritional Value of Fibrous Feed Ingredients in Swine Diets Fed in Dry or Liquid Form

by
Kory Nathaly Moran Ramirez

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Animal Science

Raleigh, North Carolina

2015

APPROVED BY:

Dr. Eric van Heugten Committee Chair	Dr. Peter Ferket
Dr. Vivek Fellner	

DEDICATION

A Dios, por guiar siempre mis pasos y bendecir mi vida.

A mi compañero de vida, mi esposo Mauricio. “Gracias” por confiar siempre en mí, por todo el amor, apoyo y compresión en todos los proyectos personales y profesionales que hemos decidido emprender juntos.

A toda mi familia por su incondicional apoyo y por compartir siempre su sabiduría y amor conmigo.

BIOGRAPHY

Kory Moran was born on November 20, 1986 in Pajan, Ecuador. She spent her entire childhood in a rural town in Ecuador where she became passionate about agriculture and animal production. In 2003, she decided to study abroad at Zamorano University, which is located in Honduras and is a well-known agriculture school in Latin America. After four years of an intensive program at Zamorano, she obtained her BSc degree in Agriculture and Animal Science. After graduating from college, she went back to Ecuador where she worked for two and a half years in a dairy farm as a production manager. In 2009, she was granted a scholarship to pursue a Master degree in Business Administration at INCAE business school, which is located in Costa Rica. After achieving her MBA degree, Kory married Mauricio Diaz on September 3, 2011. Then she moved to the United States, where she found the opportunity to pursue further education. In 2013, she was admitted in the Department of Animal Science at North Carolina State University in order to pursue a Master of Science. Kory worked under the direction of Dr. Eric van Heugten for whom she is very thankful for making this opportunity possible.

ACKNOWLEDGMENTS

This accomplishment would not have been possible without the motivation and unconditional support of my husband and my great family. I would like to especially thank Dr. Eric van Heugten, for giving me the opportunity to be part of his research group and for his personal and professional guidance during these two years. I would like to thank Dr. Vivek Fellner and Dr. Peter Ferket for being part of my graduate committee and for their mentoring during the graduate program. I would also like to appreciate Dr. Kees de Lange and Dr. Peter Wilcock for their valuable input and contribution to this research project.

I would like to express my sincere gratitude to my friends, David Rosero, Santa Maria Mendoza and Wilmer Pacheco for their unconditional support and friendship before and during the program. I would like to appreciate my fellow undergraduate and graduate students for their help and friendship, especially Oswaldo Medina, Symphony Roberts, Petra Chang, Ana Sevarolli and Adsos Passos.

Thanks to the Feed Mill and Swine Unit teams for all their support through my research project. Special thanks to Sarah McLeod, Karen Murphy and Ramon Malheiros for their patience and guidance in many lab procedures. My special gratitude to Marian Correll for her great assistance, support and friendship during the graduate program. Finally, my sincere gratitude to friends and relatives that have supported me and are not mentioned here.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

CHAPTER II

LIST OF FIGURES

CHAPTER I

CHAPTER I: Literature review

1. Introduction

The main goal of diet formulation is to maximize profits considering that the cost of feed represents more than 60% of the total cost in pork production (Noblet and Van Milgen, 2013). To increase economic efficiency, it is necessary to supply indispensable nutrients as close as possible to meet the requirements of the pigs, avoiding excesses and deficiencies.

The increasing industrial demand for feed grains, partly for biofuel production, has raised the long-term price forecast for feed grains; therefore, there is a great need for developing cost-effective feeding strategies for pigs. Alternative feedstuffs are a short-term solution for commercial swine production to control feed cost, but it is important to recognize that those alternative feed ingredients have different feeding values because of variation in nutrient content and other factors such as bioavailability, anti-nutritional factors and palatability. In order to include potential feedstuffs in swine diets as alternative sources it is necessary to use all available fundamental and applied nutritional knowledge.

The inclusion of alternative feed ingredients in swine diets is not a novelty; they have been used in traditional swine production where pigs were housed in small numbers and fed with leftover human food products (Pond and Lei, 2001). However, modern swine production requires high growth rates and safe and consistent pork products, relying on a supply of affordable feed grains and protein sources to produce pork competitively (Pond and Lei, 2001).

Nowadays in North America, the use of alternative feedstuffs in the commercial swine industry is considered beneficial during periods of high prices for more common feed

ingredients. There are products available in the market such as dried distillers grains with soluble (DDGS), corn gluten meal, corn gluten feed, corn germ, wheat middlings, and soy hulls. Unfortunately, such alternative feedstuffs have a high fiber content (lignin and non-starch polysaccharides, NSP) and low energetic value to pigs. Pigs can obtain energy from fiber but only after microbial fermentation of the fiber in the gastrointestinal tract and subsequent absorption of volatile fatty acids, which may contribute to the energy status of pigs (Noblet and Van Milgen, 2013). The greater the concentration of fiber, the lower the overall digestibility of energy in the diet. In addition, fiber may reduce the digestibility of amino acids, lipids, and some minerals. As a consequence growth rate and feed efficiency in pigs are reduced as dietary fiber level is increased (Kass et al., 1980).

Animals produce enzymes to digest feed; they can be produced by the animal itself or by the microorganisms that are present in its gastrointestinal tract. However, this process is not 100% efficient. Pigs and poultry cannot digest 15-25% of the feed they eat, because the feed ingredients contain indigestible and anti-nutritional factors that interfere with the digestive process and the animal lacks specific enzymes that break down certain components in the feed (Barletta, 2010). Therefore, the supplementation of animal feed with enzymes to increase the efficiency of digestion can be viewed as an extension of the animal's own digestive process (Steen, 2001).

Addition of exogenous enzymes in animal nutrition offers promise for improving the nutritional value of high fiber feedstuffs because feed enzymes help to target specific anti-nutrients in certain feed ingredients allowing pigs and poultry to extract more nutrients from feed and improve feed efficiency (Barletta, 2010). Fiber-degrading enzymes allow

nutritionist greater flexibility in the types and levels of alternative feedstuffs that can be used in feed formulation.

The method of feeding and applying enzymes to the feed may impact the efficiency of the enzyme. The process of applying exogenous enzymes in liquid feeding may allow the enzyme to act and penetrate the substrate more efficiently. An important aspect of liquid feeding is soaking of ingredients, which is likely to be more beneficial when using ingredients that contain higher levels of fiber, such as barley and wheat. Previous studies have shown that liquid feeding wheat and wheat middling based diets improved feed efficiency in pigs (de Lange and Zhu, 2012). However, the response of enzymes in liquid feeding systems has differed between studies (Zhu et al., 2011; de Lange and Zhu, 2012). Thus, the application of enzyme in liquid feeding systems needs to be explored in order to refine and validate this technique.

2. Dietary Fiber in Swine Nutrition

The term Dietary Fiber (DF) is defined as a heterogeneous group of plant polysaccharides and lignin that are not hydrolyzed by mammalian enzymes in the digestive system (Souffrant, 2001; Wenk, 2001). Fiber is a common component in pig feed because it is present in most ingredients and at high level in many alternative feedstuffs. The amount and composition of DF differ over a wide range between and within feed ingredients.

Dietary fiber is predominantly found in plant cell walls and consists of non-starch polysaccharides (NSP), oligosaccharides and lignin. According to Elia and Cummings (2007), the term NSP is related to dietary fiber but does not cover all components that can be

classified as dietary fiber; NSP does not include oligosaccharides and lignin. Oligosaccharides generally contain only 3 to 9 monosaccharide units; in contrast, NSPs contain more than 10 monosaccharides units. Both may have similar or different monosaccharides in their chains, various linkages structures, and may be linear or branched (Grieshop et. al, 2001). NSP and oligosaccharides are not hydrolyzed by mammalian enzymes; therefore, they are fermented and partly degraded by the micro flora residing in the intestinal tract of animals. Lignin is not considered a polysaccharide and it does not have any nutritional value for pigs because it is indigestible.

2.1 Non-starch polysaccharides (NSP)

Non-starch polysaccharides represent a wide group of heterogeneous monosaccharides which differ considerably in chemical composition and physical properties both within and between plant sources (de Lange, 2000). Non-ruminant animals depend on microbial fermentation in the intestinal lumen for the digestion and utilization of energy contained in the NSP because they lack the enzymes to break down the β-glycosidic bond in NSP structures. The digestibility of NSP is affected by several factors which include animal species, age of the animal, solubility, chemical structure, and the inclusion level in the diet (Choct et al., 2010). Moreover, the digestibility of NSP is lower than other carbohydrates such as sugar and starch. According to de Lange (2000), the efficiency of utilization of energy from fermented NSP is about 30% lower than that of digested starch and sugars.

NSP also encapsulate starch, protein, oil, and other nutrients inside the plant. NSP create an impermeable cell wall, which is a physical obstacle between the intestinal enzymes

and the cell components, and avoids full use of the nutrients in grain. This effect is called "the cage effect", and refers to the fact that nutrients entrapped within the structure are inaccessible to endogenous enzymes. The energy gained from the complete utilization of NSP contained in these cell walls is insignificant when compared to that from the release of the cell contents (Ward, 2008).

The most abundant plant cell wall NSP include cellulose, hemicellulose and pectins. The NSP, because of their large size and structure, encompass a wide range of chemical and physical properties (Bach-Knudsen et al., 1997). NSP can represent about 90% of the cell wall of plants; the predominant polysaccharides found in cell walls include cellulose, the pentoses (arabinose and xylose), hexoses (glucose, galactose and mannose), the 6-deoxyhexoses (rhamnose and fucose), and hexauronic acids (galacturonic acids) (Selvendran, 1984). But the most common NSPs found in feed ingredients of plant origin are cellulose, arabinoxylans, β-glucans, xyloglucans, xylans and arabinogalactans (de Lange 2000). The complexity of NSP is due to the multiple combinations of potential monosaccharides and their impact on the chemical and physical structure of the NSP within and between dietary sources.

Dietary NSP has considerable effects on gut microflora, viscosity, and water holding capacity of the digesta. Consequently NSP affects the digestibility of other dietary nutrients, voluntary feed intake, metabolism of nutrients after absorption, and gut health (de Lange, 2000).

2.2 Physico-chemical properties of DF

The physical and chemical location of polysaccharides in the plant cell wall have a large influence on the physico-chemical properties of cell wall polysaccharides and consequently their action in the gastrointestinal tract (Bach-Knudsen, 2001). According to Urriola et al. (2013), the main physico-chemical properties that impact animal nutrition include: solubility, water holding capacity, viscosity and fermentability.

2.2.1 Solubility

Solubility refers to the ability of DF to dissolve in water, dilute acid, dilute base, buffer or enzyme solution (Cho et al., 1997; Oakenfull, 2001). The solubility process starts when the polymer is swelling and the water spreads the molecules until they are fully extended and dispersed. In linear structures such as cellulose or linear arabinoxylan, the link between the monomers allows for an ordered structure preventing the entrance of water, thus making cellulose and linear arabinoxylan insoluble. Soluble fibers are believed to be the active compound in the regulation of digestion and absorption in the fore-gut. On the other hand, insoluble fibers mainly act in the large intestine where, due to its physical presence, it increases fecal bulk, dilutes colonic contents and decreases transit time (Bach-Knudsen, 2001).

2.2.2 Water holding capacity

Water holding capacity reflects the ability of a fiber source to incorporate water within its matrix (Bach-Knudsen, 2001). The morphological structure and composition of the fiber have a main effect in the strength of binding and the amount of water bound to the fiber

(Urriola et. al, 2013). Soluble fiber has a greater water holding capacity than insoluble fiber. In general, hemicellulose is associated with high water holding capacity and cellulose and lignin with low water binding capacity.

2.2.3 Viscosity

Soluble fiber tends to dissolve and thicken solutions or form gels in solution. This effect is called viscosity; almost all water-soluble polysaccharides produce viscous solutions. Viscosity is caused by physical interactions between polysaccharide molecules in solution (Oakenfull, 2001). The inclusion rate of DF affects viscosity. At low concentrations of soluble DF, the molecules in a solution are separated and are able to flow, but at high concentrations, molecular movement becomes difficult and physical limitations of dietary fiber molecules occurs (Dikeman and Fahey, 2006).

2.2.4 Fermentability

Dietary fiber is fermented in the gut by the microbial population and this property is influenced by solubility and water binding capacity. Soluble fiber absorbs water and swells, which increases the surface area of the polysaccharide for microbial action. This is the primary reason that soluble fiber is fermented faster than insoluble fiber. Microbes start breaking down polysaccharides into small polysaccharides during the fermentation process. The main products of fiber fermentation are short chain fatty acids (acetate, propionate and butyrate), carbon dioxide, methane and hydrogen. The effects of soluble and insoluble DF on selected properties in the gastrointestinal tract are summarized in Table 1.

Table 1. Effects of soluble, insoluble and total dietary fiber on digestion and absorption processes in pigs[a].

	Dietary fiber		
	Soluble	Insoluble	Total
Stomach			
Viscosity	+++	+	++
Small intestine			
Viscosity	++	-	+
Large intestine			
Fermentation	++++	+++	+++
Bulking	+	++++	++++
Mean transit time	-	+++	+++

[a] -: No relation, + to ++++: relative strengths of relation. Adapted from Bach-Knudsen, 2001.

2.3 Effect of DF on the digestive process

The gastrointestinal tract of pigs is incapable of degrading fiber; they rely on the symbiotic association with gastrointestinal micro flora for the utilization of fiber polysaccharides (Graham and Aman, 1991). According to data published by Bach-Knudsen (2001), the coefficient of digestibility of NSP in the small intestine of pigs fed cereals and peas diets ranged from 0.10 to 0.70 with an average of 0.20. These data demonstrate that a small fraction of NSP are digested before the end of the ileum.

Endogenous secretions, including losses of enzymes, mucus and sloughing of mucosal cells in the small intestine are likely increased when intake of NSP is increased (de Lange, 2000). According to a study conducted by Wenk (2001), increasing the content of DF (mainly soluble DF) from 50 to 180 g kg^{-1} triggered a doubling of secretion of saliva and

gastric juice in pigs of 50 kg body weight; the increased amount of secreted digestive fluids implies an extra metabolic effort for the pig.

Dietary fiber may alter intestinal morphology as well as the rate of intestinal cell turnover in pigs, which can affect the capacity of the gut to absorb nutrients. Jin et al. (1994) studied the effect of DF on cell growth, cell proliferation, cell death, and morphology of the intestinal epithelium of growing pigs and they found that feeding growing pigs with high DF (10% wheat straw) increased depth of intestinal crypts in jejunum, ileum and colon. This led to an increase in cell proliferation rate, thus increased the rate of turnover of intestinal cells. They concluded that high rates of energy expenditure are associated with high rates of cellular turnover.

