ABSTRACT

ADAMI DOS PASSOS, ADSOS. Application of Feed Enzymes in Pig Nutrition. (Under the direction of Dr. Sung Woo Kim).

The research hypothesis is that feed enzymes targeting insoluble NSP change viscosity of digesta which will improve nutrient digestibility of feedstuffs fed to pigs and blending feed enzymes can further enhance nutrient utilization of feedstuffs by pigs.

The first study (Chapter 2) used 36 pigs based on a randomized complete block design to evaluate dose effects of xylanase (0, 700, and 1,400 LXU/kg) on viscosity of jenunal digesta and ileal digestibility of nutrients. Increasing xylanase supplementation yielded linear improvements ($P < 0.05$) in AID of dry matter (DM), energy and neutral detergent fiber (NDF), and a quadratic change ($P < 0.05$) in viscosity of jejunum digesta. The lower NDF content in ileal digesta due to xylanase supplementation suggests NSP degradation. This study indicated that viscosity changes responded differently than ileal digestibility of nutrients when using increasing levels of xylanase. Therefore viscosity of digesta may not correlate well with nutrient digestibility in pigs.

The second study (Chapter 3) consisted of series of experiments evaluating the effects of phytase (Phy), protease (Pro), xylanase (Xyl), and their combinations (Phy+Pro, Phy+Xyl, Pro+Xyl, and Phy+Pro+Xyl) on nutrient digestibility and growth performance of pigs. Pro supplementation was evaluated in an ileal collection experiment to measure apparent ileal digestibility (AID) of protein and amino acids of a corn-soybean meal-DDGS based diet. Pigs were fed a low energy diet containing 1,000 FYT of phytase/kg of diet (LD Phy) or LD Phy+Pro (Pro, 15,000 PRO of protease/kg of diet). The experiment used 16 pigs based on a randomized complete block design. There was no effect of protease on AID of N and amino acids expect for proline ($P = 0.020$) and histidine ($P = 0.038$).

Four metabolism experiments (Chapter 3) were conducted to determine the dry matter digestibility (DMD), DE, ME, apparent total tract digestibility (ATTD) of N (ND), P (ATTDP), and nitrogen retention (NR), of corn (metabolism experiment 1), soybean meal (metabolism experiment 2), DDGS (metabolism experiment 3), and a corn-soybean meal-DDGS based diet (metabolism experiment 4) supplemented with feed enzymes. The diet treatments were no enzyme (CON), Phytase (Phy, 1,000 FYT/kg), Protease (Pro, 15,000 PRO/kg), Xylanase (Xyl, 200 FXU/kg), Phy+Pro, Phy+Xyl, Pro+Xyl, and Phy+Pro+Xyl and each metabolism experiment received the same treatments. In total, 64 pigs (30.1 ± 3.7 kg), divided into groups of 16 pigs per metabolism experiment, were assigned to treatment diets in a four 4 × 4 Latin squares design. The combination of Phy+Xyl and Phy+Pro improved nutrient digestibility of DDGS and soybean meal. Single use of Phy or combinations Phy+Pro and Phy+Pro can improve ATTDP. The combinational use of Phy+Pro+Xyl improved ND of a corn-soybean meal-DDGS based diet.

The growth performance (Chapter 3) experiment used 144 pigs in 48 pens based on a randomized complete block design. This experiment was to evaluate weight gain, feed intake, gain to feed ratio, and caloric efficiency (kcal of ME intake / kg of weigh gain) of pigs fed a diet formulated to meet the nutrient requirements of swine (ND) and a diet deficient in ME (LD). Diets were supplemented with feed enzymes (ND, ND Phy, LD, LD Phy, LD Phy+Pro, LD Phy+Xyl) and fed to the pigs. There were no effect of treatments on weigh gain and caloric efficiency. Feed intake was reduced ($P < 0.05$) in pigs receiving ND Phy compared with pigs receiving LD diets. Gain to feed ratio (G:F) was greater ($P < 0.05$) in pigs receiving ND Phy than pigs in other treatments. The use of LD Phy+Xyl did not affect G:F of pigs compared with ND.

The third study (Chapter 4) used 32 barrows in metabolic cages according to a 2 x 2 Latin square design replicated four times to evaluate the nutrient digestibility of bermudagrass, forage sorghum, and sweet sorghum supplemented with an enzyme combination (phytase, protease, xylanase, cellulose, and glucanase). The determined ME content of bermudagrass, forage sorghum, and sweet sorghum were 845, 1,511, and 1,061 kcal/kg respectively. Nitrogen was not utilized by the pigs, the determined ATTD of N of bermudagrass, forage sorghum, and sweet sorghum were -16.5, -0.72, and -75.47%. The enzyme combination tended to enhance (P = 0.081) energy utilization of bermudagrass, however no effect was observed on forage sorghum and sweet sorghum. Pigs could utilize energy from forages and, feed enzymes have potential to further improve nutrient utilization.

In conclusion, feed enzymes targeting insoluble NSP linearly increase nutrient digestibility however viscosity can not predict digestibility. Feed enzyme combinations improved digestibility of feedstuffs and could increase the potential inclusion of forages in swine diets.

Application of Feed Enzymes in Pig Nutrition

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

Animal Science & Poultry Science and Nutrition

Raleigh, North Carolina

2014

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DEDICATION

To my family, Tomás dos Passos, Domitila Romero, Roberto dos Passos, Izabel Adami dos Passos, Madla Adami dos Passos, Ériza Adami dos Passos, and Neuracy Gomes. Their personal support helped me to engage into the graduate program.

BIOGRAPHY

Adsos Adami dos Passos, son of Roberto dos Passos and Izabel Adami dos Passos was born on March 09, 1976 in Francisco Beltrão, in the southern state of Paraná, Brazil. He graduated from Positivo High School in 1993. Pursuing undergraduate studies Adsos was accepted at São Paulo State University (Unesp) in Botucatu, São Paulo, Brazil. He received his major in Veterinary Medicine in 2000. During the fall of 2000 Adsos participated in an internship program at Brazilian Agricultural Research Corporation (Embrapa) and decided to follow to a graduate program. In 2000 Adsos was approved at Pelotas Federal University in Capão do Leão, Rio Grande do Sul, Brazil. He obtained the Master of Science degree in 2004, at this time already working in the industry. From 2002 until 2011 Adsos worked in the industry gaining experience with in technical services. In 2011, seeking for higher education Adsos was accepted in the Department of Animal Science of North Carolina State University under the direction of Dr. Sung Woo Kim.

ACKNOWLEDGMENTS

I would like to thank to Dr. Sung Woo Kim for accepting me as his student and for his guidance during the graduate program. I also would like to thank the committee members, Dr. Eric van Heugten, Dr. Peter Ferket, and Dr. Mark Knauer for the scientific contribution on this project.

I am thankful to my lab-mates Alexandra Chaytor Weaver, Gwendoline Voilqué, Inkyung Park, Jiyao Guo, Yan Bin Shen, and Yawang Sun for the friendship, support, and help during the graduate program. Thanks also to Carla Andrade, Josh Quin, Lourdes Cámara García, and Tiago Pasquetti, and other students who provided help during the lab work and collection procedures.

Special thanks to Dr. Ramon Malheiros for his guidance in many lab procedures and for the great work environment that I had at the Scott Hall. Many thanks to Dr. Consuelo Arellando for all the support with statistics. I also would like thank Clay Bird, Charles Salmon, and Kayla Howard at the Swine Education Unit and Randy Moore, Morris Dunston, and James Pope at Swine Evaluation Station who were a great asset dealing with the animals for the experiments. Thanks also to Carl Whisenant and Shawn Bradshaw for the support preparing the diets at the Feed Mill. Many thanks to Marian Correl for her great assistance during the graduate program.

I also would like to thanks Dr. Gustavo Lima, Dr. Dirlei Berto, Dr. Fernado Rutz, and Ricardo Cazes. My previous work with them influenced me to pursue a higher education degree.

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LITERATURE REVIEW

Introduction

Corn, soybean meal and distillers dried grains with solubles (DDGS) are important ingredients to the feed industry in the United States. The feed industry consumes 122 million metric tonnes of corn, 27 million metric tonnes of soybean meal, and 26 million metric tonnes of DDGS (AFIA, 2012)**.** The swine industry consumes 14 % of the total feed production needing 23 million tonnes of feed to sustain pork production (IFIF, 2012). Traditionally, between 65 and 70% of the swine production cost are related to feed cost and efforts to improve nutrient digestibility can have significant effects on pork industry profitability (National Pork Board, 2012). Corn is the main ingredient in typical swine diets, and it is also consumed for ethanol production. Consequently its price fluctuates dramatically increasing from $3/bushel in 2008 to $7/bushel in 2013 (USDA, 2013a). Soybean meal is the main protein source in swine dites, and its price fluctuates from $10/bushel in 2008 to $14/bushel 2013 (USDA, 2013b). Price fluctuations of feed ingredients significantly affect the economy of swine production.

Yellow dent corn (IFN 4-02-861, AAFCO, 1992) is an important souce of energy in the swine diets, as it has 3,395 kcal/kg of metabolizable energy (ME), 62.55% of it is starch (NRC, 2012). Regarding the indigestible components corn contains 9.7% of non-starch polysaccharides (NSP, Knudsen, 1997) and 0.21% of phytate P (NRC, 2012). The arabinoxylans are the main NSP accounting for 4.3% of the corn composition (Ward et al., 2008). Soybean meal (IFN 5-04-612, AAFCO, 1992) has 47.73% of crude protein (CP), and the apparent ileal digestibity (AID) of CP is 82% (NRC, 2012). Therefore it is an important source of protein to the swine diets. Regarding the undigestible components, soybean meal contains 21.7% NSP (Knudsen, 1997) and 0.38% phytate P (NRC, 2012). Soybean meal also contains 3.78% raffinose and 7.33% stachyose (NRC, 2012), both considered as

flaclulance-producing factors (Liener et al., 1994). DDGS (IFN 5-02-843, AAFCO, 1992) contains 3,396 kcal ME/kg, 27.36% CP, and 8.9% ether extract, and it serves as source of energy and protein in pig diets (NRC, 2012). Regarding the indigestible components DDGS contains 19.24% NSP (Widyaratne and Zijlstra, 2007) and 0.26% of phytate P (NRC 2102). The arabinoxylans accounts for 16.72% of the DDGS composition (Kim et al., 2010).

Pigs do not produce endogenous enzymes in the gastro-intestinal tract to digest the total dietary fiber and phytate P (Hartman et al., 1961; Schlemmer et al., 2001). The presence of total dietary fiber can limit the digestibility of nutrients (Moeser et al., 2002; Urriola and Stein, 2010). However, the capability of fiber fermentation in the large intestine to produce volatile fatty acids as a source of energy is limited to 18% of the available energy (Anguita et al., 2006; Gutierrez et al., 2013). Phytate P degradation by microorganisms that occurs in the large intestine due to microorganisms (Schlemmer et al., 2001) does not enable pigs to utilize P from phytate. Therefore, feed enzymes have been studied in order to degrade the indigestable components of the diets and improve nutrient digestibility (Pettey et al., 2002; Kim et al., 2003; Ji et al., 2008; Li et al., 2010; Jo et al., 2012, Almeida and Stein, 2012) aiming to provide economic benefits to the swine industry.

The use of enzymes that target polymeric carbohydrates is being studied in swine nutrition (Cozanet et al., 2012; Nortey et al., 2007; Woyengo et al., 2008). NSP limits the nutrient digestibility (Choct and Annison, 1992, Choct et al., 2010). Based on the composition of corn (Ward et al., 2008; Knudsen, 1997) and DDGS (Kim et al., 2010) the main NSP in a typical swine diet are arabinoxylans. They represent between 3 and 6% of a corn-soybean meal based diet or a corn-soybean meal-DDGS based diet respectively. Non-ruminant animals have limitations to degrade those components in the diet (Hartman et al., 1961; Lindemann et al., 1986; Huguet et al., 2006). There is growing interest in using

supplemental enzymes to degrade NSP in order to mitigate their negative effect on nutrient digestibility (Choct and Annison, 1992, Choct et al., 2010). The total non-phytase enzymes market accounts for to 40% of the total enzyme market (Adeola and Cowieson, 2011).

It is estimated that a typical swine diet contains 0.24% of phytate P which represents between 56 to 67% of the total amount of phosphorus provided by a typical corn-soybean meal-DDGS based diet or a corn-soybean meal based diet respectively. Considering that pigs need between 0.18 and 0.36% of ATTD P (NRC 2012), the intestinal absorption of P from phytate P can contribute as a significant nutritional source of P to pigs. Therefore, there are several studies about phytase improving P digestibility in pigs (Almeida and Stein, 2010; Almeida and Stein, 2012; Akinmusire and Adeola, 2009; Yáñez et al 2011) and this enzyme accounts for 60% of the total feed enzymes included in the diets (Adeola and Cowieson, 2011).

More recently, reports observed that the use of protease could improve protein and amino acid digestibility (Guggenbuhl et al., 2012; Mc Alpine et al., 2012b). These data indicated that there may still be an opportunity to augment digestion of protein by supplementing exogenous protease and it could be an alternative to reduce feed cost.

This literature review will focus on the primary feed enzymes available for supplementation in swine diets. The objective is to analyze the available information regarding mode of action and nutrient digestibility and finally proposes a hypothesis that requires scientific investigation.

Non-starch polysaccharides and phytate

Xylans

Corn has 9.7% of NSP and D-xylopyranose (xylose) is the main component of NSP in this ingredient (Knudsen, 1997). This cereal contains 3.0% xylose (Knudsen, 1997) as

arabinoxylan and the total amount of arabinoxylan is 4.3% (Ward et al., 2008). The fermentation of corn to ethanol increases the concentration of fiber in the residual DDGS (NRC, 2012). Corn DDGS contains 16.72% of arabinoxylan (Kim et al., 2010). Soybean meal contains xylose as xyloglucans and xylose as xylans in it's hulls (Karr-Lilienthal et al., 2005). The xylan structure (Figure 1) is composed of 1,4-β-linked D-xylopyranose. Arabinoxylans are composed of a xylan back bone with L-arabinose attached to xylose units in the endosperm and pericarp tissues of the grain (Ebringerova and Heinze, 2000). The arabinoxylan of corn is characterized to be branched with L-arabinose, glucoronic acid (Huisman et al., 2000), and ferulates (Grabber et al., 1998). Under low pH, similar to that in the stomach, L-arabinose can be partially released from corn arabinoxylans (Zhang et al., 2003). Comparing different ingredients, corn and soybean meal yield less viscous solutions than rye, barley, oats, and wheat (Mathlouthi et al., 2002). The differences on viscosity are related to amount of water extractable arabinoxylan and other NSPs (Mathlouthi et al., 2002).

The non-ruminant animals do not produce enzymes to degrade the arabinoxylan, therefore one mode of action proposed for xylanases involves degradation of the arabinoxylans in the cell wall enabling digestive enzymes to digest the nutrients inside the cell wall (Tervila-Wilo et al., 1996; Masey O'Neil et al., 2014). The NSP increases intestinal endogenous losses of nitrogen (Grala et al., 1998) and affect intestinal morphology (Montagne et al., 2005; Willamil et al., 2012). It also increases viscosity of digesta (Choct and Annison, 1992) which can be related to nutrient digestibility (Choct et al., 1999). However, the high viscosity of digesta is not always related to lower digestibility in pigs. Owusu-Asiedu et al. (2006) indicate that by adding 7% of guar gum or cellulose to the diet increased viscosity of ileal digesta, slowed the passage rate of digesta through the small

intestine, and decreased apparent total tract digestibility (ATTD) of protein and digestible energy. However, it was observed that high viscous and low fermentable fiber can reduce passage rate and increase AID of dry matter, energy, and protein (Hooda et al., 2011). Including different sources of DDGS (wheat DDGS, corn DDGS, and wheat corn DDGS) to the swine diets Yang et al. (2010) observed that wheat corn DDGS yielded the highest digesta viscosity and also the highest AID of amino acids compared to the other sources. The nutrient digestibility of pigs seems to be influenced by other factors such as digesta passage rate and intestine bacterial activity (Bartelt et al., 2002).

Galactosides and galactomannans

Soybean meal contains 4.1% of galactose (Knudsen, 1997), 3.78% of raffinose, 7.33% of stachyose (NRC, 2012). It also contains manose as 1% of β-mannans (Hsiao et al., 1995). Corn contains little amount of galactose (0.5%), manose (0.3%), raffinose (0.2%), and stachyose (0.1%) compared to soybean meal (Knudsen, 1997). Due to lack of digestive enzymes to degrade α-1,6-galactosyl and β-1,4-mannosyl bonds, pigs cannot degrade those components in the small intestine (Hartman et al., 1961; Lindemann et al., 1986; Huguet et al., 2006). The indigestible galactosides from soybean meal are known as flatulence-producing factors because the microorganism in the large intestine will degrade them and produce gases such as carbon dioxide and methane (Liener et al., 1994). Reviewing the antinutritional factors of galactosides, Martinez-Villaluenga et al. (2008) commented about the osmotic changes that lead to diarrhea, microbial imbalance, abdominal pain, reduction of ME, and lower amino acids digestibility. Studying different samples of soybean meal, van Kempen et al. (2006) observed that the stachyose composition has a negative correlation with AID of dry matter and energy. It is also reported that mannans form viscous solutions

and reduce the intestinal absorption of glucose (Rainbird et al., 1984; Nunes and Malmlof, 1992) and water (Rainbird et al., 1984).

Phytate

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate) is the storage form of P in cereal grains and oil seeds (Cheryan, 1980). The corn grain will store P as phytin (phytic acid bound to Ca and Mg) mainly in the germ, but there is also phytin in the endosperm and in the hull (O'Dell et al., 1972). The soybean meal will have phytin stored together with protein (Erdman, 1979). Corn, soybean meal, and DDGS will have 0.21%, 0.36%, and 0.26% of Phytate P, respectively (NRC, 2012), thus limiting the phosphorus utilization by the pigs (Schlemmer et al., 2001).

Upon dissociation, the phytic acid will leave negative charges that can bind to cations such as Ca, Zn, Cu, Fe, Mn, Mg (Maenz et al., 1999). Phytate can also bind to protein (Rajendran and Prakash, 1993; Kies et al., 2006), and a high phytate diet decreases the absorption of amino acids (Liao et al., 2005). There is evidence that phytate interacts with fats, forming complexes of Ca, lipids, and phytate (Cosgrove, 1966), which have a negative effect on AID of energy (Liao et al., 2005).

Feed enzymes

Xylanase

The enzyme endo-1,4-β-xylanase (xylanase) carry enzyme commission identifier 3.2.1.8 and catalyzes the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans (International Union of Biochemistry and Molecular Biology, 1992). The xylanases are classified into glycoside hydrolase families based on the catalytic domains, structure, and molecular mechanism (Collins et al., 2005). The xylanases utilized by the feed industry belong to the glycosidic hydrolase families 10 and 11 and both have glutamate in the catalytic site

(Palohelmo et al., 2011). The family 11 works exclusively on substrates containing D-xylose and family 10 can be active in other substrates such as cellulose (Collins et al., 2005). Most of xylanases act on pH between 4 and 6 and there are thermostable xylanases available for feed application (Palohelmo et al., 2011).

