ABSTRACT

CHEN, HONGYU. Use of Feed Enzyme on Growth and Health of Pigs. (Under the direction of Dr. Sung Woo Kim).

The research hypothesis is that feed enzymes degrade their corresponding substrates including nutrients and anti-nutritional compounds in the feed, and enhance nutrient digestion, intestinal health, and growth of pigs. Therefore, keratinase, mannanase, xylanase, and glucanase targeting kafirin, mannan, xylan, and β-glucan, respectively in feedstuffs, have been used to investigate their effects on digesta viscosity, nutrient digestibility, gut health, and growth performance of pigs.

Experiment 1 (Chapter 2) evaluated effects of supplemental protease on nursery pigs when fed diets with sorghum. A total of 144 pigs (18.4 ± 2.3 kg at 6 wk of age) were allotted to 4 treatments in a 2 × 2 factorial arrangement (corn or sorghum basal diet, and 0 or 0.05% protease as 2 factors). This study found that use of sorghum fully replacing corn in nursery diets could be beneficial to nursery pigs with enhanced feed intake and growth of nursery pigs, potentially by reducing oxidative stress. Supplementation of protease improved protein digestion and gut health, irrespective of sorghum or corn based diets.

Three experiments (Chapter 3) investigated effects of dietary mannanase and xylanase on nursery pigs fed diets with corn distillers dried grains with solubles (DDGS). In Exp. 1, eighty-four pigs (17.6 ± 2.8 kg initial BW at 6 wk of age) were allotted to 2 treatments in a 40-d trial: corn-soybean meal-20% DDGS based diet with or without 0.05% mannanase. In Exp. 2, forty pigs (10.7 ± 1.2 kg initial BW at 6 wk of age) were allotted to 4 treatments in a 2 × 2 factorial arrangement (0 or 30% DDGS, and 0 or 0.01% xylanase as 2 factors) in a 21-d trial. In Exp. 3, thirty-two pigs (6.2 ± 0.8 kg) at 3 wk of age were allotted to 4 dietary treatments in a 20-d trial: CON (control diet, corn-soybean meal with 15% DDGS),

MAN (CON with 0.05% mannanase), XYL (CON with 0.01% xylanase), and XYL+MAN (CON with both enzymes). Collectively, feeding a diet with 30% DDGS to nursery pigs for 3 wk had no negative effect on growth performance, but could be potentially harmful to gut health of pigs. Adding mannanase and xylanase had little improvement on growth performance, but could improve gut health of nursery pigs.

Experiment 5 (Chapter 4) evaluated effects of supplemental glucanase on nursery pigs fed diets with DDGS. Sixty pigs (10.2 ± 1.3 kg initial BW at 5 wk of age) were randomly allotted to 6 dietary treatments based on a 2 × 3 factorial arrangement. Dietary DDGS (15 or 30%) and glucanase (0, 150, or 450 U/kg feed) were 2 factors. This study showed that increasing dietary DDGS from 15% to 30% did not affect the overall performance during 21 days, but impaired gut health in nursery pigs. Supplemental glucanase has the potential to improve growth and gut health of pigs fed diets with DDGS, by decreasing digesta viscosity and oxidative stress, and improving NDF digestibility and gut morphology.

Experiment 6 and 7 (Chapter 5) investigated effects of supplemental NSP degrading enzymes on nursery and grower pigs fed low nutrient diets. One hundred and eighty pigs were used in each 42-d experiment, and randomly assigned to 3 treatments: a positive control diet (PC), a low nutrient diet, and a low nutrient diet with NSP enzymes. In the nursery study, the low nutrient diet reduced NE and standardized ileal digestible (SID) Lys by 8% and 35%, respectively, while in the grower study, it reduced SID Lys by 35%. In both experiments, pigs fed low nutrient diets had poorer growth performance than those fed with PC. Supplementing NSP enzymes did not significantly improve growth performance of pigs fed with low nutrient diets.

In conclusion, sorghum is a good substitute of corn in swine diets by improving daily gain and gut health. As a high-NSP ingredient, DDGS did not cause any negative effect on growth performance, however, it impaired gut health of pigs. Although feed enzymes used in these experiments did not show any great improvements on growth performance, it enhanced nutrient digestibility and gut health of pigs, potentially by decreasing digesta viscosity, oxidative stress, and inflammatory response, as well as improving gut morphology and permeability.

Use of Feed Enzyme on Growth and Health of Pigs

by
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DEDICATION

To my family, Tianbao Chen, Xingping Zhang, and Xiao Tu, for their unconditional support and love.

BIOGRAPHY

Hongyu Chen was born and grew up in Sichuan, China. As one of the largest pork producing province, Sichuan has long history of feed and pig production. Three feed mills have been located in the district where Hongyu was living, and now, two of them are the leading feed companies in China. That might have influenced her unconsciously on college major choice. In the fall of 2015, Hongyu started her undergraduate life at the China Agricultural University (CAU), Beijing, majoring in Animal Science. In 2009, she was recommended to continue studying in Animal Nutrition and Feed Technology in the graduate school at CAU. The solid knowledge foundation and academic training received during those years inspired her a lot. After receiving her M.S. degree in 2011, Hongyu accepted a position in Asian Agribusiness Consulting (AAC) Company, as a marketing specialist. That was a great chance for her to get close to feed mills and intensive farms for poultry and pigs. In the August of 2012, she passed the Civil Service Examination, and started working at the Bureau of Animal Husbandry and Food Safety in Suining, Sichuan. Through working in government and AAC Company, she realized her passion for the animal producing industry. In the spring of 2014, Hongyu Chen came to North Carolina State University (NCSU) for her Ph.D. program under the direction of Dr. Sung Woo Kim. She has thoroughly enjoyed her courses, research, and practical training at NCSU. Her education and working experience has prepared her for a career in the related area.

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LITERATURE REVIEW

1. Introduction

The Animal producing industry has expanded as a result of increased human population and the increasing demand for high quality protein (Speedy, 2002). The growth in demand for animal products indicates that there will be a consequent increase in demand for animal feed. Feed expense is the major cost during pig production, accounting for about 60 to 75% of the total cost. In the U.S., traditional feedstuffs are corn and soybean meal (SBM), thus feed costs are very sensitive to changes in prices of corn and soybean meal. However, this situation might be improved by the diversification of feedstuffs, such as other cereal grains and co-products, when the profit of animal products decreases with relatively high prices of corn and SBM. This practice might be applicable to the industry when the quantities of these alternative ingredients are sufficient to support the industrialized pig production.

However, potential risks along with these plant-based ingredients are that most of them contain relatively high levels of less digestible components, fiber, and anti-nutritional factors, which could reduce nutrient digestion and absorption, affect normal functions in the digestive tract, and result in lower nutritive values of feedstuffs (Soetan and Oyewole, 2009; Lindberg, 2014; Patience et al., 2015). Usually, mechanic processing technology is a good way to reduce these negative effects by improving feed efficiency, such as grinding, heating, or pelleting (Soetan and Oyewole, 2009; Patience et al., 2015). Supplementation of exogenous enzymes can be considered as another effective method to solve the problems (Kiarie et al., 2013; Strube et al., 2013).

This literature review describes several alternative plant-based ingredients that could be used in swine industry, and some potential problems that exist in the utilization of these ingredients, especially how they are involved in affecting gut health. The review also discusses the nutritional benefits of feed processing technology (grinding and pelleting) and dietary supplementation of exogenous enzymes (protease and non-starch polysaccharides degrading enzymes) in swine diets. Lastly, this review indicates some questions and concepts that have not been clearly stated by previous research, and proposes some hypotheses that require scientific validation.

2. Alternative plant-based ingredients

In 2015, global corn production was 966.4 million tons, with 345.5 million tons produced in the U.S.; global SBM production was 219.2 million tons, with 40.5 million tons in the U.S. (USDA, 2016). It is well recognized that corn and SBM are two main ingredients used in swine diets in the U.S., while Asia and Europe have more options to use other cereals and co-products from milling or biofuel industry. In this section, several ingredients will be introduced, and they may completely or partially replace corn or SBM.

2.1 Cereal grains

2.1.1 Sorghum

The sorghum plant is more resistant to heat and drought compared with corn. Its annual global production in 2015 was 60.16 million tons, with 15.2 million tons in the U.S. (USDA, 2016). According to the Sorghum Checkoff website, the Sorghum Belt in the U.S. covers five central states, from South Dakota to Texas, mainly on dryland acres. Four types of sorghum

are cultivated in the U.S., including grain sorghum, forage sorghum, biomass sorghum, and sweet sorghum. The most often used sorghum in swine diets is grain sorghum. The grain can be milled into fresh flour, and its co-products also make excellent animal feed. Whole grains of sorghum contain approximately 89 to 90% DM, 8.9 to 15% CP, 2.1 to 2.3% crude fiber, and 71.7 to 72.3% nitrogen free extract on as-fed basis (Etuk et al., 2012). Compared with corn, sorghum contains 98 to 99% of gross energy and higher protein content, but is still deficient in Lys and Trp (NRC, 2012).

2.1.2 Wheat

Wheat grass usually grows well in cold temperate regions. Globally, the production of wheat was about 734.2 million tons in 2015, with 55.8 million tons produced in the U.S. (USDA, 2016). Three questions need to be identified for wheat classes: when it's harvest (spring or winter wheat), what is the color of bran (red or white wheat), and whether it has higher gluten content (hard or soft wheat). Spring wheat are planted in spring, and harvested in late summer, while winter wheat is grown in the fall, and harvest in the following spring. No major differences exist in the nutrient profiles between spring and winter wheat, except spring wheat has a higher CP level; hard wheat contains higher protein and less starch level, while soft wheat has lower protein but higher starch content (Rosenfelder et al., 2013). Compared with corn, wheat grain has higher concentrations of CP and AA, but lower energy (starch and crude fat) and greater content of fiber (NDF and ADF) according to NRC (2012). Since wheat is largely used for flour making and alcohol production, the residual is not sufficient to support constant feed production, but it still has a positive role in integrated

farms as well as commercial feed mills when its harvested location is close to the feed manufacturers.