Soluble fiber causes more water binding capacity in the stomach than insoluble fiber. Consequently, high amounts of soluble fiber in diets can lead to an increased volume of digesta in the stomach and therefore, reduces both satiety and digesta transit time in this organ (Wenk, 2001).

In the large intestine, the flow rate of the digesta is low. The transit time ranges between 20 to 40 hours (Bach-Knudsen, 2001). Additionally, in the large intestine there is low oxygen concentration and high moisture content; these conditions create an ideal environment for the microbial ecosystem, which plays an important role in the fiber fermentation process.

The bacterial population in the large intestine is responsible for the fermentation of dietary residues that escape digestion in the small intestine (Figure 1). The products of fermentation of DF are short chain fatty acids (SCFA), which are absorbed into the portal

vein by passive diffusion (Urriola et al., 2013) and used as substrate for intestinal cell growth and renewal, or excreted in feces. According to Anguita et al. (2006), energy absorbed as SCFA account for 67% to 74% of the total energy absorbed in the hindgut of pigs fed high fiber diets and the contribution of absorbed SCFA to the total available energy in pigs ranges from 7.1% to 17.6%. However, the utilization of energy absorbed as SCFA from the large intestine is lower than the energy absorbed as glucose from the small intestine, primarily due to losses of energy as fermentative gases (Bach-Knudsen et al., 2013).

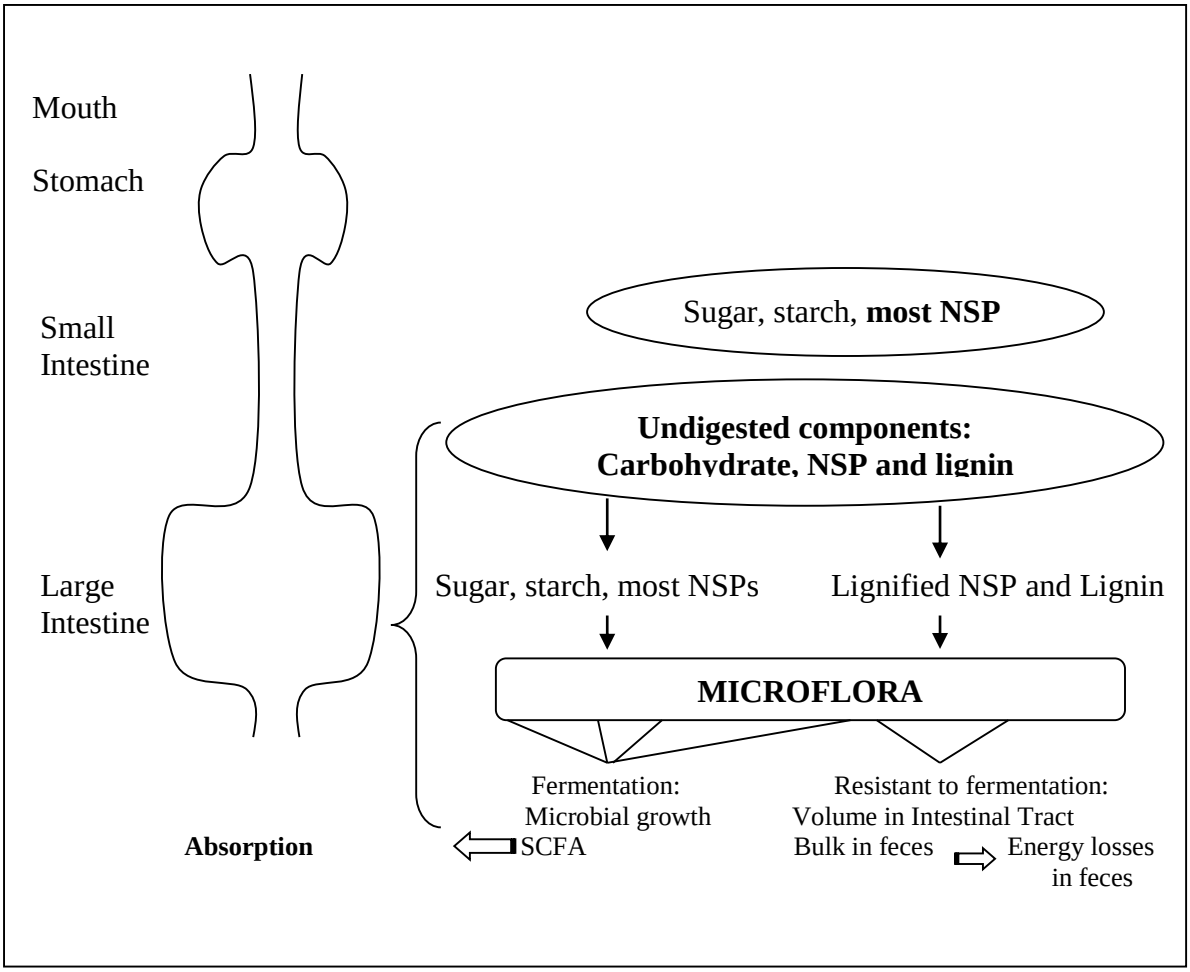

Figure 1. Illustration of degradation of undigested residues in the large intestine. Adapted from Bach-Knudsen et al. (2013).

2.4 Undesirable effect of DF on nutrient digestibility

The digestibility of energy and amino acids consistently decreases with increasing fiber content in the diets, but the magnitude of this negative impact depends on the type and origin of the fiber source, feeding level, age and live weight of the pigs (Fernández and Jørgensen, 1986).

The capacity of the pig to digest DF improves with age and live weight, therefore, the digestibility of DF is greater in adult animals than in young animals. The most likely explanation is that the hindgut size as a proportion of live weight or relative feed intake increases markedly with live weight, with subsequent lower rate of passage of digesta and prolonged time for fermentation of residues from the ileum (Noblet and Le Goff, 2001).

Thus, an increased intake of DF will reduce total tract digestibility and increase the proportion of energy digested in the large intestine. This results in more energy derived from SCFA and less energy from glucose absorbed in the small intestine. It has been estimated that 1% additional energy digested in the large intestine reduces the utilization of metabolizable energy by 0.27% (Bach-Knudsen, 2001). Dietary fiber also may have a detrimental effect on the efficiency of nitrogen utilization by increasing secretion of endogenous nitrogen, reducing dietary nitrogen absorption or increasing bacterial nitrogen excretion (Grieshop et. al, 2001).

3. Energy and fiber in corn distiller's dried grains with solubles (DDGS) and wheat middling

Corn DDGS is produced from the fuel ethanol industry and with the rapid growth of this industry, greater quantities of DDGS are available for livestock rations, including for swine diets in all phases of production. Maximum recommended dietary DDGS inclusion rates to support excellent performance are: up to 30% for nursery pigs weighing more than 7 kg, growing-finishing pigs, and lactating sows; and levels of up to 50% of the diets for gestation sows (U.S. Grains council, 2012). During the ethanol production process, most of

the starch in corn is fermented to produce ethanol, thus only small amounts of starch are present in DDGS. However, the fiber in corn is not converted to ethanol so the concentration of fiber is relative high in DDGS. According to an analysis of 46 samples of DDGS, the average content of NDF (neutral detergent fiber) and ADF (acid detergent fiber) in DDGS is 25.3% and 9.9%, respectively (Stein and Shurson, 2009). This shows that DDGS has a large hemicellulose component as calculated by the difference between NDF and ADF. Gross energy in DDGS averages 5,434 kcal/kg DM and is greater than the concentration of GE in corn which is 4,496 kcal/kg DM. But the apparent total tract digestibility of energy in DDGS is lower than in corn (76.8% vs. 90.4%, respectively) (Stein, 2007). Currently, the inclusion of DDGS in diets for all swine production categories is a common practice, when it is economically attractive. Usually, nutritionists include up to 35% in nursery and growing pig diets, but in finishing diets the inclusion level is often limited to 20% because one of the mayor issues in using greater amounts or DDGS in finishing diets is the effect on carcass fat quality. Feeding ingredients greater in unsaturated fatty acids, such as DDGS, changes the proportions of fatty acids tissues and increased the risk of producing pigs with soft bellies (which reduces bacon slicing yield) (Stein, 2007).

The profile and levels of NSP in corn DDGS samples from different source across the USA has been assessed (Table 2). According to Ward (2008) the total NSP in corn DDGS represents between 23.1% of dry matter and most of them are insoluble, which is not surprising because during the ethanol production process most of the soluble fraction is fermented; the estimated arabinoxylan fraction was 11.7% (arabinose and xylose portion).

Table 2. Non-starch polysaccharide (NSP) profile of corn DDGS (% DM)

NSP Component	Average, %	Range, %
Arabinose	4.98	4.09 - 6.08
Xylose	6.42	4.81 - 7.78
Glucose	7.86	6.72 - 9.68
Mannose	1.62	1.16 - 2.44
Galactose	1.61	1.19 - 2.08
Rhamnose	0.08	0.05 - 0.09
Ribose	0.11	0.06 - 0.20
Fucose	0.06	0.01 - 0.18

Adapted from Ward et al. 2008.

Wheat middlings are common cereal by-products used in commercially pelleted pig feeds because it has good pellet binding properties and its cost competitiveness with other feed ingredients. Wheat middlings consist of fine particles of wheat bran, wheat shorts, wheat germ and wheat flour (AAFCO, 2000). Wheat by-products have higher content of NSP than the wheat grain, resulting in reduced digestibility of energy and amino acids. The inclusion level of middlings can be up to 10% in corn-soybean meal based diets for growing pig when fed in meal form (dust prohibits a high inclusion rate), and up to 35% of the diets if it is pelleted (Thaler and Holden, 2010).

Cromwell et. al. (2000) evaluated 14 sources of wheat middlings and reported a mean composition of CP and NDF of 16.2% and 36.9%, respectively. The energy value averages 3,080 kcal/kg DE (Thaler and Holden, 2010). Wheat has arabinoxylans in its bran and seed coat and these compounds are concentrated in wheat middlings, thus the high fiber and arabinoxylan content in wheat middlings reduces nutrient digestibility (Feoli et. al., 2006).

According to Bach Knudsen (1997) total NSP compromises 19% and arabinoxylans 11.5% of dry matter in wheat middlings.

4. Use of exogenous enzyme in pig diets

Enzymes have found extensive application in animal diets based predominantly upon wheat, oats, barley, triticale or rye. Since the late 1980's, feed enzymes have played a major role in helping to improve the efficiency of meat and egg production by enhancing the nutritional value of feed ingredients and increasing the efficiency of digestion (Barletta, 2010). This improvement in the digestibility of energy, proteins and minerals allows feed manufacturers to decrease feed costs by reformulating feed considering improved nutrient availability when enzymes are used. The value of adding enzymes depends on the cost of the enzyme versus the cost of energy sources (e.g. grains and fat), protein sources (e.g. soybean meal) and inorganic phosphorus sources (e.g. dicalcium phosphate), when the price of these nutrient sources increases, the addition of enzymes becomes more profitable.

Enzymes can be used to improve the efficiency of feed utilization in several ways. First, improving the digestive capacity of the host. Second, degrading of specific non-starch gel forming polysaccharides, plant cell walls and proteins. Third, breakdown of anti-nutritional substances in raw materials (Dierick, 1989). Enzymes can be added to dry feed, liquid and wet feed. The hydration provides potentially excellent conditions for the enzymes because it allows better interaction between the enzyme and its specific substrate.

Enzymes are classified according to the substrates they act upon. Currently, the types of enzymes used in animal diets are those that break down carbohydrates (fiber and starch),

proteins, and phytate. Carbohydrases help to break down carbohydrates into shorter chains and simple sugars; this enzyme category can be divided into those that target fiber (NSP) and starch. A listing of key enzymes and substrates upon which they act are described in Table 3.

Table 3. Enzyme activity and associative substrate

Enzyme	Substrate
Xylanase	Arabino-xylose (NSP)
β-Glucanase	β-glucans (NSP)
Mannase	Oligo-mannans (Mannose)
α-Galactosidase	α-Galactosyl moieties
Cellulase	Cellulose (NSP)
Pectanase	Pectins (NSP)
Amylase	Amylose

Adapted from Kerr and Shurson (2013).

4.1 Fiber degrading enzymes: nutrient utilization and efficiency

The main fiber degrading enzymes used in animal feed are xylanase and β-glucanase. Xylanases break down arabinoxylans, mainly present in grains and their by-products. β-glucanases break down β-glucans that are prevalent in barley and oats and their by-products. There are other fiber degrading enzymes currently used in animal nutrition, but to a lesser extent, such as β-mannanase, pectinase, and α-galactosidase.

Fiber degrading enzymes (xylanase and β-glucanase) have provided a clear benefit in poultry due to an improvement in feed efficiency and bird uniformity (Barletta, 2010). Conversely, the response of pigs to xylanase and β-glucanase reported in the literature has been inconsistent. The differences in response between pigs and poultry have been attributed to several factors. The main beneficial effect of exogenous enzyme in poultry diets have been

associated with reduced viscosity of the digesta, which leads to more efficient nutrient absorption and better growth performance. However, in pigs the negative effect of NSP is not related to viscosity, but it may be due to its role as a physical barrier that encapsulate nutrients and keeps them protected from enzymatic activity (Grieshop et al. 2001). Other factors such as enzyme source, level of inclusion, ingredient variety and environment under which the ingredient was grown, stored and processed into animal feed, age of animal, interaction with other dietary ingredients and health status have been shown to affect significantly the animal response (Bedford and Schulze, 1998).

Jacela et al. (2010) reported that the inclusion of β-mannanase, β-glucanase, cellulase, proteases and xylanase in growing pig diets containing 15%, 30%, 45% and 60% of DDGS, did not have any positive effects on growth performance in pigs. Similarly, Feoli et al. (2009) did not find any effect of supplemental xylanase on growth performance and nutrient digestibility in finishing pigs fed diets containing 30% wheat middlings. In general, previous research on the use of carbohydrases in pig diets has produced inconsistent results. Use of individual enzymes or cocktails of enzymes that target NSP in feedstuffs improved nutrient digestibility and/or growth performance in several studies (O'connell et al., 2005; Carneiro et al., 2008; Woyengo et al., 2008; Yoon et al., 2008), but not in others (Feoli et al., 2009; Jacela et al., 2010).

4.2 Xylan

Xylans are the mayor components of hemicellulose and after cellulose is the second most common polysaccharide in nature (Paloheimo et al., 2011). Xylans are polymers

composed of 1,4-β-linked-D-xylopyranose units. In the cell walls of annual plants and cereals, xylans are commonly found in the form of arabinoxylans. Arabinoxylan structures are composed of a xylan back bone with L-arabinose in the endosperm and pericarp tissues of the grains (Ebringerova and Heinze, 2000; Paloheimo et al., 2011). There are two main forms of arabinoxylans: highly branched and without uronic acid substitutions (found in cereal endosperm); and much less branched and substituted with uronic acid and/or with 4-*O*-methyl ether and galactose (found in lignified tissues) (Bhat and Hazlewood. 2001). Arabinoxylan is the main NSP present in corn DDGS and wheat middlings. Arabinoxylan represents 10.4% to 11.7% of dry matter in corn DDGS and 11.5% to 15.9% of dry matter in wheat middlings (Bach Knudsen, 1997; Ward, 2008; Kim et al., 2010).