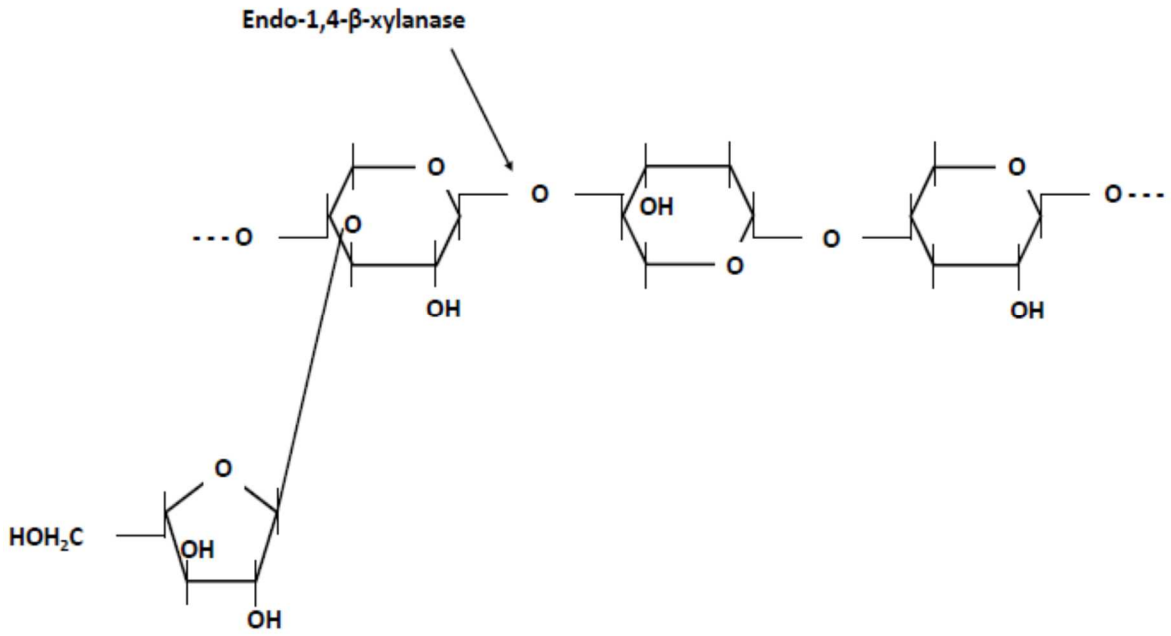

Figure 1. Structure of xylan and sites for endo-1,4-β-xylanase (adapted from Subramaniyan and Prema, 2002).

The utilization of feed enzymes in animal nutrition involves degradation of the cell wall in the ingredients and reduction of digesta viscosity associated with NSP (Masey O'Neil et al., 2014). Studying different xylanases, Choct et al. (2004) demonstrated that dietary xylanase supplementation of xylanase reduced the the amount of NSP in the jejunum and in the ileum

of broilers. Choct and Annison (1992) reported that dietary arabinoxylans increased digesta viscosity and dietary supplementation of xylanase reduced the effect of arabinoxylans on viscosity. Liu et al. (2011) reported that the use of xylanase in a diet composed of 10 or 20% of corn DDGS improved the hemicellulose digestibility by 20% and the ME by 145 kcal/kg in poultry.

The supplementation of xylanase improved digestibility of a wheat-based diet fed to pigs (Woyengo et al., 2008; Moehn et al., 2007, Nortey et al., 2007). However, these data was not supported by Olukosi et al. (2007) who observed no effect of xylanase in wheat based diets. No improvements were observed on growth performance of pigs due to xylanase supplementation in a corn-soybean meal-DDGS based diets (30 to 60% of DDGS) (Jacela et al., 2009). This data was supported by Yáñez et al. (2011) who did not observed an effect of dietary xylanase supplementation on nutrient digestibility of a co-fermented corn and wheat DDGS. However, by supplementing a blend of enzymes composed of xylanase, glucanase, protease and amylase in a corn-soybean meal-DDGS based diet (40% of corn or sorghum DDGS) the ATTD of DM, N, and GE of the diets were improved (Feoli et al., 2008).

Galactosidase and mannanase

The enzyme β-galactosidase (galactosidase) carry the enzyme commission number 3.2.1.23 and catalyzes the hydrolysis of the terminal non-reducing β-D-galactose residues in β-D-galactosides (International Union of Biochemistry and Molecular Biology, 1992). The enzyme endo-1,4-β-mannanase (mannanase) carry the enzyme commission number 3.2.1.78 and catalyzes the random hydrolysis of (1→4)-β-D-mannosidic linkages in mannans (International Union of Biochemistry and Molecular Biology, 1992).

The feed supplementation of enzymes targeting α-1,6-galactosides and β-galactomannans was reported in pigs. Kim et al. (2003) studied α-1,6-galactosidase and β-

1,4-mannanase in corn-soybean meal diets fed to nursery pigs. They observed improvements on AID of GE, lysine, threonine, and tryptophan due to enzyme supplementation. Moreover, the pigs fed a diet supplemented with galactosidase and mannanase had a greater G:F ratio, and raffinose and stachyose concentration in the small intestine was reduced. Pettey et al. (2002) reported improved G:F ratio in nursery pigs fed a corn-soybean meal based diet supplemented with β-1,4-mannanase. Studying β-1,4-mannanase in corn-soybean meal-DDGS based diet Yoon et al. (2010) observed increased ADG, and improved ATTD of dry matter, GE, and protein in finisher pigs.

Glucanase

Cellulose is a water insoluble β-glucan consisting of linear molecule of D-anhydroglucopyranose linked by β-(1-4) bond. Corn contains 2.2% of cellulose and soybean meal contain 6.2% (Knudsen et al., 1997). The cereal β-glucan is soluble mixed linkage 1-3, 1-4 β-D-glucan. The 1-3 linkage makes the structure soluble. Corn and soybeans contain little amount of glucans (Knudsen et al., 1997; Ko and Lin, 2004).

The enzyme endo -1,4- β-glucanase (cellulose) carries the enzyme commission number 3.2.1.4 and catalyzes the endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, and cereal β-D-glucans. The enzyme 1,3(4)- β-glucanase carries the enzyme commission number 3.2.1.6 and catalyzes the reaction endohydrolysis of (1→3)- or (1→4)-linkages in β-D-glucans. (International Union of Biochemistry and Molecular Biology, 1992). The dietary supplementation of glucanase has been tested in pigs. Kong and Adeola (2012) fed a barley-corn-soybean meal based diet to growing pigs supplemented with glucanase. There was no effect of glucanase on ATTD and AID of DM, GE, and N. However, it was reported that supplementation of glucanase increase AID of starch by 1.8% in a diet formulated to

contain 87% barley (Graham et al., 1989). Li et al (1996) observed that glucanase in a barley-based diet can increased digestible energy by 3% and ATTD of CP by 6%.

Amylase

Although pigs can digest carbohydrate from the diet, the interest in amylase supplementation relies on the digestion of the resistant starch that is not digestible by the animals (Isaksen et al., 2011).The enzyme α-amylase (amylase) catalyzes the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units (International Union of Biochemistry and Molecular Biology, 1992). It was observed that amylase form *Bacillus amyloliquefaciens* hydrolyses amylopectin faster than pancreatic amylase (Bijttebier et al., 2010). Supplementation of amylase in corn-soybean meal based diet improved weight gain by 9% and feed conversion by 4% in poultry (Gracia et al., 2003). However, no improvement was observed on growth performance and ATTD of nutrients in growing pigs due to amylase supplementation to a corn-soybean meal based diet (Jo et al., 2012).

Phytase

The enzymes myo-inositol (1,2,3,4,5,6) hexakiphosphate phosphohydrolases (phytases) carry the enzyme commission identifiers 3.1.3.8 (3-phytase), 3.1.3.2 (4-phytase), 3.1.3.72 (5-phytase), or 3.1.3.26 (6-phytase). These enzymes catalyze the reaction: myo-inositol hexakiphosphate phosphohydrolases + H_2O = myo-inositol pentakiphosphate + phosphate (International Union of Biochemistry and Molecular Biology, 1992). The phytases can be classified based on the optimum pH (acid or alkaline), based on the carbon in the myo-inositol ring of phytate where the phosphorylation starts (3- phytases, 5 phytases, or 6 phytases), or based on the catalytic mechanism (histidine phytase, cysteine phytase, purple acid phytase, or β-propeler phytase). The phytases utilized by the feed industry belong to

the histidine acid phytase (Greiner and Konietzny, 2011). Their utilization in animal nutrition depends of their proteolitic resistance (Wyss et al., 1999), pH of optimum activity (Boyce and Walsh, 2006), and thermal tolerance (Garret et al., 2004).

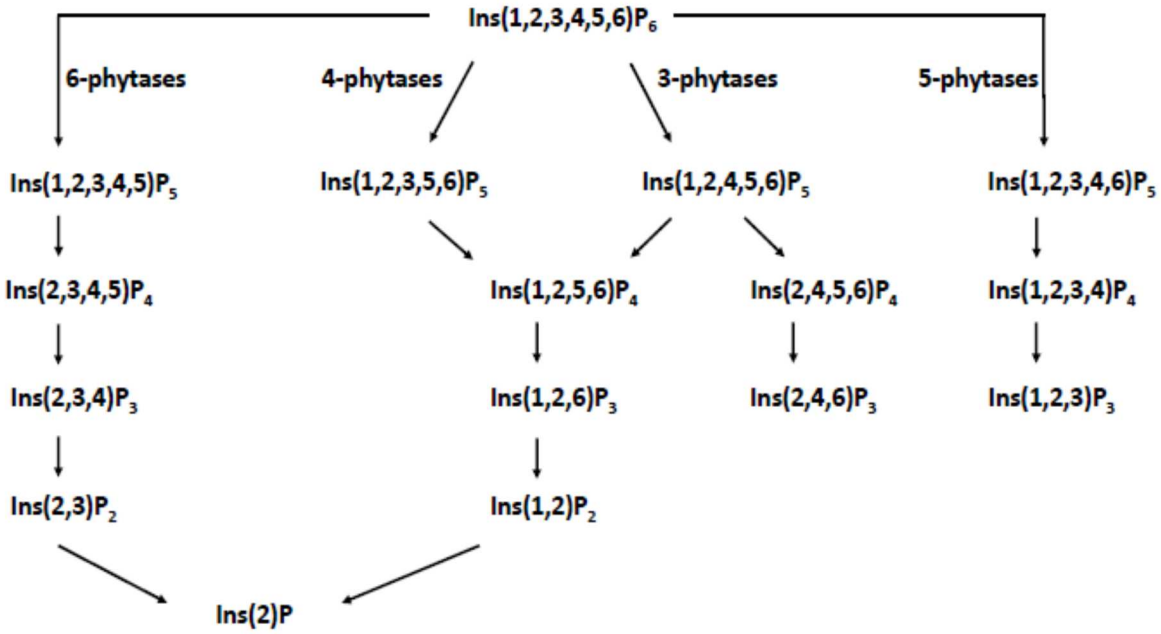

Figure 2. Dephosphorylation of myo-inositol hexakiphosphate by phytases (adapted from Greiner and Konietzny, 2011).

The dephosphorylation of myo-inositol hexakiphosphate (phytate) by phytases (Figure 1) involves sequential removal of phosphate groups (Greiner et al., 2002). The removal of P from phytate and the further P absorption in the small intestine (Jones et al., 2010; Guggenbuhl et al., 2012; Rojas and Stein, 2012) is the main reason for phytase supplementation to the swine diets.

As previously mentioned, it was indicated that phytate can bind to protein (Rajendran and Prakash, 1993; Kies et al., 2006) and the phytate supplementation decreased absorption of protein and amino acids (Liao et al., 2005). But the literature regarding the benefits of phytase on amino acid digestibility is not consistent. Some studies indicated benefits due to phytase supplementation (Kemme et al., 1999; Zeng et al., 2014), while others did not (Traylor et al., 2001; Liao et al., 2005). The inconsistent results in the literature (Adeola and Sands, 2003) could be related to the source of protein provided in the diet (Pomar et al., 2008).

As mentioned before, there is evidence that phytate interact with fats forming complexes of Ca, lipids, and phytate (Cosgrove, 1966), which have a negative impact on the AID of energy (Liao et al., 2005). There are also suggestions that phytase could improve energy digestibility in pigs. Johnston et al. (2004) observed increased AID of energy and DM due to phytase supplementation to a corn-soybean meal based diet formulated with low Ca and P levels. Shelton et al. (2003) studied the effect of phytase on growth performance of pigs and observed an increase in the back fat deposition, but not on the ADG, ADFI and G:F. Selle and Ravidran, 2007 speculated that phytase can improve fat digestibility and evidence in poultry supported this hypothesis (Liu et al., 2010). However other studies concluded that dietary phytase supplementation had no effect of phytase on AID of energy (Liao et al., 2005; O'Quinn et al., 1997).

Protease

Protease is the general term for enzymes that degrade proteins. The majority of the proteases are classified as serine proteases because the amino acid serine is in the active site (Hedstrom, 2006). Serine proteases catalyze the hydrolysis of peptide bonds (Figure 3) and carry the enzyme commission number 3.4.21 (International Union of Biochemistry and

Molecular Biology, 1992). Effective utilization of supplemental protease in diets depends upon resistance to low pH and the ability to degrade soybean meal if it is the major protein ingredient in swine diets (Glitsø et al., 2012). Pedersen et al. (2012) studied different commercial proteases and observed that they are active between pH 5.5 and 7.0. The first use of protease in pig nutrition was reported by Cunningham and Brisson (1957) where they predigested feed ingredients with the enzymes, but no improvement of growth performance was observed. Recent studies regarding dietary supplementation of protease are not consistent. Guggenbuhl et al. (2012) supplemented protease to corn-soybean meal based diet and observed an improvement in AID of protein in nursery pigs. Similar positive effects of dietary protease supplementation was observed in finishing pigs, but reduced daily gain was observed in growing pigs fed diets containing protease (Mc Alpine, 2012b).

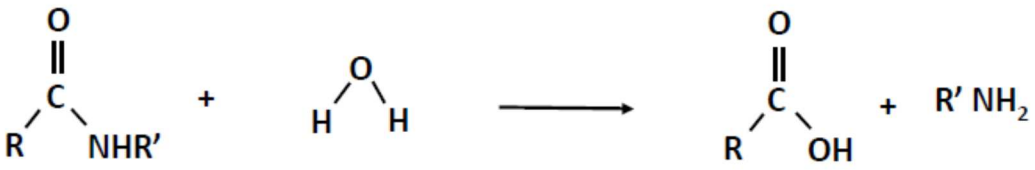

Figure 3. Catalysis of peptide hydrolysis (adapted from Hedstrom, 2006).

Application of enzyme combinations

Wheat has 87% of the phytate P stored in the aleurone layer (O'Dell et al., 1972) and the xylanase can increase the aleurone permeability (Parkkonen et al., 1997). The combination of phytase and xylanase could improve the phytate degradation and phosphorus digestibility. The use of phytase and xylanase was tested in several studies in wheat based

diets with different outcomes. Some studies indicated additive effect on nutrient digestibility by combining phytase and xylanase (Olukosi et al., 2007; Nortey et al., 2007). But other reports did not demonstrate an additive effect of phytase and xylanase supplementation (Moehn et al., 2007; Woyengo et al., 2008). Olukosi et al (2007) evaluated the supplementation of phytase or an enzyme blend (xylanase, protease, and amylase) in a diet composed of wheat, wheat middlings, soybean meal, and canola meal. This study indicated that the individual use of xylanase or phytase had no effect on ME, but the combination of both enzymes improved this parameter by 10%. This study also observed that the P retention improved by 26% in low phosphorus diets by supplementing the combination of phytase and xylanase. Nortey et al. (2007) also studied the effect of phyate and xylanse in diets formulated with wheat millrun, wheat, and soybean meal. It was observed that the use of both enzymes improved ATTD of P, however no interaction was observed on AID of energy and total tract digestibility of energy due to enzyme combination. Other studies indicated no benefits due to phytase and xylanase (Moehn et al., 2007; Woyengo et al., 2008). The study conducted by Moehn et al. (2007) utilized a diet formulated with wheat and wheat millrun and observed that phytase improved P digestibility by 4.1% and xylanase improved ATTD of nitrogen by 0.6%, however no improvements due to enzyme combination was observed. Woyengo et al. (2008) reported that xylanase improved AID of lysine, leucine, phenylalanine, threonine, glycine, and serine. Phytase improved ATTD of P by 50% but no interaction between the 2 enzymes was observed (Woyengo et al. 2008). Reviewing studies with feed enzymes Adeola and Cowieson (2011) suggested that the variation among studies could be related to differences among type of enzymes, inclusion rates, type of cereal grains utilized in the formulations, and nutrient composition of the diets.

The combined use of phytase and xylanase supplementation was evaluated on ground (383 µm) DDGS cofermented with wheat and corn (Yáñez et al., 2011). The use of single enzymes or the combination of phytase + xylanase did not improve the ATTD of energy and AID of amino acids.

Other supplemental enzyme combinations were also tested in diets fed to pigs. Mannanase, amylase, protease, or amylase + protease were supplemented to a corn-soybean meal based diet and fed to growing pigs (Jo et al., 2012). This study observed that mannanase + amylase + protease improved G:F ratio by 4.9%. Other enzyme combinations did no differ from the control group not receiving feed enzymes in the diet. The use of enzyme phytase, an enzyme blend (amylase, protease, and xylanase), and the combination phytase + blend was also tested in vitro in corn-soybean meal based diets (Li et al., 2010). It was observed that the blend of enzyme or its combination with phytase improved digestibility of energy, protein, and NDF by 39, 19, and 68% respectively.

A recent study evaluated the supplementation of protease and xylanase on AID of nitrogen and energy in grower-finisher pigs (Mc Alpine et al., 2012b). The diet was formulated utizing wheat distillers, rapessed meal, wheat, and barley and it was observed that protease improved AID of nitrogen, but no effect on AID of energy was observed. However the combinationed use of protease and xylanase reduced the AID of energy indicating that these enzymes together may not be effective. The interaction between protease and xylanase was further observed in poultry (Barekatain et al., 2013) in a diet formulated with corn soybean meal and sorghum DDGS. This study indicated that xylanase decreased the total amount of insoluble NSP in the ileum of poultry. However, the effect on insoluble NSP was mitigated by combining protease with xylanase.

Scope of the current research

The literature reviewed indicated that most of the non-phytase studies were done to evaluate the effect of feed enzymes supplemented to diets. The diets utilized a variety of formulations and it is not possible to make conclusions about the effect of enzyme supplementation on a specific ingredient. Moreover, it is not possible to predict the enzyme effect if the diet composition changes. The effect of viscosity on nutrient digestibility was evaluated in viscous ingredients with soluble NSP. However the typical ingredients utilized in swine diets contain insoluble NSP that forms low viscous solutions. Phytase demonstrated consistent effect on phorphorus digestibility. However, there is little information about the effect of combining supplemental feed enzymes on growth performance and nutrient utilization in pigs. Therefore this dissertation aims to study the effect of feed enzymes on viscosity of digesta and nutrient digestibility of ingredients. The research hypothesis tested is that feed enzymes targeting insoluble NSP change viscosity of digesta which will improve nutrient digestibility of feedstuffs fed to pigs and the combinationed use of feed enzymes can further enhance nutrient utilization of feedstuffs by pigs. The first study (chapter 2) measured viscosity of digesta and apparent ileal digestibility of nutrients in pigs fed diet supplemented with xylanase. The second study (chapter 3) measured the apparent ileal digestibility of nitrogen and viscosity of digesta of pigs fed diet supplemented with protease.This study aldo measured energy, nitrogen, and phosphorus digestibility of ingredients and growth performance of pigs fed diets supplemented with enzyme combinations. The last study (chapter 4) measure nutrient digestibility of forages supplemented with an enzyme combination.