2.2 Milling co-products

2.2.1 Wheat bran

Wheat grain is mainly used for human consumption, like making flour. In the U.S., 25.1 million tons of wheat were ground for flour in 2015, producing about 19.2 million tons of flour and 5.9 million tons of co-products (NASS, 2016a). The nutrient content are similar among co-products produced from wheat milling, but they are generally identified by the fiber contents. Among these co-products generated from commercial milling process, wheat bran is mainly the coarse outer layers of wheat kernel, with small amount of endosperm (AAFCO, 2000). Wheat bran has a higher fiber content than other co-products (Rosenfelder et al., 2013), with total non-starch polysaccharides (NSP) accounting for 46%. The main NSP present are arabinoxylans, accounting for 70% of total NSP of the bran (Stevenson et al., 2012). Such high fiber content resulted in lower digestibility of energy and fiber in growing pigs, and the less fiber digestion was associated with decreased heat increment and hindgut fermentation (Jaworski, et al., 2016).

2.2.2 Broken rice

Like wheat, rice is also mainly used for human consumption in many areas of the world. Globally, milled rice (without husks) production was 470.9 million tons in 2015, with 6.1 million tons produced in the U.S. (USDA, 2016). The U.S. has long history of exporting rice at half of its production, and was the fourth exporter behind Thailand, India, and Vietnam in

2015 (USDA, 2016). This crop mainly grows in flooded areas, so the main production states are either close to the coast or Mississippi river. The paddy or rough rice goes through cleaning, dehusking, paddy separation, de-stoning, polishing, grading and mixing, in order to become white rice (Serna-Saldivar, 2010). This process will produce 60 to 72% of white rice, and the remaining part are co-products, such as broken rice, rice bran, and rice hull (Singh et al., 2013). Broken rice is much smaller than the undamaged rice, about 25% or less of a whole kernel (Casas et al., 2015), which is not qualified for human consumption but has the same nutritional value as polished rice. In broken rice, the levels of starch and ME, as well as digestibility of AA are all higher than that in corn, and the content of phytate is lower in broken rice (Stein et al., 2016). Replacing corn with broken rice during early weaning stage resulted in a better growth performance on weaning pigs (Liu et al., 2016).

2.3 Biofuel co-products

2.3.1 Corn distillers dried grains with solubles (DDGS)

Corn DDGS is the main co-product of dry milling ethanol production. In the U.S., total corn consumed for alcohol production in 2015 was 148.3 million tons, with 118.6 million tons used for dry milling ethanol production, resulting in 22.3 million tons of corn DDGS (NASS, 2016b). These ethanol plants are primarily located where the corn is harvested. Inconsistency in the nutrient profiles of corn DDGS has been attributed to different fermentation methods, distillation and drying methods, ethanol plants, and grain sources. Corn DDGS is rich in protein, about 25 to 30% CP on as-fed basis, and can successfully replace a portion of SBM in the swine diets. Compared with corn, corn DDGS has similar

DE and ME, higher concentrations of AA with lower digestibility, and increased phosphorus level and fiber content (Stein and Shurson, 2009). If compare with SBM, corn DDGS has much lower CP and less digestible AA, and higher NSP content (Knudsen Adedokun et al., 2008; Choct et al., 2010, Pedersen et al., 2014). The users need to balance the price and protein level when substituting SBM with corn DDGS, and also pay attention to the risks of mycotoxin in DDGS (Khatibi et al., 2014).

2.3.2 Wheat DDGS

Unlike the situation in the U.S., wheat is the primary source of starch to produce alcohol in Europe, Canada, and Australia. In 2015, the estimated amounts of wheat used for fuel ethanol production were 2.6, 1.0, and 0.46 million tons in Europe, Canada, and Australia, respectively (GAIN, 2016a, b, c). Because starch in the wheat grain is removed during the fermentation process, wheat DDGS has a much higher level of minerals, NSP, fat, and protein than wheat grain (Nuez Ortín and Yu. 2009). Compared with their original grains, the increase in CP level of both wheat and corn DDGS is not only due to the removal of starch but the presence of yeast protein during the fermentation process (Moreau et al., 2011). Compared to corn DDGS, wheat DDGS has higher levels of CP and NSP, and lower content of DE (Widyaratne and Zijlstra, 2007; Nuez Ortín and Yu. 2009), which might be due to the nutritional difference between original wheat and corn kernels.

3. Potential problems with plant-based ingredients

3.1 Storage protein in seed

In general, there are two kinds of seed storage protein depending on their solubility in specific solvent. One type, such as globulin or certain albumin, can dissolve in several solutions, while the other, like prolamins, can only dissolve in alcoholic solutions (Shewry and Halford, 2002). Prolamins are very unique in cereal grains, and contribute 50 to 60% of the total protein in the endosperm (Lopes and Larkins, 1993). They are more variable in structure than globulins (Shewry and Halford, 2002). Prolamins, except α-zein in the corn, contain repeated sequence, and can be divided into different classes by cereal species and their amino acid sequences (Shewry and Tatham. 1990).

Kafirin is the prolamin storage protein in sorghum grains. There are four types of kafirins at different levels in the seed. They are α-, β-, γ-, and δ-kafirins, and the major one is α-kafirin, about 66% to 84% of total kafirins in the sorghum grain. They can also form different secondary and tertiary structures depending on their amino acid profiles and disulfide bonds. For example, β-kafirins are rich in Met and Cys; γ-kafirins are rich in Pro, Cys, and His. Both of them can form inter-molecular and intra-molecular disulfide bonds, while α-kafirins primarily form intramolecular bonds (Belton et al., 2006; De Mesa-Stonestreet et al., 2010).

3.1.1 Limiting factor of using sorghum in animal diets

Tannin used to be the first limiting factor that prevented sorghum from being used in animal feed, however, a number of countries are producing tannin-free sorghum. In that case,

kafirin may be the main reason that limits the application of sorghum in swine diets (Duodu et al., 2003). Healy et al. (1994) observed that nursery pigs fed sorghum based diets had lower feed intake and weight gain, and were less efficient than those fed corn based diets. They also reported reduced apparent digestibilities of N, DM, and GE of sorghum-fed pigs. Louis et al. (1991) reported decreased apparent N digestibility, and lower feed consumption in lactating sows fed with sorghum based diets. The poor performance of pigs might be attributed to the complicated structure of kafirin, low solubility, and unbalanced AA profiles of sorghum grains (De Mesa-Stonestreet et al., 2010). Nevertheless, growth performance and nutrient digestibility were similar between growing pigs fed low-tannin sorghum based and corn based diets in many studies (Cousins et al., 1981; Lin et al., 1987; Etuk et al., 2012).

3.2 Non-starch polysaccharides

Structurally, a seed has the function of providing sufficient nutrients to embryo development and plant growth after germination, as well as the function of preventing seed from damages or hurt from insects or microorganism, in order to assure the growth of a seed into a plant. At the level of kernel structure, cereal grains and oilseed are good sources of animal feed for these nutrients stored in the endosperm, but the out layer of a seed is rich in fiber. At the level of plant cell, cell content are mainly protein, starch, lipid, and water, while polysaccharides make up most of the cell wall, and also serve as seed storage carbohydrates although starch is the major one in the seed (Scheller and Ulvskov, 2010; Gilbert, 2010). The seeds, including cereals and oil seeds are usually processed to obtain germ or endosperm for human consumption. The co-products containing major part of pericarp are used for feed

production. When cereal grains, oilseed, and their co-products are utilized in the animal diets, it is necessary to ensure not only their nutrient levels, but also fiber contents.

3.2.1 Concepts related to fiber and NSP

There are plenty of terms to describe the fiber content in raw ingredients and compound feed, such as crude fiber, dietary fiber, NDF, ADF, hemicellulose, and NSP. As a measurement in proximate feed analysis, crude fiber represents organic matter remaining after a series of acid and alkaline extractions, including lignin, the majority of cellulose, and some other insoluble polysaccharides (Choct, 2015). This method was developed by Henneberg and Sttohmann during the 1860s. Dietary fiber is made up of the polysaccharides and lignin that cannot be digested by the endogenous enzymes of monogastric animals, and it is a physiologic definition because these fractions are not easy to be clearly quantified (Raninen et al., 2011). Neutral detergent fiber is the residue or insoluble fraction remaining after boiling in neutral detergent solution with a heat stable α-amylase (Robertson and Van Soest, 1980), which is considered as an estimate of the total fiber constituents of feedstuffs since it measures the content of cellulose, hemicellulose, lignin, silica, tannins, and cutins. Then in the sequence analysis, the ADF residue can be fractionated by boiling the NDF in acid detergent solution, and it represents the least digestible fiber portion, including lignin, cellulose, silica, and insoluble forms of nitrogen, but not hemicellulose. So, the hemicellulose is solubilized during this procedure, while lignin and cellulose still remain insoluble. Generally, the amount of hemicellulose can be calculated by subtracting ADF from the amount of NDF.

Literally, non-starch polysaccharides include all the polysaccharides other than starch in plants, and are a dominant part of dietary fiber in the grains (Englyst, 1989). These polysaccharides are long polymeric chains, which have several hundred thousand monomeric units. They can be grouped into several categories, usually based on the type of monomeric sugar and the linkages between them. Their properties are still depending on the presence of branch chains, and specific groups present in the chain (Tungland and Meyer, 2002).

3.2.2 Types of NSP

Non-starch polysaccharides can be classified into three groups: pectic polysaccharides, cellulose, and non-cellulosic polymers (Bailey, 1973). The main chain of pectic polysaccharide is composed of galacturonic acid and L-rhamnopyranosyl residues, linked by α-1,4- and α-1,2-glycosidic bonds, respectively (Kumar et al., 2012; Patova et al., 2014). Pectic polysaccharide makes up 10 to 35% of cell wall polysaccharides, and the contents of pectic polysaccharides in the cell wall decrease during maturation of the cell wall. Cellulose is very important to maintain the structure of cell walls in cereal grains, and is made up of glucose units linked by β-1,4-glycosidic bonds. Cellulose is also highly insoluble in water, because the cellulose chains can bind with each other through their intra- and inter-molecular hydrogen bonds (Kumar et al., 2012).