4.3 Xylanase

The enzyme endo-1,4-β-xylanase (EC 3.2.1.8) (International Union of Biochemistry and Molecular Biology, 1992), attacks randomly the internal xylosidic linkages of the xylan backbone, producing non-substituted or branched xylo-oligosaccharides (Wong et al., 1988). When xylanase acts on cereal arabinoxylans, oligosaccharides of xylose or xylose and arabinose residues are released (Paloheimo et al., 2012). Multiple xylanases have been reported in various organism such as algae, protozoa, snails, crustaceans and seed of terrestrial plants (Dornez et al., 2009: Wong et al., 1988). According to Paloheimo et al. (2012), most of the commercial xylanases used as feed additive are of fungal origin. The organisms that produce this enzymes include: Tricoderma, Humicola, Talaromyces, Aspergillus, Penicillium and Thermomyces species.

It has been suggested that the use of xylanase as a feed additive in animal nutrition could improve the nutritional value of feedstuffs that contain high amounts of arabinoxylan. Xylanase can disrupt the arabinoxylan structures present in cell walls, resulting in elimination of the encapsulating effects of nutrients by the cell wall and consequently increase the digestion of energy and protein (Dierick and Decuypere, 1994). However, the complete cleavage of xylan is very complex and involves a diversity of xylanolytic enzyme systems. These enzymes include endo-1,4-β-D-xylanases (EC 3.2.1.8) which breaks the xylan backbone, β-D-xylosidases (EC 3.2.1.37) which breaks xylose monomers from the non-reducing end of xylo-oligosaccharides and the side groups are cleaved by α-L-arabinofuranosidases (EC 3.2.1.55), acetylxylan esterases (EC 3.1.1.72), feluric acid esterases and p-coumaric acid esterases (Collins et al., 2005). However, it should be mentioned that the monomers arabinose and xylose that can be released by xylanases, have little direct value, because these pentose sugars are poorly metabolized by pigs. They may be absorbed but cannot be metabolized and may be excreted in urine (de Lange, 2000; Yule and Fuller, 1992).

5. Liquid feeding and its potential to improve nutrient utilization.

Feeding liquid diets to pigs is a practice that has been applied especially in areas where there is availability of liquid co-products from the human food industry, because liquid feeding represents an opportunity to recycling liquid residues as animal feed (Brooks et al., 2001). Traditionally the availability of liquid residues may have been the main reason for installing a liquid feed system. However, this feeding method has gained additional interest

due to several advantages over dry feed such as: use of inexpensive co-products from the food and alcohol industry; reduction of feed loss as dust during handling and feeding; improvement of gut health in pigs; increased availability of phosphorus, and improved accessibility to substrates by digestive enzymes (Brooks et al., 2001; Choct et al., 2004; de Lange et al., 2006).

Liquid feeding implicates the use of diets prepared from a mixture of liquid food industry residues and conventional dry feed, or from dry raw materials mixed with water (Missotten et al., 2010). Usually a liquid diet contains 200-300 g dry matter per kg (Brooks et al., 2001). It is important to understand the differences between non-fermented and fermented liquid feed, the first is when dry feed and water are mixed immediately before feeding the animal. Conversely, the fermented diet is a mixture of feed and water stored in a tank at a certain temperature and for a specific period of time before it is fed to the animals (Canibe and Jensen, 2003). The fermentation process results in variable microbial and nutritional characteristics of the mixture as fermentation develops. The initial phase is characterized by low levels of lactic acids, lactic acid bacteria and yeasts; high pH and high number of entero-bacteria. In the second phase, the steady state is reached and in this phase lactic acid bacteria become dominant and lactic acid levels are high and pH decreases; the entero-bacteria counts and other possible pathogens are reduced (Canibe and Jensen, 2003).

An important nutritional consideration in fermented diets is the loss of essential nutrients from the feed, such as vitamins and free amino acids, specifically lysine (Missotten et al., 2010). During the fermentation process the lysine level decreases mainly due to decarboxylation of free Lysine with a consequent production of biogenic amines, such as

cadaverine; this may impair the nutritional value and palatability of fermented diets (Canibe and Jensen, 2012). Niven (2006) found that the loss of free lysine and subsequently production of cadaverine in fermented diets is caused by the metabolism of *E.coli*. One strategy to ameliorate this negative effect is fermenting only the dietary cereals and the subsequent combination of this fermented mixture with the protein rich ingredients prior to feeding the animals.

Dry matter and energy losses can take place also during fermentation because there is an alteration in nutrient composition, for example, conversion of sugars to lactic acid and carbon dioxide and ammonia production (Canibe and Jensen, 2012). The effect of steeping and fermentation on digestibility of other nutrients has been observed in several studies. Dung et al. (2005) found that fermented diets (24 h) improved the total tract digestibility of organic matter and crude protein over dry diets in growing-finish pigs. Similarly, Lyberg et al. (2006) found an improvement in ileal digestibility of organic matter and nitrogen in growing pigs when they were fed with fermented diets. However, others (de Lange et al., 2006) detected a decrease in total tract digestibility of energy, crude protein and crude fat when growing pigs were fed liquid corn and soybean meal diets with fermented corn distiller solubles. Although the effects of fermented diets on nutrient digestibility have been inconsistent, it is believed that there is a potential for improving the nutritional value of feeding ingredients following fermentation. A potential reason that fermented diets (low pH) can improve protein digestibility is that the low gastric pH stimulates proteolytic activity in the stomach and decreases the gastric emptying rate, which allows more time for digestion.

Also, low gastric pH inhibits the proliferation of pathogens in the gastrointestinal tract (Canibe and Jensen, 2012).

A potential benefit of liquid feeding that recently has gained interest is that it represents the opportunity for modifying raw materials prior to feeding, such as by-products that usually have low nutritional value. Simply steeping materials in water for a period can activate naturally occurring enzymes. In addition, a liquid medium produces more opportunities for using exogenous enzymes to target specific substrates in raw materials and, the nutritional value of these ingredients could increase. However, it is important to consider that when materials are in liquid form, the natural micro flora present on the ingredients will be activated and can make changes that can be either beneficial or harmful.

Most of the raw materials have natural flora, primarily lactic acid bacteria and yeasts; the dominant micro flora that grow in liquid diets are lactic acid bacteria which are beneficial because they produce organic acids, mainly lactic acid. Lactic acid has showed positive effects on the feed intake, daily gain and feed conversion ratio in piglets (Brooks et al., 2001). However, raw materials also have undesirable micro flora like coliforms, salmonella and molds that can be harmful to the animals.

Addition of exogenous enzymes to liquid feed can be considered a strategy to improve the digestibility and fermentability of nutrients because enzymes need a liquid medium to be active. Also it allows the exogenous enzyme to act on its substrates before the animal consumes the feed (Canibe and Jensen, 2012). However, the literature about the amount of water required for an optimum enzyme activity is limited. According to Denstadli et al. (2006), the degradation of inositol 6-phosphate in a wheat and soybean mixture with

added endogenous phytase (2500 FTU/kg) was most efficient when the feed was incubated at 45% of moisture and 45 ºC. However, very low hydrolysis of inositol 6-phosphate was observed at 25 and 35% moisture.

Phytases, which are naturally present in the pericarp of some cereal grains and seeds, are activated when these feedstuffs are steeped in water, increasing phytate hydrolysis and consequently increasing phosphorus availability. But, when exogenous phytase is added the effect is greater (Brooks et al., 2001). According to de Lange et al. (2006) when high moisture corn and corn are steeped in water with exogenous pythase, 85% or more of phytate phosphorus is released quickly, showing that the use of phytase in liquid diets can be more effective than in conventional dry diets.

In an in vitro study conducted by Christensen et al. (2007), liquid diets with and without addition of fiber degrading enzymes (xylanase and β-glucanase) were fermented for 8 hours after which the diets were exposed to in vitro digestion. Enzyme supplementation increased solubility of NSP and dry matter, which represents a benefit for pigs because the solubilization of NSP helps to improve fermentation of these components in the large intestine of the pig. The use of phytase in liquid feed has demonstrated a clear benefit, increasing the degradation of phytate during fermentation and soaking. The effects of addition of other enzymes in liquid feed have shown positive effects, but has not been extensively investigated.

6. **Current research focus**

The application of fiber degrading enzymes in animal nutrition represents an alternative to provide pigs with additional nutrients resulting in cost effective, adequate growth performance and a sustainable swine production system. Therefore, the main focus of the present research was to improve the nutritional value of corn DDGS and wheat middlings, which are high-fiber co-products from the food and bio-fuel industries. Improvement in nutritional value was pursued through supplementation of xylanase and application of liquid feeding.

References

AAFCO. 2000. Official Publication of Association of American Feed Control Officials, Oxford, IN.

Anguita, M., N. Canibe, J. F. Pérez, and B. B. Jensen. 2006. Influence of the amount of dietary fiber on the available energy from hindgut fermentation in growing pigs: Use of cannulated pigs and in vitro fermentation. J. Anim. Sci. 84:2766-2778.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin content of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319-338.

Bach Knudsen, K. E. 2001. The nutritional significance of “dietary fiber” analysis. Anim. Feed Sci. Technol. 90: 3-20.

Bach Knudsen, K. E, Lærke, H. N. and Jørgensen, H. 2013. Carbohydrates and Carbohydrate Utilization in Swine. In: L.I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publish Ltd., Oxford, UK. p. 109-137.

Barletta, A. 2010. Introduction: current market and expected developments. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 1-11

Bedford, M. R. and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. Nutr. Research Reviews 11:91-114.

Bhat, M. K., and G. P. Hazlewood. 2001. Enzymology and other characteristics of cellulases and xylanases. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 11-13.

Brooks, P. H., J. D. Beal and S. Niven. 2003. Liquid feeding of pigs: Potential for reducing environmental impact and for improving productivity and food safety. Animal Science Papers and Reports.

Canibe, N., and B. B. Jensen. 2003. Fermented and nonfermented liquid feed to growing pigs: effect on aspects of gastrointestinal ecology and growth performance. J. Anim. Sci. 81:2019-2031.

Canibe, N., and B. B. Jensen. 2012. Fermented liquid feed-Microbial and nutritional aspects and impact on enteric diseases in pigs. Anim. Feed Sci. Techn. 173:17-40.

Carneiro, M. S. C., M. M. Lordelo, L. F. Cunha, and J. P. B. Freire. 2008. Effects of dietary fiber source and enzyme supplementation on fecal apparent digestibility, short chain fatty acid production and activity of bacterial enzymes in the gut of piglets Anim. Feed Sci. Techn. 146:124-136.

Cho S., J. W. De Vries, and L. Prosky. 1997. Dietary fiber analysis and applications. AOAC Intl., Gaithersburg, MD.

Choct, M., E. A. D. Selby, D. J. Cadogan, and R. G. Campbell. 2004. Effect of liquid to feed ratio, steeping time, and enzyme supplementation on the performance of weaner pigs. Crop Pasture Sci. 55: 247-252.

Choct, M., Y. Dersjant-Li, J. McLeish, and M. Peisker. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australasian J. Anim. Sci. 23:1386-1398.

Christensen, P., V. Glitsø, D. Pettersson, and B. Wischmann. 2007. Fiber degrading enzymes and *Lactobacillus plantarum* influence liquid feed characteristics and the solubility of fiber components and dry matter *in vitro*. Livest. Sci. 109:100-103.

Collins, T., C. Gerday, and G. Feller. 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbial. Reviews. 29:3-23.

Cromwell, G. L., T. R. Cline, J. D. Crenshaw, T. D. Crenshaw, R. A. Easter, R. C. Ewan, and C. R. Hamilton. 2000. Variability among sources and laboratories in analyses of wheat middlings. NCR-42 Committee on Swine Nutrition. J. Anim. Sci. 78:2652-2658.

de Lange, C. F. M. 2000. Characterization of the non-starch polysaccharides in feeds. In: Moughan P., M. Verstegen, and I. Marijke, editors, Feed evaluation-principles and practice. Wageningen Press. The Netherlands. p. 77-92.

de Lange, C. F., C. H. Zhu, S. Niven, D. Columbus, and D. Woods. 2006. Swine liquid feeding: Nutritional considerations. In: Proc. Western Nutrition Conference, Winnipeg, MB, Canada. p. 1-13.

de Lange, C. F. M., and C. H. Zhu. 2012. Liquid feeding corn-based diets to growing pigs: practical considerations and use of co-products. In: J. Patience, editor, Feed Efficiency in Pigs. Wageningen Academic Publisher. Wageningen, The Netherlands. p. 63-80.

Denstadli, V., R. Vestre, B. Svihus, A., Skrede, and T. Storebakken. 2006. Phytate degradation in a mixture of ground wheat and ground defatted soybeans during feed processing: Effects of temperature, moisture level, and retention time in small-and medium-scale incubation systems. J. Agric. Food Chem. 54:16, 5887-5893.

Dierick, N. A. 1989. Biotechnology aids to improve feed and feed digestion: enzymes and fermentation. Arch. Anim. Nutr. 39: 241-261.

Dierick, N., and J. Decuypere. 1996. Mode of action of exogenous enzymes in growing pig nutrition. Pig News Info. 17:41-48.

Dikeman, C. L., and G. C. Fahey Jr. 2006. Viscosity as related to dietary fiber: a review. Critical reviews in food science and nutrition, 46:649-663.

Dung, N. N. X., L. H. Manh, and B. Ogle. 2005. Effects of fermented liquid feeds on the performance, digestibility, nitrogen retention and plasma urea nitrogen (PUN) of growing-finishing pigs. Livest. Research Rural Development, 17:8.

Ebringerova, A., and T. Heinze. 2000. Xylan and xylan derivatives–biopolymers with valuable properties. Naturally occurring xylans structures, isolation procedures and properties. Macromolecular rapid communications. 21:542-556.

Elia, M., and J. H. Cummings. 2007. Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. Eur. J. Clin. Nutr. 61(Suppl. 1): 40-74.

Feoli, C., C. R. Monge, C. L. Jones, C. W. Starkey, and J. D. Hancock. 2009. Effects of xylanase and wheat middlings in diets for finishing pigs. p. 124-127. http://krex.k-state.edu/dspace/handle/2097/1874. (Accessed 10 July 2014).

Fernández, J., and J. N. Jørgensen. 1986. Digestibility and absorption of nutrients as affected by fiber content in the diet of the pig. Quantitative aspects. Livest. Prod. Sci. 15:53-71.