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CHAPTER 2

EFFECT OF DIETARY SUPPLEMENTATION OF XYLANASE ON APPARENT ILEAL DIGESTIBILITY OF NUTRIENTS, VISCOSITY OF DIGESTA, AND INTESTINAL MORPHOLOGY OF GROWING PIGS FED CORN AND SOYBEAN MEAL BASED DIETS

Abstract: This study was to determine apparent ileal digestibility of ADF, NDF, DM, energy, OM, crude ash, digesta viscosity, and gut morphology in nursery pigs fed diets containing xylanase (Lohmann Animal Health GmbH, Cuxhaven, Germany). The diet (61% corn, 35% soybean meal, 1% poultry fat, and 3% minerals and vitamins) was mixed with 3 levels of xylanase (0, 700, and 1400 LXU/kg). Thirty-six barrows (17.6 ± 3.3 kg) received one of 3 treatment diets based on a randomized complete block design with the initial BW as a block. Pigs were individually housed and received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs ($0.09 \times BW^{0.75}$ kg). Pigs were fed diets for 10 d, and chromium oxide (0.3%) was added to the diets from d 6 as an indigestible external marker. Pigs were euthanized at the end of d 10 for the collection of digesta and tissues. Jejunal digesta were centrifuged to measure viscosity using a viscometer (Brookfield Engineering Laboratories, Stoughton, MA). Diets and freeze-dried ileal digesta were used to measure ADF, NDF, and chromium to calculate apparent ileal digestibility of ADF and NDF. Villus height and crypt depth of jejunum were measured using a microscope (Fisher Scientific, Hampton, NH). Data were analyzed using polynomial contrasts in the MIXED procedure of SAS Software (Cary, NC). Morphological measurements and ileal ADF digestibility were not affected by increasing xylanase. However, increasing xylanase supplementation from 0 to 1400 LXU/kg enhanced ileal digestibility of NDF ($P < 0.042$, linear) from 27.9 to 40.3%, DM ($P < 0.006$, linear) from 55.4 to 64.6%), OM ($P < 0.006$, linear) from 59.2 to 67.7%, and energy ($P < 0.003$, linear) from 58.8 to 68.0%. Viscosity of jejunal digesta decreased ($P < 0.023$) in a non-linear manner from 2.9 to 2.5 centipoises. In conclusion, the usage of xylanase in corn and soybean meal based pig diets linearly enhanced digestibility of nutrients and affected viscosity of digesta in a non-linear manner.

Key words: digestibility, pigs, viscosity, xylanase

Introduction

Feed makes up more than 70% of the swine production cost and efforts to improve nutrient digestibility by the pigs can have effects on profitability of the pork industry (National Pork Board, 2012). Corn and soybean meal are typical fed ingredients in pigs diets and studies indicate that non-starch polysaccharides (NSP) in those ingredients negatively affect nutrient digestibility (Moeser et al., 2002; van Kempen et al., 2006). Swine do not produce enzymes to digest NSP components in the feed. Arabinoxylans are fiber components found in pericarp and aleurone of the corn grain (Landis et al., 2001). Whole corn grain contains 27 to 32 g of xylose/kg (Knudsen, 1997). Xylan is found as xyloglucan in the structural polysaccharides in soybean meal (Karr-Lilienthal et al., 2005). Soybean meal contains 18 to 19 g of xylose/kg (Irish and Balnave, 1993; Knudsen, 1997).

Feed enzymes effect was previously reported on feedstuffs such as corn (Li et al., 2010; Cozanet et al., 2012) and soybean meal (Cozanet et al., 2012), as well as a complete feed based on corn and soybean meal fed to pigs (Pettey et al., 2002; Kim et al., 2003; Ji et al., 2008; Jo et al., 2012). Endo-1,4-β-xylanase (xylanase) carries the enzyme commission identifier 3.2.1.8 and catalyzes endohydrolysis of 1,4-β-D-xylosidic linkages in xylans (International Union of Biochemistry and Molecular Biology, 1992). Feed application of xylanase has been evaluated to improve nutrient digestibility in non-ruminant animals (Moehn et al., 2007; Nortey et al., 2007; Woyengo et al., 2008). Xylanase yields oligosacharides from corn and wheat fiber (Katapodis et al., 2003; Katapodis and Christakopoulos, 2008). The mechanism proposed to explain the dietary effect of fiber degrading enzymes in non-ruminant animals involves and degradation of polysaccharides in the cell wall (Adeola & Cowieson, 2011; Masey et al., 2014). The use of feed enzyme can reduce digesta viscosity (Mathlouthi et al., 2002; Garcia et al., 2008) and increase nutrient

release due to degradation of NSP in cell wall of ingredients fed to poultry (Meng et al., 2005). However the outcome might be different in pigs. Large intestine of pigs are bigger relative to their size than that of poultry, the microbial population is different yielding a greater methane production (Jensen, 1996). Furthermore, viscosity might not be the most important factor affecting nutrient digestibility in pigs (Bartelt et al., 2002) once intestinal fermentation and type of fiber should be is considered (Hooda et al., 2010).

The hypothesis of this study is that supplementation of xylanase in corn-soybean meal based diets reduces digesta viscosity and thus enhances digestibility of nutrients. The objective of this study is to measure viscosity of jejunum digesta, intestinal morphology, and ileal digestibility of DM, energy, protein, ADF, NDF, and crude ash of a corn-soybean meal based diet supplemented with xylanase fed to pigs.

Materials and method

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee.

Experimental diets and pigs

The experiment was conducted at the Swine Educational Unit at the North Carolina State University (Raleigh, NC). Pigs were used to evaluate digestibility of DM, energy, protein, ADF, NDF, and crude ash of a diet (Table 1) supplemented with feed enzyme. Corn was ground to 400 µm. Xylanase (Carboflex, Lohmann Animal Health GmbH, Cuxhaven, Germany) was supplemented at 0 mg/kg of diet (C), 100 mg/kg of diet (T1), and 200 mg/kg of diet (T3) to provide 0, 700, and 1,400 LXU of xylanase/kg of diet respectively. LXU is the amount of enzyme which releases one micromole of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C (EURL, 2013).

Thirty six barrows (17.6 ± 3.3 kg) were placed in metabolic cages (0.6 m wide, 1.8 m long) equipped with stainless-steel feeder attached to the front of the pen, nipple water drinker next to the feeder, and slatted flooring. There were 12 cages available for the study and 3 groups of 12 pigs were allotted in the metabolism room. Pigs received one of the 3 treatment diets based on a randomized complete block design with initial body weight as block. The experimental period consisted of 10 days. Ileum content of ADF and NDF, ileal digestibility of ADF and NDF, villus height/crypt depth in jejunum and digesta viscosity were measured.

Experimental procedures, chemical analyses, and digesta viscosity

Pigs received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs (0.09 x $BW^{0.75}$ kg). Dietary treatments were fed to pigs for 10 days. Chromium oxide was added to experimental diets (0.3%) from day 6 as an indigestible external marker for calculation of ileal digestibility. Pigs were euthanized via captive-bolt stunning and exsanguination at day 10 for sample collection 8 hours after the last meal. Immediately after the euthanasia, an ileal portion (a portion of 20 cm prior to ileo-cecal connection) of small intestine was used to obtain digesta in ileum. Digesta from ileum was stored in sterile container and kept frozen at -20^{o}C. Jejunum tissue sample (3cm) was collected and stored in formaline for further histological analysis. Intestine (20 cm) from distal portion of jejunum was also used to obtain digesta to measure viscosity. Jejunal contents were emptied into 50-mL tubes, samples were kept on ice and viscosity was measured immediately after the collection.

Frozen Ileal digesta were freeze-dried (24D x 48, Virtis, Gardiner, NY) for storage and chemical analysis. Diets and freeze dried digesta were analyzed for moisture (Method 934.01, AOAC, 2006), acid detergent fiber (Method 973.18, AOAC, 2006), neutral detergent

fiber (Van Soest et al., 1991), ash (Method 942.05, AOAC, 2006), chromium (Williams et al., 1962), and energy using a calorimeter (6200, Parr Instrument Company, Moline, IL). Apparent ileal digestibility (AID, %) of ADF and NDF were calculated using the chromium concentration in the diets and digesta by using the AID = 100 − ([ND/NF] × [CrF/CrD] × 100) where ND is the nutrient concentration present in the ileal digesta, NF is the nutrient concentration in the diet, CrF is the chromium concentration in the feed, and CrD is the chromium concentration in the ileal digesta.

Viscosity was done using a viscometer (Brookfield Digital Viscometer, Model DV-II Version 2.0, Brookfield Engineering Laboratories Inc., Stoughton, MA). The tubes were centrifuged at 3,000 rpm for 5 minutes and then 2 ml of the supernatant was centrifugated at 12,500 rpm for 5 minutes. Viscometer was set at 25^{o}C, 0.5 ml of digesta supernatant was placed in the viscosimeter. Viscosity measurement was the average between 45.0 sec $^{-1}$ and 22.5 sec $^{-1}$ shear rates.

Histology

Jejunum morphology were analyzed according to Fan et al. (2001) to obtain villus height, crypt depth and the relation villus height to crypt depth. Jejunum samples (2 sections per pig) were fixed in formaline and sent to North Carolina State University histology laboratory for hematoxylin and eosin staining and sectioning according to standard histological technique. The sections were dehydratated and embedded in paraffin. Staining was done using hematoxylin and eosin dyes (Junqueira and Carneiro, 2005).

Villus height, crypta depth, and relation villus height and crypt depth were measured in the microscope (Micromaster, Fisher Scientific International Inc., Pittsburgh, PA). For each section, 15 measurements of adjacent villus height and crypt depth were obtained. The measurements were done with ImageJ software (NIH, 2013) and transferred to Microsoft

Excel software. The relation villus height to crypt depth of each measurement was calculated. The averages of the 30 measurements per pig were calculated and reported as one number per pig.

Statistical analysis

Data were analyzed using polynomial contrasts in the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The experiment was a randomized complete block design using initial BW and group of pigs allotted in the metabolism as blocking factor. The experimental unit was the individual pig. Initial BW and group of pigs were considered random effect. Statistical differences were considered significant with $P < 0.05$. Probabilities less than 0.10 and equal or greater than 0.05 were considered as a tendency.

Results

The average BW of pigs utilized on this study was 17.6 kg and ADFI was 757 g/d (Table 2). The weight gain of the pigs during the metabolism study was 352 g/d and the G:F ratio was 0.47 in average. Increasing the level of xylanase in the diet (0 to 1,400 LXU/kg) did not affect ($P > 0.10$) growth performance of pigs individually house in the metabolism cages. Pigs received a limited amount of feed based on their BW and this study was not designed to measure growth performance.

Increasing xylanase in the diet from 0 to 1,400 LXU/kg did not affect histological measurements (Table 3) including villus height, crypt depth, and villus height/crypt depth relation. Increasing xylanase resulted in a quadratic change ($P = 0.023$) in viscosity of jejunal digesta from 2.94 to 2.52 cP when xylanase increased from 0 to 700 LXU/kg and from 2.52 to 3.20 when xylanase increased from 700 to 1,400 LXU/kg respectively.

Increasing xylanase (0 to 1,400 LXU/kg) yielded greater AID of DM (linear increase from 55.43 to 64.58%, $P = 0.006$), OM (linear increase from 59.19 to 67.70%, $P = 0.006$), and

energy (linear increase from 58.78 to 68.04%, $P = 0.003$). Similarly, AID of crude ash increased by 16% (quadratic increase from 18.71% to 34.34%, $P = 0.045$) and AID of NDF by 12% (linear increase from 27.91 to 40.32%, $P = 0.042$). However AID of ADF was not affected by supplementation of xylanase ($P > 0.10$).

Discussion

The digesta viscosity obtained on this study ranged from 2.52 to 3.20 cP. The viscosity can be affected by the type of ingredient in the diet (Willamil et al., 2012). Digesta viscosity in the ileum was reported to be 2.8 cP for a corn-soybean meal based diet (Willamil et al., 2012), 1.7 cP in corn-soybean meal-DDGS based diet (Agyekum et al., 2012), 4.6 cP in a wheat based diet (Mavromichalis et al., 2000), and 7.0 cP in a rye-wheat based diet (Bartelt et al., 2002). Corn was the major ingredient in the diet of the present study, and it has lower content of soluble NSP than wheat, rye, barley, and oats (Knudsen, 1997) yielding low viscous solutions (Mathlouthi et al., 2002).

This study indicated that by increasing the use of xylanase yields a quadratic change in the viscosity of the digesta in pigs fed corn-soybean meal based diets. Corn grain NSP contains arabinoxylans (Landis et al., 2001), and contains 30 g total xylose/kg of xylose (Knudsen, 1997). Soybean meal contais 18 to 19 g xylose/kg of xylose (Irish and Balnave, 1993; Knudsen, 1997) as xyloglucan (Karr-Lilienthal et al., 2005), therefore the main substrate for xylanse in a corn-soybean meal-based diet will be the arabinoxylans in the corn. The effect of xylanase on corn fiber was previously demonstrated by *in vitro* studies (Grabber et al., 1998; Saha, 2001; Hu et al., 2008). The limitations regarding the xylanase activity on corn fiber (Rose and Inglett, 2011) involve the arabinose side-chains in the xylan back-bone of the arabinoxylan (Doner et al., 2001; Rose et al., 2010). However, arabinofuranosyl groups attached to xylan can be partially released under acidic pH

conditions in the stomach (Zhang et al., 2003). In addition, the corn fiber utilization on xylanase production increases the number of side activity enzymes (β xylosidase and α-L-arabinofuranosidase) that enhance the release of arabinose and xylose from arabinoxylans (Saha, 2001). Arabinoxylans can form viscous solutions (Izydorczyk and Biliaderis, 1992; Izydorczyk and Biliaderis, 1992) and increase viscosity of digesta (Choct and Annison, 1992). Xylanase can break arabinoxylans (Grabber et al., 1998; Pedersen et al., 2012) and reduce viscosity of *in vitro* solutions (Mathlouthi et al., 2002) and also digesta viscosity (Yin et al., 2001, Adeola and Bedford, 2004).

Increasing supplementation of xylanase yields a quadratic response on digesta viscosity. Corn contains a greater proportion of xylose in the insoluble NSPs (Knudsen, 1997) and some xylanases have affinity to insoluble xylan (Connerton et al., 1999; Sun et al., 1998). There is evidence that xylanases can degrade insoluble NSP into soluble NSP increasing digesta viscosity (Choct et al., 2004). Therefore one can speculate that at greater dosages of xylnase (as treatment T2 in the study reported herein), the insoluble NSP become more soluble, and thus increase digesta viscosity. The NDF result of this study supports the degradation of corn NSP, however it needs further investigation.

This study observed that by increasing the dietary supplementation level of xylanase, there will be a linear increase in the ileal digestibility of DM, OM, energy, and NDF (Table 4). The mode of action of xylanase on enhancing nutrient digestibility may involve the degradation of the cell wall NSPs, thus enabling endogenous digestive enzymes to access nutrients trapped. (Adeola and Cowieson, 2011; Masey O'Neil et al., 2014). The greater NDF digestibility can be explained by the method utilized to analyze NDF. Xylanase release oligosaccharides (xylobiose to xylopentose) from arabinoxylans (He et al., 2010; Rajagopalan et al., 2013). The filter bags utilized in the NDF analysis procedure have pore

sizes of 25 microns (F57, ANKOM, Macedon, NY) and the smaller particles of xylotriose to xylopentose released by xylanase might not be retained by the filter bags. There are nutritional benefits of NSP degradation (Choct and Annison, 1992). The use of xylanase in corn-soybean meal based diets improved ileal digestibility of energy by 2% (Nian et al., 2011) and also an enzyme blend containing xylanse, protease, and amylase improved protein digestibility (Zanella et al., 1999). Improvement in NDF, DM, GE, and starch digestibility were observed utilizing *in vitro* and *in vivo* digestibility methods in pigs when an enzyme blend composed of xylanase, protease, and amylase was added to the diet (Li et al., 2010). The present study indicated that as dietary level of xylanase increased, digestibility of DM, OM, energy, NDF, and crude ash increased by 9.2%, 8.5%, 9.3%, 12.4%, and 10.7%, respectively.

Dietary level of NSP can affect intestinal morphology (Montagne et al., 2003). Diets with high content of NSP from wheat and barley affected villus height and the relation villus height/crypt depth in the ileum of pigs compared to diet formulated with corn and soybean meal (Willamil et al., 2012). The use of feed enzyme can also mitigate the negative effect of NSP from wheat and barley on intestinal morphology, however it does not affect intestinal morphology in corn-soybean meal based diet (Willamil et al., 2012). Similarly, there was no siginificant effect of dietary xylanase supplementation of corn-soybean meal diet on intestinal morphology measured in the study reported herein.

Conclusion

The ileal nutrient digestibility of a corn-soybean meal based diet improved when dietary xylanase supplementation level increased from 0 to 1,400 LXU/kg. There was a quadratic change in viscosity of jejunum digesta, but no effect on intestinal morphology. The results

confirms our hypothesis that xylanase can be supplemented to swine diets in order to improve nutrient digestibility.

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Table 1. Ingredient composition of experimental diets (as-fed basis)

	Xylanase, LXU[1]/kg		
Item	0	700	1,400
Ingredients, %			
Yellow corn, ground	61.12	61.11	61.10
Soybean meal	35.30	35.30	35.30
Limestone	1.10	1.10	1.10
Monocalcium phosphorus	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Trace mineral premix[2]	0.15	0.15	0.15
Vitamin premix[3]	0.03	0.03	0.03
Xylanase	0.00	0.01	0.02
Calculated composition			
ME, kcal/kg	3,349	3,349	3,349
SID[4] Lys, %	1.01	1.01	1.01
SID Met + Cys,%	0.59	0.59	0.59
SID Thr, %	0.63	0.63	0.63
SID Trp, %	0.21	0.21	0.21
Ca, %	0.89	0.89	0.89
P total, %	0.61	0.61	0.61
P available, %	0.33	0.33	0.33
Analyzed composition			
DM, %	91.77	91.83	91.66
GE, kcal/kg	4,027	4,035	3,983
CP, %	20.36	20.73	21.01
Fat,%	2.55	2.91	2.39
Ca,%	0.76	0.71	0.78
P,%	0.56	0.58	0.56
Ash,%	4.64	4.82	4.81
Xylanase, LXU/kg	<200	674	1,231

[1] LXU is the amount of enzyme which releases one micromole of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C.

[2] Trace mineral premix supplied per kg of feed: 16.5 mg/kg of Cu as copper sulfate, 165.3 mg/kg of Fe as ferrous sulfate, 39.60 mg/kg of Mn as manganous oxide, 165.30 mg/kg of Zn as zinc sulfate, 0.30 mg/kg of I as ethylenediamine dihydroiodine and 0.30 mg/kg of Se as sodium selenite.

[3] Vitamin premix supplied per kg of feed: 6171 IU of vitamin A as vitamin A acetate, 880 IU of vitamin D as cholecalciferol, 35 IU of vitamin E as tocopheryl acetate, 0.02 mg/kg of vitamin B12 as cyanocobalamin, 0.18 mg/kg of biotin, 2.91 mg/kg of vitamin K as menadione sodium bidulfite, 4.40 mg/kg of riboflavin, 17.64 mg/kg of pantothenic acid as

Table 1. Continued
calcium pantothenate, 26.45 mg/kg of niacin as nicotidamide, 1.32 mg/kg of folate as folic acid.
[4] Standardized ileal digestibility

Table 2. Initial BW (kg), ADFI (g/d), ADG (g/d), and G:F of pigs housed individually in metabolism cages with daily feed allowance of 0.09 x $BW^{0.75}$ kg of corn-soybean meal based diets supplemented with xylanase

	Xylanase, LXU[1]/kg				*P* value	
Item	0	700	1,400	SEM	Linear	Quadratic
Initial BW, kg	17.4	17.4	17.7	1.0	0.145	0.370
ADFI, g/d	753	756	763	28	0.249	0.799
ADG, g/d	340	353	364	57	0.408	0.964
G:F	0.458	0.471	0.482	0.093	0.498	0.952

[1]LXU is the amount of enzyme which releases one micromole of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C.

Table 3. Jejunum villus height (µm), crypt depth (µm), villus height/crypt depth, and viscosity of jejunal digesta (cP[1]) in pigs fed corn-soybean meal based diets supplemented with xylanase

	Xylanase, FXU[1]/kg				*P* value	
Item	0	700	1,400	SEM	Linear	Quadratic
Villus height, um	431	407	403	18	0.263	0.652
Crypt depth, um	233	212	226	14	0.642	0.171
Villus height/crypt depth	1.89	1.97	1.80	0.14	0.553	0.295
Viscosity,	2.94	2.52	3.20	0.12	0.314	0.023

[1] cP = centipoise (1 cP = 1/100 dyne s/cm[2])

[2]LXU is the amount of enzyme which releases one micromole of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C.