The major polysaccharides of non-cellulosic polymers are β-glucans, heteroxylans, xyloglucan, and mannans. Among them, β-glucans and heteroxylan predominate in cereal grain cell walls (Burton and Fincher, 2014). The extent of heterogeneity results in the difference in their physical and chemical properties (Muralikrishna and Rao, 2007).

β-glucans, also called β-1,3/1,4-glucans, is composed of glucose units linked by β-1,4- and β-1,3-bonds. Compared with cellulose, the β-1,3-linkages disturb the uniform structure of the β-1,4-glycosidic bonds and result in the hydrophilicity of β-glucans. Therefore, β-glucans can be partly soluble in water and form the gel-like structures (Burton and Fincher, 2014). They are widely present in plants, mainly cereals (Synytsya and Novak, 2014). It is relatively high in barley and oat, compared with corn.

Heteroxylans, including arabinoxylans and glucuronoarabinoxylans, have a main chain composed of xylose units linked by β-1,4-glycosidic bonds, which are usually attached by L-arabinofuranose and D-glucuronosyl residues as side branches. The substitution of the xylosyl backbone with arabinofuranosyl residues could inhibit the aggregation of the xylan chains into insoluble microfibrils, and results in that heteroxylan can partly dissolve in water and can form gel-like fluid (Collins et al., 2010). Arabinoxylans are the dominant non-cellulosic polysaccharides in the cell wall of endosperm (Burton and Fincher, 2014). For example, arabinoxylans comprise about 70% of the endosperm cell wall polysaccharides in wheat, while it makes up 40% of the bran in corn (Muralikrishna and Rao, 2007).

Xyloglucan, almost found in every land plant, has a backbone composed of glucose units linked by β-1,4-glycosidic bonds, which is like cellulose. But the difference is that xylopyranosyl units are attached to the glycosyl residues as side chains (Tungland and Meyer, 2002). Xyloglucan mainly exists in the primary cell wall of plants at a relative low level, but it is very important to the biosynthesis of primary and secondary wall (Scheller and Ulvskov, 2010).

Mannans, can be divided into four groups: galactomannan, glucomannan, galactoglucomanan, and linear mannans (Moreira and Filho. 2008). They all have a backbone composed of mannose units linked by β-1,4-glycosidic bonds or inserted by glucose residues. Moreover, galactose residues can be attached to this main chain linked by α-1,6-glycosidic bonds (Scheller and Ulvskov, 2010). Galactomannans, especially highly galactose-branched ones, are commonly found in plant seeds, which are water soluble, can be reserved as a source of carbon for germination. Particularly, galactomannans from legume endosperm is relatively high, about 2% of the dry weight of the seed (Buckeridge et al., 2000).

In summary, the amount and composition of NSP vary between plant species and tissues of kernels (Bach Knudsen, 2014). In commelinid monocotyledonous plants, like cereal grains, the major NSP are arabinoxylans, cellulose, and β-glucans, while in dicotyledonous plant, pectic polymers content are higher (Harris and Bronwen, 2006). The content of NSP in different plant-based ingredients are listed in Table 1. Variety, location, and year of harvest are also the factors that determine the actual content of NSP in cereal grains.

3.2.3 Solubility and viscosity of NSP

The solubility of these polysaccharides is different depending on the structure and conformation. For example, if some polysaccharide has an irregular, highly branched, ionic charged structure, is at low molecular size, or is linked by 1,3 or 1,6-glycosidic linkage instead of 1,4-bond, it would be more likely to be soluble (Whistler, 1973). Cellulose does not dissolve in water, while pectic polysaccharides and non-cellulosic polymers are partially soluble in water (Choct et al., 2010). Viscosity is another important property of NSP in

solutions, indicating the intermolecular linkage in the fluid. The factor that could affect the viscosity of intestinal digesta are molecular weight of NSP, concentration of NSP in the diet, and processing condition including temperature, pH and milling process when these ingredients have been produced (Dikeman and Fahey, 2006). It is also recognized that viscosity is an issue related to soluble NSP rather than insoluble NSP. However, insoluble polysaccharides can also exhibit different degree of viscosity. Replacing sodium with potassium could keep the waxy corn starch sulfate insoluble, but the sodium salt was more viscous than potassium salt (Whistler, 1973). So, solubility and viscosity are not necessarily associated with each other, and it should be evaluated case by case.

3.2.4 Anti-nutritional effects of NSP

It is clear that NSP cannot be broken down by monogastric animals themselves without the help from intestinal microflora, because they are lacking corresponding digestive enzymes. The digestibility of NSP is determined by multiple factors, including species and age of animals, chemical and physical properties of NSP, and their content in the diet (Bach Knudsen et al., 2012; Ivarsson et al., 2014). Feeding NSP usually cause negative effects in nutrient digestion and gut health of monogastric animals.

Nutrient digestion. In general, soluble NSP inhibits the rate of diffusion of nutrients and digestive enzymes, and decreases their effective access at the mucosal surface. Besides, it slows down gastric emptying and increases intestinal transit time. Moreover, non-starch polysaccharide in the diet increases the endogenous losses through the intestinal wall. They might also impair nutrient absorption in the small intestine. On the other hand, insoluble NSP

decreases transit time, enhances water holding capacity, and increases fecal bulking in poultry and pigs (Morel et al., 2003; Kumar et al., 2012; Montagne et al., 2003). Högberg and Lindberg (2006) reported that nursery pigs had slower gain and lower digestibility of OM, CP, and energy when fed with high NSP diets. In growing pigs, high dietary NSP reduced growth performance and apparent total tract digestibility of energy (Agyekum et al., 2014; Ngoc et al., 2013). Furthermore, Gutierrez et al. (2014) evaluated the correlation between 11 fiber components and nutrient digestibility, and found that most of the variation in apparent digestibility of corn co-products can be explained by the content of arabinoxylan and xylose in the NSP fraction, mainly because arabinoxylan is the most abundant NSP in corn and its co-products.

Gut Health. It is very important for the growth and health of animals, since gastrointestinal tract is significant in nutrient absorption, protection from the pathogens, signaling delivery, and endocrinal regulation of other organs. Gut health is a comprehensive and complex definition, which is hard to access. It might be approached through the following aspects: sufficient nutrients, intact gut barrier, balanced microbiota, healthy immune status, and balanced micro-environment in the gut (Montagne et al., 2003; Bischoff, 2011). These components interplay with each other, and keep a dynamic equilibrium in healthy animals.

It is well known that the majority of degradation of NSP is caused by fermentation in the gut, and the products are short chain fatty acids (SCFA), such as acetate, propionate, and butyrate. After absorbed from the gut lumen, the SCFA are metabolized in difference tissues,

such as colonic epithelial cells, liver, or principal tissues (Argenzio and Southworth, 1974). Aacetate is mainly transported around and metabolized by the periphery. Propionate partially serves the colonocytes, but mainly by the liver. Butyrate is locally used by colonocytes in humans and pigs (Den Besten et al., 2013). Other biological effects of SCFA is that butyrate can exert several modulatory effects on nuclear proteins, decrease gene expression of tumor necrosis factor-α (TNF-α) induced factor B, and inhibit activation of pro-inflammatory cytokine induced NF-κB (Andoh et al., 2003), which can reduce inflammatory and immune responses.

The SCFA provide energy to the gut tissue, and its production is mainly related with the substrates and the composition of microbiota in the gut (Ehle et al., 1982; Anguita et al., 2006). So, changes in dietary NSP drive changes in the profile of SCFA, the intestinal structure, and the microbial profile. Increased NDF level (by 35%) in the diet decreased crypt density, and increased villus width in ileum of growing pigs (Ngoc et al., 2012). Increased NSP in the diet (from 6.5 to 12.2 % DM) reduced the proliferation of epithelial cells in jejunum of nursery pigs, and affected enzyme activity and global cell number in the small intestine (Hedemann et al., 2006). Furthermore, adding high viscous NSP carboxylcellulose resulted in increased global cells in ileum without changing ileal morphology and apparent digestibility of weaning pigs (Piel et al., 2005).

Research has been conducted to determine the relationship between the occurrence of intestinal diseases and fiber components. For example, the intake of NSP from oat hulls and DDGS, all of which are rich in insoluble NSP, have been related to a decrease in the

colonization of hemolytic *Escherichia coli* in the intestine and a faster recovery from post-weaning colibacillosis (PWC) (Bertschinger et al., 1978; Perez-Mendoza, 2010; Mateos et al., 2006). Meanwhile, diets containing NSP from sources like guar gum and carboxymethylcellulose (CMC), which has relatively high level of soluble and viscous NSP, have been related with a higher susceptibility to enteric desease including PWC and swine dysentery (Durmic et al., 1998; McDonald et al., 2001). It has also been suggested that the microflora located at mucosa may have greater potential to modulate intestinal disease than the flora in the lumen (Krogfelt, 1991). The adherence of bacteria to the gut mucosa may result in weakened mucous barrier and induce sustained inflammation (Tysk et al., 1991). Further study on soluble NSP such as inulin, which does not increase viscosity, indicated that viscosity might contribute to the negative effects of NSP on gut health of pigs instead of solubility of NSP (Wellock et al., 2008).

Therefore, dietary NSP has an impact on nutrient digestion and gut health in monogastic animals, which could further affect their growth performance and health.

4. Feed processing

The goal of feed industry is to provide nutrients to animals in the farm by processing raw ingredients, mixing and pelleting the diet, and delivering the finish products to the farm. These technologies used in the feed mills can change the physical and chemical properties of ingredients, and potentially affect the digestion and fermentation of feed in the gastrointestinal tract, which may influence the growth and health of pigs.