Graham, H., K. Hesselman, and P. Aman. 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116:242-251.

Graham, H., and P. Aman. 1991. Nutritional aspects of dietary fiber. Anim. Feed Sci. Techn. 32:143-158.

Grieshop, C. M., D. E. Reese, and G. C. Fahey Jr. 2001. Non-starch Polysaccharides and Oligosaccharides. In: L. Southern and J. Lewis, editors, Swine nutrition. CRC Press, Boca Raton, FL. p. 107-130.

International Union of Biochemistry and Molecular Biology. 1992. Enzyme nomenclature: recommendations of the nomenclature committee of International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes. Acad. Press. New York, NY.

Jacela, J. Y., S. S. Dritz, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2010. Effects of supplemental enzymes in diets containing distillers dried grains with solubles on finishing pig growth performance. The Professional Animal Scientist. 26:412-424.

Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton, and J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72:2270-2278.

Kass, M. L., P. J. Van Soest, and W. G. Pond. 1980. Utilization of dietary fiber from alfalfa by growing swine. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. J. Anim. Sci. 50:192–197.

Kerr, B. J., and G. C. Shurson. 2013. Strategies to improve fiber utilization in swine. J. Anim. Sci. Biotech. 4(1):11.

Missotten, J. A., J. Michiels, A. Ovyn, S. De Smet, and N. A. Dierick. 2010. Fermented liquid feed for pigs. Arch. Anim. Nutrit. 64:437-466.

Noblet, J., and G. Le Goff. 2001. Effect of dietary fiber on the energy value of feeds for pigs. Anim. Feed Sci. Techn. 90:35-52.

Noblet, J. and Van Milgen, J. 2013. Energy and Energy Metabolism in Swine. In: L.I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publish Ltd., Oxford, UK. p. 23-57.

Oakenfull, D. 2001. Physicochemical properties of dietary fiber: Overview. Food Sci. Technol. 95-206.

O'Connell, J. M., T. Sweeney, J. J. Callan, and J. V. O'Doherty. 2005. The effect of cereal type and exogenous enzyme supplementation in pig diets on nutrient digestibility, intestinal microflora, volatile fatty acid concentration and manure ammonia emissions from finisher pigs. Anim. Sci. 81:357-364.

Paloheimo, M., J. Piironen, and J. Vehmaanperä. 2011. Xylanases and cellulases as feed additives. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 12-25.

Pond, W.G., and Lei, X. G. 2001. Of pigs and people. Swine nutrition. In: L. Southern and J. Lewis, editors, Swine nutrition. CRC Press, Boca Raton, FL. p. 3-18.

Selvendran, R. R. 1984. The plant cell wall as a source of dietary fiber: chemistry and structure. Ame. J. Clin. Nutr.

Souffrant, W. B. 2001. Effect of dietary fiber on ileal digestibility and endogenous nitrogen losses in the pig. Anim. Feed Sci. Technol. 90:93-102.

Steen, P. 2001. Liquids application systems for feed enzymes. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 353-376.

Stein, H. H. 2007. Distillers dried grains with solubles (DDGS) in diets fed to swine. http://livestocktrail.illinois.edu/uploads/porknet/papers/DDGS%20in%20Swine%20Diets-Stein.pdf. (Accessed 15 July 2014).

Stein, H. H., and G. C. Shurson. 2009. Board-Invited Review: The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci. 87:1292-1303.

Thaler, B., and P. Holden. 2010. By-product Feed Ingredients for Use in Swine Diets. In: D. J. Meisinger, editor, National swine nutrition guide. U.S. Pork Cent. Excellence, Ames, IA. p. 301-312.

U.S. Grains Council. 2012. A guide to Distiller's Dried Grains with Soluble (DDGS). Handbook 3rd ed.

Urriola, P. E., S. K. Cervantes-Pahm, and H. H. Stein. 2013. Fiber in swine nutrition. In: L.I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publish Ltd., Oxford, UK. p. 23-57. p. 255-276.

Ward, N. E. 2008. Non-starch Polysaccharide enzymes for poultry. In: Proc. 6th Mid-Atlantic Nutr. Conference, Timonium, MD. p. 41.

Wenk, C. 2001. The role of dietary fiber in the digestive physiology of the pig. Anim. Feed Sci. Techn. 90:21-33.

Woyengo, T. A., J. S. Sands, W. Guenter, and C. M. Nyachoti. 2008. Nutrient digestibility and performance responses of growing pigs fed phytase-and xylanase-supplemented wheat-based diets. J. Anim. Sci. 86:848-857.

Yáñez, J. L., E. Beltranena, M. Cervantes, and R. T. Zijlstra. 2011. Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles cofermented from wheat and corn in ileal-cannulated grower pigs. J. Anim. Sci. 89:113–123.

Yule, M. A., and M. F. Fuller. 1992. The utilization of orally administered D-xylose, L-arabinose and D-galacturonic acid in the pig. Inter. J. Food Sci. Nutr. 43: 31-40.

Yoon, S. Y., Y. X. Yang, P. L. Shinde, J. Y. Choi, J. S. Kim, Y. W. Kim, and K. Yun. 2010. Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of grower-finisher pigs. J. Anim. Sci 88:181-191.

Zhu, C., L. M. Rudar, D. Wey, and C. F. M. de Lange. 2011. Glucanase, xylanase and microbial inoculants improve feeding value of DDGS for liquid-fed finishing pigs. J. Anim. Sci. 89 (Suppl. 1):78. (Abstr.)

CHAPTER II: Enzyme Supplementation to Improve the Nutritional Value of Fibrous Feed Ingredients in Swine Diets Fed in Dry or Liquid Form

ABSTRACT:

Co-products are a short-term solution for commercial swine production to control feed cost, but these co-products have substantial amounts of fiber that decrease nutrient and energy digestibility in pigs. Application of fiber degrading enzymes and liquid feeding methods may help to improve the nutritional value of fibrous feed ingredients. This study evaluated the effect of endo-1,4-β-xylanase supplementation (Xyl; with or without), feeding method (dry or liquid) and feedstuff (corn DDGS or wheat middlings) on apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of nutrients, intestinal morphology, caecal pH and VFA (volatile fatty acids) concentration in growing pigs. Sixty-four pigs (BW 25.9 ± 0.38 kg) were blocked by BW and sex, placed in individual pens and randomly assigned to 8 dietary treatments. Within each feedstuff, diets were fed either liquid or dry, without or with Xyl (24,000 BXU/kg feed). DDGS and wheat middlings-based diets contained 3.32 and 3.19 Mcal/kg ME and 1.03 and 1.07% SID (Standardize Ileal Digestible) lysine, respectively. Pigs were fed isocaloric amounts of ME per day and restricted at 3 times maintenance energy requirements (197 kcal ME/kg $BW^{0.60}$). The daily ration was fed in 2 equal meals. Liquid diets were prepared by steeping DDGS or wheat middlings with water (1:3 w:v) with or without Xyl for 24 h, followed by mixing with the respective basal diet and water to achieve a final ratio of 1:2.5 w:v. Diets were fed for 16 days and then pigs were euthanized. An interaction between feeding method, Xyl supplementation and feedstuff ($P < 0.10$) was observed for AID of GE and NDF. When Xyl was added in dry wheat middlings-based diets an increase in AID of GE and NDF was observed as compared to dry wheat middlings-based diets without Xyl (64.50 vs. 54.67% and 52.88 vs. 31.69%, respectively).

However, supplementation of Xyl did not impact AID of GE and NDF when liquid wheat middlings-based diets were offered. Supplementation of Xyl in liquid DDGS-based diets enhanced the AID of NDF as compared to liquid DDGS-based diets without Xyl, but Xyl did not affect AID of NDF in dry DDGS diets. Addition of Xyl to wheat middlings-based diets improved ATTD of GE and N as compared to wheat middlings diets without Xyl (80.37 vs. 78.07% and 80.23 vs. 77.94%, respectively); however, there was no effect of Xyl in DDGS diets (feedstuff by Xyl interaction, $P < 0.05$). DDGS diets in liquid form reduced ATTD of GE as compared to DDGS diets offered in dry form (81.10 vs. 82.97%); however, no effects on ATTD of GE were observed when wheat middlings diets were offered, regardless of feeding method (78.89 vs. 79.55%; feeding method by feedstuff interaction, $P = 0.010$).

Pigs fed DDGS diets had greater concentrations of butyrate in the cecum ($P = 0.001$) compared to pigs fed wheat middlings-based diets (27.55 vs. 20.44 mmol/L). Pigs fed DDGS-based diets with Xyl had deeper crypts in the jejunum than pigs fed DDGS diets without Xyl (98.20 vs. 86.16 µm). However, there was no effect of Xyl in pigs fed wheat middlings-based diets.

Under the conditions of this experiment, liquid feeding and the application of Xyl demonstrated a limited potential to enhance nutrient digestibility in pigs fed corn DDGS-based diets. However, the supplementation of Xyl in wheat middlings-based diets improved the AID of NDF and ATTD of GE and N; but liquid feeding as a pretreatment did not enhance further the nutritional value of wheat middling based diets.

1. Introduction

Feed cost accounts for the largest proportion of the total cost of pig production (65-75%), and growing-finishing pigs account for approximately 80% of feed consumed. Costs of feed ingredients have increased substantially in recent years, thus there is a great need for developing cost-effective feeding strategies for growing-finishing pigs. Alternative feedstuffs are a short-term solution for commercial swine production to control feed cost, but it is important to recognize that those alternative feed ingredients have different feeding values because of variations in nutrient content and other factors, such as bioavailability, anti-nutritional factors and palatability. These common co-product ingredients to corn and soybean meal include dried distillers grains with solubles (DDGS), corn gluten meal, corn gluten feed, corn germ, wheat middlings, and soy hulls.

DDGS are a good source of some nutrients that are required in animal production. DDGS contains 3.396 kcal ME/kg, 27.4% CP, and 8.9% ether extract, and it is used as an energy and protein source in pig diets (NRC, 2012). In addition, DDGS have relatively high digestible phosphorus content. Based on the NRC (1994) report, about 54% of the total phosphorus in corn DDGS is non-phytate phosphorus. The total NSP content in corn DDGS compromises 23.1% of the dry matter, with the arabinoxylan fraction (arabinose and xylose portion) being 11.7% (Ward, 2008). Wheat middlings are another cereal by-product used in commercially pelleted pig feeds because it has good pellet binding properties and its cost competitiveness with other feed ingredients. Wheat middlings consist of fine particles of wheat bran, wheat shorts, wheat germ and wheat flour (AAFCO, 2000). Wheat by-products have higher content of NSP than the wheat grain, resulting in reduced digestibility for

nutrients such as amino acids and energy. Wheat middlings contain 2,968 kcal/kg ME, 15.8% CP, and 3.15% ether extract (NRC, 2012); the NSP in wheat by-products are mainly arabinoxylans and cellulose (Zijlstra et al, 1999). Total NSP range from 19% to 31.8% of dry matter in wheat middling and the arabinoxylans range from 11.5% to 15.9% of the dry matter (Bach Knudsen, 1997).

Unfortunately, both alternative feedstuffs have a high fiber content (lignin and non-starch polysaccharides, NSP) and low energetic value for pigs. Pigs can obtain energy from fiber but only after microbial fermentation of the fiber in the gastrointestinal tract and subsequent absorption of volatile fatty acids, which may contribute to the energy status of pigs (Noblet and Van Milgen, 2013). In addition, fiber may reduce the digestibility of amino acids, lipids, and some minerals. As a consequence growth rate and feed efficiency in pigs are reduced as dietary fiber level is increased (Kass et al., 1980).

Addition of exogenous fiber degrading enzymes in animal nutrition offers promise for improving the nutritional value of high fiber feedstuffs because feed enzymes help to target specific anti-nutrients in certain feed ingredients allowing pigs to extract more nutrients from feed and improve feed efficiency (Barletta, 2010).

The method of feeding and applying enzymes to the feed may impact the efficiency of the enzyme. Because enzymes need a liquid medium to be active, the process of applying exogenous enzymes in liquid feeding may allow the enzyme to act and penetrate the substrate more efficiently and the nutritional value of these ingredients could increase. However, the literature about the amount of water required for optimum enzyme activity is limited. According to Denstadli et al. (2006), the degradation of inositol 6-phosphate in a wheat and

soybean mixture with added endogenous phytase (2500 FTU/kg) was most efficient when the feed was incubated at 45% of moisture and 45 ºC. However, very low hydrolysis of inositol 6-phosphate was observed at 25 and 35% moisture.

Previous studies have shown that liquid feeding wheat and wheat middling based diets improved feed efficiency in pigs (de Lange and Zhu, 2012). However, the response of enzymes in liquid feeding systems has differed between studies (Zhu et al., 2011; de Lange and Zhu, 2012). Thus, the application of enzyme in liquid feeding systems needs to be explored in order to refine and validate this technique. Therefore, the main focus of the present study was to improve the nutritional value of DDGS and wheat middlings through supplementation of xylanase and application of liquid feeding.

2. Materials and Methods

Animal use protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee.

This experiment was conducted using a total of 64 growing pigs, with an average initial BW of 25.87 ± 0.38 kg. Pigs were blocked by BW and sex, and randomly assigned within blocks to 1 of 8 dietary treatments. Pigs were placed into a temperature-controlled room at the Research Educational Unit, Raleigh, NC. They were housed in individual pens (0.91 m by 1.82 m) using 64 pens (8 replicates per treatment). Each pen was equipped with a stainless steel feeder and a nipple drinker. Pigs were limit fed and allowed ad libitum access to water throughout the experiment. In this study, pigs were fed the experimental diets for 16 d to evaluate the impact of feedstuffs, feeding methods and xylanase supplementation on

growth performance, nutrient digestibility, morphology in the jejunum, pH and concentration of VFA at ileum and caecum level.

Two feedstuffs (DDGS and wheat middlings), two feeding methods (dry and liquid), two enzyme supplementation treatments (without or with xylanase) were combined to create 8 experimental diets in a 2 x 2 x 2 factorial arrangement. The source of xylanase used was endo-1,4-β-xylanase (Econase XT, ABvista) and was included at 150g/1000 kg of finished feed to reach an activity of 24,000 BXU/kg of feed.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit. Diets were manufactured by creating two basal mixes first that contained all ingredients with the exception of the DDGS, wheat middlings, or xylanase. Dry diets were subsequently manufactured from the appropriate basal mix by combining either 30% DDGS or 30% wheat middlings with or without xylanase with 70% of basal mix. Preparation of the liquid diets began twenty-four hours before feeding the animals. The DDGS or wheat middling were steeped separately with water (1:3 weight:volume) in the absence or presence of xylanase for about 24 hours and then mixed with the respective basal diet in a 11 kg commercial mixer, which was then fed to the pigs in a final weight:volume ratio of 1:2.5 (de Lange and Zhu, 2012). This method of feed manufacturing ensured that the diets were identical with the exception of fiber source and enzyme addition (Table 4).