Table 4. Apparent ileal digestibility (%) of DM, OM, energy, ash, NDF, and NDF in pigs fed corn-soybean meal based diets supplemented with xylanase.

	Xylanase, FXU[1]/kg				*P* value	
Item	0	700	1,400	SEM	Linear	Quadratic
DM	55.43	66.80	64.58	2.46	0.006	0.020
OM	59.19	69.80	67.70	3.60	0.006	0.021
Energy	58.78	69.00	68.04	2.80	0.003	0.038
Crude ash	18.71	34.34	29.40	4.04	0.062	0.045
NDF	27.91	43.63	40.32	4.80	0.042	0.070
ADF	1.54	13.20	8.77	7.90	0.430	0.312

[1]LXU is the amount of enzyme which releases one micromole of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50°C.

CHAPTER 3

NUTRIENT UTILIZATION OF CORN, SOYBEAN MEAL, AND DISTILLERS DRIED GRAINS WITH SOLUBLES SUPPLEMENTED WITH FEED ENZYMES

Abstract: A series of experiments were conducted to evaluate the hypothesis that blended feed enzymes improve nutrient digestibility of feed ingredients and diets fed to pigs. Initially an ileal collection experiment was done to evaluate apparent ileal digestibility (AID) of protein, amino acids and viscosity of jejunum digesta of a corn-soybean meal-DDGS based diet supplemented with protease (Pro). In total 16 pigs with 39.5 ± 4.5 kg were allotted in 2 treatments according to a randomized complete block design. There were two treatments, control diet (LD Phy) formulated with phytase (1,000 FYT of phytase/kg of diet) and diet supplmemented with protease (LD Phy Pro, 15,000 PRO of protease/kg of diet). The ileal digestibility of protein (P = 0.263) and amino acids (P > 0.050) were not affected due to protease supplementation except for proline (P = 0.020) and histidine (P = 0.038). The supplementation of Pro did no affect viscosity of jejunum digesta. Four metabolism experiments were conducted to determine the dry matter digestibility (DMD), DE, ME, apparent total tract digestibility (ATTD) of N (ND), P (ATTDP), and nitrogen retention (NR), of corn (metabolism experiment 1), soybean meal (metabolism experiment 2), DDGS (metabolism experiment 3), and a corn-soybean meal-DDGS based diet (metabolism experiment 4) supplemented with feed enzymes. The diet treatments were no enzyme (CON), Phytase (Phy, 1,000 FYT/kg), Protease (Pro, 15,000 PRO/kg), Xylanase (Xyl, 200 FXU/kg), Phy+Pro, Phy+Xyl, Pro+Xyl, and Phy+Pro+Xyl and each metabolism experiment received the same treatments. In total, 64 pigs (30.1 ± 3.7 kg), divided into groups of 16 pigs per metabolism experiment, were assigned to treatment diets in a repeated 4 × 4 Latin squares design. The metabolism experiment 1 indicated that corn DE (3,316 kcal/kg), ME (3,263 kcal of/kg), ND (81.27%), and NR (64.35%) were not affected (P > 0.100) by feed enzyme supplementation. The corn ATTDP (28.54%) was improved (P < 0.001) to 43.59% due to Phy supplementation, combined or not with other enzymes. The metabolism

experiment 2 indicated that DMD (92.39%), DE (3,427 kcal.kg), ME (3,404 kcal/kg), and ND (88.89%) of soybean meal increased ($P < 0.05$) to 96.55%, 3,477 kcal/kg, 3,376 kcal/kg, and 89.50% respectively due to Phy+Pro supplementation. The ATTDP of soybean meal (48.47%) improved ($P = 0.003$) to 58.26% due to Phy, Phy+Pro, or Phy+Xyl supplementation. The metabolism experiment 3 indicated that DMD (64.71%), DE (2,855 kcal/kg), ME (2,707 kcal/kg), and ND (76.81%) of DDGS improved ($P < 0.05$) to 70.57%, 3,039 kcal/kg, 2,869 kcal/kg, and 79.36% respectively due Phy+Xyl supplementation. The ATTDP of DDGS (64.65%) improved ($P < 0.001$) in average to 72.21% due to Phy, Phy+Pro, or Phy+Xyl supplementation. The metabolism experiment 4 indicated that ND of the complete diet (83.56%) improved to 86.58% due to Phy+Pro+Xyl ($P = 0.024$).The growth performance experiment evaluated 2 corn-soybean meal-DDGS based diets with equal nutrient composition but different energy (ND, 3,282 kcal/kg or LD, 3,110 kcal/kg). Enzymes were then supplemented to these diets to produce the following dietary treatmens: ND, ND Phy, LD, LD Phy, LD Phy+Pro, LD Phy+Xyl. In total, 144 pigs (72 males and 72 females) with 19.7 ± 2.8 kg were allotted in 48 pens during 3 wk according to a randomized complete block design. ADG was not affected ($P = 0.366$) due to enzyme supplementation. ADFI was lower for ND and ND Phy compared to LD ($P = 0.023$). The ND Phy had greater ($P < 0.05$) G:F compared to ND. LD Phy+Xyl did not differ ($P > 0.05$) from ND. In conclusion, protease had a minor effect on AID of amino acids. The combination of Phy+Xyl and Phy+Pro improved energy digestibility of DDGS and soybean meal. Single use of Phy or combinations Phy+Pro and Phy+Pro can improve ATTDP. The combinational use of Phy+Pro+Xyl improved nutrient digestibity of a complete diet. However combining Phy+Pro+Xyl was not the optimal enzyme blend to improve nutrient digestibility of

ingredients. The combination Phy+Xyl improved nutrient digestibility of ingredients and compensated the ME reduction in the diet of the growth performance study.

Key words: corn, soybean meal, DDGS, feed enzymes, pigs.

Introduction

The swine industry consumes 23.59 million tons of feed utilizing corn, soybean meal, and distillers dried grains with solubles (DDGS) as typical ingredients in the diets (AFIA, 2012). These ingredients contain phytate (NRC, 2012) and NSPs (Knudsen, 1997; NRC, 2012; Kim et al., 2010) that mitigate digestibility of nutrients (Liao et al., 2005; Moeser et al., 2002; van Kempen et al., 2006). Pigs do not produce digestive enzymes to degrade phytate and NSPs (Hartman et al., 1961; Lindemann et al., 1986; Huguet et al., 2006), therefore feed enzymes are being studied to improve digestibility of corn (Cozanet et al., 2012; Almeida and Stein, 2010; Almeida and Stein, 2012), soybean meal (Almeida and Stein, 2010; Cozanet et al., 2012), DDGS (Almeida and Stein, 2012, Yáñez et al., 2011), and corn-soybean meal based diets (Pettey et al., 2002; Kim et al., 2003; Ji et al., 2008; Li et al., 2010; Jo et al., 2012).

It is estimated that phytases and carbohydrases (mainly xylanase and glucanase) account for 90% of the global feed enzyme market (Adeola and Cowieson, 2011). Feed enzymes are available as single component enzymes with a main enzyme activity, or as multiple component enzymes having more than one enzyme activity produced in a single fermentation (Masey O'Neill et al., 2014). Single component enzymes can also be blended to contain 2 or 3 main enzymes activities (Masey O'Neill et al., 2014), although enzyme interactions might occur (Nortey et al., 2007; Barekatain et al., 2013). There is extensive research about single enzymes such as phytase (Boyce and Walsh., 2006; Akinmusire and O. Adeola, 2009; Woyengo et al., 2010; Almeida and Stein, 2012; Guggenbuhl et al., 2012; Torrallardona et al., 2012), xylanase (Woyengo et al., 2008; Moehn et al., 2007, Nortey et al., 2007; Yáñez et al., 2012; Mc Alpine et al., 2012b), and protease (Guggenbuhl et al., 2012; Mc Alpine et al., 2012a). The mode of action proposed for carbohydrases involves

degradation of the cell wall for further digestion of nutrients traped by the NSPs (Tervila-Wilo et al., 1996; Adeola and Cowieson, 2011; Masey O'Neil et al., 2014). Corn germ contains 88% of the phytate P (O'Dell et al., 1972) stored in the kernel and the NSPs of corn germ are mostly arabinose and xylose (Doner et al., 2001). Therefore it can be speculated that phytase and xylanase can further improve phosphorus digestibility. Dietary supplementation of protease can improve digestibility of protein (Guggenbuhl et al., 2012; McAlpine, 2012b). However, there is evidence that protease can interact with other enzymes (Saleh et al., 2004) and mitigate the effect of carbohydrases (Barekatain et al., 2013; McAlpine et al., 2012b). Therefore more studies regargind combinations of enzymes are necessary to elucidade the potential effect of enzyme combination on typical ingredients utilized in swine diets.

The hypothesis of this study is that blended feed enzymes improve nutrient digestibility of feed ingredients and diets fed to pigs. This study measured the effect of protease in a diet containing phytase on AID of nitrogen and amino acids. Protease was combined with phytase, and xylanase to measure the nutrient digestibility of corn, soybean meal, DDGS, and a complete diet. Finally, the most favorable enzyme combinationds to improve growth performance of pigs was determined.

Materials and method

The study was conducted at the Swine Educational Unit at the North Carolina State University (Raleigh, NC). The experimental protocol was approved by North Carolina State University Animal Care and Use Committee. The main feed ingredients utilized on the experiments were corn (IFN 4-02-861, AAFCO, 1992), soybean meal (IFN 5-04-612, AAFCO, 1992), and DDGS (IFN 5-02-843, AAFCO, 1992). The feed enzymes utilized on the experiments were phytase (myo-inositol hexakiphosphate phosphohydrolase, Ronozyme

HiPhos, DSM, Parsippany, NJ), protease (serine endopeptidase, Ronozyme ProAct, DSM, Parsippany, NJ), and xylanase (endo-1,4-β-xylanase, DSM, Parsippany, NJ). Phytase (Phy) inclusion was 100 mg/kg (1,000 FYT/kg), protease (Pro) inclusion was 200 mg/kg (15,000 PRO/kg), and xylanase (Xyl) inclusion 200 mg/kg (200 FXU/kg). One FYT is defined as the amount of phytase that releases 1 μmol of inorganic phosphate from phytate per minute under reaction conditions with a phytate concentration of 5.0 mM at pH 5.5 and temperature 37°C (EFSA, 2012a). One PRO is defined as the amount of protease that liberates 1 μmol para-nitroaniline (pNA) from 1mM Suc-Ala-Ala-Pro-PhepNA ($C_{30}H_{36}N_6O_9$) substrate per minute at pH = 9.0 and at 37°C (EFSA, 2009). One FXU unit is defined as the amount of xylanase that liberates 7.8 micromoles of reducing sugars (xylose equivalents) per minute from azo-wheat arabinoxylan at pH 6.0 and 50 °C (EFSA, 2012b).

Ileal digestibility

The ileal collection experiment evaluated the AID of nitrogen and amino acids of a diet supplemented with Pro. In total, 16 pigs (8 males and 8 females) with 39.5 ± 4.5 kg were alloted in pens (4.0 x 1.4 m) equipped with stainless-steel feeder attached to the front of the pen, nipple water drinker, and concrete flooring. There were 2 treatments:

- LD Phy: the control diet (LD, Table 3) formulated to meet the nutrient requirement of swine (NRC, 1998) containing Phy.
- LD Phy Pro: the LD Phy diet supplemented with Pro.

Pigs received the dietary treatments during 28 days according to a randomized complete block design assigning sex (male and females) and BW (4 groups based on initial BW) as blocking factors. On d 23 the diets were mixed with 0.5% of chromium oxide as an indigestible exogenous marker for the calculation of digestibility. On d 28, pigs were fasted

from 1300 h and re-fed *ad libitum* from 0100 h of d 29. After 8 hours of their last meal, the pigs were euthanized for samples collection. Immediately after euthanasia, ileum digesta was obtained from an ileal portion (a portion of 20 cm prior to ileo-cecal connection) of small intestine. Digesta from ileum was stored in sterile containers and kept frozen at -20^{o}C. Jejunum tissue samples (3cm) were collected and stored in formaline for further histological analysis. Intestinal sections (20 cm) from distal portion of jejunum were also sampled to obtain digesta for viscosity measurement. Jejunal contents were emptied into 50-mL tubes, samples were kept on ice, and viscosity was measured immediately after the collection.

Frozen Ileal digesta were freeze-dried (24D x 48, Virtis, Gardiner, NY) for storage and subsequent chemical analysis. Diets and freeze dried digesta were analyzed for moisture (Method 934.01, AOAC, 2006), chromium (Williams et al., 1962), and complete amino acid content (Method 982.30, AOAC, 2006). Nitrogen was obtained by combustion method (FP528, Leco, St Joseph, MI) to calculate crude protein content (Method 992.15, AOAC, 2006). Apparent ileal digestibility (AID, %) of protein and amino acids were calculated using the chromium concentration in the diets and digesta as follow: AID = 100 − ([ND/NF] × [CrF/CrD] × 100), where ND is the nutrient concentration present in the ileal digesta, NF is the nutrient concentration in the diet, CrF is the chromium concentration in the feed, and CrD is the chromium concentration in the ileal digesta.

Digesta viscosity was measured using a viscometer (Brookfield Digital Viscometer, Model DV-II Version 2.0, Brookfield Engineering Laboratories Inc., Stoughton, MA). The tubes were centrifuged at 3,000 rpm for 5 minutes and then 2 ml of the supernatant was centrifuged at 12,500 rpm for 5 minutes. Viscometer was set at 25°C, 0.5 ml of digesta supernatant was placed in the viscosimeter. Two viscosity readings were done at 45.0 sec $^{-1}$

and 2 at 22.5 sec $^{-1}$, disk rotation speed, and the average of the 4 readings were used for the statistical analysis.

Jejunum morphology was evalauted according to Fan et al. (2001) to determine obtain villus height, crypt depth, and calculate villus height to crypt depth ratio. Jejunum samples (2 sections per pig) were fixed in formaline and sent to the North Carolina State University histology laboratory for hematoxylin and eosin staining and sectioning according to standard histological techniques. The sections were dehydratated and embedded in paraffin. Staining was done using hematoxylin and eosin dyes (Junqueira and Carneiro, 2005).

Villus height, crypta depth, and villus height:crypt depth ratio were measured microscopically (Micromaster, Fisher Scientific International Inc., Pittsburgh, PA). For each section, 15 measurements of adjacent villus height and crypt depth were obtained. The measurements were made using ImageJ software (NIH, 2013) and transferred to Microsoft Excel software for further data management and the calculation of villus height:crypt depth ratio. The averages of the 15 measurements per pig were calculated and reported as one value per pig.

Metabolism experiments

A total of 4 metabolism experiments were conducted to evaluate nutrient digestibility of ingredients and a diet fed to pigs:

Experiment 1: this experiment was conducted to evaluate the nutrient digestibility of corn. A corn diet (Table 2) was formulated to contain 95% of corn (Table 1). The average weight of the 16 pigs utilized in this experiment was 39.2 ± 2.4 kg.

Experiment 2: this experiment was conducted to evaluate the nutrient digestibility of soybean meal. The soybean meal metabolism experiment was done by formulating a soybean meal diet (Table 2) containing 26% of soybean meal (Table 1) and 71.5% of corn

(same corn from experiment 1). The average weight of the 16 pigs utilized in this experiment was 23.5 ± 4.1 kg.

Experiment 3: this experiment was conducted to evaluate the nutrient digestibility of DDGS. The DDGS metabolism experiment was done by formulating a DDGS diet (Table 2) containing 35% of DDGS (Table 1) and 61.6% of corn (same corn from experiment 1). The average weight of the 16 pigs utilized in this experiment was 24.0 ± 3.7 kg.

Experiment 4: this experiment was conducted to evaluate the nutrient digestibility of a complete diet (Table 2) formulated with the same ingredients utilized in the previous experiments. The average weight of the 16 pigs utilized in this experiment was 27.5 ± 4.7 kg.

Particle size of the ingredients was measured accordint to ASAE (1993). The geometric mean diameter (Dgw) of the corn particles was 451 microns. The standard deviation of geometric mean diameter (Sgw) was 3.9. The Dgw of the soybean meal particles was 872 microns and Sgw was 2.5. The Dgw of the DDGS particles was 969 microns and Sgw was 2.0 Sgw.

Mixing feed enzymes to the diets (Table 2) yielded the treatments CON (no enzyme), Phy, Pro, Xyl, Phy+Pro, Phy+Xyl, Pro+Xyl, and Phy+Pro+Xyl. Each metabolism experiment received the same treatments. The enzyme analysis on the feed samples averaged 1,073 FYT/kg for the samples receiving Phy, 16,635 PRO/kg for the samples receiving Pro, and 146 FXU/kg for the samples receiving Xyl.

Pigs of each experiment were assigned to dietary treatment diets based on four 4 × 4 Latin square designs, with 4 pigs evaluated during 4 periods. The first Latin square received the treatments CON, Phy, Xyl, and Phy+Xyl. The second Latin square received the treatments CON, Phy, Pro, and Phy+Pro. The third Latin square received the treatments

CON, Pro, Xyl, and Pro+Xyl. The fourth Latin square received the treatments Phy+Xyl, Phy+Pro, Pro+Xyl, Phy+Xyl+Pro. Each period consisted of 8 days (4 d adaptation, 3 d collection, and 1 d transition).

Pigs of each experiment were allotted in the metabolism cages on d 0 at 0700 h and received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs (0.09 x $BW^{0.75}$ kg). On d 3 if each period, chromium oxide (0.5%) was added to the evening meal as an external marker for fecal collection. Sampling of feces and urine were done during 3 consecutive days. Fecal collection was initiated when green color from chromium oxide was observed in the feces after feeding a meal with chromium oxide, whereas urine sampling was initiated from the time of feeding a meal with chromium oxide. On d 6 of each experiment, chromium oxide (0.5%) was added to the evening meal again and fecal sampling was terminated when green color was observed in the feces in the following day. Urine collection was terminated at the time of the evening meal on d 6 of each period. Pigs were weighed at the end of each period to adjust feed allowance for a subsequent period. Urine samples were collected in a plastic bucket with 20 mL concentrated HCl (5 *N*). Volume of urine was measured each day during the collection period and 150 mL of urine sample was daily sub-sampled. Fecal samples were weighed at the end of each day during the collection period. Urine and fecal samples were frozen (-20ºC) immediately after collection.

Fecal samples were oven dried in air forced oven at 65 °C. Fecal and feed samples were analyzed for moisture (Method 934.01, AOAC, 2006). Nitrogen was obtained by combustion method (FP528, Leco, St Joseph, MI) to calculate protein (Method 992.15, AOAC, 2006). Gross Energy was determided using adiabatic bomb calorimeter (C2000, IKA, Wilmington, NC). Total ash (Method 942.05, AOAC, 2006), calcium (method

968.08, AOAC, 2006), and phosphorus (method 946.06, AOAC, 2006) were also analyzed for the experiments done with corn, soybean meal, and DDGS. Phytate (Ray et al., 2012) was analyzed in the study done with the complete diet. Urine samples were freeze-dried (24D x 48, Virtis, Gardiner, NY) and analyzed for nitrogen and gross energy as previously described.