4.1 Grinding

Particle size is one of the important characteristics of feed, which could influence nutrient digestion and gut health of animals. Grinding raw ingredients into smaller particle size (from 1,000 to 400 μm) improved feed efficiency and ATTD of energy, but increased the stomach lesion and keratinization in finishing pigs without affect their growth performance (Wondra et al., 1995). However, studies focusing on gut health showed that fine particle size increased crypt depth, cell proliferation, mucin staining volume in colon, and the sensibility of pigs to pathogens (Brunsgaard, 1998, Mikkelsen et al., 2004). Smaller particle size indicates more surface area, and this is good for nutrients to mix with digestive enzymes in the gut lumen. It also stimulates their contact with mucosa layer, and affects nutrient absorption as well as morphology and micro-environment of the intestine. In that case, we need to balance its positive and negative effects in either nutritional or economic aspects, by controlling the particle size between a reasonable range (such as 400 to 600 μm), in order to improve feed efficiency and maintain gut health.

4.2 Pelleting

Since most of the commercial feed mills are producing pelleted compound feed, it seems the benefit of pelleting has been recognized by the industry. The most advantage of pelleted feed for commercial feed mills might be relatively constant distribution of nutrients in the feed after long distance delivery. In the farms, pelleting can reduce wasted feed and improve feed flowability. Pelleted feed resulted in higher feed efficiency and ATTD of DM, N, and GE in finishing pigs (Wondra et al., 1995). The process of pelleting itself could

reduce bacteria of feed due to the heat treatment. However, feeding pelleted diet increased the mucin staining area of villi in small intestine of growing pigs, and potentially allowed more adhesion of *Salmonella enterica*, which is not good for gut health (Hedemann et al., 2005). Although it is hard to detect the effect of fiber content on the pellet quality due to multiple factors involved in pelleting, generally, soluble NSP might improve pellet durability, while the insoluble fraction make the pellet easier to break (Thomas et al., 1998).

5. Feed enzymes

Exogenous enzyme supplementation is another effective way of reducing the negative effects caused by planted-based ingredients. According to the global enzyme report from Market Data Forecast website, the value of feed enzymes was estimated to be 85.5 billion in 2016, where carbohydrase (including starch- and NSP-degrading enzymes) was the most used enzymes, and poultry industry was the largest segment. The largest user was Europe, and the biggest rise occurred in the Asian-Pacific region. Other enzymes, designed towards improving breakdown of protein, lipids, and phytate, are also available at the market (Ravindran and Son, 2011).

Enzymes naturally exist and are produced by living organisms to catalyze chemical reactions, in order to maintain normal functions of all living things. Enzymes were first introduced into the feed industry in the 1980s in Europe, and such enzymes were fiber-degrading enzymes (Sheppy, 2001). The main purpose of supplementing enzymes to the animal diets is to increase efficiency and bioavailability of nutrients in the diet, by 1) breaking down anti-nutritional factors that cannot be digested by endogenous digestive

enzymes, 2) supplementing enzymes to young animals due to the limited secretion by their immature digestive system, 3) breaking down some chemical bonds in raw materials, thus releasing more nutrients, and 4) benefiting animals through other mechanism instead of digestibility improvement (Sheppy, 2001; Choct, 2006). However, the dominant portion of available enzyme supplements in the market, have been utilized in poultry diets, and have typically been supplemented with ingredients of barley, oats, peas, rye, or wheat, with fewer studies that have evaluated their use in corn based diets. There are three major categories of commercial enzyme products: protease, carbohydrase, and phytase, targeting on protein, starch or fiber, and phytate substrates, respectively.

Protease, also called peptidases or proteolytic enzymes, are the enzymes that catalyze the hydrolysis of peptide bonds in protein and polypeptides. It can be divided into six groups: serine, cysteine, aspartic, glutamic, threonine, and metalloproteases, based on the property of amino acid residues located at the active site of the enzyme (Rao, et al., 1998). Proteases supplemented in the feed can increase protein digestion and growth performance (Upadhaya et al., 2016; Tactacan et al., 2016). Furthermore, protease supplementation can also be used on some feedstuffs with low quality protein, through degrading protein bound complex and releasing other nutrients as well as protein (Wang et al., 2008). Additionally, it might stimulate gut growth and maturation in young animals (Prykhodko et al., 2015).

Carbohydrases, are a group of enzymes that catalyze breaking down of carbohydrates into oligosaccharides and monosaccharides. They can be divided into starch degrading and NSP degrading enzymes. Starch degrading enzymes are not often used in swine diets,

because monogastric animals can produce this type of enzymes, like α-amylase, and are capable of digesting starch. Non-starch polysaccharide degrading enzymes include, but are not limited to, xylanase, mannanase, and glucanase, targeting xylan, mannan, and β-glucan, respectively. Problems caused by dietary NSP may be alleviated by supplementation of NSP degrading enzymes. The global market of NSP enzymes is dominated by xylanase and glucanase, which is in agreement with the fact that arabinoxylan and glucan are the predominant types of NSP in the feed ingredients (Adeola and Cowieson, 2011). The mechanism of dietary NSP degrading enzymes has been explored, and can be summarized in the following aspects. Firstly, it can hydrolyze NSP existing in feed ingredients into oligosaccharides, considering that most enzymes are "endo" acting. The released oligomers are still indigestible, so the benefit of NSP enzymes needs to be achieved through different modes of action. Secondly, NSP enzymes can reduce NSP induced digesta viscosity (Adeola and Bedford, 2004; Vahjen et al., 2007). Thirdly, it could increase energy efficiency by changing the amount of produced VFA and absorption of monomeric sugars at the small intestine (Li et al., 1996), and releasing nutrients, such as protein, starch, and minerals, by hydrolysis of encapsulating cell walls (Meng and Slominski, 2005; Nortey et al., 2007; Yin et al., 2010). Fourthly, NSP enzymes improve intestinal microbial population by supporting growth of beneficial bacteria (Vahjen et al., 1998; He et al., 2010). Fifthly, it also enhances gut morphology, such as reduced relative weight of organs in the digestive system and increased villus height (Hopwood et al., 2004; Sieo et al., 2005).

Phytases were, first introduced into feed industry around 1990's, due to the environmental pressure to reduce phosphorous excretion from pigs and poultry. Phytases dephosphorylate insoluble phytic acid into orthophosphate and inositol phosphates. They can be classified into two types: 3- or 6- phytase, on their active site of initial dephosphorylation. Direct effects of supplemental phytase will be the improvement of phosphorus digestion, and indirect effects include improved digestibility of other dietary nutrients and reduced endogenous loss (Simons et al., 1990; Onyango et al., 2005; Cowieson and Ravindran, 2007).

6. Scope of the current research

It is well known that the feed industry is sustainably growing and large portions of plant-based ingredients are used in swine diets. Feed efficiency is one of the important considerations in this area, however, there is another growing concern for animal health, which has expanded the opportunity to utilize exogenous enzymes. Especially as fermentation and transgenic technologies are being improved, the cost of adding enzyme products to the animal diet is decreasing. Now, the application of enzymes in the diet seems more promising in pig producing industry. Although a lot of research has been conducted to evaluate effects of supplemental exogenous enzymes in pigs, they have mainly focused on nutrient utilization and growth performance. Additionally, the reported effects of enzymes are not consistent, varying between different studies. Therefore, the objective of this dissertation is to evaluate effects of feed enzymes on specific ingredients, and significantly, to investigate how they affect growth and gut health of pigs, in order to provide more details on the utilization of exogenous enzymes in the pig producing industry. The first study

(chapter 2) investigated effects of protease (keratinase) on nursery pigs fed diets supplemented with sorghum. The second study (chapter 3) included three experiments, and evaluated effects of mannanase and xylanase (single use or combination) on nursery pigs fed diets supplemented with DDGS. The third study (chapter 4) explored effects of glucanase on nursery pigs fed diets with DDGS, and the fourth study (chapter 5) investigated effects of NSP degrading enzymes on nursery and grower pigs fed with low nutrient (energy or AA) diets, focusing on growth performance.

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Table 1. The content of non-starch polysaccharides (NSP) in cereal grains and co-products that are mainly used in the swine diets (% DM)

Ingredient	Arabinoxylan	Mannan	β-glucan	Soluble NSP	Total NSP
Corn	3.5-5.0	0.6-1.0	0.6-0.8	0.2-1.0	6.7-9.1
Corn DDGS	15.3-17.2	3.2-4.1	2.2-2.9	1.8-3.7	31.3-33.7
Sorghum	3.0	0.4	1.0	0.4	6.6
Wheat	6.5-7.0	0.8-1.0	1.1-1.7	2.3-2.5	10.8-13.6
Wheat Bran	24.6-28.6	0.9-1.3	0.4-3.0	2.9-6.4	18.3-41.6
Soybean meal	2.7-4.5	5.4	0.6-0.7	2.7-13.9	19.2-30.3

Data source: Choct et al. (2010), Rosenfelder et al. (2013), Pedersen et al. (2014), Jaworski et al. (2015)

CHAPTER 2

EFFECTS OF SUPPLEMENTAL PROTEASE ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND GUT HEALTH IN NURSERY PIGS FED DIETS WITH CORN OR SORGHUM

Abstract: A total of 144 pigs (18.4 ± 2.3 kg initial BW at 6 wk of age) were used in a 40-d trial to evaluate effects of protease (300,000 U/kg feed, BioResource International. Inc., Durham, NC) on growth performance, apparent ileal digestibility (AID) of nutrients, and gut health of nursery pigs fed diets with sorghum. Pigs were randomly allotted to 4 treatments (12 pens per treatment, 3 pigs per pen) in a 2 × 2 factorial arrangement (corn or sorghum basal diet, and 0 or 0.05% protease as 2 factors) with sex and initial BW as blocks. Experimental period had phase 1 (d 1 to 21) and phase 2 (d 22 to 40). About 65 % (phase 1) and 72% (phase 2) of cereal grains were used in corn or sorghum based diets. Both cereals were ground to 400 µm. Body weight and feed intake were recorded weekly. On d 35, serum was collected to quantify tumor necrosis factor-alpha (TNF-α) and malondialdehyde (MDA). Titanium dioxide (0.3%) was added as an indigestible marker for an additional 4 d feeding. On d 40, 32 pigs (8 pigs per treatment) were euthanized to collect digesta from jejunum and ileum (for viscosity and AID), tissues (for morphology) and mucosa samples (for TNF-α and MDA) samples from duodenum, jejunum, and ileum. Replacing corn with sorghum in the diet increased ($P < 0.05$) overall ADG (from 756 to 787 g/day) and ADFI (1374 to 1473 g/day), reduced ($P < 0.05$) overall G:F (from 0.553 to 0.537), and did not affect nutrient digestibility. Pigs fed diets with sorghum had lower ($P < 0.05$) MDA content in serum (from 14.61 to 6.48 µM) and jejunum (1.42 to 0.91 µmol/g protein), and reduced ($P < 0.05$) villus height (from 492 to 396 µm) and crypt depth (from 310 to 257 µm) in jejunum. Dietary protease improved ($P < 0.05$) AID of CP (from 81.8% to 86.0%), decreased MDA level (from 1.20 to 0.98 µmol/g protein) in duodenum, and increased ($P < 0.05$) ratio of villus

height to crypt depth (from 1.08 to 1.21) in duodenum. Overall, use of sorghum fully replacing corn in nursery diets could be beneficial to nursery pigs with enhanced feed intake and growth of nursery pigs, potentially by reducing oxidative stress. Supplementation of protease improved protein digestion and gut health, irrespective of sorghum or corn based diets.