Diets were representative of current commercial practices and consisted mainly of corn and soybean meal as basal ingredients. Diets met NRC (2012) requirements for all nutrients for 25 kg pigs and they were fed in meal form.

Daily feed allowance was restricted to 3 times maintenance (3 x 197 kcal ME/kg $BW^{0.60}$; NRC 2012), which was fed in two equal meals per day (8:00 and 16:00). Additionally, diets contained 0.3% of titanium dioxide as an indigestible marker to calculate the apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of nutrients.

2.1 Measurements

Average daily gain (ADG), average daily feed intake (ADFI), and G:F (gain:feed) ratio were measured. During the final three days of the study, fecal samples were collected from each pen by sampling of freshly voided feces or from fresh feces present in the pen. Pens in this facility were separated by solid partitions and, therefore, samples in each pen were specific to the pig in that pen. Fecal samples were frozen in plastic bags at -20ºC for subsequent analyses.

At the end of the experiment, pigs were fasted for 6 to 8 h and then euthanized using captive bolt followed by exsanguination. The abdominal cavity was opened and a section of the jejunum was collected, cleaned with deionized water and fixed in 10% formalin solution for measurement of mucosal histology. The digesta content of ileum and caecum were removed immediately and the pH determined. Samples of dry and liquid diets, ileum and feces were prepared for analyses by drying for 3 d at 55 ºC. The samples were ground using a kitchen blender.

2.2 Chemical Analyses

The chemical composition of the experimental diets were analyzed for crude protein, crude fat, crude fiber, ADF, NDF, soluble and insoluble fiber (The University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO) using AOAC (2005) procedures. Concentration of titanium dioxide in the diets and in the fecal and ileal samples were determined according to Myers et al. (2004) with a minor modification. The modification consisted of the addition of 50 µL of stabilized 30% hydrogen peroxide into all wells of the microplate, 30 min before reading to ensure orange color of the reaction. Concentrations were determined relative to a standard curve at 410 nm using a microplate reader (Synergy HT Multi-detection, Bio-Teck Instruments, Inc., Winooski, VT).

Gross energy (GE) was determined by dynamic bomb calorimetry (C5000 Calorimetric System, IKA, Wilmington, NC), calibrated using benzoic acid. Nitrogen (N) was measured using the combustion method (LECO, St Joseph, MI). The NDF (Neutral detergent fiber) content was determined according to Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Fairport, NY). The ATTD and AID of GE, N and NDF were obtained using the index ratio procedure (Adeola, 2001).

$$\text{Nutrient Digestibility} = 100 - [100 * \left(\frac{\text{Marker in diet} * \text{Nutrient in feces}}{\text{Marker in feces} * \text{Nutrient in diet}}\right)]$$

The VFA concentration was measured by gas liquid chromatography (Varian GC model CP-3380, Walnut Creek, CA). Jejunum samples were sent to North Carolina State

University histology laboratory for hematoxylin and eosin staining. Villus height, villus width and crypt depth were measured using a microscope (Micromaster, Fisher Scientific International Inc., Pittsburgh, PA). Ten villi that were well positioned were randomly selected and measured for each pig and then the data were averaged to provide equal morphological representation per pig.

2.3 Statistical Analysis

Statistical analysis was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model included block, feedstuff, feeding method, xylanase supplementation, and their interactions as fixed effects. The initial BW was used as covariate to analyze final BW. Least squares means were reported and differences were considered statistically significant at $P \leq 0.05$, with tendencies at $0.05 < P \leq 0.10$.

3. Results

3.1 Growth performance

Feeding method (dry or liquid), enzyme supplementation (with or without Xyl), and feedstuff addition (DDGS or wheat middlings) did not produce statistical differences ($P > 0.30$) in final BW, ADG and G:F ratio (Table 5; Appendix A). A main feeding method effect ($P = 0.001$) was observed for ADFI, indicating that pigs fed liquid diets had lower ADFI than pigs fed dry diets (1.29 vs. 1.34 kg/d). Also, ADFI depended on the feedstuff used ($P = 0.0001$). Pigs fed DDGS-based diets had lower feed intake than pigs fed wheat middlings-based diets (1.29 vs. 1.34 kg/d).

3.2 Nutrient digestibility

A tendency for an interactive effect between feeding method, Xyl supplementation and feedstuff (P = 0.056) was observed for AID of GE (Table 6; Appendix B). Xylanase supplementation to dry wheat middlings-based diets tended to improve AID of GE compared to dry wheat middlings-based diets without Xyl (64.50 vs. 54.67%); however, Xyl supplementation to liquid wheat middlings-based diets did not affect AID of GE (62.88 vs. 59.94%). Xylanase supplementation to DDGS-based diets did not improve AID of GE, regardless of feeding method.

An interaction between feeding method, Xyl supplementation and feedstuff (P = 0.010) was also observed for AID of NDF. When Xyl was supplemented to dry wheat middlings-based diets, an increase in AID of NDF was observed as compared to dry wheat middlings-based diets without Xyl (52.88 vs. 31.69%). Supplementation of Xyl had no effect when liquid wheat middlings-based diets were offered (29.99 vs. 33.34%). Supplementation of Xyl in dry DDGS-based diets did not affect AID of NDF as compared to dry DDGS-based diets without Xyl (46.87 vs. 49.76%), but the addition of Xyl in liquid DDGS-based diets enhanced AID of NDF as compared to liquid DDGS-based diets without Xyl (46.54 vs. 31.58%).

The AID of N depended on the feeding method (P = 0.013), where pigs fed liquid diets had greater AID of N than pigs fed dry diets (76.20 vs. 71.96%).

An interaction between feedstuff and Xyl supplementation was observed (P = 0.001) for ATTD of GE. Addition of Xyl to the wheat middlings-based diets improved ATTD of GE

as compared to the wheat middlings-based diets without Xyl (80.37 vs. 78.07%). However, addition of Xyl to the DDGS-based diets did not impact ATTD of GE (81.55 vs. 82.51%).

Moreover, an interaction between feeding method and feedstuff was detected (P = 0.010) for ATTD of GE. When DDGS-based diets were offered in liquid form, a reduction in ATTD of GE was observed as compared to the DDGS-based diets offered in dry form (81.10 vs. 82.97%). However, no effects on ATTD of GE were observed when wheat middlings-based diets were offered either in dry or liquid forms (78.89 vs. 79.55%).

There was an interaction (P < 0.0001) between feeding method, Xyl supplementation and feedstuff for the ATTD of NDF. The addition of Xyl in the dry wheat middlings-based diets increased the ATTD of NDF as compared to the dry wheat middlings-based diets without Xyl (67.55 vs. 62.28%). In contrast, supplementation of Xyl in liquid wheat middlings-based diets decreased ATTD of NDF as compared to liquid wheat middlings-based diets without Xyl (57.24 vs. 65.08%). Supplementation of Xyl in the dry DDGS-based diets did not impact ATTD of NDF as compared to dry the DDGS-based diets without Xyl (70.40 vs. 73.70%). Likewise, addition of Xyl in the liquid DDGS-based diets did affect ATTD of NDF as compared to liquid DDGS-based diets without Xyl (65.44 vs. 64.17%).

The effect of Xyl on ATTD of N depended on the type of feedstuff (P = 0.027). Pigs fed wheat middlings-based diets with Xyl had greater ATTD of N than those fed wheat middlings-based diets without Xyl (80.23 vs. 77.94%). However, the addition of Xyl to DDGS-based diets did not improve ATTD of N as compared to DDGS-based diets without Xyl (81.04 vs. 82.19%).

3.3 pH and VFA

No significant differences in ileal pH were found among treatments (Table 7; Appendix C). However, a main feedstuff effect (P = 0.004) was observed for cecum pH, indicating that pigs fed diets containing wheat middlings had greater cecum pH than pigs fed the diets containing DDGS (5.76 vs. 5.62).

The concentration of VFA in ileum samples were low and below the detection level of the instrument used in this study, therefore data were not reported. The concentration of acetate, propionate and total VFA in the cecum were not significantly different among the treatments (P > 0.66). However, the concentration of butyrate in cecum depended on the type of feedstuff (P = 0.001): pigs fed the DDGS-based diets had greater concentrations of cecum digesta butyrate than pigs fed the wheat middlings-based diets (27.55 vs. 20.44 mmol/L).

The molar proportions of each VFA relative to the total VFA were analyzed. The ratio of acetate:total VFA tended to be affected by Xyl supplementation (P = 0.061), indicating that pigs fed diets supplemented with Xyl had a higher acetate:total VFA ratio compared to pigs fed diets without Xyl (0.52 vs. 0.50).

An interaction was observed between feeding method and Xyl supplementation for the propionate:total VFA ratio (P = 0.032). The addition of Xyl in the liquid diets decreased the propionate:total VFA ratio as compared to liquid diets without Xyl (0.33 vs. 0.37). In contrast, addition of Xyl in dry diets did not have a significant effect on propionate:total VFA ratio as compared to dry diets without Xyl (0.36 vs. 0.35).

The butyrate:total VFA ratio depended significantly on the type of feedstuff (P = 0.011). Pigs fed diets containing DDGS had greater butyrate:total VFA than pigs fed diets containing wheat middlings (0.16 vs. 0.12).

3.4 Morphology

An interaction was observed between feeding method, Xyl supplementation and feedstuff for villi height (P = 0.018; Table 8; Appendix D). Addition of Xyl in liquid DDGS-based diets increased villi height as compared to the liquid DDGS-based diets without Xyl (319.56 vs 276.29 µm). But, no statistical differences were observed in villi height when Xyl was added to the dry DDGS-based diets as compared to the dry DDGS-based diets without Xyl (322.96 vs 350.42 µm). Supplementation of Xyl in the liquid wheat middlings-based diets did not affect villi height as compared to liquid wheat middlings-based diets without Xyl (295.45 vs. 327.46 µm). Likewise, no differences were observed in villi height when Xyl was added to the dry wheat middlings-based diets as compared to the wheat middlings-based diets without Xyl (289.65 vs. 305.21 µm).

Jejunum villi width was affected by an interaction between feeding method and feedstuff (P = 0.032). Villi width of pigs fed liquid wheat middlings-based diets was greater than the villi width of pigs fed dry wheat middlings-based diets (141.9 vs. 122.91 µm). However, villi width of pigs fed dry DDGS-based diets was not was not statistical different from pigs fed liquid DDGS diets (138.22 vs. 133.38 µm).

An interaction between feedstuff and Xyl supplementation (P = 0.007) was detected for crypt depth. Pigs fed DDGS-based diets with Xyl had greater crypt depth than pigs fed

DDGS-based diets without Xyl (98.20 vs. 86.16 µm). However, the crypt depth in pigs fed wheat middlings-based diets with Xyl was not statistically different from pigs fed wheat middlings-based diets without Xyl (91.62 vs. 101.92 µm).

4. Discussion

Pigs on all treatments grew well during the 16 days of the experiment. This experiment was not designed to evaluate growth performance per se due to the small number of animals used in the study. However, ADFI, ADG, and G:F ratio were measured to verify that pigs were eating and growing normally. Pigs fed the liquid diets had a lower ADFI than pigs fed the dry diets, which was due to feed refusal of liquid feed at the start of the study. Also, ADFI depended on the type of feedstuff used because the ME density between DDGS and wheat middlings-based diets was different and pigs were fed equal amounts of ME. Inconsistent results on growth performance from the use of xylanase in DDGS and wheat middlings-based diets have been reported. Jacela et al. (2010) did not observe any significant effects on growth performance when xylanase was added in growing-finishing pigs diets containing 30% DDGS. Similarly, Feoli et al. (2006) did not observe improvements in growth performance when finishing pigs were fed diets containing 30% wheat middlings and supplemented with xylanase. On the other hand, de Lange et al. (2013) found that the inclusion of xylanase and glucanase in liquid diets containing 30% corn DDGS improved ADG and F:G ratio in finishing pigs as compared to liquid diet without enzyme supplementation. Additionally, de Lange et al. (2013) evaluated the efficacy of xylanase and glucanase in dry and liquid diets that contained 40% wheat shorts in finishing pigs. They

reported that pigs fed liquid diets performed better than pigs fed dry diets, but the response to added enzymes was not significant.

Co-products of cereal grains, such as the ones used in this experiment (DDGS and wheat middlings), have a high content of NSP. Pigs cannot produce the endogenous enzymes to digest NSP, therefore, supplementation of NSP-degrading enzymes represents one approach to alleviate detrimental effects of NSP and enhance the nutritional value for young pigs (Nortey et al., 2007). A potential strategy to enhance the efficiency of exogenous enzymes is the use of liquid feeding, because enzymes need a liquid medium to be active and it gives more opportunities for exogenous enzymes to target specific substrates in raw materials before the animal consumes the feed (Canibe and Jensen, 2012).

The total NSP content in corn DDGS comprises 23.1% of dry matter, and the water insoluble portion represents about 88% of this total (Ward, 2008). A large proportion of insoluble NSP in corn DDGS is expected because the soluble portion is degraded during fermentation and ethanol production. The arabinose and xylose portion provide an estimate of the arabinoxylan fraction, which is 11.7% of dry matter (Ward, 2008). Similarly, the NSP in wheat co-products are mainly arabinoxylans and cellulose (Zijlstra et al., 1999); total NSP and arabinoxylan content in wheat middlings range from 19% to 31.8% and from 11.5% to 15.9% of the dry matter, respectively. The NSP entrap nutrients and act as a physical barrier to effective nutrient hydrolysis and absorption. It has been proposed that supplementation of xylanase may improve the nutritional value of NSP diets by partially hydrolyzing soluble and insoluble NSP, breaking NSP-containing cell walls and thereby liberating their contents for enzymatic hydrolysis (Dierick and Decuypere, 1996; Diebold et al., 2005). Xylanase

randomly breaks the arabinoxylan backbone into smaller chains and reduces their molecular weight (Tapingkae et al., 2008). Therefore, feedstuffs with greater arabinoxylan content will have more encapsulated nutrients and thus derive greater benefit from xylanase supplementation (Nortey et al., 2008).