The laboratory results of DM, GE, N, total ash, Ca, and P were utilized for calculations (Olukosi and Adeola, 2009) of dry matter digestibility (DMD), DE, ME, apparent total tract digestibility of nitrogen (ND), nitrogen retention (NR), apparent total tract digestibility of ash (ATTDash), Ca (ATTDCa), and P (ATTDP). Results of GE, DE, and ME were expressed in kcal/kg while DMD, ND, NR, ATTDash, ATTDCa, ATTDP were expressed in %, and Phytate degradation in µg/kg. Dry matter digestibility (DMD = ((DM intake – fecal DM)/DM intake)*100), DE (DE = (GE intake – fecal GE)/DM intake), and ME (ME = (GE intake – fecal GE – urine GE)/DM intake), ND (ND = ((N intake – fecal N)/N intake)*100), NR (NR = ((N intake – fecal N – urine N)/N intake)*100) were calculated for the corn diet, soybean meal diet, DDGS diet, and the complete diet. The ATTDash, ATTDCa, ATTDP and phytate degradation were calculated by respectively replacing N in the ND formula.

The calculation for each of the tested ingredients (TI) was done considering the diet ingredient composition of each experiment. The corn metabolism experiment considered corn as TI, while the amino acids, limestone and monocalcium phosphorus were considered basal ingredients (BI). Assumptions about energy and nutrient digestibility of amino acids were done based on Rostagno et al. (2011). Assumptions about P bioavailability of monocalcium phosphorus was done based on NRC (1998). Calcium digestibility of each ingredient was not calculated because there is limited information on ATTDCa of limestone and monocalcium phosphorus. The soybean meal and DDGS metabolism experiments

considered soybean meal and DDGS as TI while corn, amino acids, limestone, and monocalcium phosphorus were considered as BI. Initially, the gross energy in the diet contributed from the basal ingredient was calculated by:

$$\% \text{ of GE from BI} = \frac{[\text{GE of BI} \times \text{BI \%}]}{([\text{GE of TI} \times \text{TI \%}]) + ([\text{GE of BI} \times \text{BI \%}])} \times 100$$

The gross energy in the diet contributed from the test ingredient was calculated by:

$$\% \text{ of GE from TI} = \frac{[\text{GE of TI} \times \text{TI \%}]}{([\text{GE of TI} \times \text{TI \%}]) + ([\text{GE of BI} \times \text{BI \%}])} \times 100$$

The amount of DE in the diet contributed from the BI was calculated by: DE from BI = DE of BI x % GE contributed from BI. The calculation of the amount of DE in the diet contributed from the TI was calculated by: DE from TI = DE of the diet – DE from BI. Finally the calculation of DE of the test ingredient was calculated by: DE of TI = DE from TI / % GE from TI.

Similar formulations were utilized for DMD, ME, ND, NR, ATTDash, ATTDCa, and ATTDP to obtain values for each of the tested ingredients (corn, soybean meal, and DDGS).

Growth performance

The growth performance experiment evaluated the most favorable enzyme combination to improve growth performance of pigs. In total 144 pigs (72 males and 72 females) with 19.7 ± 2.8 kg were allotted in 48 pens (3 pigs per pen). The pens (4.0 x 1.4 m) were equipped with stainless-steel feeder attached to the front of the pen, nipple water drinker, and concrete flooring. The corn-soybean meal-DDGS based diets (Table 3) were formulated with the same ingredients (Table 1) utilized in the metabolism study. The normal energy diet (ND) was formulated to have 3,282 kcal/kg of ME and meet the requirements of ME, CP, amino acids, minerals, and vitamins (NRC, 1998). The low energy diet (LD) had similar

nutrient composition however 3,110 kcal/kg of ME. The ME of ND and LD (Table 3) were calculated based on the corn ME (Table 4), soybean meal ME (Table 5), and DDGS ME (Table 6) obtained from the previous metabolism experiments 1, 2, and 3. The ME of poultry fat was assumed to be 8,197 kcal/kg (Carter, 2010) and the ME of crystalline L-Lysine HCL ME was assumed to be 4,559 kcal/kg (Rostagno, 2011). The enzymes (Phy, Pro, and Xyl as previously described) were mixed to the diets and the treatments (ND, ND Phy, LD, LD Phy, LD Phy+Pro, LD Phy+Xyl) were fed to the pigs according to a randomized complete block design, assigning sex (male and females) and BW (4 groups based on initial BW) as blocking factors. The pigs received the treatments during 3 weeks and ADFI, ADG, G:F, and caloric efficiency (CE) were measured. Caloric efficiency was calculated based on ME (kcal/kg) intake divided by kg of weight gain in the period.

Statistical analysis

The data of experiments were analyzed using the Glimmix procedure of SAS (SAS Inst. Inc., Cary, NC).

- Ileal digestibility experiment: each pig as experimental unit. Gender was considered as fixed effect and initial BW blocks as random effect.
- Metabolism experiments: the individual pig was the experimental unit in each of the metabolism experiments. Period and Latin square were included as fixed effects and pig nested to Latin square was included as random effect.
- Growth performance experiment: pen was the experimental unit in the growth performance study. Gender was included as a fixed effect and block of initial BW as a random effect.

Statistical differences were considered significant with $P < 0.05$. Probabilities less than 0.10 and equal or greater than 0.05 were considered as a tendency.

Results

Ileal digestibility

The supplementation of Pro (Table 9) did not affect viscosity of jejunum digesta ($P = 0.396$), villus height ($P = 0.229$), crypt depth ($P = 0.402$), and the villus height:crypt depth ratio ($P = 0.724$). The ileal collection (Table 10) indicated that supplementation of Pro to LD Phy diet did not affect ileal digestibility of protein ($P = 0.263$) and amino acids ($P > 0.050$) except for proline ($P = 0.020$) and histidine ($P = 0.038$).

Metabolism experiments

Experiment 1

The results of the corn diet (Table 4) not supplemented with feed enzymes were 90.56%, 3,366 kcal/kg, 3,307 kcal/kg, 83.23%, and 68.73% for DMD, DE, ME, ND, and NR, respectively. The feed enzyme supplementation did not affect DMD ($P = 0.517$), DE ($P = 0.646$,) ME ($P = 0.566$), ND ($P = 0.766$), and NR ($P = 0.731$) of the corn diet. However, the ATTDP was 60.70% and it increased ($P < 0.001$) due to enzyme supplementation to 68.98%, 70.99%, 66.18%, and 66.99% due to supplementation of Phy, Phy+Pro, Phy+Xyl, and Phy+Xyl+Pro, respectively, when compared to the CON. The results of corn digestibility was calculated from the results of corn diet digestibility after discount the nutrient contribution from the basal ingredients. There were no effects of feed enzyme supplementation on DE ($P = 0.657$), ME ($P = 0.548$), ND ($P = 0.783$), and NR ($P = 0.688$) of corn. The corn not supplemented with feed enzymes had 3,316 kcal/kg, 3,263 kcal/kg, 81.27%, and 64.35% of DE, ME, ND, and NR, respectively. The ATTDP was 28.54%, and

like the results in the corn diet, it increased (P < 0.001) to 43.59, 47.26, 38.50, and 39.99% due to Phy, Phy+Pro, Phy+Xyl, and Phy+Pro+Xyl, respectively.

Experiment 2

The results of the soybean meal diet (Table 5) not supplemented with feed enzymes were 90.26%, 3,402 kcal/kg, 3,296 kcal/kg, 88.20%, 74.35%, and 69.96% for DMD, DE, ME, ND, NR, and ATTDP respectively. The DMD of the Pro (93.78%), Phy+Pro (94.42%), or Phy+Xyl (94.63%) was greater (*P* = 0.001) than CON. The DE of Pro (3,438 kcal/kg), Phy+Pro (3,436 kcal/kg) and Phy+Xyl (3,443 kcal/kg) were greater (*P* = 0.030) than the CON. The ND of the diet supplemented with single use of Pro (89.20%) was greater (*P* = 0.021) than the combination Pro+Xyl (87.31%). The ATTDP improved (*P* = 0.002) to 76.61, 73.97, and 74.54% on the diets supplemented with Phy, Phy+Pro, and Phy+Xyl respectively when compared to the CON (69.95%), Pro (69.68%), or Pro+Xyl (68.55%). The ME (*P* = 0.240) and NR (*P* = 0.162) were not affected by enzyme supplementation in the soybean meal diet.

The results of soybean meal digestibility was calculated from the results of soybean meal diet digestibility after discount the nutrient contribution from the basal ingredients. The results of soybean meal digestibility (Table 5) not supplemented with feed enzymes were 92.39%, 3,427 kcal/kg, 3,304 kcal/kg, 88.59%, 74.85%, and 48.47% for DMD, DE, ME, ND, NR, and ATTDP respectively. The DMD of Pro (95.91%), Phy+Pro (96.55%), Phy+Xyl (96.76%) were greater (P = 0.001) than the CON. The DE of Pro (3,479 Kcal/kg), Phy+Pro (3,477 kcal/kg), and Phy+Xyl (3,478 kcal/kg) were greater (P = 0.029) than the CON. The ND of soybean meal supplemented with single use of Pro (89.70%) was greater (*P* = 0.010) than the combination Pro+Xyl (87.61%). The soybean meal receiving Phy (58.26%), Phy+Pro (54.36%), Phy+Xyl (55.20%) had superior (*P* = 0.003) ATTDP than CON (48.47%),

Pro (48.05%), and Pro+Xyl (46.40%). The enzyme supplementation did not change ME (P = 0.108) and NR (P = 0.165) of soybean meal.

Experiment 3

The results of the DDGS diet (Table 6) not supplemented with feed enzyme in the were 82.90%, 3,162 kcal/kg, 3,082 kcal/kg, 79.82%, 61.40%, and 67.78% for DMD, DE, ME, ND, NR, and ATTDP, respectively. The DMD increased ($P < 0.001$) due to supplementation of Pro (84.96%), Xyl (84.76%), Phy+Pro (83.87%), and Phy+Xyl (84.80%) compared to CON (82.90%) and Phy+Pro+Xyl (82.49%). The DE also increased ($P < 0.001$) due to supplementation of Pro (3,251 kcal/kg), Xyl (3,242 kcal/kg), and Phy+Xyl (3,236 kcal/kg) compared to CON (3,162 kcal/kg) and Phy+Pro+Xyl (3,120 kcal/kg). The ME was greater ($P < 0.001$) in the DDGS diet supplemented with Pro (3,155 kcal/kg), Xyl (3,139 kcal/kg), and Phy+Xyl (3,134 kcal/kg) than CON (3,082 kcal/kg), Pro+Xyl (3,072 kcal/kg) and Phy+Pro+Xyl (3,014 kcal/kg). The supplementation of Pro (82.19%), Xyl (81.66%), and Phy+Xyl (81.32%) improved ($P = 0.001$) the ND of DDGS diet as compared to CON (79.82%) and Phy+Pro+Xyl (78.98%). The supplementation of Pro (63.57%) tended to improve ($P = 0.081$) nitrogen retention of DDGS diet compared to CON (61.40%), Phy (58.72%), Pro+Xyl (58.57%) and Phy+Pro+Xyl (58.53%). The ATTDP was greater ($P < 0.001$) in the DDGS diet receiving Phy (74.73%), Pro (71.35%), Xyl (71.20%), Phy+Pro (73.40%), and Phy+Xyl (74.01%) than in the DDGS diet receiving CON (0.31%) and Pro+Xyl (0.31%).

The results of DDGS digestibility was calculated from the results of DDGS diet digestibility after discount the nutrient contribution from the basal ingredients. The results of the DMD, DE, ME, ND, NR and ATTDP in the DDGS (Table 6) not supplemented with feed enzymes were 64.71%, 2,855 kcal/kg, 2,707 kcal/kg, 76.81%, 55.67%, and 64.65%,

respectively. The DMD of DDGS increased ($P < 0.001$) by supplementing Pro (71.03%), Xyl (70.44%), Phy+Pro (67.69%), and Phy+Xyl (70.57%) as compared to CON (64.71%) and Phy+Pro+Xyl (63.41%). The DE improved ($P < 0.001$) due to supplementation of Pro (3,075 kcal/kg), Xyl (3,052 kcal/kg), Phy+Xyl (3,039 kcal/kg) compared to CON (2,855 kcal/kg) and Phy+Pro+Xyl (2,751 kcal/kg). The ME of DDGS was affected ($P < 0.001$) due to feed enzyme supplementation and increased to 2,931, 2,893, and 2,869 kcal/kg in the Pro, Xyl, and Phy+Xyl supplementation, respectively, when compared to CON (2,707 kcal/kg) and Phy+Xyl+Pro (2,576 kcal/kg). The ND of DDGS supplemented with Pro (80.85%), Xyl (79.94%) and Phy+Xyl (79.36%) was greater ($P = 0.001$) than CON (76.81%) and Phy+Pro+Xyl (75.38%). The NR of DDGS supplemented with Pro (59.36%) tended to be greater ($P = 0.081$) than Phy (51.09%), Pro+Xyl (50.85%), and Phy+Pro+Xyl (50.44%). The ATTP of DDGS in the CON was 64.65% and it increased to 72.21, 68.53, 68.37, 70.76, and 71.42% due to supplementation of Phy, Pro, Xyl, Phy+Pro and Phy+Xyl, respectively.

Experiment 4

The results of the complete diet (Table 7) DMD, DE, ME, ND, and NR were 86.68%, 3,515 kcal/kg, 3,398 kcal/kg, 83.56%, and 71.03%, respectively. The DMD of Phy+Xyl (87.42%) and Phy+Pro+Xyl (87.35%) tended to be greater ($P = 0.061$) than CON (86.38%). The DE of Pro+Xyl (3,549 kcal/kg) and Phy+Pro+Xyl (3,560 kcal/kg) tended to be greater ($P = 0.067$) than Phy (3,495 kcal/kg) and Phy+Pro (3,493 kcal/kg). The feed enzyme supplementation did not affect NR ($P = 0.172$) of the complete diet. The ND of compete diet (83.56%) was affected by feed enzymes and Phy+Pro+Xyl improved ($P = 0.024$) ND to 86.58% if compared to the CON. The ME of the complete diet supplemented with Phy+Pro+Xyl (3,426 kcal/kg) was greater ($P = 0.044$) than Phy+Pro (3,360 kcal/kg). The phytate degradation of the CON was 2,817 µg/kg. The supplementation with Phy, Xyl,

Phy+Pro, and Phy+Xyl improved (*P* = 0.020) phytate degradation to 2,832, 2,831, 2,829, and 2,831 µg/kg respectively if compared to CON.

Growth performance

The ADG was not affected (P = 0.366) by dietary due to treatments in the growth performance study (Table 8). The ADFI decreased (*P* = 0.023) from 1.694 kg/d in the LD treatment to 1.540 kg/d in the ND Phy treatments, respectively. The G:F of ND (0.590) and ND Phy (0.620) was greter (*P* < 0.001) than LD (0.562), LD Phy (0.558), and LD Phy+Pro (0.558), however ND was not different (*P* > 0.05) than LD Phy+Xyl (0.571). The pigs receiving ND Phy had a better (*P* < 0.001) G:F ratio than pigs receiving ND. The CE was not affected by dietary treatments (P = 0.241).

Discussion

Ileal digestibility

The ileal collection indicated that the protease did not change the AID of amino acids except proline and histidine. This experiment is in accordance with the complete diet metabolism study, where the individual use of protease did not affect the digestibility of nitrogen as compared to the CON treatment. In the metabolism experiments, dietary protease supplementation improved the ATTD of N of DDGS and SBM, however differences were not observed in the ileal digestibility of amino acids. Protease supplementation improved AID of N in nursery pigs (Guggenbuhl et al., 2012) and our study analyzed AID of amino acid in growing pigs with 77 days of age and 39.5 kg BW. Therefore, the differences among studies might be related to the age of pigs, since older pigs can better digest protein than youger pigs (Wilson and Leibholz, 1981; Lindemann et al., 1986). Dietary supplementation of protease did not affect amino acid digestibility in finishing pigs (McAlpine et al., 2012b); however, it increased the NH_3 manure emissions and production of valeric

acid, isovaleric acid, and isobutyric acid, indicating protein degradation in the large intestine (McAlpine et al., 2012a). Therefore, one can speculate that that improvements on ATTD of N due to protease supplementation may have contribution from NH_3 emissions, however it needs further investigation.

The gelation of soy protein by proteases is being studied in the development of soy foods (Zhong et al., 2007) and whey products (Creusot and Gruppen, 2007). This gelling mechanism involves the hydrolysis of protein by proteases to form small soluble peptides (Creusot and Gruppen, 2007) wich positively affects on viscosity (Zhong et al., 2007). This study however showed no effect of protease on viscosity of jejunum digesta in pigs.

Metabolism experiments

Experiment 1

Dietary supplementation of enzymes did not improve energy and nitrogen digestibility of corn. Comparing the nutrient composition of the ingredients (Table 1), corn is low in protein and fiber (ADF and NDF) (NRC, 2012). Therefore, corn has little substrate for protease and xylanase as compared to other ingredients. No improvements on DE, ME, ND and NR of corn were observed. A different outcome was observed on phosphorus. The ATTDP of corn improved due to phytase supplementation in agreement with previously published studies (Almeida and Stein, 2010; Almeida and Stein, 2012). In addition, the combination with other enzymes did not change the phytase effect on ATTDP. In summary, 42.4% of the corn phosphorus was digested in the treatments receiving phytase.

Experiment 2

This study indicated that DMD and DE of soybean meal can be improved by dietary Pro supplementation as it can improve AID of amino acid in nursery pigs (Guggenbuhl et al., 2012). Proteolitic activity was reported in the gastro-intestinal tract due to dietary protease

supplementation (McAlpine et al., 2012a). Despite the improvements due to Pro and its combination with Phy, no improvements on digestibility of nutrients were observed when protease was combined to xylanase. The response to treatments Pro+Xyl and Phy+Pro+Xyl were not significantly different from CON, indicating that it may not be an effective combination in soybean meal. It was previously reported that the individual use of protease improves methionine digestibility, while individual use of xylanase reduces the concentration of insoluble non-starch polysaccharides (NSPs) in the ileum of poultry (Barekatain et al., 2013). However, combining both enzymes mitigate the effect of protease on amino acid digestibility and the effect of xylanase on insoluble NSPs (Barekatain et al., 2013). McAlpine (2012b) observed that the combination of protease and xylanase reduced GE ileal digestibility in finishing pigs. Saleh et al (2004) reported that protease inhibited other carbohydrases (cellulase, xylanase, glucanase) in an in vitro digestibility experiment studying enzymes added to a corn-soybean meal based diet. The present study indicated that the combination of Pro and Xyl was not effective in improving nutrient digestibility of soybean meal.

The combination Phy+Xyl improved DMD and DE in soybean meal although the individual enzymes did not have effect on these parameters. The main NSP components in soybean meal consists of 162 g cellulose/kg $^{-1}$, 41 g galactose/kg $^{-1}$, and 48 g uronic acids /kg $^{-1}$ (Knudsen, 1997), while it contains only 18 to 19 g xylose /kg $^{-1}$ soybean meal (Irish and Balnave, 1993; Knudsen, 1997). Xylose in soybean meal exist as xyloglucan and xylanase has no effect on this component (Huisman et al., 2000). The degradation of soybean meal NSPs can be done with other enzymes such as endo-glucanase (Huisman et al., 2000) endo-galactanase, endo-arabinanase, arabinofuranosidase (Huisman et al., 1999) arabinogalactanase, and endopolygalacturonase (Vahjen et al., 2005). It is known that

during fermentative enzyme production process, side activities enzyme are produced (Saha, 2001). Moreover, the combination of Phy and other carbohydrases can effectively to improve nutrient digestibility in pigs (Nortey et al., 2007; Olukosi et al., 2007). Therefore, one can speculate that side activities of different enzymes could degrade the NSPs of the soybean meal, as and this study apparently recovered phytase, protease, and xylanase enzyme activities.

The result of ATTDP in the soybean meal indicated that 48.5% of the phosphorus was digested in the CON treatment. The Phy supplementation improved ATTDP by 9.8%. The effect of phytase on soybean meal was previously reported (Akinmusire and Adeola, 2009; Almeida and Stein, 2010, Rojas and Stein, 2012). The effect of dietary Phy supplementation ATTDP was not different than Phy+Pro and Phy+Xyl treatments, suggesting that the efficacy of phytase is not influenced affected by other single enzymes. However, the combination of Phy+Pro+Xyl did not improve ATTDP of soybean meal, similar to the observations of other nutrients.