Key words: growth performance, gut health, protease, nursery pigs, sorghum

INTRODUCTION

Sorghum can be cultivated under drier conditions when compared to maize, suggesting it could be more available to the feed manufacturers located in the dry lands. The global production of sorghum grains in 2015 was 60.16 million tons, with 15.2 million tons in the U.S. (USDA, 2016). Sorghum can substitute other cereal grains used in swine diets, showing that growth performance of pigs fed sorghum based diets may not always be comparable to that of corn based diet (Lin et al., 1987; Jondreville et al., 2001; Nyannor at al., 2007). Low-tannin sorghum was used in these experiments, indicating that tannins were not responsible for the decreased growth performance. Thus, kafirin might be the main factor rather than tannin, limiting the use of sorghum in non-ruminant species. Kafirin, as the prolamin storage protein in sorghum grains, has relatively low levels of basic amino acids, especially Lys (De Mesa-Stonestreet et al., 2010). Moreover, sorghum has poor protein digestibility, due to the hydrophobicity and disulfide crosslinking of kafirins (Duodu et al., 2003).

Keratinase, a class of proteolytic enzymes, has the capacity to cleave disulfide bonds, and hydrolyze soluble casein, insoluble keratin, and other proteins crosslinked by disulfide bonds (Brandelli et al., 2010). Keratinase supplementation in corn-based diets improved growth performance of pigs and poultry (Odetallah et al., 2005; Wang et al., 2011). It is hypothesized that keratinase supplementation could have greater benefit in pigs fed sorghum based diets by hydrolyzing kafirin and thus improving protein digestibility. However, most studies of keratinase application in sorghum based diets were conducted in poultry and showed improved apparent ileal digestibility (AID) of protein and AA (Selle et al., 2010; Liu

et al., 2013b). Additionally, the effect of sorghum based diets or protease supplementation was inconsistent in pigs (Zamora et al., 2011; Liu et al., 2013a). Therefore, the objective of this study was to determine the effect of protease on growth performance, nutrient digestibility, and gut health in nursery pigs fed sorghum based diets.

MATERIALS AND METHODS

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee (Raleigh, NC).

Animals and Experimental Design

The experiment was conducted at the North Carolina Swine Evaluation Station (Clayton, NC). A total of 144 barrows and gilts (18.4 ± 2.3 kg) at 6 wk of age were allotted to 4 dietary treatments in a 2 × 2 factorial arrangement (corn or sorghum basal diet, and 0 or 300,000 U keratinase /kg feed as 2 factors) based on sex and initial BW. Therefore, there were 4 dietary treatments with 12 replicate pens per treatment (6 male pens and 6 female pens), with 3 pigs per pen. The experiment period was 40 d, and was divided into 2 phases: phase 1 (1 to 21 d) and phase 2 (22 to 40 d). Four diets in each phase were made separately at the North Carolina State University Feed Education Unit (Raleigh, NC). Both corn and sorghum were ground to 400 μm. The analyzed nutrient value of corn and sorghum were listed in Table 1. The source of protease used in this study was versazyme (BioResource International. Inc., Durham, NC). The inclusion ratio of such enzyme product was 0.05% by replacing corn or sorghum in the basal diet, so it needed to be premixed with about 5 kg ground corn or sorghum before feed mixing. The calculated values of essential nutrients in

four experimental diets of each phase were adequate (NRC, 2012). The diet composition was summarized in Table 2. The diets were all mash feed. Pens (4.0 × 1.4 m) with solid concrete floor were equipped with a nipple drinker and a 1-hole steel self-feeder. Pigs had free access to feed and water. Body weight and feed intake were recorded weekly. Feed efficiency was calculated as G:F. On d 35, titanium dioxide (0.3%) was added as an indigestible marker to all diets for an additional 4 d feeding.

Sample Collection

On d 35, blood samples were collected from the jugular vein with BD sterile vacutainers (BD, Franklin Lakes, NJ) for serum. Blood samples were centrifuged at 3,000 × g for 15 min at 4°C to obtain the supernatant. Serum samples were stored at -80°C until analyzed for concentrations of tumor necrosis factor-α (TNF-α) and malonedialdehyde (MDA).

On d 40, 32 pigs (1 pig per pen, 8 pens per treatment) representing a median BW of each pen were selected and euthanized by using captive bolt. Digesta from ileum (about 20 cm before the ileal-cecal junction) was collected and stored at -20°C for AID measurement. Mucosa sample from duodenum (2 cm after the pyloric-duodenal junction until the loop ends), jejunum (around 100 cm before the ileal-cecal junction), and ileum were stored in -80°C for concentrations of TNF-α and MDA. Tissue sample from duodenum, jejunum, and ileum were flushed with saline solution, and stored in 10% formalin buffer at room temperature for histology evaluation.

Chemical Analysis

Diets and ileal digesta were stored at -20°C, until being freeze-dried (24D x 48, Virtis, Gardiner, NY). Diet and freeze-dried digesta samples were ground and analyzed for dry matter (Method 934.01, AOAC, 2006). Titanium dioxide concentration was measured at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). Nitrogen in the feed and digesta samples was quantified using TruSpec N Nitrogen Determinator (LECO Corp., St. Joseph, MI) to calculate crude protein (6.25 × N). Gross energy was determined using a calorimeter (Model 6200, Parr Instrument Company). Samples of feed and ileal digesta were analyzed sequentially for NDF and ADF using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp, Fairport, NY). Apparent ileal digestibility of DM, GE, CP, NDF, and ADF were calculated using titanium concentration in the feed and digesta. The digestibility was calculated with the following equation:

$$\text{AID, \%} = \left(1 - \frac{Ti_{feed} \times N_{digesta}}{Ti_{digesta} \times N_{feed}}\right) \times 100\%,$$

where Ti_{feed} represents the titanium concentration in the feed, $Ti_{digesta}$ is the titanium concentration in the ileal digesta, N_{feed} represents the nutrient concentration in the feed, and $N_{digesta}$ is the nutrient concentration in the ileal digesta.

ELISA Measurement

Mucosa samples were homogenized (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL) on ice. The homogenate was centrifuged at 15,000 × g at 4°C for 30 min to

collect supernatant. The supernatant was used to determine concentrations of total protein, TNF-α, and MDA.

Total protein of serum and mucosa samples were analyzed with Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc. Rockford, IL). Concentrations of TNF-α in serum and mucosa from duodenum, jejunum, and ileum, were analyzed using Porcine TNF-α Immunoassay ELISA Kit (R&D System Inc. Minneapolis, MN). The detection limit range for TNF-α ELISA was 2.8 to 5.0 pg/mL. Concentrations of TNF-α in serum and mucosa samples were expressed as ng/mL and ng/mg protein, respectively. Concentrations of MDA in serum and mucosa samples from duodenum, jejunum, and ileum, were analyzed using Thiobarbituric Acid Reactive Substance (TBARS) Assay Kit (Cell Biolabs, Inc. San Diego, CA) following the instructions of Weaver et al. (2014). Concentrations of MDA in serum and mucosa samples were expressed as μM and μmol/g protein, respectively.

Histology

Tissue samples from duodenum, jeunum, and ileum were fixed in formalin buffer and sent to North Carolina State University histology laboratory (Raleigh, NC) for dehydration, embedment and staining according to their internal standard protocol. Staining was done using hematoxylin and eosin dyes. Villus height and crypt depth were measured under an Infinity 2-2 digital CCD camera attached to an Olympus CX31 microscope (Lumenera Corporation, Ottawa, Canada). Then, the ratio of villus height to crypt depth was calculated. Lengths of 10 well-oriented intact villi and their associated crypts were measured in each slide. One person executed all the analysis of intestinal morphology.

Statistical Analysis

Data were analyzed using Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The experiment was a randomized complete block design using initial BW and sex as blocking factors. The experimental unit was the pen for growth performance, while the individual pig for other measurements. Initial BW block was considered as a random effect, while ingredient (corn or sorghum), enzyme supplementation (0 or 0.05% protease), the interaction between ingredient and enzyme, and the block of sex were considered as fixed effects. Statistical differences were considered significant with $P < 0.05$. Probabilities less than 0.10 and equal or greater than 0.05 were considered as tendencies.

RESULTS

Growth Performance

The average initial BW of each treatments were not significantly different from each other (Table 3). During wk 1, 3, and 5, ADG was not affected by either cereal base or enzyme supplementation. During wk 4, pigs fed sorghum based diets had an increased ($P < 0.05$) ADG. So, phase 1 and overall ADG were improved ($P < 0.05$) by supplemental sorghum.

During wk 1, ADFI tended to be increased ($P = 0.073$) by supplemental sorghum. From wk 2 to 5, ADFI was significantly increased ($P < 0.05$) by supplemental sorghum. Regardless of the diet type, dietary protease improved ($P < 0.05$) ADFI during wk 3.