The results of the present study indicated that when DDGS-based diets were fed, neither addition of Xyl nor feeding method appeared to improve the total tract and ileal digestibility of nutrients. However, the ATTD of GE and NDF were reduced when DDGS-based diets were fed in liquid form. The lack of an effect of Xyl in DDGS-based diets may be associated with the insoluble arabinoxylans in corn DDGS that were inaccessible to Xyl due to the fiber fraction in corn DDGS being composed of highly substituted glucoarabinoxylans that are cross-linked with lignin and cellulose within the cell wall matrix (Vries et al., 2014). According to in vitro digestion and fermentation studies conducted by de Vries et al. (2014), the cell wall structure of DDGS was barely affected when different processing technologies (wet-milling, extrusion, autoclaving and mild hydrothermal acid treatment) in combination with exogenous enzymes (endo-1,4-β-xylanase and endo-1,4-β-glucanase) were applied in corn DDGS. In a subsequent study, de Vries et al. (2014) evaluated the effect of hydrothermal maleic acid treatment on the degradability of corn DDGS-based diets in growing pigs, and they found that this processing technology helped to improve degradation of NSP at the ileum level and shifted fermentation from caecum to more proximal gastrointestinal sections. However, the total tract degradation of NSP was not affected. These findings confirm that the cell wall structure presents in corn DDGS are complex structures highly resistant to degradation. Moreover, Jha et al. (2015) conducted an in vitro

fermentation study where they found that the extent of the heat damage associated with the DDGS production process affects the fermentation of the complex fiber fraction of DDGS and also affects the efficiency of supplemental carbohydrate enzymes.

The results found in the current study are supported by Choct et al. (2004), who reported a negative impact of xylanase addition to liquid feed fermented for 1 h on energy digestibility in weaned pigs. Most of the studies that evaluated the supplementation of NSP enzymes in DDGS-based diets in growing pigs did not report improvements in nutrient digestibility (Mc Alpine et al., 2012; de Vries et al., 2014; Diebold et al., 2004; Kerr et al., 2010; Kerr and Shurson, 2013; Zijlstra et al., 2004).

In the present study, liquid feeding as pretreatments did not appear to be an advantageous strategy to enhance digestibility of nutrients in wheat middlings-based diets. However, supplementation of Xyl had a clear positive effect on ATTD of GE and N and on AID of NDF when supplemented to wheat middlings-based diets. This positive response of Xyl supplementation when fed wheat middlings-based diets is supported by several studies. Diebold et al. (2004) reported a positive effect on the AID of GE and NDF with Xyl supplementation in weanling pigs fed wheat based diets. Similarly, Nortey et al. (2007) found that inclusion of Xyl in wheat based diets containing wheat millrun improved AID and ATTD of GE in grower pigs. The increase in nutrient digestibility due the supplementation of Xyl could be due the arabinoxylan hydrolysis by Xyl, exposing the enclosed intracellular nutrients to digestive enzymes within the gut lumen permitting more complete digestion. The lack of effects of Xyl in liquid wheat middlings-based diets may be associated with the presence of natural xylanase inhibitors. Cereal grains such as wheat, rye, and barley contain

proteins that can inhibit xylanase activity (Bonnin et al., 2005; Debyser et al., 1999 and Paloheimo et al., 2011). Perhaps the steeping process of wheat middlings with water and supplemental Xyl, not only stimulates Xyl activity as more substrate is accessed (improved mobility of Xyl), but it also could stimulate the activity of xylanase inhibitors (increased mobility of xylanase inhibitors). Enzyme inhibition is a natural phenomenon that occurs in plant seeds to act as defense mechanism and regulate plant metabolic processes (Nortey et al., 2008). The presence of inhibitors can therefore negate the effects that can be achieved by adding xylanase to swine diets that contain wheat. The effects of endogenous xylanase inhibitors and xylanase have been studied more extensively in the food industry, especially in bread making (Debyser et al., 1999).

Wheat grain contains endogenous arabinoxylan degrading enzymes and they have been detected in wheat flour and in wheat bran (Bonnin et al, 2005; Gys et al., 2004). In contrast, no active endogenous enzymes are present in corn DDGS because during its production process, DDGS are drying and the endogenous enzymes are deactivated. Thus, the natural enzyme activity present in wheat middlings may have masked, in part, the effects of supplemental xylanase, especially when diets were fed in liquid form.

The steeping process employed in the current study involved the thorough mixing the ingredients with water after which the mixture was allowed to steep for 24 h. Thus, no further agitation occurred which could have limited the efficient interaction of the enzymes with the substrate.

Desirable effects of supplementation of fiber degrading enzymes include increased VFA production, which represents an energy source for pigs. However in the present study,

no statistically significant differences were found in the concentration of acetate, propionate and total VFA in caecum digesta among the treatments. However, the concentration of butyrate was greater in pigs fed the DDGS-based diets than those fed the wheat middlings-based diets. Butyrate is an important metabolite because it serves as an energy source for the epithelium but it also regulates cell proliferation and differentiation in the gastrointestinal tract (Pryde et al., 2002). The ability of gut micro flora to produce butyrate can depend considerably on diets composition (Knudsen et al., 2003). In monogastric species, dietary sugars usually do not reach the large intestine due to digestion and absorption in the small intestine, but dietary fiber in combination with slowly degradable starch stimulate the production of butyrate in the large intestine (Plöger et al., 2012). Thus, the higher concentration of butyrate in the cecum of pigs fed corn DDGS-based diets may be due to more undigestible fiber and non-structural carbohydrates reaching the caecum when corn DDGS-based diets were offered compared as wheat middlings-based diets. According to Jin et al. (2000), butyrate has a beneficial effect in the gut because it promotes the intestinal colonization by *Lactobacillus* to the detriment of *Escherichia coli* bacteria colonization; thus, the health of the caecum and colon epithelium may be improved when there is a greater production of VFA, especially butyrate. However, the microbial population in the gut was not evaluated to validate this effect in the current study. The pH observed in this study decreased from the ileum to the caecum, as has been reported in the literature (Horberg and Lidberg, 2004, Bach Knudsen et al., 2013).

Dietary fiber may alter intestinal morphology as well as the rate of intestinal cell turnover in pigs, which can affect the capacity of the gut to absorb nutrients (Jin et al., 1994).

The villi are mainly responsible for absorption of nutrients. Villus height is an indicator of intestinal health status. Damaged villi are shorter and the enterocytes at the villi tip are more immature. Crypt depth is another indicator of intestinal health, as crypts will deepen to produce more cells when cell turnover rates are high (Min et al., 2012). In the present study, greater crypt depth was observed in pigs fed DDGS diets with Xyl and wheat middlings diets with or without Xyl. According to Yason et al. (1987) and Paulus et al. (1992) epithelial regeneration begins from the villi crypt, so a deep crypt indicates a rapid enterocyte turnover and greater mucosal tissue maintenance requirements. The accelerated enterocyte proliferation and the epithelial cell turnover rate greatly impacts protein and energy requirements of the small intestinal mucosa (Simon, 1989). The results observed on crypt depth in the jejunum are similar to the finding of Jin et al. (1994), who reported that feeding growing pigs a high fiber diet (i.e. 10% wheat straw) increased depth of intestinal crypts in jejunum, ileum and colon, which led to an increase in cell proliferation rate, thus increased the rate of villi enterocytes intestinal.

Previous studies (Hurst et al., 2001; Scholten et al., 2002) have shown that liquid diets promotes longer villus in the epithelium of the intestine of pigs. However, that effect was not observed in the present study. Scholten et al. (2002) found that piglets fed a liquid diet containing wheat fermented for 24 hours had increased jejunum villus height. Similarly, Hurst et al. (2001) found that finishing pigs fed liquid feed had greater villus height and better feed efficiency than pigs fed dry diets.

Under the conditions of this experiment, the combination of liquid feeding method and the application of Xyl are not enough to disrupt the cell wall structures of DDGS because

no improvement in apparent ileal and apparent total tract digestibility of GE, N and NDF was observed in growing pigs fed diets containing 30% corn DDGS combined with Xyl and in liquid form. Likewise, liquid feeding did not improve nutrient digestibility in pigs fed wheat middlings-based diets, but the addition of Xyl enhanced the AID of NDF and the ATTD of GE and N in growing pigs fed diets containing 30% of wheat middlings.

In conclusion, liquid feeding method and the application of Xyl demonstrated a limited potential to enhance degradation and feeding value of the fiber fraction in corn DDGS-based diets. On the other hand, the addition of Xyl improved the nutritional value of wheat middlings-based diets but the liquid feeding as a pretreatment did not enhance further the nutritional value of wheat middling based diets. Different pretreatments and enzyme combinations should be explored to target the degradation of the complex and highly substituted arabinoxylans present in the cell wall matrix of corn DDGS and wheat middlings and their effect on the gastrointestinal microbiota and metabolism in pigs.

References

AAFCO. 2000. Official Publication of Association of American Feed Control Officials, Oxford, IN.

Adeola, O. 2001. Digestion and balance techniques in pigs. In: L. Southern and J. Lewis, editors, Swine nutrition. CRC Press, Boca Raton, FL. p. 906.

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th ed. Assoc. Offic. Anal. Chem., Gaithersburg, MD.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Techn. 67:319-338.

Bach Knudsen, K. E., A. Serena, N. Canibe, and K. S. Juntunen, K. S. 2003. New insight into butyrate metabolism. Proc. Nutr. Society. 62:81-86.

Bach Knudsen, K. E, H. N. Laerke and H. Jørgensen. 2013. Carbohydrates and Carbohydrate Utilization in Swine. In: L.I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publish Ltd., Oxford, UK. p. 109-137.

Barletta, A. 2010. Introduction: current market and expected developments. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 1-11

Bonnin, E., S. Daviet, K. Gebruers, J. A. Delcour, A. Goldson, N. Juge, and L. Saulnier. 2005. Variation in the levels of the different xylanase inhibitors in grain and flour of 20 French wheat cultivars. J. Cereal Sci. 41:375-379.

Canibe, N., and B. B. Jensen. 2012. Fermented liquid feed-Microbial and nutritional aspects and impact on enteric diseases in pigs. Anim. Feed Sci. Techn. 173:17-40.

Choct, M., E. A. D. Selby, D. J. Cadogan, and R. G. Campbell. 2004. Effect of liquid to feed ratio, steeping time, and enzyme supplementation on the performance of weaner pigs. Crop Pasture Sci. 55: 247-252.

de Lange, C. F. M., and C. H. Zhu. 2012. Liquid feeding corn-based diets to growing pigs: practical considerations and use of co-products. In: J. Patience, editor, Feed Efficiency in Pigs. Wageningen Academic Publisher. Wageningen, The Netherlands. p. 63-80.

de Lange, C. F. M., C. H. Zhu, D. Wey, J. Guimaras, D. Colombus, M. Rudar and M. Or-Rashid. 2013. Swine Liquid Feeding Research II: Increase co-product usage, reduce energy input cost, impact on meat quality, and gut health and environment.

De Vries, S. 2014. Fiber fermentation in pigs and poultry: sense and nonsense of its manipulation. PhD Diss. Univ. of Wageningen.

Debyser, W., W. J. Peumans, E. J. M. Van Damme, and J. A. Delcour. 1999. *Triticum aestivum* Xylanase Inhibitor (TAXI), a new class of enzyme inhibitor affecting breadmaking performance. J. Cereal Sci. 30:39-43.

Diebold, G., R. Mosenthin, H. P. Piepho, and W. C. Sauer. 2004. Effect of supplementation of xylanase and phospholipase to a wheat-based diet for weanling pigs on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces. J. Anim. Sci. 82:2647-2656.

Diebold, G., R. Mosenthin, W. C. Sauer, M. E. Dugan, and K. A. Lien. 2005. Supplementation of xylanase and phospholipase to wheat-based diets for weaner pigs. J. Anim. Physiol. Anim. Nutr. 89:316-325.

Dierick, N., and J. Decuypere. 1996. Mode of action of exogenous enzymes in growing pig nutrition. Pig News Info. 17:41-48.

Feoli, C., C. R. Monge, C. L. Jones, C. W. Starkey, and J. D. Hancock. 2006. Effects of xylanase and wheat middlings in diets for finishing pigs. In: Kansas State University Swine Day 2006. Report of Progress 966. Kansas State University. http://krex.k-state.edu/dspace/bitstream/handle/2097/1874/Effects%20of%20Xylanase%20and%20

Wheat%20Middlings%20in%20Diets%20for%20Finishing%20Pigs%20-%20Swine%20Day%202006.pdf?sequence=1. (Accessed 01 July 2014.)

Gys, W., K. Gebruers, J. F. Sørensen, C. M. Courtin, and J. A. Delcour. 2004. Debranning of wheat prior to milling reduces xylanase but not xylanase inhibitor activities in wholemeal and flour. J. Cereal Sci. 39:363-369.

Högberg, A., and J. E. Lindberg. 2004. Influence of cereal non-starch polysaccharides and enzyme supplementation on digestion site and gut environment in weaned piglets. Anim. Feed Sci. Technol. 116:113-128.

Hurst, D., I. J. Lean, and A. D. Hall. 2001. The effects of liquid feed on the small intestine mucosa and performance of finishing pigs at different water to feed ratios. In: Proc. of the British Society of Animal Science. p. 161.

Jacela, J. Y., S. S. Dritz, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2010. Effects of supplemental enzymes in diets containing distillers dried grains with solubles on finishing pig growth performance. The Professional Animal Scientist 26:412-424.

Jha, R., T. A. Woyengo, J. Li, M. R. Bedford, T. Vasanthan, and R. T. Zijlstra. 2015. Enzymes enhance degradation of the fiber-starch-protein matrix of distillers dried grains with solubles as revealed by a porcine in vitro fermentation model and microscopy. www.animalsciencepublications.org/publications/jas/view/first-look/jas2014-7910.pdf. (Accessed 01 February 2015.)

Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton, and J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72:2270-2278.

Kass, M. L., P. J. Van Soest, and W. G. Pond. 1980. Utilization of dietary fiber from alfalfa by growing swine. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. J. Anim. Sci. 50:192–197.

Kerr, B. J., T. E. Weber, P. V. Anderson, and G. C. Shurson. 2010. Enzymes for use in high DDGS swine diets. In: 71st Minn. Nutr. Conf. Proc., Owatonna, MN. p. 129–152.

Kerr, B. J. and G. C. Shurson. 2013. Strategies to improve fiber utilization in swine. J. Anim. Sci. Biotech. 4(1):11.

Mc Alpine, P. O., C. J. O'Shea, P. F. Varley, and J. V. O'Doherty. 2012. The effect of protease and xylanase enzymes on growth performance and nutrient digestibility in finisher pigs. J. Anim. Sci. 375-377.

Min, Y. N., H. L. Li, L. Li, Z. Y. Niu, J. J. Wang, S. K. Liu, J. Zhang, and F. Z. Liu. 2012. Effects of Dietary Distillers Dried Grains with Solubles (DDGS) Concentrations on Intestinal Morphology of Broiler Chicken. J. Anim. Vet. Adv. 12: 6-9.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179-183.

Noblet, J. and Van Milgen, J. 2013. Energy and Energy Metabolism in Swine. In: L.I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publish Ltd., Oxford, UK. p. 23-57.