Experiment 3

Similar to the observation of soybean meal, Pro improved DMD, DE, ME, and ND of DDGS. The combination Phy+Pro improved DMD of DDGS. Previous studies reported that the use of exogenous protease in pigs improved on AID of amino acids in corn-soybean meal based diet fed to nursery pigs (Guggenbuhl et al., 2012). Protease can increase on AID of nitrogen in a wheat distillers-rapeseed meal-wheat-barley based diet fed to pigs (McAlpine et al., 2012b). Similar to our observation with the soybean meal diet, the combinations Pro+Xyl or Phy+Pro+Xyl were not effective. As mentioned before, previous literature indicate that combining dietary supplementation of Pro and Xyl might not be

effective on improving nutrient digestibility (Mc Alpine 2012b; Barekatain et al., 2013) due to the potential protease interaction with other carbohydrases (Saleh et al., 2004).

The limitations regarding the xylanase activity on corn NSPs (Rose and Inglett, 2011) involves the arabinose side chains in the xylan back bone of the arabinoxylan in corn (Doner et al., 2001; Rose et al., 2010) and DDGS (Dien et al., 2008) . However, there is evidence that arabinofuranosyl groups attached to corn xylan can be partially released under acidic pH conditions as in the stomach (Zhang et al., 2003), thus improving the degradation of xylan back bone by xylanase (Craeyveld et al., 2009). It is also known that enzyme production from fermentation with corn fiber will produce accessory enzymes such as (β-xylosidase and α-L-arabinofuranosidase), which improve efficacy of xylanase on corn xylan (Saha, 2001). Based on the evidence of this study, accessory enzyme activities can be speculated to work together with the main enzyme activities.

Previous reports showed no effect of xylanase on energy digestibility of ground DDGS (383 microns of Dgw) cofermented from wheat and corn (Yáñez et al., 2011). Our study utilized a non-ground DDGS (969 microns of Dgw and 2.0 Sgw) and observed that supplementation with Xyl or the combination Phy+Xyl improved DMD, DE, ME, and ND of DDGS. The differences among the studies could be due to the particle size, since lower particle size improves digestibility of DDGS (Liu et al., 2012) and feed enzymes may be less effective on feed ingredients with low particle size (Mavromichalis et al., 2000). Dietary supplementation of Phy+Xyl was not different than Xyl supplementation alone. Considering that Phy did not significantly affect DMD, DE, ME and ND, the improvements observed by dietary Phy+Xyl supplementation can be attributed largely to the Xyl activity.

Studies regarding the effect of dietary supplementation of phytase on phosphorus digestibility of DDGS are contradictory. Yáñez et al (2011) reported a 10% improvement in

ATTDP due to dietary phytase supplementation. Almeida and Stein (2012) reported a marginal 6% improvement in ATTDP, while Almeida and Stein (2010) observed no effect. The DDGS has 0.26% of phytate P (NRC, 2012) and our study indicated that phytase improved ATTDP by 7.6%. Although the objective of our study was not to compare differences among ingredients, the data indicated that the improvement on ATTDP due to phytase supplementation was 11.6 % on DDGS, 54.0% on corn, and 20.2% on soybean meal. The phytate P will represent 35% of the total phosphorus in DDGS and this proportion is 80% in corn and 54% on soybean meal (NRC, 2012). The corn fermentation reduces phytate concentration (Lopez et al., 1983; Liu and Han, 2011) and it might explain the lower improvement observed on DDGS. Our study also observed the effect of Pro and Xyl on ATTDP. Based on this results, it is suggested that protease and xylanse are releasing non-phytate phosphorus trapped by undigested protein and arabynoxylans.

Experiment 4

Different than the results of experiments 1, 2, and 3 done with ingredients, the combination of Phy+Pro+Xyl improved ND of the complete diet in the experiment 4. This diet was formulated with the same batch of ingredients utilized in the previous metabolism experiments, the only difference was the additional poultry fat. It is known that dietary fat supplementation in the diet can prolong gastric empting (Frost et al., 2003; Little et al., 2007) and this mechanism could enhance the effect of enzymes like phytase that has optimal activity in acidic (pH 2-4) environment (Boyce and Walsh, 2007; Naves et al., 2012). Reviewing the effect of different commercial xylanases Polizeli et al (2005) reported the optimal pH activity of xylanases is 5.4 ± 3.8, with some of xylanases working a lower pH between 2 and 4. Proteases, however, have optimal activity at pH 7 (Pedersen et al. 2012). A longer retention time of digesta in the stomach could favor the enzymes, like phytase and

xylanase, that have optimal pH activities at low pH. In contrast, protease may not be active in the low pH becoming more active in the higher pH of the small intestine. The longer retention time in the stomach would enable a longer activity time for the xylanase and phytase to have their effect before protease become active.

The analysis of the complete diet included phytate degradation in the gastro-intestinal tract. The Phy, Xyl and the combinations Phy+Pro and Phy+Xyl increased phytate degradation as compared to the CON treatment. This apparent effect of phytase was expected, based on the previous experiments with the separate ingredients and observations reported by other researchers (Akinmusire and Adeola, 2009; Almeida and Stein, 2010; Almeida and Stein, 2012; Yáñez et al., 2011). The Xyl also increased phytate degradation, however in the previous experiments, Xyl did not improve ATTDP. Pigs can hydrolyze phytate in the large intestine due to alkaline phosphatases produced by intestinal microorganisms (Schlemmer et al., 2001). It is therefore likely that xylanase supplementation enhances phytate P utilization due to associated increased microbial fermentation in the large intestine. Previous literature reported that the combination of phytase and xylanase improved ATTDP in wheat (Nortey et al, 2007) suggesting that xylanase may play a role in the phytate degradation. Arabinoxylans are present in the pericarp and aleurone layer of the grain (Doner et al., 2001). It was also reported that xylanase will degrade cell wall of wheat (Tervila-Wilo et al., 1996), as demonstrated starch removal in an image of ground maize that was incubated in xylanase (Masey O'Neil et al., 2014). This supports the mechanism of degradation of NSPs in cell wall and further digestion of nutrients trapped in the cell wall (Adeola and Cowieson, 2011; Masey O'Neil et al., 2014).

Growth performance

The enzyme treatments Phy+Pro and Phy+Xyl of this experiment were selected based on the ME results of soybean meal and DDGS experiments respectively. The effect of dietary energy on performance of pigs was previously described (Lawrence et al., 1994; Beaulieu et al., 2006), thus explaining the better G:F of ND as compared to LD.

The ND diet was formulated with 4% supplemented of poultry fat, while the LD was formulated with 0.5% supplemental poultry fat. The Phy supplementation to ND diet improved G:F. The fat inclusion in the complete diet of the metabolism experiment was half of the fat in the diet of the growth performance experiment. The metabolism study measured digestible energy, while other reports indicated a greater AID of fat or energy by dietary to phytase supplementation (Johnston et al., 2004; Liu et al., 2010; Zaefarian et al., 2013). Therefore this study coud not fully explain the better G:F due to Phy supplementation to ND diet. It was reported that phytase can increase AID of energy and fat in poultry (Liu et al., 2010; Zaefarian et al., 2013), however, dietary phytase supplementation did not affect apparent metabolizable energy, which also accounts for caecal micro-organisms fermentation (Zaefarian et al., 2013). Differences on fat digestibility may be related to lipase activity. Liu et al (2010) observed a reduction of lipase activity in diets with high phytate phosphorus (0.4%) content in comparison to diets with low phytate phosphorus (0.2%) in poultry. It is also suggested that Ca-phytate can bind to lipids to form soaps that negatively affect the fat digestibility (Selle and Ravindran, 2007). However, this hypothesis needs further investigation in pigs.

Conclusion

This study demonstrated that protease had a minor effect on AID of amino acids. The combination of Phy+Xyl and Phy+Pro improved energy digestibility of DDGS and soybean

meal. By combinating supplemental enzymes does not change the effect of Phy on ATTD of P in corn. The combination of Phy+Pro+Xyl improved nutrient digestibity of a complete diet. However, combining Phy+Pro+Xyl was not the optimal enzyme blend to improve nutrient digestibility of ingredients. The combination Phy+Xyl improved nutrient digestibility of ingredients and compensated the ME reduction in the diet of the growth performance study. Therefore, the combination Phy+Xyl can potentially be supplemented to swine diets in order to reduce feed cost.

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

Item	Corn	Soybean meal	DDGS
DM,%	90.2	91.4	94.2
GE, Kcal/kg	3,855	4,057	4,550
CP, %	7.25	46.82	28.06
Fat, %	2.57	0.74	7.50
NDF, %	9.07	6.36	28.53
ADF, %	2.99	5.13	13.61
Total ash, %	1.09	6.25	6.20
Ca, %	0.01	0.25	0.02
P, %	0.22	0.67	0.91

Table 2. Ingredient composition, calculated composition, and analyzed composition (as-fed basis) of the diets

Item	Corn diet	Soybean meal diet	DDGS diet	Complete diet
Ingredient, %				
Yellow corn, ground	95.24	71.50	61.60	54.34
Soybean meal	-	26.00	-	21.00
DDGS	-	-	35.00	20.00
Poultry fat	-	-	-	2.00
L-Lys HCl	0.85	0.05	0.79	0.20
DL-Met	0.19	-	0.05	-
L-Thr	0.33	-	0.22	-
L-Trp	0.12	-	0.08	-
L-Val	0.27	-	0.08	-
L-Ile	0.24	-	0.07	-
L-Phe	-	-	-	-
Limestone	1.10	1.05	1.35	1.30
Monocalcium phosphorus	0.90	0.64	-	0.40
Salt	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.06	0.06	0.06	0.06
Trace mineral premix[2]	0.30	0.30	0.30	0.30
Calculated composition				
ME, kcal/kg	3,340	3,328	3,149	3,305
CP, %	9.70	18.33	16.26	20.29
SID[3] Lys, %	0.83	0.83	0.83	0.85
SID Met + Cys,%	0.47	0.52	0.47	0.54
SID Thr, %	0.52	0.16	0.53	0.52
SID Trp, %	0.15	0.52	0.15	0.16
SID Val, %	0.56	0.70	0.56	0.72
SID Ile, %	0.45	0.63	0.45	0.64

Table 2. Continued

SID Phe, %	0.31	0.75	0.57	0.81
Ca, %	0.60	0.60	0.60	0.67
P total, %	0.46	0.50	0.24	0.53
P available, %	0.23	0.23	0.45	0.28
Analyzed composition				
DM, %	90.81	93.20	93.18	94.15
GE, kcal/kg	3,825	3,832	3,960	4,165
CP, %	9.53	18.43	16.61	21.07
Fat, %	2.37	1.58	4.09	4.55
NDF, %	8.24	6.04	15.45	13.10
ADF, %	2.96	2.91	6.89	5.77
Total ash, %	3.03	4.15	4.25	4.84
Ca, %	0.60	0.64	0.59	0.62
P, %	0.37	0.45	0.46	0.58

[1]Vitamin premix supplied per kg of feed: 12,341 IU of vitamin A as vitamin A acetate; 1,759 IU of vitamin D as cholecalciferol; 70.50 IU of vitamin E as tocopheryl acetate; 0.04 mg/kg of vitamin B12 as cyanocobalamin; 0.35 mg/kg of biotin; 5.82 mg/kg of vitamin K as menadione sodium bisulfite; 8.80 mg/kg of riboflavin; 35.27 mg/kg of pantothenic acid as calcium pantothenate; niacin, 52.91 mg/kg of niacin as nicotinamide; 2.65 mg/kg of folate as folic acid.

[2]Trace mineral premix supplied per kg of feed: 33 mg/kg of Cu as copper sulfate; 331 mg/kg of Fe as ferrous sulfate; 79 mg/kg of Mn as manganous oxide; 330 mg/kg of Zn as zinc sulfate; 0.59 mg/kg of I as ethylenediamine dihydroiodide; 0.60 mg/kg of Se as sodium selenite.

[3] Standardized ileal digestibility

Table 3. Ingredient composition, calculated composition, and analyzed composition (as-fed basis) of complete diets formulated to meet the energy requirement (ND) or below the energy requirement of growing pigs (LD)

Item	ND	LD
Ingredient, %		
Yellow corn, ground	52.27	55.77
Soybean meal	21.00	21.00
DDGS	20.00	20.00
Poultry fat	4.00	0.50
L-Lys HCl	0.30	0.30
DL-Met	-	-
L-Thr	-	-
L-Trp	-	-
L-Val	-	-
L-Ile	-	-
L-Phe	-	-
Limestone	1.40	1.40
Monocalcium phosphorus	0.45	0.45
Salt	0.40	0.40
Vitamin premix[1]	0.03	0.03
Trace mineral premix[2]	0.15	0.15
Calculated composition		
ME, kcal/kg	3,280	3,110
CP, %	20.09	20.37
SID[3] Lys, %	1.00	1.00
SID Met + Cys,%	0.56	0.57
SID Thr, %	0.59	0.59
SID Trp, %	0.18	0.18
SID Val, %	0.78	0.79
SID Ile, %	0.68	0.69
SID Phe, %	0.84	0.85
Ca, %	0.67	0.67
P total, %	0.50	0.26
P available, %	0.26	0.51
Analyzed composition		
DM, %	93.34	92.91
CP, %	20.61	20.68
Fat, %	7.13	3.72
NDF, %	8.71	11.86

Table 3. Continued

ADF, %	4.48	4.67
Ca, %	0.75	0.73
P, %	0.51	0.51

[1] Vitamin premix supplied per kg of feed: 6,171 IU of vitamin A as vitamin A acetate; 880 IU of vitamin D as cholecalciferol; 35 IU of vitamin E as tocopheryl acetate; 0.02 mg/kg of vitamin B12 as cyanocobalamin; 0.18 mg/kg of biotin; 2.91 mg/kg of vitamin K as menadione sodium bidulfite; 4.40 mg/kg of riboflavin; 17.64 mg/kg of pantothenic acid as calcium pantothenate; 26.45 mg/kg of niacin as nicotidamide; 1.32 mg/kg of folate as folic acid.

[2] Trace mineral premix supplied per kg of feed: 16.5 mg/kg of Cu as copper sulfate; 165.3 mg/kg of Fe as ferrous sulfate; 39.60 mg/kg of Mn as manganous oxide; 165.30 mg/kg of Zn as zinc sulfate; 0.30 mg/kg of I as ethylenediamine dihydroiodine; 0.30 mg/kg of Se as sodium selenite.

[3] Standardized ileal digestibility

Table 4. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), apparent total tract digestibility of nitrogen digestibility (ND, %), nitrogen retention (NR, %), apparent total tract digestibility of ash (ATTDash, %), apparent total tract digestibility of Ca (ATTDCa, %), and apparent total tract digestibility of P (ATTDP, %) of corn diet and corn supplemented with feed enzyme as-fed basis.

	Enzyme									
Item	CON[1]	Phy[2]	Pro[3]	Xyl[4]	Phy+Pro	Phy+Xyl	Pro+Xyl	Phy+Pro+Xyl	SEM	*P* value
Corn diet										
DMD, %	90.56	90.95	91.80	90.49	91.21	90.83	90.38	90.18	0.180	0.517
DE, kcal/kg	3,366	3,379	3,383	3,373	3,391	3,374	3,359	3,341	8.635	0.646
ME, kcal/kg	3,307	3,302	3,322	3,314	3,324	3,303	3,294	3,270	8.805	0.566
ND, %	83.23	83.68	84.24	83.51	84.74	84.13	83.57	83.81	0.396	0.776
NR, %	68.73	68.37	69.43	68.26	69.61	66.69	68.38	68.81	0.722	0.731
ATTDash, %	65.51^{c}	70.01^{ab}	66.62^{bc}	64.38^{c}	70.91^{a}	67.88^{abc}	65.09^{c}	68.18^{abc}	0.762	0.011
ATTDCa, %	75.75^{bc}	79.03^{a}	77.10^{ab}	73.80^{c}	79.92^{a}	75.70^{bc}	74.72^{bc}	80.81^{a}	0.734	<.0001
ATTDP, %	60.70^{d}	68.98^{ab}	62.20^{cd}	59.84^{d}	70.99^{a}	66.18^{bc}	59.81^{d}	66.99^{abc}	0.966	<.0001
Corn										
DE, kcal/kg	3,316	3,328	3,334	3,325	3,341	3,323	3,309	3,291	8.973	0.657
ME, kcal/kg	3,263	3,257	3,279	3,274	3,280	3,258	3,250	3,224	9.150	0.548
ND, %	81.27	81.43	82.33	81.24	82.72	82.07	81.41	81.72	0.458	0.783
NR, %	64.35	63.98	65.07	64.01	65.37	61.74	64.23	64.33	0.839	0.688
ATTDP, %	28.54^{d}	43.59^{ab}	31.28^{cd}	26.98^{d}	47.26^{a}	38.50^{bc}	26.94^{d}	39.99^{abc}	1.756	<.0001

[1] No enzyme supplemented
[2] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[3] Ronozyme WX (DSM, Parsippany, NJ), 200 mg/kg (200 FXU/kg)
[4] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

Table 5. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), apparent total tract digestibility of nitrogen digestibility (ND, %), nitrogen retention (NR, %), apparent total tract digestibility of ash (ATTDash, %), apparent total tract digestibility of Ca (ATTDCa, %), and apparent total tract digestibility of P (ATTDP, %) of soybean meal diet and soybean meal supplemented with feed enzyme as-fed basis.

	Enzyme									
Item	CON[1]	Phy[2]	Pro[3]	Xyl[4]	Phy+Pro	Phy+Xyl	Pro+Xyl	Phy+Pro+Xyl	SEM	*P* value
Soybean meal diet										
DMD, %	90.26^{d}	92.10^{abcd}	93.78^{abc}	91.69^{bcd}	94.42^{ab}	94.63^{a}	90.13^{d}	91.13^{cd}	0.476	0.001
DE, kcal/kg	3,402^{b}	3,419^{ab}	3,438^{a}	3,415^{ab}	3,436^{a}	3,443^{a}	3,397^{b}	3,405^{ab}	5.720	0.030
ME, kcal/kg	3,296	3,291	3,323	3,310	3,347	3,336	3,291	3,298	6.916	0.240
ND, %	88.20^{abcd}	87.96^{bcd}	89.20^{a}	87.70^{cd}	89.01^{ab}	88.83^{abc}	87.31^{d}	88.06^{abcd}	0.229	0.021
NR, %	74.35	67.37	66.22	74.53	75.21	74.11	70.81	74.63	1.140	0.162
ATTDash, %	67.52^{bc}	69.88^{ab}	68.29^{bc}	68.36^{bc}	72.08^{a}	71.86^{a}	66.19^{c}	69.03^{abc}	0.614	0.007
ATTDCa, %	73.51^{bc}	77.54^{a}	73.80^{bc}	73.79^{bc}	78.49^{a}	77.85^{a}	72.44^{c}	76.84^{ab}	0.682	0.002
ATTDP, %	69.96^{c}	76.614^{a}	69.68^{c}	71.50^{bc}	73.97^{ab}	74.54^{ab}	68.55^{c}	70.24^{bc}	1.194	0.003
Soybean meal										
DMD, %	92.39^{d}	94.23^{abcd}	95.91^{abc}	93.82^{bcd}	96.55^{ab}	96.76^{a}	92.26^{d}	93.26^{cd}	0.476	0.001
DE, kcal/kg	3,427^{b}	3,425^{b}	3,479^{a}	3,440^{ab}	3,477^{a}	3,478^{a}	3,421^{b}	3,430^{ab}	8.424	0.029
ME, kcal/kg	3,304	3,268	3,344	3,317	3,376	3,351	3,296	3,306	9.960	0.108
ND, %	88.59^{abc}	87.72^{c}	89.70^{a}	87.92^{bc}	89.50^{a}	89.12^{ab}	87.61^{c}	88.40^{abc}	0.255	0.010
NR, %	74.85	65.97	68.16	74.48	76.32	74.30	70.83	75.15	1.253	0.165
ATTDP, %	48.47^{c}	58.26^{a}	48.06^{c}	50.73^{bc}	54.36^{ab}	55.20^{ab}	46.40^{c}	48.87^{bc}	1.755	0.003

[1] No enzyme supplemented
[2] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[3] Ronozyme WX (DSM, Parsippany, NJ), 200 mg/kg (200 FXU/kg)
[4] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

Table 6. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), apparent total tract digestibility of nitrogen digestibility (ND, %), nitrogen retention (NR, %), apparent total tract digestibility of ash (ATTDash, %), apparent total tract digestibility of Ca (ATTDCa, %), and apparent total tract digestibility of P (ATTDP, %) of DDGS diet and DDGS supplemented with feed enzyme as-fed basis.