From wk 1 to 4, G:F was not affected by diet type or protease supplementation, but was greatly reduced ($P < 0.05$) by supplemental sorghum in wk 5 (0.467 to 0.427), which resulted in a lower G:F in phase 2 and the overall period when pigs were fed sorghum based diet.

Apparent Ileal Digestibility

AID of nutrients were not influenced by completely replacing corn with sorghum (Table 4). However, supplementation of protease tended to increase ($P < 0.10$) AID of DM, GE, and NDF, and improved ($P < 0.05$) AID of CP. There were no interactions observed.

Immune and Oxidative Status

Concentrations of TNF-α in serum or mucosa samples were not affected by cereal base or supplementing protease (Table 5). Pigs fed sorghum basal diets had lower ($P < 0.05$) MDA content in serum and jejunum mucosa. Malondialdehyde level in duodenum mucosa was reduced ($P < 0.05$) by supplementation of protease. No interactions were observed.

Histology

Dietary protease increased ($P < 0.05$) the ratio of villus height to crypt depth in duodenum. Pigs fed sorghum based diets had lower ($P < 0.05$) villus height and crypt depth in jejunum (Table 6).

DISCUSSION

Sorghum

In the current study, completely replacing corn with sorghum greatly increased ADG and ADFI, and resulted in a lower feed efficiency in pigs. According to the analyzed GE content, corn based diets had slightly higher GE, by about 0.2%. During phase 2, pigs fed

corn based diets had lower ADFI than these fed with sorghum based diets, by about 7.5%. This led to an increased amount of energy intake in pigs fed sorghum based diets (7,301 vs. 6,763 kcal/d), and thus improved ADG. However, nutrient digestibility of pigs was not affected by diet type. In other studies, finisher pigs fed sorghum based diets tended to have increased ADFI and ADG, but unaffected G:F (Paulk et al., 2015). However, Healy et al. (1994) reported that nursery pigs fed diets with low-tannin sorghum consumed less feed, gained slower, and were less efficient than pigs fed corn based diets. Such results corresponded with its decreased apparent total tract digestibility (ATTD) of nutrients. Louis et al. (1991) observed similar results that feed intake of lactating sows fed with low-tannin sorghum based diet were lower than those fed with corn based diet, and ATTD of nutrients were not significantly affected by diet type. Kim et al. (1998) observed that pigs fed sorghum based diets had unchanged feed intake and daily gain, but a lower feed efficiency and reduced ATTD of nutrients. It seems that feed efficiency was not always consistent with apparent digestibility of nutrients. During the nursery stage, feed efficiency was not that critical due to the smaller feed intake than grower and finisher pigs. So, in this study, the effect of sorghum in improving daily gain was much more important to animal growth than decreasing feed efficiency.

Sorghum diet contains less digestible protein (Mariscal-Landín et al., 2010), but we did not observe reduced nutrient digestibility caused by replacing corn with sorghum in the diets. Such response can be attributed to two reasons. One is the particle size of cereal grains. Previous research concluded that feed efficiency and nutrient digestibility of pigs can be

improved by reducing the particle size of sorghum (Healy et al., 1994; Paulk et al., 2015). At the particle size of 600 um, AID of AA were lower in growing pigs fed sorghum when compared with those fed corn based diets (Pedersen et al., 2007). Particle size of sorghum and corn used in this study were about 400 μm. So, reducing particle size of grains improved the efficiency of energy utilized in both corn and sorghum based diets. The other reason is supplementation of crystalline AA based on the standardized ileal digestible (SID) AA values. Compared with corn, sorghum has a higher CP level, but lower in Lys, Thr, and Met (Wall and Paulis, 1978). Formulating diets based on SID values also did not cause decreased ADG in the study conducted by Paulk et al. (2015), thus it would be possible to expand the use of sorghum in swine diets.

Sorghum contains many phytochemicals, such as phenolic acids and flavonoids, and tannins are a type of flavonoid compound (Awika and Rooney, 2004). These phytochemicals have different physiological effects. Ethanolic extract of black sorghum bran significantly hindered the production of the pro-inflammatory cytokines TNF-α (Burdette et al., 2010). However, this effect was not observed in non-tannin sorghum (Moraes et al., 2012). Sorghum used in the current study was low tannin cultivar. This corresponded with our finding that sorghum diets had no influence on TNF-α levels of pigs. Then, methanol extract of sorghum has antioxidant capacities (Taylor et al., 2014). Anti-oxidant activities of sorghum correlated positively with levels of total phenolic acids (Awika and Rooney, 2004; Burdette et al., 2007). Oxidative stress is caused by production of oxidants, and would leads to cellular damage to the cell components, such as the oxidation of proteins and lipids in the cell

(Schieber and Chandel, 2014). The level of oxidative stress can be evaluated by the content of lipid peroxidation, expressed as MDA concentrations (Liu et al., 2000). In our study, replacing corn with sorghum decreased MDA levels in serum and jejunum in pigs. Therefore, sorghum utilized in the diets exerted a positive effect on gut health by reducing oxidative stress rather than regulating inflammation response.

In this study, villus height and crypt depth of jejunum were decreased by supplemental sorghum. The change in gut morpgology of pigs may be associated with the tannin content in the diet. For example, tannin supplementation (up to 4.5 g/kg feed per day) reduced crypt depth of the ileum in weaned pigs (Biagi et al., 2010), and increasing tannin level in the diet reduced villus height and crypt depth of jejunum in broilers (Nyamambi et al., 2007). In the present study, the reduction in villus height and crypt depth might be associated with small amounts of tannin in the diet. As one of the anti-nutritional factors, tannin might be responsible for the changes in the gut morphology and function in pigs (Pluske et al., 1997). However, we did not measure the actual tannin content in our study. There might be a range of tannin in the diet that is not sufficient to affect immune status, but still could change gut morphology of pigs, which needs further research.

Protease

The protease supplemented in this study is produced by *Bacillus Licheniformis* PWD-I, and has the ability of degrading keratins and a wide range of proteins (Wang et al., 2006). It is well recognized for attacking highly cross-linked and recalcitrant structural proteins, and used as a feed enzyme to improve nutritional value of proteins existing in the diet (Gupta et

al., 2013). In our study, dietary supplementation of protease improved ADFI only during the third week, but did not significantly affect ADG and G:F. Previous studies showed that protease supplementation to corn-soybean meal (SBM) diets improved growth performance of pigs (Guo et al., 2014; Park et al., 2015; Wang et al., 2011). Dietary protease increased AID of CP in both types of diet in the present study. Similarly, AID of CP and most AA in growing pigs were improved by adding protease to corn based diets (Wang et al., 2011). The effect of protease in corn-SBM diets might be attributed to the hydrolysis of cystine disulfide bonds found in soybean proteins, such as glycinin and β-conglycinin, and thus the improved protein digestion (Hou and Chang, 2004). Studies in poultry showed that adding protease to sorghum based diets improved amino acid and protein digestibility in chickens (Selle et al., 2010; Liu et al., 2013b), which might be due to similar mechanism that protease hydrolyzes the less digestible proteins such as kafirin, to make them more available to animals. However, such improvement did not result in increased feed efficiency in this study. This might be because of an unbalanced digestible AA/energy ratio, and these released AA could not be utilized for protein deposition (Van Lunen et al., 1996). However, such improvement may improve the weight gain in a longer period of feeding study, which needs further investigation.

In our study, dietary protease did not affect the levels of TNF-α in serum and mucosa samples. However, reduced TNF-α levels were observed in serum, duodenum, and jejunum of pigs fed diets with protease (Guo et al., 2014; Park et al., 2015). Other pro-inflammatory cytokines, such as interleukin-1 and -6, were inhibited by protease supplementation (Wang et

al., 2011). Interestingly, dietary protease reduced MDA level in duodenum. Guo et al. (2014) also reported adding protease decreased MDA level in serum of nursery pigs fed corn and 30% SBM based diets. In broilers, supplementing protease decreased MDA level in serum and ileum of birds fed with corn, SBM, and DDGS based diets. As an indicator of lipid peroxidation levels, MDA also represents levels of reactive oxygen species, indicating the oxidative stress in the tissue of animals. Stimuli of oxidative stress could cause up-regulation of inflammatory response and cell proliferation. The reduced MDA level in duodenum corresponded with an improved ratio of villus height to crypt depth in the duodenum. Similar improvement in morphology of small intestine were also observed by Guo et al. (2014) and Park et al. (2015). Dietary factors in the lumen will lead to relatively quick changes in the mucosa due to the interaction between the mucosal surface and the intestinal digesta. The hydrolysis of dietary protein might contribute to such improvement. An increase in the ratio of villus height to crypt depth was associated with better nutrient absorption, better gastrointestinal health, and improved growth performance (Wang et al., 2008).

Overall, completely replacing corn with sorghum was not a big concern to nursery pigs. On the contrary, sorghum based diets might be potentially beneficial due to the increased feed intake and reduced oxidative stress. Supplementation of protease improved protein digestion and gut health by decreasing oxidative stress and enhancing morphology, irrespective of sorghum or corn based diets.