Nortey, T. N., J. F. Patience, P. H. Simmins, N. L. Trottier, and R. T. Zijlstra. 2007. Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower pigs fed wheat-based diets containing wheat millrun. J. Anim. Sci. 85:1432-1443.

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.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient Requirements of Swine. 11 th ed. Natl. Acad. Press, Washington, DC.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 1:125-134.

Paloheimo, M., J. Piironen, and J. Vehmaanperä. 2011. Xylanases and cellulases as feed additives. In: M. Bredford and G. Partridge, editors, Enzymes in farm animal nutrition. Wallingford, UK. p. 12-25.

Paulus, U., C.S. Potten, and M. Loeffler. 1992. A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation. Cell Prolif. 25:559–578.

Plöger, S., F. Stumpff, G. B. Penner, J. D. Schulzke, G. Gäbel, H. Martens, and J. Aschenbach. 2012. Microbial butyrate and its role for barrier function in the gastrointestinal tract. Ann. NY Acad. Sci. 1258:52-59.

Pryde, S. E., S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint. 2002. The microbiology of butyrate formation in the human colon. FEMS microbiology letters. 217:133-139.

Scholten, R. H. J., C. M. C. Van der Peet-Schwering, L. A. Den Hartog, M. Balk, J. W. Schrama, and M. W. A. Verstegen. 2002. Fermented wheat in liquid diets: effects on gastrointestinal characteristics in weanling piglets. J. Anim. Sci. 80:1179-1186.

Simon, O. 1989. Metabolism of proteins and amino acids. Protein Metabolism and Farm Animals. Evaluation, Digestion, Absorption and Metabolism. Oxford University Press. p. 271–336.

Tapingkae, W., M. Yachai, W. Visessanguan, P. Pongtanya, and P. Pongpiachan. 2008. Influence of crude xylanase from *Aspergillus niger* FAS128 on the in vitro digestibility and production performance of piglets. Anim. Feed. Sci. Technol. 140:125-138.

Van Soest, P. V., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Ward, N. E. 2008. Non-starch Polysaccharide enzymes for poultry. In: Proc. 6th Mid-Atlantic Nutr. Conference, Timonium, MD. p. 41.

Yason C. V., B.A. Summers, and K.A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. American J. Vet. Res. 6:927–938.

Zhu, C., L. M. Rudar, D. Wey, and C. F. M. de Lange. 2011. Glucanase, xylanase and microbial inoculants improve feeding value of DDGS for liquid-fed finishing pigs. J. Anim. Sci. 89:78.

Zijlstra, R. T., C. D. 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.

Zijlstra, R. T., S. Li, A. Owusu-Asiedu, P. H. Simmins, and J. F. Patience. 2004. Effect of carbohydrase supplementation of wheat-and canola-meal-based diets on growth performance and nutrient digestibility in group-housed weaned pigs. Can. J. Anim. Sci. 84:689-695.

Table 4. Composition of the experimental diets, as feed basis[1]

	Dry diets				Liquid diets			
	DDGS		MIDDS		DDGS		MIDDS	
Enzyme supplementation	-	+	-	+	-	+	-	+
Ingredient, %								
Corn, yellow dent	47.79	47.79	48.62	48.62	47.79	47.79	48.62	48.62
Soybean meal, 47.5% CP	17.47	17.47	16.42	16.42	17.47	17.47	16.42	16.42
Corn DDGS, 6 - 9% oil	30.00	30.00	-	-	30.00	30.00	-	-
Wheat middlings, <9.5% fiber	-	-	30.00	30.00	-	-	30.00	30.00
Poultry fat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-lysine HCl	0.45	0.45	0.46	0.46	0.45	0.45	0.46	0.46
DL-methionine	0.03	0.03	0.11	0.11	0.03	0.03	0.11	0.11
L-threonine	0.08	0.08	0.17	0.17	0.08	0.08	0.17	0.17
Monocalcium phosphate	0.70	0.70	0.80	0.80	0.70	0.70	0.80	0.80
Limestone	1.49	1.49	1.43	1.43	1.49	1.49	1.43	1.43
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
G/F vitamin premix[2]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Titanium Dioxide[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Xylanase[5]	-	0.0015	-	0.0015	-	0.0015	-	0.0015
Calculated composition								
ME, Mcal/kg	3.32	3.32	3.19	3.19	3.32	3.32	3.19	3.19
NDF %	14.93	14.93	16.46	16.46	14.93	14.93	16.46	16.46
Total Lysine %	1.26	1.26	1.16	1.16	1.26	1.26	1.16	1.16
Analyzed composition								
CP, %	22.67	22.11	18.36	19.63	25.62	25.63	19.58	21.2
CF, %	3.83	4.21	4.55	4.21	4.66	4.73	4.68	4.27
ADF, %	6.36	6.55	6.2	5.71	6.65	7.24	5.39	5.61
NDF, %	14.21	16.07	17.5	15.46	14.32	1671	13.87	12.68
Soluble Fiber, %	0.19	0.23	0.23	0.20	0.22	0.24	0.18	0.18
Insoluble Fiber, %	17.29	18.86	20.76	18.63	18.29	19.64	16.98	17.54

[1]Diets were formulated based on NRC (2012) requirements.
[2]Supplied per kg of complete diet: 8,819 IU of vitamin A, 1123 IU of vitamin D_3 as D-activated animal sterol, 26.6 IU of vitamin E, 0.04 mg of vitamin B_{12}, 6.13 mg of riboflavin, 35.3 mg of niacin, 24.6 mg of d-pantothenic acid as calcium pantothenate, 3.4 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, and 0.09 mg of d-biotin.
[3]Supplied per kg of complete diet: 16.5 mg of copper as copper sulfate, 0.3 mg of iodine as ethylenediaminedihydroiodide, 165 mg of iron as ferrous sulfate, 40 mg of manganese as manganous oxide, 0.3 mg of selenium as sodium selenite, and 165 mg of zinc as zinc sulfate.
[4]Titanium dioxide as indigestible marker.
[5]Econase XT AB-vista

Table 5. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation (Xyl) on growth performance in growing pigs[1]

	Dry				Liquid				
	DDGS		Wheat Middlings		DDGS		Wheat Middlings		SEM
Xylanase supplementation[2]	-	+	-	+	-	+	-	+	
Initial body weight, kg	26.30	25.67	26.48	25.61	26.32	25.65	25.95	25.25	0.39
Final body weight, kg	35.38	35.96	35.80	35.34	35.26	35.53	35.33	35.63	0.43
ADG, kg	0.61	0.66	0.64	0.62	0.61	0.63	0.62	0.64	0.03
ADFI,[3] kg (as fed basis)	1.30	1.32	1.37	1.36	1.27	1.26	1.34	1.29	0.02
G:F	0.47	0.50	0.47	0.46	0.48	0.50	0.46	0.50	0.02

[1]Values are means of 8 observations per treatment.

[2]With xylanase (+) or without xylanase (-)

[3]Significant main effect of FM and FS ($P < 0.01$)

Table 6. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation (Xyl) on apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of GE, NDF, and N[1]

	Dry				Liquid				
	DDGS		Wheat Middlings		DDGS		Wheat Middlings		SEM
Xylanase supplementation[2]	-	+	-	+	-	+	-	+	
AID, %									
GE[3]	65.68	62.19	54.67	64.50	58.72	67.19	59.94	62.88	3.39
NDF[4]	49.76 a	46.86 a	31.69 b	52.88 a	31.58 b	46.53 a	33.34 b	29.99 b	5.58
N[5]	72.37	71.76	69.85	73.85	72.96	76.52	78.29	77.03	2.30
ATTD, %									
GE[6]	83.85	82.08	77.49	80.30	81.18	81.01	78.65	80.44	0.66
NDF[7]	73.70 a	70.40 ab	62.27 d	67.55 bc	64.17 cd	65.43 cd	65.08 cd	57.23 e	1.21
N[8]	83.17	81.12	77.44	79.59	81.20	80.96	78.44	80.86	1.06

[a – e] Values within a row with the same letter are not different (P > 0.05)

[1]Values are means of 8 observations per treatment.

[2]With xylanase (+) and without xylanase (-)

[3]Tendency FM × FS × E interaction (P = 0.056)

[4]Significant FM × FS × Xyl interaction (P = 0.01)

[5]Significant main effect of FM (P = 0.013)

[6]Significant FM × FS and FS × Xyl interaction (P < 0.01)

[7]Significant FM × FS × Xyl interaction (P < 0.0001)

[8]Significant FS × Xyl interaction (P = 0.027)

Table 7. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation (Xyl) on pH and VFA in ileal and cecal contents at slaugther[1]

	Dry				Liquid				
	DDGS		Wheat Middlings		DDGS		Wheat Middlings		SEM
Xylanase supplementation[2]	-	+	-	+	-	+	-	+	
Ileum									
pH	6.38	6.55	6.41	6.42	6.47	6.48	6.43	6.52	0.12
Caecum									
pH[3]	5.54	5.66	5.71	5.80	5.64	5.63	5.75	5.77	0.06
VFA concentration mmol/L									
Acetate	89.44	100.51	94.85	92.51	89.65	97.44	86.99	88.14	9.39
Propionate	61.46	67.24	66.17	66.23	64.68	54.03	70.75	63.10	7.07
Butyrate[4]	25.85	24.09	22.29	18.38	28.84	31.43	21.47	19.63	2.82
Total VFA	176.74	191.85	183.31	177.12	183.18	182.89	179.21	170.87	16.07
VFA molar proportion									
Acetate/total VFA[5]	0.51	0.52	0.52	0.53	0.48	0.52	0.48	0.52	0.01
Propionate/total VFA[6]	0.34	0.35	0.35	0.36	0.35	0.29	0.39	0.36	0.01
Butyrate/total VFA[7]	0.15	0.13	0.13	0.11	0.17	0.18	0.13	0.12	0.02

[1]Values are means of 8 observations per treatment.
[2]With xylanase (+) and without xylanase (-)
[3]Significant main effect of FS (P = 0.004)
[4]Significant main effect of FS (P = 0.0008)
[5]Tendency main effect of Xyl (P = 0.061)
[6]Significant FM × Xyl interaction (P = 0.031)
[7]Significant main effect of FS (P = 0.011)

Table 8. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation (Xyl) on intestinal morphology[1]

	Dry				Liquid				SEM
	DDGS		Wheat Middlings		DDGS		Wheat Middlings		
Xylanase supplementation[2]	-	+	-	+	-	+	-	+	
Jejunum									
Villi height, µm[3]	350.42 a	322.95 ac	305.20 bcd	289.64 cd	276.29 d	319.56 abc	327.45 ab	295.45 bcd	12.56
Villi width, µm[4]	124.38	152.06	116.61	129.22	131.54	135.22	148.82	135.00	7.65
Crypt depth, µm[5]	84.85	94.63	91.06	84.35	87.47	101.77	112.78	98.90	5.59

[a – d] Values within a row with the same letter are not different (P > 0.05)

[1]Values are means of 8 observations per treatment.

[2]With xylanase (+) and without xylanase (-)

[3]Significant FM × FS × Xyl interaction (P = 0.018)

[4]Significant FM × FS interaction (P = 0.032)

[5]Significant FS × Xyl interaction (P = 0.007)

CHAPTER III: General Discussion

Co-products such as wheat middlings and corn DDGS were initially primarily used in diets for ruminant species. However, due to the recent increased production and price competitiveness of co-products, they have become attractive in swine diets to reduce high feed costs. Those co-products ingredients are partially made up of the residual seed coats or bran of the parent commodity and therefore contain levels of fiber that are two to three times higher than their parent grain (Lachey, 2010). The addition of fibrous feedstuffs in swine diets had stimulated the interest in the nutritional aspects of fiber and their effects on the animal.

The objective of this thesis was to improve the nutritional value of corn DDGS and wheat middlings through the supplementation of Xyl and application of liquid feeding. In the first chapter, the literature review described the role of fiber as a component of animal feed and its effects on animal performance and metabolism. In addition, the use of fiber degrading enzymes as feed additives and the potential benefit of liquid feeding were reviewed. The exact mechanisms of action of enzymes in affecting intestinal microflora populations and digesta composition to benefit gut health status are not well understood. (Zijlstra et al., 2010).

In the second chapter, the digestibility study was described and the results suggested that liquid feeding method and the application of Xyl had a limited potential to enhance degradation of the fiber fraction in corn DDGS-based diets. The structure of NSP in cereal co-products often restricts their accessibility to microbial and endogenous enzymes, and thereby their degradation. The fiber fraction present in corn DDGS is composed of complex and highly substitute glucoarabinoxylans that are cross-linked with cellulose and lignin

within the cell wall (de Vries et al., 2014). To disrupt the cell wall of this specific feedstuff more aggressive pretreatments or enzyme technologies may need to be applied.

The supplementation of Xyl in wheat middlings-based diets improved the AID of NDF and ATTD of GE and N; but the liquid feeding as a pretreatment did not enhance further the nutritional value of wheat middling based diets. This fact may be attributed, in part to the presence of natural inhibitors of xylanase in wheat and wheat co-products (Nortey et al., 2008) that may decrease the activity of exogenous Xyl during the steeping process when wheat middlings-based diets were offered in liquid form.

Future research should explore different pretreatments or processing technologies that help to modify the complex cell wall architecture found in fibrous feedstuffs to improve the accessibility of fiber degrading enzymes to NSP. According to de Vries et al. (2014), modifications in chemical structures and physicochemical properties of NSP produced by processing technologies are not well understood and depend mainly on the type of NSP and process condition. Due to the large diversity and concentration of fiber fraction existing among fibrous feedstuffs, the addition of exogenous enzymes also depends on the interactions between the fiber characteristics of the feedstuffs and the enzyme activity.

Principally, the enzyme must match the target substrate and a “multi-enzyme complex” may be required to effectively disrupt the complex matrixes of fibrous carbohydrates structures (Kerr et al., 2010). Supplemental enzymes in the appropriate combination and proportion can play an essential role in efficient and effective nutrient use of fibrous co-products in swine diets.

In conclusion, future research should aim to evaluate different pretreatments or processing technologies that help to modify the complex cell wall architecture to improve the accessibility of fiber degrading enzymes to NSP structures. Additionally, the effect of a cocktail of fiber degrading enzymes should be explored instead of a single enzyme. Finally, the interaction between the enzyme activities and the fiber characteristics of the most common fibrous feedstuff used in the swine diets should be evaluated to enhance not only the digestive utilization of nutrients but also their effect on microbial populations and gut health.

References

De Vries, S. 2014. Fiber fermentation in pigs and poultry: sense and nonsense of its manipulation. PhD Diss. Univ. of Wageningen.

Kerr, B. J., T. E. Weber, P. V. Anderson, and G. C. Shurson. 2010. Enzymes for use in high DDGS swine diets. In: Proc. 71st Minn. Nutr. Conf. Proc., Owatonna, MN. p. 129-152.