	Enzyme									
Item	CON[1]	Phy[2]	Pro[3]	Xyl[4]	Phy+Pro	Phy+Xyl	Pro+Xyl	Phy+Pro+Xyl	SEM	*P* value
DDGS diet										
DMD, %	82.90^{d}	83.42^{cd}	84.96^{a}	84.76^{ab}	83.87^{bc}	84.80^{ab}	83.52^{cd}	82.49^{d}	0.175	<0.001
DE, kcal/kg	3,162^{bc}	3,178^{bc}	3,251^{a}	3,242^{a}	3,185^{b}	3,236^{a}	3,182^{b}	3,120^{c}	8.289	<0.001
ME, kcal/kg	3,082^{c}	3,085^{c}	3,155^{a}	3,139^{ab}	3,094^{bc}	3,134^{ab}	3,072^{c}	3,014^{d}	7.898	<0.001
ND, %	79.82^{d}	80.22^{cd}	82.19^{a}	81.66^{ab}	80.52^{bcd}	81.32^{abc}	80.43^{bcd}	78.98^{d}	0.315	0.001
NR, %	61.40^{ab}	58.72^{b}	63.57^{a}	61.14^{ab}	61.66^{ab}	60.59^{ab}	58.57^{b}	58.33^{b}	0.617	0.081
ATTDash, %	67.68^{c}	72.03^{a}	71.59^{a}	70.63^{ab}	71.64^{a}	72.06^{a}	68.56^{bc}	68.30^{bc}	0.3959	<.0001
ATTDCa, %	80.93^{c}	85.74^{a}	83.39^{ab}	85.56^{a}	85.97^{a}	85.66^{a}	82.44^{bc}	81.66^{bc}	0.584	0.0002
ATTDP, %	67.78^{d}	74.73^{a}	71.35^{bc}	71.20^{bc}	73.40^{ab}	74.01^{ab}	67.87^{d}	69.29^{cd}	0.621	<.0001
DDGS										
DMD, %	64.71^{d}	66.30^{cd}	71.03^{a}	70.44^{ab}	67.69^{bc}	70.57^{ab}	66.63^{cd}	63.41^{d}	0.538	<0.001
DE, kcal/kg	2,855^{bc}	2,894^{bc}	3,075^{a}	3,052^{a}	2,912^{b}	3,039^{a}	2,906^{b}	2,751^{c}	20.555	<0.001
ME, kcal/kg	2,707^{cd}	2,739^{cd}	2,931^{a}	2,893^{ab}	2,769^{bc}	2,869^{ab}	2,734^{cd}	2,576^{d}	20.426	<0.001
ND, %	76.81^{d}	77.48^{cd}	80.85^{a}	79.94^{ab}	78.01^{bcd}	79.36^{abc}	77.83^{bcd}	75.38^{d}	0.538	0.001
NR, %	55.67^{ab}	51.09^{b}	59.36^{a}	55.23^{ba}	56.12^{ba}	54.30^{ba}	50.85^{b}	50.44^{b}	1.053	0.081
ATTDP, %	64.65^{d}	72.21^{a}	68.53^{bc}	68.37^{bc}	70.76^{ab}	71.42^{ab}	64.75^{d}	66.30^{cd}	0.676	<.0001

[1] No enzyme supplemented
[2] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[3] Ronozyme WX (DSM, Parsippany, NJ), 200 mg/kg (200 FXU/kg)
[4] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

Table 7. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), apparent total tract digestibility of nitrogen digestibility (ND, %), nitrogen retention (NR, %), phytate intestinal degradation (phytate, μg/kg) of the complete diet supplemented with feed enzyme as-fed basis.

	Enzyme									
Item	CON[1]	Phy[2]	Pro[3]	Xyl[4]	Phy+Pro	Phy+Xyl	Pro+Xyl	Phy+Pro+Xyl	SEM	*P* value
Complete diet										
DMD, %	86.38^{c}	86.46^{bc}	86.47^{bc}	86.91^{bac}	86.47^{bc}	87.09^{ba}	87.42^{a}	87.35^{ba}	0.119	0.061
DE, kcal/kg	3,515^{ba}	3,495^{b}	3,513^{ba}	3,536^{ba}	3,493^{b}	3,530^{ba}	3,549^{a}	3,560^{a}	5.958	0.067
ME, kcal/kg	3,398^{ab}	3,368^{ab}	3,378^{ab}	3,417^{a}	3,360^{b}	3,412^{a}	3,417^{a}	3,426^{a}	6.116	0.044
ND, %	83.56^{bc}	82.81^{c}	83.31^{bc}	84.36^{abc}	82.76^{c}	83.76^{bc}	84.94^{ab}	86.58^{a}	0.264	0.024
NR, %	71.03	69.39	71.52	71.15	69.97	70.46	72.73	74.17	0.503	0.172
Phytate, μg/kg	2,817^{b}	2,832^{a}	2,820^{ab}	2,831^{a}	2,829^{a}	2,831^{a}	2,825^{ab}	2,819^{ab}	2.151	0.020

[1] No enzyme supplemented
[2] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[3] Ronozyme WX (DSM, Parsippany, NJ), 200 mg/kg (200 FXU/kg)
[4] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

Table 8. ADG (kg/d), ADFI (kg/d), G:F, and caloric efficiency (CE, kcal of ME intake / kg of weight gain) of pigs fed low energy diet (LD) or normal energy diet (ND) supplemented with phytase (Phy[1]), protease (Pro[2]), and xylanase (Xyl[3]).

	Enzyme							
Item	LD[1]	LD Phy[2]	LD Phy+Pro[3]	LD Phy+Xyl[4]	ND	ND Phy	SEM	*P* value
ADG, kg/day	0.946	0.928	0.915	0.942	0.947	0.952	0.019	0.355
ADFI, kg/day	1.694[a]	1.663[a]	1.640[a]	1.655[a]	1.612[ab]	1.540[b]	0.054	0.022
G:F	0.562[c]	0.558[c]	0.558[c]	0.571[bc]	0.590[b]	0.620[a]	0.0133	<0.001
CE, kcal/kg	5,559	5,577	5,582	5,467	5,582	5,299	131.49	0.241

[1] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[2] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)
[3] Ronozyme WX (DSM, Parsippany, NJ), 200 mg/kg (200 FXU/kg)

Table 9. Jejunum villus height (µm), crypt depth (µm), villus height/crypt depth, and viscosity (centipoise) of jejunum digesta, in pigs fed a complete diet (LD) containing enzymes (Phy[1] and Pro[2]).

	Enzyme			
Item	LD Phy	LD Phy+Pro	SEM	*P* value
Viscosity	2.28	2.13	0.343	0.396
Villus height, um	621.24	674.62	22.933	0.229
Crypt depth, um	342.30	362.16	11.231	0.402
Villus height/crypt depth	1.83	1.88	0.063	0.724

[1] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[2] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

Table 10. Ileal digestibility (%) of DM, N, and amino acids in pigs fed with diets containing phytase (LD Phy) or phytase and protease (LD Phy+Pro) during the growing period.

Item	LD Phy [1]	LD Phy+Pro [2]	SEM	*P* value
Aspartic Acid	51.17	60.22	2.887	0.126
Threonine	33.45	48.21	4.734	0.128
Serine	45.96	59.28	3.626	0.069
Glutamic Acid	57.01	63.86	2.338	0.1547
Proline	42.89	58.22	3.355	0.020
Glycine	25.83	38.81	4.126	0.125
Alanine	44.04	55.59	3.184	0.073
Cysteine	35.96	44.87	3.247	0.184
Valine	47.14	48.63	3.219	0.828
Methionine	54.84	63.80	2.718	0.106
Isoleucine	54.11	59.20	2.592	0.348
Leucine	51.38	60.48	2.806	0.113
Tyrosine	58.98	64.41	2.426	0.283
Phenylalanine	54.77	61.79	2.711	0.212
Lysine	58.89	66.10	2.630	0.184
Histidine	55.88	65.86	2.411	0.038
Arginine	67.86	73.30	1.725	0.124
Tryptophan	55.10	61.23	3.350	0.383
CP	44.62	51.99	3.160	0.263
DM	28.76	36.94	3.548	0.344

[1] Ronozyme Hiphos (DSM, Parsippany, NJ), 100 mg/kg (1,000 FYT/kg)
[2] Ronozyme ProAct (DSM, Parsippany, NJ), 200 mg/kg (15,000 PRO/kg)

CHAPTER 4

NUTRIENT UTILIZATION OF BERMUDA GRASS, FORAGE SORGHUM, AND SWEET SORGHUM FED TO PIGS AND THE USE OF FEED ENZYMES TO ENHANCE NUTRIENT DIGESTIBILITY

Abstract: This study determined DE, ME, apparent total tract digestibility of nitrogen (ND), and nitrogen retention (NR) of ground spray field forages (bermudagrass, forage sorghum, and sweet sorghum) fed to pigs and the effects of the supplemental feed enzyme Allzyme SSF (Alltech, Nicholasville, KY) on energy and nitrogen utilization of these forages. The study had 4 sets of quadruplicated 2 × 2 Latin square design using 32 barrows (38.7 ± 11.9 kg). Each Latin square consisted of 2 treatments and 2 periods. Each period was 14 d (10 d adjustment and 4 d collection). Particle grind size was 400 and 600 µm (sgw?) for corn and forages, respectively. The basal diet (BA) contained 94% corn with 4% amino acids, minerals, and vitamins. Test diets contained 85% BA + 15% Bermuda grass, forage sorghum, or sweet sorghum. For the basal diet and each test diet, carbohydrases were supplemented (0 or 200 mg Allzyme SSF/kg). Allzyme SSF was composed of phytase (1,434 SPU/g), protease (11,584 HUT/g), cellulase (178 CMCU/g), glucanase (749 BGU/g), and xylanase (520 XU/g).Pigs received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs (0.09 × $BW^{0.75}$ kg). On d 10, chromium oxide (0.5%) was added to the diet at 1700 h as an external marker to indicate initiation of fecal collection. Fecal and urine samples were collected during 4 consecutive days. Gross energy of feed, urine, and feces was measured using an adiabatic bomb calorimeter (IKA, Wilmington, NC) to calculate DE and ME. Nitrogen was measured using combustion method (LECO, St Joseph, MI). The basal diet contained 3,850 kcal DE/kg, 3,769 kcal ME/kg, 86.06% ND and 71.10% NR, and were not affected by enzyme supplementation. The bermudagrass contained 893 kcal DE/kg, 845 kcal ME/kg, -16.50% ND, and -37.49% NR, and tended to be improved by enzyme supplementation to 1,211 kcal DE/kg (*P* = 0.098), 1,185 kcal ME/kg (*P* = 0.081), and -10.54% NR (*P* = 0.076). The ND of bermudagrass increased to 0.08% (*P* = 0.018) by Allzyme SSF supplementation. The forage sorghum

contained 1,520 kcal DE/kg, 1,511 kcal ME/kg, -0.72% ND, and -16.99% NR. The sweet sorghum contained 1,086 kcal DE/kg, 1,061 kcal ME/kg, -75.47% ND, and -49.22% NR. Allzyme SSF supplementation did not improved energy digestibility of the sorghums, and the nitrogen on those ingredients was poorly utilized. In conclusion, bermudagrass, forage sorghum, and sweet sorghum grown in spray fields of pig farms have potential application in pig production as alternative feedstuffs. Pigs can utilize nutrients in bermudagrass, forage sorghum, and sweet sorghum whereas their contained protein was poorly digested. The use of Allzyme SSF tended to enhance both energy and nitrogen utilization in bermudagrass.
Key words: bermudagrass, enzymes, forage sorghum, pigs, sweet sorghum

Introduction

Forages are typically fed to ruminants. Swine farms grow forages in spray fields to recycle nutrients from swine manure. Bermudagrass (*Cynodon dactylon*) is often chosen to grow in spray fields due to its high ability to take up nitrogen (Conrad-Acuña et al., 2013). It is reported that bermudagrass can take up 166 kg ha^{-1} yr^{-1} of N, 26 kg of P ha^{-1} yr^{-1} and 195 kg of K ha^{-1} yr^{-1} (Guretzky et al., 2010), and it can tolerate between 300 and 600 kg of N ha^{-1} yr^{-1} from swine manure (Burns et al. 1990). Sorghum (*Sorghum bicolor*) is an annual grass that is also chosen to grow in spray fields (McLaughlin et al., 2004). It is cultivated for forage (sudangrass), syrup production (sweet sorghum) or grain production (grain sorghum) (Smith and Frederiksen, 2000). It is reported that the annual nutrient removal by different sorghum cultivars is 112 kg ha^{-1} of N, 22 kg ha^{-1} of P and 176 kg ha^{-1} of K (Powell and Hons, 1992). Forages are not typically utilized in swine diets because pigs cannot produce the necessary digestive enzymes to degrade non starch polysaccharides (NSP). However microorganisms in the large intestine can utilize NSP in some extent. Volatile fatty acids from microbial fermentation in the large intestine are shown to provide between 7.1 and 17.6% of total available energy to pigs (Anguita et al., 2006). Bermudagrass and sorghum can take nutrients from swine manure (McLaughlin et al. 2004). Therefore, dietary utilization of forages could recycle nutrients into the farm and improve sustainability of swine production.

Dietary supplementation of fibrolytic enzymes have been studied in ruminants (Yang et al., 2000; Giraldo et al., 2008) and monogastric animals (Yin et al., 2000; Olukosi et al., 2007; Carneiro et al., 2008; Yáñez et al., 2011). Bermudagrass, forage sorghum, and sweet sorghum are high in fiber (Worker and Marble, 1968; Billa et al., 1997; NRC, 2001), providing substrates for fibrolytic enzymes. Feed enzymes can improve intestinal fiber

fermentation in pigs (Yin et al., 2000) and improve total tract digestibility of NDF and ADF (Carneiro et al., 2008).

This study will test the hypothesis that pigs can utilize energy from bermudagrass, forage sorghum, and sweet sorghum and dietary supplementation of an appropriate enzyme product targeting NSP can further improve energy digestibility of these spray field forages fed to pigs. The objective of this study is to measure, dry matter digestibility, energy digestibility, and metabolizable energy from bermudagrass, forage sorghum, and sweet sorghum supplemented with feed enzyme fed to pigs.

Materials and method

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee.

Preparation of forages

Bermudagrass (BG), forage sorghum (FS), and sweet sorghum (SS) were chopped with a forage chopper (5400 Chopper, John Deere, Moline, IL) and dried to less than 8% moisture content. The material passed twice through a hammer mill, initially through a 3/8'' screen and then through a 3/64'' screen. The particle size was measured according to ASAE (1993). The geometric mean diameter (Dgw) of bermudagrass particles was 453 microns and the standard deviation of geometric mean diameter (Sgw) was 3.0. The Dgw of forage sorghum particles was 549 microns and Sgw was 2.97. The Dgw of sweet sorghum particles was 355 microns and Sgw was 2.44.

Experimental diets and pigs

The experiment was conducted at the Swine Educational Unit at the North Carolina State University (Raleigh, NC). Pigs were used to evaluate the nutrient digestibility, DE, ME, and nitrogen retention of 3 forages (Table 1) supplemented with a commercial supplemental

enzyme product. The DGW of corn was 314 microns (Sgw was 3.1) and a basal diet (BA) was formulated (Table 2). Three test diets were prepared by mixing 85% of the basal diet with 15% of BG, FS, or SS. To the basal diet and each test diet, a commercial feed enzyme (Allzyme SSF, Alltech, Nicholasville, KY) was supplemented at 0 or 200 mg/kg. Allzyme SSF contains was composed of phytase (1,434 SPU/g), protease (11,584 HUT/g), xylanase (520 XU/g), cellulase (178 CMCU/g), and glucanase (749 BGU/g) (Santos, 2011).

Thirty two barrows (38.7 ± 11.9 kg) were placed in metabolic cages (0.6 m wide, 1.8 m long) equipped with stainless-steel feeder attached to the front of the pen, nipple water drinker next to the feeder, and slatted flooring. Room temperature during the experimental period was 24.0 ± 10.5ºC. Four sets of quadruplicated 2 x 2 Latin Square design for the basal diet and each of the ingredients were used to measure nutrient digestibility, DE, ME, and nitrogen retention with and without feed enzyme. For each 2 x 2 Latin Square, there were 2 treatments (0 or 200 mg of Allzyme SSF/kg of feed) and 2 periods. Each period was 14 d, consisting of 10 d adjustment and 4 d collection. Each treatment had 8 replicates from 4 Latin squares and 2 pigs per Latin square. This study was conducted in two groups of 16 pigs each. Thus, the entire animal study lasted 9 wk (4 wk for group 1, 1 wk transition, and 4 wk for group 2).

Experimental procedures and chemical analyses

Pigs received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs (0.09 x $BW^{0.75}$ kg). Pigs were weighed at the end of each period to adjust feed allowance for a subsequent period. On d 10, chromium oxide (0.5%) was added to the evening meal as an external marker for fecal collection. Sampling was done during 4 consecutive days. Fecal collection was initiated when green color from chromium oxide was observed in the feces after feeding a meal with chromium oxide, whereas urine sampling

were initiated from the time of feeding a meal with chromium oxide. On d 14, chromium oxide (0.5%) was added to the evening meal again and fecal sampling was terminated when green color was observed in the feces in the following day. Urine collection was terminated at the time of evening meal on d 14. Urine samples were collected in a plastic bucket with 20 mL concentrated HCl (5 *N*). Volume of urine was measured each day during the collection period and 150 mL of urine sample was daily sub-sampled. Fecal samples were weighed at the end of each day during the collection period. Urine and fecal samples were frozen (-20ºC) immediately after collection.

Fecal samples were oven dried in air forced oven at 65 °C. Fecal and feed samples were analyzed for moisture (Method 934.01, AOAC, 2006). Nitrogen was obtained by combustion method (FP528, Leco, St Joseph, MI) to calculate protein (Method 992.15, AOAC, 2006). Gross energy was obtained using calorimetric bomb (C2000, IKA, Wilmington, NC). Urine samples were freeze dried (24D x 48, Virtis, Gardiner, NY) and analyzed for nitrogen and gross energy as previously described.