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Table 1. Analyzed nutrient profile of corn and sorghum (as-fed basis)

Item	Corn	Sorghum
DM, %	87.03	86.09
Ash, %	1.17	1.40
CP, %	7.20	9.83
NDF, %	8.39	8.26
ADF, %	2.76	4.33
Crude fat, %	3.43	2.61
Ca, %	0.01	0.05
P, %	0.25	0.26

Table 2. Composition of corn- and sorghum-based diets

Item	Phase 1 (d 1 to 21)		Phase 2 (d 22 to 40)	
	Corn-based diet	Sorghum-based diet	Corn-based diet	Sorghum-based diet
Ingredient, %				
Corn	65.00	0.00	72.00	0.00
Sorghum	0.00	65.00	0.00	72.00
Soybean meal	30.00	30.00	24.00	24.00
Keratinase[1]	0.05	0.05	0.05	0.05
L-Lys HCl	0.40	0.45	0.30	0.35
DL-Met	0.12	0.15	0.10	0.12
L-Thr	0.12	0.12	0.08	0.07
Poultry fat	1.91	1.91	1.00	1.09
Salt	0.22	0.22	0.22	0.22
Vitamin premix[2]	0.03	0.03	0.03	0.03
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Dicalcium phosphate	1.00	0.90	1.07	0.92
Limestone	1.00	1.02	1.00	1.00
Total	100.00	100.00	100.00	100.00
Calculated nutrient values				
ME, kcal/kg	3,374	3,340	3,334	3,304
Lys[4], %	1.23	1.24	1.00	1.01
Met + Cys[4], %	0.69	0.68	0.62	0.60
Trp[4], %	0.21	0.21	0.18	0.18
Thr[4], %	0.73	0.74	0.61	0.61
Ca, %	0.71	0.70	0.71	0.68
Available P, %	0.34	0.34	0.34	0.33

Table 2. Continued

Analyzed nutrient values				
DM, %	88.86	87.77	91.82	91.82
CP, %	19.41	20.48	16.00	17.50
GE, kcal/kg	-	-	3,925	3,919
NDF, %	6.70	10.51	9.47	16.28
ADF, %	2.90	4.28	3.00	4.58
TiO_2, %	-	-	1,700	1,780

[1]Keratinase source was Versazyme (BRI Inc., RTP, NC) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase/kg feed.

[2]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

[3]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[4]Standardized ileal digestible

Table 3. Growth performance of pigs fed corn or sorghum based diets supplemented with and without keratinase

Ingredient	Corn		Sorghum		SEM	*P*-value[1]		
Keratinase	0	0.05%	0	0.05%		Ing	Enz	Ing × Enz
BW, kg								
Initial	18.36	18.42	18.43	18.44	0.67	0.608	0.726	0.808
Wk 1	22.94	22.82	22.99	23.03	0.88	0.487	0.827	0.674
Wk 2	27.30	26.56	27.00	27.59	1.01	0.189	0.793	0.017
Wk 3	32.41	31.92	32.45	33.18	1.17	0.081	0.744	0.101
Wk 4	38.11	37.89	38.80	39.54	1.35	0.015	0.590	0.308
Wk 5	44.28	43.64	44.57	45.53	1.49	0.060	0.780	0.163
ADG, g/day								
Wk 1	654	649	652	672	32	0.527	0.648	0.431
Wk 2	623	535	593	651	37	0.198	0.656	0.033
Wk 3	742	765	779	800	43	0.168	0.393	0.938
Wk 4	815	863	907	943	43	0.006	0.159	0.840
Wk 5	881	846	824	855	35	0.450	0.943	0.296
Phase 1 (wk 1 to 3)	673	650	675	707	26	0.039	0.727	0.051
Phase 2 (wk 4 to 5)	848	854	866	899	29	0.155	0.359	0.525
Overall (wk 1 to 5)	760	752	770	803	23	0.020	0.327	0.103
ADFI, g/day								
Wk 1	1003	1007	1029	1057	50	0.073	0.427	0.557
Wk 2	1112	1033	1113	1171	50	0.034	0.741	0.038
Wk 3	1346	1343	1381	1525	60	0.003	0.047	0.038
Wk 4	1536	1631	1735	1754	78	0.013	0.356	0.537
Wk 5	1881	1844	1958	2003	81	0.040	0.938	0.462
Phase 1 (wk 1 to 3)	1154	1128	1175	1251	49	0.002	0.251	0.024

Table 3. Continued

Phase 2 (wk 4 to 5)	1708	1737	1846	1879	71	0.007	0.534	0.975
Overall (wk 1 to 5)	1375	1372	1443	1502	55	0.001	0.307	0.246
G:F								
Wk 1	0.655	0.642	0.635	0.637	0.009	0.164	0.577	0.414
Wk 2	0.558	0.514	0.538	0.553	0.027	0.638	0.473	0.153
Wk 3	0.553	0.575	0.563	0.536	0.029	0.488	0.905	0.246
Wk 4	0.534	0.532	0.528	0.543	0.022	0.897	0.762	0.661
Wk 5	0.472	0.461	0.421	0.433	0.014	0.008	0.999	0.414
Phase 1 (wk 1 to 3)	0.585	0.578	0.577	0.570	0.018	0.425	0.462	0.997
Phase 2 (wk 4 to 5)	0.499	0.494	0.469	0.484	0.014	0.034	0.629	0.285
Overall (wk 1 to 5)	0.555	0.551	0.535	0.539	0.009	0.029	0.993	0.571

[1]Ing: main effect of sorghum; Enz: main effect of keratinase; Ing x Enz: interaction effect between sorghum and keratinase

Table 4. Apparent ileal digestibility (AID) of DM, CP, GE, NDF, and ADF in pigs fed corn or sorghum based diets supplemented with and without keratinase

Ingredient	Corn		Sorghum		SEM	*P*-value[1]		
Keratinase	0	0.05%	0	0.05%		Ing	Enz	Ing x Enz
AID, %								
DM	82.1	84.9	82.5	83.9	1.3	0.966	0.078	0.462
CP	81.7	85.7	81.8	86.2	1.7	0.868	0.014	0.898
GE	84.5	87.2	85.5	86.8	1.1	0.784	0.063	0.532
NDF	41.9	44.8	42.6	43.7	1.1	0.838	0.071	0.405
ADF	31.5	33.3	30.3	31.7	1.5	0.345	0.299	0.877

[1]Ing: main effect of sorghum; Enz: main effect of keratinase; Ing x Enz: interaction effect between sorghum and keratinase

Table 5. Tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA) in serum and mucosa samples of pigs fed corn or sorghum based diets supplemented with and without keratinase

Ingredient	Corn		Sorghum		SEM	*P*-value[1]		
Keratinase	0	0.05%	0	0.05%		Ing	Enz	Ing x Enz
TNF-α,								
Serum, pg/ml	91.46	80.33	88.71	84.74	12.86	0.949	0.562	0.783
Duodenum, pg/mg protein	8.92	9.51	8.34	8.86	0.69	0.379	0.429	0.956
Jejunum, pg/mg protein	7.03	6.19	7.01	5.71	0.64	0.454	0.208	0.469
Ileum, pg/mg protein	5.61	5.77	5.55	4.59	0.42	0.203	0.355	0.160
MDA,								
Serum, µM	15.62	13.59	6.73	6.23	1.83	0.001	0.496	0.680
Duodenum, umol/g protein	1.20	0.99	1.19	0.97	0.06	0.855	0.023	0.985
Jejunum, umol/g protein	1.40	1.43	1.00	0.81	0.12	0.003	0.496	0.372
Ileum, umol/g protein	0.79	0.75	0.72	0.79	0.07	0.844	0.760	0.488

[1]Ing: main effect of sorghum; Enz: main effect of keratinase; Ing x Enz: interaction effect between sorghum and keratinase

Table 6. Villus height (H), crypt depth (D), and the ratio of villus height to crypt depth (H:D) of duodenum, jejunum, and ileum in pigs fed corn or sorghum based diets supplemented with and without keratinase

Ingredient	Corn		Sorghum		SEM	*P*-value[1]		
Keratinase	0	0.05%	0	0.05%		Ing	Enz	Ing x Enz
Duodenum								
H, µm	473	557	499	495	25	0.478	0.128	0.095
D, µm	437	443	474	431	14	0.402	0.201	0.096
H:D ratio	1.09	1.27	1.06	1.15	0.06	0.246	0.038	0.470
Jejunum								
H, µm	497	486	389	403	24	0.004	0.963	0.611
D, µm	322	297	248	266	15	0.002	0.818	0.158
H:D ratio	1.57	1.64	1.58	1.52	0.08	0.479	0.940	0.411
Ileum								
H, µm	423	409	404	434	23	0.836	0.682	0.310
D, µm	234	235	254	235	12	0.409	0.468	0.430
H:D ratio	1.86	1.73	1.60	1.86	0.09	0.484	0.476	0.044

[1]Ing: main effect of sorghum; Enz: main effect of keratinase; Ing x Enz: interaction effect between sorghum and keratinase

CHAPTER 3

EFFECTS OF SUPPLEMENTAL MANNANASE AND XYLANASE ON GROWTH PERFORMANCE, DIGESTA VISCOSITY, NUTRIENT DIGESTIBILITY, AND GUT HEALTH IN NURSERY PIGS FED DIETS WITH CORN DISTILLER DRIED GRAINS WITH SOLUBLES

Abstract: The objective of these experiments was to investigate the effects of dietary mannanase and xylanase on growth performance, digesta viscosity, nutrient digestibility, and gut health in nursery pigs fed diets with corn distillers dried grains with solubles (DDGS). In Exp. 1, eighty-four pigs (17.6 ± 2.8 kg initial BW at 6 wk of age) were used in a 40-d trial and allotted to 2 treatments (14 pens per treatment, 3 pigs per pen) based on a randomized complete block design with sex and initial BW as blocks. Experimental diets were corn, soybean meal, and 20% DDGS based diet with or without 400 U mannanase/kg feed. Mannanase increased ($P < 0.05$) the overall G:F, pH of colon digesta, and apparent ileal digestibility (AID) of NDF and ADF. Additionally, mannanase reduced ($P < 0.05$) viscosity of jejunal digesta, improved ($P < 0.05$) ratios of villus height to crypt depth in jejunum and ileum, and tended to reduce ($P = 0.078$) TNF-α concentration in jejunum. In Exp. 2, forty pigs (10.7 ± 1.2 kg initial BW at 6 wk of age) were used in a 21-d trial, individually housed and randomly allotted to 4 treatments in a 2 × 2 factorial arrangement (0 or 30% DDGS, and 0 or 1,500 EPU/kg xylanase as 2 factors) based on sex and initial BW. In wk 3, supplemental DDGS increased ($P < 0.05$) ADFI. Xylanase increased ($P < 0.05$) the overall ADG. Use of DDGS increased ($P < 0.05$) viscosity of jejunal digesta, whereas xylanase reduced ($P < 0.05$) it. The AID of DM and GE were decreased ($P < 0.05$) by supplemental DDGS, while AID of GE and NDF were improved ($P < 0.05$) by xylanase. Plasma TNF-α and peptide YY were decreased ($P < 0.05$) by xylanase. Use of DDGS reduced ($P < 0.05$) the ratio of villus height to crypt depth whereas xylanase increased ($P < 0.05$) crypt depth in duodenum. In Exp. 3, thirty-two pigs (6.2 ± 0.8 kg) at 3 wk of age were utilized in a 20-d trial. On d 6, these pigs

were randomly allotted to 4 dietary treatments based on BW and sex: CON (control diet), MAN (CON with 400 U mannanase/kg feed), XYL (CON with 1,500 EPU xylanase/kg feed), and XYL+MAN (CON with both enzymes). The CON contained 40% corn, 23% soybean meal, and 15% DDGS. Supplementation of mannanase tended to decrease (P = 0.057) MDA level in jejunum, and increased ($P < 0.05$) expressions of occludin. Xylanase improved ($P < 0.05$) villus height in duodenum and proliferation rate in the jejunal crypt, and increased ($P < 0.05$) expressions of claudin, occludin, and zonula occludens-1. Collectively, feeding a diet with 30% DDGS to nursery pigs for 3 wk had no negative effect on growth performance, but could be potentially harmful to gut health of pigs. Adding mannanase and xylanase to a DDGS containing diet had little improvement on growth performance, but could improve gut health of nursery pigs.