Lackey, R. 2010. Byproduct feed ingredients for swine diets-opportunities and challenges. In: Proc. 10th. London Swine Conf. London, On, Canada. p. 136.

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.

Zijlstra, R. T., A. Owusu-Asiedu, and P. H. Simmins. 2010. Future of NSP-degrading enzymes to improve nutrient utilization of co-products and gut health in pigs. Livest. Sci. 134.1:255-257.

APPENDICES

Appendix A. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on growth performance in growing pigs.

			Initial BW[2], kg	Final BW, kg	ADG, kg	ADFI, kg	G:F
Feeding method × Feedstuff × Xyl Interaction							
Dry	DDGS	-	26.30	35.38	0.61	1.30	0.47
Dry	DDGS	+	25.67	35.96	0.66	1.32	0.50
Dry	Midds	-	26.48	35.80	0.64	1.37	0.47
Dry	Midds	+	25.61	35.34	0.62	1.36	0.46
Liquid	DDGS	-	26.32	35.26	0.61	1.27	0.48
Liquid	DDGS	+	25.65	35.53	0.63	1.26	0.50
Liquid	Midds	-	25.95	35.33	0.62	1.34	0.46
Liquid	Midds	+	25.25	35.63	0.64	1.29	0.50
	SEM		0.389	0.429	0.028	0.020	0.019
Feeding method × Xyl Interaction							
Dry	-		26.39	35.59	0.63	1.34	0.47
Dry	+		25.64	35.65	0.64	1.34	0.48
Liquid	-		26.13	35.30	0.61	1.31	0.47
Liquid	+		25.45	35.58	0.64	1.27	0.50
	SEM		0.275	0.306	0.020	0.014	0.014
Feedstuff × Xyl Interaction							
DDGS	-		26.31	35.32	0.61	1.29	0.48
DDGS	+		25.66	35.75	0.65	1.29	0.50
Midds	-		26.22	35.57	0.63	1.36	0.46
Midds	+		25.43	35.48	0.63	1.33	0.48
	SEM		0.275	0.305	0.020	0.014	0.014
Feeding method × Feedstuff Interaction							
Dry	DDGS		25.99	35.67	0.64	1.31	0.49
Dry	Midds		26.04	35.57	0.63	1.37	0.46
Liquid	DDGS		25.98	35.39	0.62	1.26	0.49
Liquid	Midds		25.60	35.48	0.63	1.32	0.48
	SEM		0.275	0.301	0.020	0.014	0.014
Main Effect of Feeding Method							
Dry			26.01	35.62	0.63	1.34	0.47
Liquid			25.79	35.44	0.62	1.29	0.48
	SEM		0.19	0.213	0.014	0.010	0.010

Appendix A. Continued

Main Effect of Feedstuffs					
DDGS	25.98	35.53	0.63	1.29	0.49
Midds	25.82	35.53	0.63	1.34	0.47
SEM	0.19	0.212	0.014	0.010	0.010
Main Effect of Xyl					
-	26.26	35.44	0.62	1.32	0.47
+	25.54	35.61	0.64	1.31	0.49
SEM	0.19	0.219	0.014	0.010	0.010
P-values					
FM × FS × Xyl	0.849	0.375	0.400	0.807	0.302
FM × Xyl	0.905	0.708	0.731	0.224	0.429
FS × Xyl	0.804	0.399	0.400	0.278	0.544
FM × FS	0.434	0.757	0.731	0.982	0.689
FM	0.420	0.545	0.639	0.001	0.475
FS	0.559	0.981	0.975	0.0001	0.161
Xyl	0.012	0.596	0.334	0.319	0.148

[a,b,c] Values within a column with the same letter are not different (P > 0.05)
[1]With xylanase (+) and without xylanase (-)
[2]Initial BW was used as covariate to analyze final BW.

Appendix B. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of nutrients.

			AID, %					ATTD, %				
			GE		NDF		N	GE		NDF		N
Feeding method × Feedstuff × Xyl Interaction												
Dry	DDGS	-	65.68	x	49.76	a	72.37	83.85		73.70	a	83.17
Dry	DDGS	+	62.19	xy	46.87	ab	71.76	82.08		70.40	ab	81.12
Dry	Midds	-	54.67	y	31.70	b	69.85	77.49		62.28	d	77.44
Dry	Midds	+	64.50	x	52.89	a	73.85	80.30		67.55	bc	79.59
Liquid	DDGS	-	58.72	xy	31.58	b	72.96	81.18		64.17	cd	81.20
Liquid	DDGS	+	67.19	x	46.54	ab	76.52	81.01		65.44	cd	80.96
Liquid	Midds	-	59.94	xy	33.34	b	78.29	78.65		65.08	cd	78.44
Liquid	Midds	+	62.88	xy	29.99	b	77.03	80.44		57.24	e	80.86
	SEM		3.40		5.59		2.31	0.67		1.22		1.07
Feeding method × Xyl Interaction												
Dry	-		60.17		40.73		71.11	80.67		67.99		80.31
Dry	+		63.34		49.88		72.80	81.19		68.98		80.35
Liquid	-		59.33		32.46		75.63	79.92		64.63		79.82
Liquid	+		65.04		38.26		76.77	80.73		61.34		80.91
	SEM		2.40		3.96		1.63	0.47		0.86		0.75
Feedstuff × Xyl Interaction												
DDGS	-		62.20		40.67		72.66	82.51	a	68.94		82.19	a
DDGS	+		64.69		46.70		74.14	81.55	ab	67.92		81.04	a
Midds	-		57.30		32.52		74.07	78.07	c	63.68		77.94	b
Midds	+		63.69		41.44		75.44	80.37	b	62.39		80.23	a
	SEM		2.40		3.95		1.63	0.47		0.86		0.75
Feeding method × Feedstuff Interaction												
Dry	DDGS		63.93		48.32		72.06	82.97	a	72.05		82.15
Dry	Midds		59.58		42.29		71.85	78.89	c	64.91		78.51
Liquid	DDGS		62.95		39.06		74.74	81.10	b	64.81		81.08
Liquid	Midds		61.41		31.67		77.66	79.55	c	61.16		79.65
	SEM		2.40		3.96		1.63	0.47		0.86		0.75
Main Effect of Feeding Method												
Dry			61.76		45.30		71.96	80.93		68.48		80.33
Liquid			62.18		35.36		76.20	80.32		62.98		80.37
	SEM		1.70		2.81		1.15	0.33		0.61		0.53

Appendix B. Continued

Main Effect of Feedstuffs						
DDGS	63.44	43.69	73.40	82.03	68.43	81.61
Midds	60.50	36.98	74.75	79.22	63.04	79.08
SEM	1.70	2.80	1.15	0.33	0.61	0.53
Main Effect of Xyl						
-	59.75	36.60	73.37	80.29	66.31	80.06
+	64.19	44.07	74.79	80.96	65.16	80.63
SEM	1.70	2.79	1.15	0.33	0.61	0.53
P-values						
FM × FS × Xyl	0.056	0.010	0.155	0.172	<.0001	0.614
FM × Xyl	0.601	0.676	0.868	0.762	0.016	0.491
FS × Xyl	0.421	0.717	0.974	0.001	0.876	0.027
FM × FS	0.563	0.863	0.343	0.010	0.048	0.150
FM	0.861	0.017	0.013	0.205	<.0001	0.962
FS	0.226	0.097	0.412	<.0001	<.0001	0.002
Xyl	0.071	0.065	0.387	0.167	0.187	0.452

[a - g] Values within a column with the same letter are not different ($P > 0.05$)
[x - y] Values within a column with the same letter show tendency ($0.05 < P \leq 0.10$)
[1]With xylanase (+) and without xylanase (-)

Appendix C. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on pH and VFA in ileal and caecal contents at slaughter.

			Ileal	Caecum								
					VFA, mmol/L[2]				VFA, molar proportions			
			pH	pH	C_2	C_3	C_4	Total VFA	C_2/Total VFA	C_3/Total VFA		C_4/Total VFA
Feeding method × Feedstuff × Xyl Interaction												
Dry	DDGS	-	6.38	5.54	89.44	61.46	25.85	176.74	0.51	0.34		0.15
Dry	DDGS	+	6.55	5.66	100.51	67.24	24.09	191.85	0.52	0.35		0.13
Dry	Midd	-	6.41	5.71	94.85	66.17	22.29	183.31	0.52	0.35		0.13
Dry	Midd	+	6.42	5.80	92.51	66.23	18.38	177.12	0.53	0.36		0.11
Liquid	DDGS	-	6.47	5.64	89.65	64.68	28.84	183.18	0.48	0.35		0.17
Liquid	DDGS	+	6.48	5.63	97.44	54.03	31.43	182.89	0.52	0.29		0.18
Liquid	Midd	-	6.43	5.75	86.99	70.75	21.47	179.21	0.48	0.39		0.13
Liquid	Midd	+	6.52	5.77	88.14	63.10	19.63	170.87	0.52	0.36		0.12
	SEM		0.12	0.07	9.40	7.07	2.83	16.07	0.02	0.02		0.02
Feeding method × Xy Interaction												
Dry	-		6.40	5.62	92.14	63.82	24.07	180.03	0.52	0.35	ab	0.14
Dry	+		6.48	5.73	96.51	66.74	21.24	184.48	0.52	0.36	ab	0.12
Liquid	-		6.45	5.70	88.32	67.72	25.16	181.19	0.48	0.37	a	0.15
Liquid	+		6.50	5.70	92.79	58.56	25.53	176.88	0.52	0.33	b	0.15
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01		0.01
Feedstuff × Xyl Interaction												
DDGS	-		6.43	5.59	89.55	63.07	27.35	179.96	0.49	0.35		0.16
DDGS	+		6.51	5.64	98.97	60.63	27.76	187.37	0.52	0.32		0.15
Midd	-		6.42	5.73	90.92	68.46	21.88	181.26	0.50	0.37		0.13
Midd	+		6.47	5.78	90.33	64.66	19.00	174.00	0.52	0.36		0.11
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01		0.01
Feeding method × Feedstuff Interaction												
Dry	DDGS		6.46	5.60	94.97	64.35	24.97	184.29	0.51	0.35		0.14
Dry	Midd		6.41	5.75	93.68	66.20	20.33	180.22	0.52	0.36		0.12
Liquid	DDGS		6.48	5.64	93.54	59.35	30.14	183.03	0.50	0.32		0.18
Liquid	Midd		6.48	5.76	87.57	66.92	20.55	175.04	0.50	0.38		0.12
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01		0.01

Appendix C. Continued

Main Effect of Feeding Method									
Dry	6.44	5.67	94.33	65.28	22.65	182.26	0.52	0.35	0.13
Liquid	6.48	5.70	90.56	63.14	25.34	179.04	0.50	0.35	0.15
SEM	0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01
Main Effect of Feedstuffs									
DDGS	6.47	5.62	94.26	61.85	27.55	183.66	0.51	0.33	0.16
Midd	6.45	5.76	90.62	66.56	20.44	177.63	0.51	0.37	0.12
SEM	0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01
Main Effect of Xyl									
-	6.42	5.66	90.23	65.77	24.61	180.61	0.50	0.36	0.15
+	6.49	5.71	94.65	62.65	23.38	180.68	0.52	0.34	0.13
SEM	0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01
P-values									
FM × FS × Xyl	0.483	0.710	0.799	0.664	0.776	0.772	0.775	0.667	0.620
FM × Xyl	0.837	0.278	0.994	0.233	0.427	0.701	0.203	0.031	0.393
FS × Xyl	0.826	0.979	0.454	0.892	0.415	0.521	0.812	0.596	0.752
FM × FS	0.770	0.740	0.726	0.570	0.222	0.864	0.669	0.069	0.210
FM	0.653	0.623	0.573	0.671	0.185	0.778	0.187	0.781	0.153
FS	0.776	0.004	0.586	0.350	0.001	0.597	0.703	0.013	0.011
Xyl	0.431	0.245	0.509	0.536	0.541	0.995	0.061	0.201	0.393

[a, b] Values within a column with the same letter are not different (P > 0.05)

[1] With xylanase (+) and without xylanase (-)

[2] C2: Acetate; C3: Propionate; C4: Butyrate; C2 + C3+ C4: Total VFA

Appendix D. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on intestinal morphology.

			µm					
			Villi Height		**Villi width**		**Crypt depth**	
Feeding method × Feedstuff × Xyl Interaction								
Dry	DDGS	-	350.42	a	124.38		84.85	
Dry	DDGS	+	322.96	ac	152.06		94.63	
Dry	Midd	-	305.21	bcd	116.61		91.06	
Dry	Midd	+	289.65	cd	129.22		84.35	
Liquid	DDGS	-	276.29	d	131.54		87.47	
Liquid	DDGS	+	319.56	abc	135.22		101.77	
Liquid	Midd	-	327.46	ab	148.82		112.78	
Liquid	Midd	+	295.45	bcd	135.00		98.90	
	SEM		12.56		7.65		5.59	
Feeding method × Xyl Interaction								
Dry	-		327.81		130.49		87.96	
Dry	+		306.30		140.64		89.49	
Liquid	-		301.87		140.18		100.13	
Liquid	+		307.51		135.11		100.33	
	SEM		8.88		5.41		3.96	
Feedstuff × Xyl Interaction								
DDGS	-		313.36		127.96		86.16	b
DDGS	+		321.26		143.64		98.20	a
Midd	-		316.33		132.71		101.92	a
Midd	+		292.55		132.11		91.62	ab
	SEM		8.88		5.41		3.96	
Feeding method × Feedstuff Interaction								
Dry	DDGS		336.69		138.22	a	89.74	
Dry	Midd		297.43		122.91	b	87.71	
Liquid	DDGS		297.93		133.38	ab	94.62	
Liquid	Midd		311.46		141.91	a	105.84	
	SEM		8.88		5.41		3.96	

Appendix D. Continued

Main Effect of Feeding Method				
Dry		317.06	130.56	88.72
Liquid		304.69	137.64	100.23
	SEM	6.28	3.82	2.80
Main Effect of Feedstuffs				
DDGS		317.31	135.80	92.18
Midd		304.44	132.41	96.77
	SEM	6.28	3.82	2.80
Main Effect of Xyl				
-		314.84	130.33	94.04
+		306.90	137.87	94.91
	SEM	6.28	3.82	2.80
P-values				
FM × FS × Xyl		0.018	0.911	0.463
FM × Xyl		0.133	0.240	0.867
FS × Xyl		0.081	0.139	0.007
FM × FS		0.005	0.032	0.100
FM		0.170	0.197	0.005
FS		0.154	0.534	0.251
Xyl		0.376	0.170	0.827

[a - d] Values within a column with the same letter are not different (P > 0.05)
[1] With xylanase (+) and without xylanase (-)