Dry matter (DM), gross energy (GE), and nitrogen (N) results from laboratory analyses were used for dry matter digestibility (DMD), DE, ME, apparent total tract digestibility of nitrogen (ATTDN), and nitrogen retention (NR) calculations (Olukosi and Adeola, 2009). GE, DE, and ME were expressed in kcal/kg while DMD, ATTDN, and NR were expressed in %. Dry matter digestibility (DMD = DM intake – fecal DM), DE (DE = GE intake – fecal GE), and ME (ME = GE intake – fecal GE – urine GE) were calculated for the basal diet and tested diets. The calculation for each of the test ingredients (BG, FS, and SS) were done considering the test diet ingredient composition of 85% basal diet and 15% test ingredient. Initially the gross energy in the test diet contributed from the test ingredient was calculated by:

$$\frac{[\text{GE test ingredient} \times \% \text{ test ingredient}]/100}{([\text{GE test ingredient} \times \% \text{ test ingredient}]/100) + ([\text{GE basal diet} \times \% \text{ basal diet}]/100)} \times 100$$

The gross energy in the test diet contributed from the basal diet was calculated by:

$$\frac{[\text{GE basal diet} \times \% \text{ basal diet}]/100}{([\text{GE test ingredient} \times \% \text{ test ingredient}]/100) + ([\text{GE basal diet} \times \% \text{ basal diet}]/100)} \times 100$$

The amount of DE in the test diet contributed from the basal diet was calculated by:

[DE of the basal diet x % GE contributed from the basal diet] / 100

The calculation of the amount of DE in the test diet contributed from the test ingredient was calculated by:

[DE of the test diet – DE from the basal diet = DE from the test ingredient]

Finally the calculation of DE of the test ingredient was calculated by:

[DE from test ingredient / % GE contributed from the test ingredient]

Similar formulations were utilized for DMD, ME, ATTDN and NR to obtain values for each of the test ingredients.

Statistical analysis

Data were analyzed by using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The experiment was based on a Latin square design, and the experimental unit was the individual pig. Period and Latin square were included as fixed effects and pig was included as random effect. Statistical differences were considered significant with $P < 0.05$. Probability less than 0.10 and equal or greater than 0.05 was considered as a tendency.

Results

The analyzed composition (Table 1) indicated that corn had 3,931 kcal/kg of GE and 7.25% CP. Forage sorghum had 3,691 kcal/kg of GE and 5.68% CP. Sweet sorghum had 4,212 kcal/kg GE and 3.54% CP. Bermudagrass had 3,557 kcal/kg GE and 6.76% CP. The

lower energy of forage sorghum and bermudagrass can be explained by the ash content in the forages. The ash content was greater in bermudagrass and forage sorghum than in sweet sorghum. It suggests high nitrogen fertilization (Mayland and Sneva, 1983), long term manure application to spray-fields (Benke et al., 2013), and soil contamination (Undersander, 2014).

The ME of the basal diet was 3,769 kcal/kg (Table 3). There was no effect of supplemental Allzyme SSF on DMD (P = 0.495), DE (P = 0.912), ME (P = 0.992), ND (P = 0.458), and NR (P = 0.768) of the basal diet. There was no effect of Allzyme SSF on DMD (P = 0.149) of BG tested diet (Table 4), however the DE (P = 0.099), ME (P = 0.080), and NR (P = 0.078) tended to be greater (1.37%, 1.50%, and 4.40% respectively) when Allzyme SSF was included in the diet. There was a 2.13% improvement on ND due to Allzyme SSF supplementation on BG tested diet (P = 0.018).

The FS test diet DMD (P = 0.373), DE (P = 0.553), ME (P = 0.703), ND (P = 0.699), and NR (P = 0.506) were not affected by Allzyme SSF supplementation (Table 5). The FS tested diet ME without the enzyme supplementation was 3,418 kcal/kg. The SS tested diet DMD (P = 0.415), DE (P = 0.156), ME (P = 0.182), and ND (P = 0.123) were not affected by Allzyme SSF supplementation (Table 6). However, the SS tested diet NR (64.26%) decreased 3.53% when Allzyme SSF was added (P = 0.025). The SS tested diet ME without the enzyme supplementation was 3,308 kcal/kg.

The basal diet results were utilized for calculations of the tested ingredients (BG, FS, and SS), since the test diets were formulated by mixing 85% of the basal diet with 15% of the test ingredient. Using calculations previously described DMD, DE, ME, ND, and NR in the test diets were obtained. Then results regarding DMD, DE, ME, ND, and NR of BG, FS and SS were calculated.

The ME of BG was 845 kcal/kg (Table 4).There was no effect of Allzyme SSF on DMD of BG (P = 0.149) however DE (P = 0.098), ME (P = 0.081), and NR (P = 0.076) tended to be greater (35.6%, 40.2%, and 71.9% respectively) when Allzyme SSF was included in the diet. The Allzyme SSF supplementation increased ND of bermudagrass from -16.50 to 0.08 (P = 0.018). The ME of FS was 1,511 kcal/kg (Table 5) and Allzyme SSF supplementation had no effect on DMD (P = 0.374), DE (P = 0.552), ME (P = 0.627), ND (P = 0.709) and NR (P = 0.513). The ME of SS was 1,061 kcal.kg (Table 6) and Allzyme SSF supplementation had no effect on DMD (P = 0.418), DE (P = 0.155), ME (P = 0.184) and ND (P = 0.121). Nitrogen in SS was not retained in the body.

Discussion

Excess excretion of N and P through pig manure is related to environment concerns (Carpenter et al., 1998). A pig with 100 kg BW can produce 6.8 L of manure per day, which includes 53 g of N and 9 g of P (USDA, 2008). Bermudagrass is often grown in the spray fields of pig farms, as it can take up greater amounts of N and P than other conventional crops such as corn and soybeans (Power et al., 1986; Ferguson et al., 1991; Mukuralinda et al., 2010; Nyiraneza et al., 2009). A long term (11 year) application of swine manure at 335 kg of N ha^{-1} yr^{-1} to spray fields with bermudagrass did not pose a hazard for N pollution of groundwater and did not increase N accumulation in the soil (King et al., 1990). Bermudagrass consumes more N than other crops, such as corn and soybeans, when high greater loading rates of N are applied to the fields (Woodard et al., 2002). Bermudagrass also increases P uptake from swine manure when loading rate increase (Burns et al., 1990). Increasing the loading rate from 300 to 600 kg of N ha^{-1} yr^{-1} increased dry matter production of bermudagrass by 35% (Burns et al., 1990). Sorghum (*Sorghum bicolor*) can also be grown on the swine manure spray fields (McLaughlin et al., 2004). Forage sorghum is more

efficient in N uptake and yields greater DM production per ha than other grain sorghum cultivars under increasing levels of N fertilization (Powell and Hons, 1992) and sweet sorghum increases sugar production when nitrogen fertilization increases (Roland et al., 2012). Therefore, bermudagrass, forage sorghum, and sweet sorghum seem to be promising crops to grow in spray fields of pig farms to agronomically handle N and P from the manure without excessive environmental emissions. Finding an effective way to utilize DM of these forages in pig production could allow nutrient recycling for sustainable pig production, thus mitigating environmental concerns.

Previous studies reported the use of forages as feedstuffs for pigs. The digestible energy of different forages ranges from 1,386 kcal/kg to 3,011 kcal/kg for European wild boars (Quijada et al., 2012). Different species of tropical forages were also evaluated utilizing *in vitro* the DM digestibility method (Kambashi et al., 2014), indicating that 25 to 53% of DM or energy was digestible and digestibility of CP ranged from 29 to 81%. The DM digestibility of grasses (25 to 30%) is lower than legumes (33 to 47%) due to their high content of NDF (Kambashi et al. 2014). The DMD of bermudagrass, forage sorghum, and sweet sorghum in this study were close to the *in vitro* DMD of forages measured by Kambashi et al. (2014). However, ND of bermudagrass, forage sorghum, and sweet sorghum were lower than *in vitro* ND of forages measured by Kambashi et al. (2014).

The forages tested in this study had a negative protein digestibility indicating that either the pigs had endogenous nitrogen losses when fed the forages or the forages negatively affected the nitrogen digestibility of the basal diet. Insoluble fiber (wheat straw) decreases nitrogen digestibility of a diet (Renteria-Flores et al., 2008) implying that insoluble fiber could have affected the nitrogen absorption from the basal diet on the present study. High fiber diet increases mucin production from goblet cells in the small intestine of pigs (Morel et al.,

2008; Hino et al., 2012), and also increases enzyme activity (enzyme units / mg of protein from intestinal mucosa) of maltase, dipeptidylpeptidase, and aminopeptidase (Hedemann et al., 2006). Since dietary fiber is related to the nitrogen losses (Morel et al., 2008), it could also explain the low nitrogen digestibility and nitrogen retention of the forages on this study.

Apparently, pigs can increase their ability to utilize energy in forages once pigs are adapted to high forage content in their feed. van Kempen et al.(2002) demonstrated that 10 d feeding of bermudagrass to pigs (110 kg BW) increased digestibility of energy and N from -15 to 33% and -88 to 0%, respectively. This response indicates the importance of an adaptation period for pigs to digest nutrient in forages. This study had 10 d adaptation period before the collection of fecal and urine samples to provide enough time for pigs to be adapted to high forage feed.

It was reported that bermudagrass contains between 178 and 226 g/kg of xylans (Lee et al., 2009; Canizo et al., 2013), 304 g/kg of cellulose, 312 g/kg of glucans (Canizo et al., 2013), and 104 g/kg of CP (NRC, 2001). It has been shown that the contents of xylan and cellulose in sorghum cultivars were between 142 and 177 g/kg (Dien et al., 2009; Stefaniak et al., 2012) and between 197 and 277 g/kg (Dien et al., 2009; Zhao et al., 2009) on DM basis, respectively. Total glucans (starch, cellulose, and soluble sugars) in 6 forage sorghums varieties were between 441 and 555 g/kg (Dien et al., 2009) and the CP content is between 33 and 78 g/kg (Dien et al., 2009; Stefaniak et al., 2012). Therefore, dietary supplementation of feed enzymes targeting xylans, cellulose, glucans, and protein might be effective for increasing nutrient digestibility of bermudagrass, forage sorghum, and sweet sorghum fed to pigs. In this study, the Allzyme SSF supplemented to the diets increased ND and tended to increase DE, ME, and NR of bermudagrass. However, the Allzyme SSF did not improve nutrient digestibility of forage sorghum and sweet sorghum.

Nutrient digestibility of can be improved by dietary supplementation of NSP degrading enzymes (Kim et al., 2003; Ji and Kim, 2004; Kim et al., 2006; Cozannet et al., 2012). These enzymes may improve nutrient digestibility by degrading the NSP in cell wall (Masey-O'Neill et al., 2014). The feed enzymes used in this study tended to improve nutrient digestibility of bermudagrass whereas without statistical improvements for forage sorghum and sweet sorghum. Sorghum hybrids contain tannin in the seeds, chaff, leaves, and stalks, which are negatively correlated with nutrient digestibility (Cummins 1971). Tannins can bind to amino acids during the digestion and decrease N digestibility in pigs (Mitaru et al., 1984). Tannins are also shown to decrease activity of digestive enzymes such as amylase, trypsin, and lipase (Al-Mamary et al., 2001). Therefore, the response of Allzyme SSF may not be clear when supplemented to feed containing forage sorghum and sweet sorghum due to tannins which warrants further investigation.

Conclusion

Collectively, this study demonstrated that pigs can utilize nutrients in bermudagrass, forage sorghum, and sweet sorghum to obtain energy. However, N in these forages was poorly utilized by pigs. Dietary supplementation of feed enzymes containing phytase, protease, xylanase, cellulose and glucanase tended to enhance energy and nitrogen utilization in bermudagrass. Bermudagrass, forage sorghum, and sweet sorghum are apparently effective crops to grow in spray fields of pig farms for potential application in pig production as alternative feedstuffs and an appropriate supplemental feed enzymes targeting NSP can help this application.

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

Item	Corn	Forage sorghum	Sweet sorghum	Bermudagrass
DM,%	89.35	96.02	95.94	94.39
GE, Kcal/kg	3,931	3,691	4,212	3,557
CP, %	7.25	5.68	3.54	6.76
Fat, %	3.81	1.04	1.01	1.40
NDF, %	6.74	70.56	75.32	68.94
ADF, %	2.45	60.36	53.82	53.47
Total ash, %	1.12	12.24	3.54	14.19
Ca, %	0.01	0.17	0.16	0.18
P, %	0.24	0.17	0.10	0.18

Table 2. Ingredient composition and calculated analysis (as-fed basis) of the basal diet

Item	Basal diet
Ingredient, %	
Yellow corn, ground	96.20
L-Lys HCl	0.63
DL-Met	0.11
L-Thr	0.24
L-Trp	0.08
L-Val	0.16
L-Ile	0.16
L-Phe	0.10
Limestone	1.00
Monocalcium phosphorus	0.80
Salt	0.34
Vitamin premix[1]	0.03
Trace mineral premix[2]	0.15
Calculated composition	
ME, kcal/kg	3,352
CP, %	9.32
SID[3] Lys, %	0.66
SID Met + Cys,%	0.39
SID Met, %	
SID Thr, %	0.43
SID Trp, %	0.12
SID Val, %	0.46
SID Ile, %	0.37
SID Phe, %	0.41
Ca, %	0.55
P total, %	0.44
P available, %	0.21
Analyzed composition	
GE, kcal/kg	3,864
CP, %	8.78
Fat, %	2.41
NDF, %	68.21
ADF, %	2.51
Ca, %	0.57
P, %	0.38

[1]Vitamin premix supplied per kg of feed: 6,170 IU of vitamin A as vitamin A acetate; 879 IU of vitamin D as cholecalciferol; 35.25 IU of vitamin E as tocopheryl acetate; 0.02 mg/kg of

Table 2. Continued
vitamin B12 as cyanocobalamin; 0.15 mg/kg of biotin; 2.91 mg/kg of vitamin K as menadione sodium bisulfite; 4.39 mg/kg of riboflavin; 17.63 mg/kg of pantothenic acid as calcium pantothenate; niacin, 26.45 mg/kg of niacin as nicotinamide; 1.32 mg/kg of folate as folic acid.
[2]Trace mineral premix supplied per kg of feed: 16.5 mg/kg of Cu as copper sulfate; 165 mg/kg of Fe as ferrous sulfate; 39 mg/kg of Mn as manganous oxide; 165 mg/kg of Zn as zinc sulfate; 0.30 mg/kg of I as ethylenediamine dihydroiodide; 0.30 mg/kg of Se as sodium selenite.
[3] Standardized ileal digestibilty

Table 3. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), nitrogen digestibility (ND, %), and nitrogen retention (NR, %) of basal diet supplemented with feed enzyme on DM basis

	Enzyme[1]			
Item	No	Yes	SEM	*P* value
Basal diet				
DMD, %	92.50	92.75	0.18	0.495
DE, kcal/kg	3,850	3,852	10.65	0.912
ME, kcal/kg	3,769	3,769	12.61	0.992
ND, %	86.06	86.88	0.62	0.458
NR, %	71.10	71.46	0.80	0.768

[1] Allzyme SSF (Alltech, Nicholasville, KY), 200 mg/kg of feed

Table 4. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), nitrogen digestibility (ND, %), and nitrogen retention (NR, %) of diet (85% basal diet, 15% bermudagrass) and bermudagrass supplemented with feed enzyme on DM basis

	Enzyme[1]			
Item	No	Yes	SEM	*P* value
Diet [2]				
DMD, %	82.33	83.34	0.30	0.149
DE, kcal/kg	3,414	3,461	18.00	0.099
ME, kcal/kg	3,338	3,388	17.98	0.080
ND, %	76.03	77.65	0.50	0.018
NR, %	60.45	63.11	0.91	0.078
Bermudagrass				
DMD, %	27.49	34.00	1.92	0.149
DE, kcal/kg	893	1,211	94.70	0.098
ME, kcal/kg	845	1,185	92.58	0.081
ND, %	-16.50	0.08	5.30	0.018
NR, %	-37.49	-10.54	10.13	0.076

[1] Allzyme SSF (Alltech, Nicholasville, KY), 200 mg/kg

Table 5. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), nitrogen digestibility (ND, %), and nitrogen retention (NR, %) of diet (85% basal diet, 15% forage sorghum) and forage sorghum supplemented with feed enzyme on DM basis

	Enzyme[1]			
Item	No	Yes	SEM	*P* value
Diet				
DMD, %	83.34	84.35	0.41	0.373
DE, kcal/kg	3,488	3,510	17.39	0.5531
ME, kcal/kg	3,418	3,432	16.49	0.7025
ND, %	77.84	78.38	0.65	0.699
NR, %	62.77	63.22	0.56	0.506
Forage sorghum				
DMD, %	34.20	40.70	2.61	0.374
DE, kcal/kg	1,520	1,667	155.63	0.553
ME, kcal/kg	1,511	1,604	155.29	0.702
ND, %	-0.72	5.32	7.53	0.709
NR, %	-16.99	-11.59	7.07	0.513

[1] Allzyme SSF (Alltech, Nicholasville, KY), 200 mg/kg

Table 6. Apparent total tract dry matter digestibility (DMD, %), DE (kcal/kg), ME (kcal/kg), nitrogen digestibility (ND, %), and nitrogen retention (NR, %) of diet (85% basal diet, 15% sweet sorghum) and sweet sorghum supplemented with feed enzyme on DM basis

	Enzyme[1]			
Item	No	Yes	SEM	*P* value
Diet				
DMD, %	81.52	82.06	0.27	0.415
DE, kcal/kg	3,381	3,431	17.93	0.156
ME, kcal/kg	3,308	3,343	18.97	0.182
ND, %	79.11	77.34	0.58	0.123
NR. %	64.26	61.99	0.87	0.025
Sweet sorghum				
DMD, %	23.72	27.10	1.68	0.418
DE, kcal/kg	1,086	1,375	108.18	0.156
ME, kcal/kg	1,061	1,257	80.938	0.184
ND, %	-75.47	-106.75	21.79	0.121
NR, %	-49.22	-89.42	16.82	0.021

[1] Allzyme SSF (Alltech, Nicholasville, KY), 200 mg/kg

CHAPTER 5

OVERALL CONCLUSION

The first study showed that increasing levels of xylanase yielded a linear improvement on ileal digestibility of nutrients and a quadratic change on viscosity of digesta. Therefore viscosity changed differently than nutrient digestibility. The reduction of NDF and changes on viscosity of digesta indicated degradation of NSP. It seems that xylanase will degrade insoluble NSP and make them soluble increasing viscosity of digesta. The diet was composed mainly of insoluble NSP and the reduction of NDF suggested that the xylanase utilized on this study have affinity for insoluble arabinoxylan. The degradation of the NDF and greater ileal digestibility of energy support the concept that xylanse is releasing nutrients trapped inside the cell wall for further digestion in the small intestine.

The second study concluded that feed ezyme combinations can improve nutrient digestibility of ingredients. However the combinations of this study had different effect on corn, soybean meal, and DDGS. It was not possible to improve energy and nitrogen utilization of corn. The optimal enzyme combination for soybean meal was Phy+Pro and for DDGS was Phy+Xyl. By combining the 3 enzymes (Phy+Pro+Xyl) no effect was observed on ingredinets however it improved nitrogen digestibility of a complete diet formulated with those 3 ingredients plus 2% poultry fat. This study concluded that it is possible to improve energy, nitrogen, and phosphorus digestibility of ingredients by using specific enzyme combinations. A possible inhibition of xylanse in presence of protease on ingredient experiments but not in the complete diet needs further investigation. The combination of the 3 ingredients in a complete diet with added fat suggested that diet compositon will change the enzyme effect compared to the results observed with individual ingredients. The phytase effect on feed efficiency was obserded in the normal energy diet but not in the low energy diet. These experiments had similar ingredient composition except by the poultry fat inclusion in the diet. The effect of enzyme on diets with different energy compositon needs

further investigation. Our experiments indicated that it might be related to the fat compositon once it was the only different ingredient added to the diet in comparison with experiments measuring ingredient digestibility.

The third study showed that pigs can utilize nutrients from bermudagrass, forage sorghum, and sweet sorghum. Forages have substantial amount of NSP which are substrates for feed enymes. A feed enzyme composed of phytase, protease, xylanse, cellulose, and glucanase tended to improve nutriend digestibility of bermudagrass. Therefore, feed enzymes could help the potential utilization of forages in swine diets.

Overall, the utilizatin of feed enzymes targeting insoluble NSP changed viscosity of digesta and nutrient digestibility, however viscosity can not predict digestibility. Combinations of feed enzymes can be useful to further improve nutrient digestibility of ingredients and help the inclusion of forages as feedstuffs to pigs.