Key words: corn distillers dried grains with solubles, growth performance, gut health, mannanase, nursery pigs, xylanase

INTRODUCTION

The production of corn distiller dried grains with solubles (DDGS) has increased as the biofuel industry has grown. Its nutritional composition indicates that DDGS is an attractive alternative for traditional energy and protein ingredients in animal feeds (Kim et al., 2008). However, after the conversion of starch to ethanol during the commercial fermentation process, other substances in DDGS, such as protein, minerals, and non-starch polysaccharides (NSP), increase approximately 3 times (Belyea et al., 2004; Pedersen et al., 2014). Non-starch polysaccharides constitute 25 to 30% of DDGS, of which two main polysaccharides are arabinoxylan and galactomannan (Pedersen et al., 2014). As one of the staple ingredients used in the feed, soybean meal contains about 20% NSP, and 1.2% of dehulled soybean meal is galactomannan (Hsiao et al., 2006; Choct et al., 2010). The anti-nutritional effect of NSP on poultry and pig generally include increasing digesta viscosity in the small intestine, decreasing nutrient digestibility and absorption, interacting with gut microflora, and modifying physiological functions of the intestine in monogastric animals (Choct, 1997). Moreover, supplementation of DDGS caused oxidative damage by increasing oxidant content (Li et al., 2012). The increased oxidative stress affected the response to inflammatory stimuli, and regulated the production of pro- and anti-inflammatory cytokines (Weber and Kerr, 2011).

Mannanase and xylanase have the potential to alleviate negative effects caused by DDGS supplementation, because they target mannan and xylan in the diet, and hydrolyze them into oligosaccharides and simple sugars. Supplemental feed enzymes could reduce digesta viscosity, improve nutrient digestion, and alter intestinal morphology (Yoon et al., 2010;

Passos et al. 2015; Swiatkiewicz et al., 2016). These effects might lead to healthier gut and improved growth performance of animals. Interestingly, supplemental xylanase also affected intestinal function through modifying the expression of gut hormones such as peptide YY (PYY) in broiler (Singh et al., 2012), but this is uncertain for species of pig. Moreover, the effect of exogenous enzymes on growth and nutrient utilization seemed less consistent, and there is little information about their effects on immune status and intestinal permeability in pigs fed diets with DDGS (Swiatkiewicz et al., 2016). Therefore, we hypothesized that mannanase and xylanase will have more potential when supplemented to the diets containing DDGS, compared with corn and soybean meal diets. The objective of these experiments was to evaluate the effects of enzymes on growth performance, digesta viscosity, nutrient digestibility, and gut health of pigs fed diets containing various concentrations of DDGS.

MATERIALS AND METHODS

These experimental protocols were approved by North Carolina State University Animal Care and Use Committee (Raleigh, NC).

Animals and Experimental Design

Experiment 1 was conducted at the North Carolina Swine Evaluation Station (Clayton, NC). Eighty-four barrows and gilts (17.6 ± 2.8 kg) at 6 wk of age were allotted to 2 dietary treatments in a randomized complete block design based on initial BW and sex. The experimental diets mainly include corn, soybean meal, and 20% DDGS, with or without mannanase (400 U/kg feed, CTCBIO Inc., Seoul, Korea). One unit of mannanase activity is defined as the amount of enzyme that released one μmol mannose from mannan per minute

under 0.2 M sodium phosphate (pH 6) at 50 °C. The experiment period was 40 d, and was divided into 2 phases: phase 1 (1 to 21 d) and phase 2 (22 to 35 d). Two treatment diets for each phase were mixed separately, and inclusion ratio of mannanase supplementation was 0.05% by replacing same amount of ground corn in the diet. The enzyme product needed premixing with ground corn before being hand-added to the diet. The diet composition was summarized in Table 1. Each treatment had 14 replicate pens (7 male pens and 7 female pens in each treatment), with 3 pigs per pen. Pens (4.0 × 1.4 m) with solid concrete floor were equipped with a nipple drinker and a 1-hole self-feeder. On d 35, titanium oxide (0.3%) was added as an indigestible marker to the diets for an additional 4 d feeding.

Experiment 2 was conducted at the Swine Education Unit of North Carolina State University (Raleigh, NC). Forty crossbred barrows and gilts (10.7 ± 1.2 kg) at 6 wk of age were randomly allotted to 4 dietary treatments based on a 2 × 2 factorial arrangement. The first factor was endo-1,4-β-xylanase (0 or 1,500 EPU/kg complete feed), and the second factor was DDGS inclusion (0 or 30%). This enzyme (Hostazym X 100 xylanase) was obtained from Huvepharma USA (Peachtree City, GA). One EPU is defined as the amount of enzyme that releases low-molecular fragments from dyed xylan in amount equal to the amount of such fragments liberated from 1 unit enzyme standard under the conditions of the assay (T 50°C and pH 4.7). Four treatment diets were mixed separately, and xylanase product was premixed before being added to the diet. The inclusion ratio of xylanase supplementation was 0.01% by replacing same amount of ground corn in the diet. The diet composition was summarized in Table 2. The experimental period was 21 d. Each treatment has 10 pens (5

male pens and 5 female pens) with 1 pig per pen. Pens (1.73 × 0.83 m) with metal screen floor were equipped with 1 nipple drinker and 1 self-feeder. On d 15, titanium dioxide (0.3%) was blended into experimental diet as an indigestible marker for calculation of apparent ileal digestibility (AID).

Experiment 3 was conducted at the Metabolism Educational Unit at North Carolina State University (Raleigh, NC). Thirty-two pigs (6.2 ± 0.8 kg) at 3 wk of age received the same starter diet (Table 3). On d 6, these pigs were randomly allotted to 4 dietary treatments: CON (control diet), MAN (CON supplemented with 400 U mannannase/kg feed), XYL (CON supplemented with 1,500 EPU xylanase/kg feed), and XYL+MAN (CON supplemented with both enzymes). The control diet was 40% corn, 23% soybean meal, and 15% DDGS based diet. The xylanase (Hostazym X 100) was obtained from Huvepharma USA (Peachtree City, GA), and the mannanase was from CTCBIO Inc. (Seoul, Korea). The diet composition and nutrient concentrations are shown in Table 3. The experimental period was 20 d. Each treatment has 8 pens (4 male pens and 4 female pens) with 1 pig per pen. Pens (1.50 × 0.74 m) with slatted floor were equipped with 1 nipple drinker and 1 self-feeder.

Pigs had free access to water and feed. All the diets were mash feed and mixed at North Carolina State University Feed Educational Unit (Raleigh, NC). All the essential nutrients in the experimental diets were adequate (NRC, 2012).

Sample Collection

Body weight and feed intake were recorded weekly. Feed efficiency was calculated as G:F. Each section of intestine was identified as follows: duodenum, 2 cm from the pyloric-

duodenal junction until the loop ends; middle jejunum, 100 cm before the ileal-cecal junction; distal jejunum, right before the jejunal-ileal junction; ileum, around 20 cm until the ileal-cecal junction; and colon, transverse part can easily be identified by eyes.

In Exp. 1, blood samples were collected from jugular vein with BD serum sterile vacutainers (BD, Franklin Lakes, NJ) for serum on d 35. On d 40, 16 pigs (1 pig per pen, 8 pens per treatment) representing a median BW of each pen were selected and euthanized to collect digesta samples from distal jejunum, ileum, and colon, as well as tissue and mucosa samples from duodenum, middle jejunum, and ileum. Serum and mucosa samples were for tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA) measurements. Digesta from distal jejunum were for viscosity and pH measurements. Digesta from ileum were for AID and pH measurements. Digesta from colon were only for pH measurement. Tissue samples were for histology evaluations.

In Exp. 2, blood samples of all pigs were collected from jugular vein with BD EDTA sterile vacutainers (BD, Franklin Lakes, NJ) for plasma on d 19. On d 21, all pigs were euthanized to collect digeata samples from distal jejunum and ileum, tissue samples from duodenum, middle jejunum, and colon, and mucosa samples from middle jejunum and colon. Tumor necrosis factor-α and MDA were determined in both plasma and mucosa samples, with PYY only for plasma samples. Digesta from distal jejunum were for viscosity. Digesta from ileum were for AID. Tissue samples were for histology evaluations.

In Exp. 3, blood samples of all pigs were collected from jugular vein with BD EDTA sterile vacutainers (BD, Franklin Lakes, NJ) for plasma on d 18. On d 20, all pigs were

euthanized to collect tissue and mucosa samples from duodenum and middle jejunum. Plasma and mucosa samples were for TNF-α and MDA measurements. Tissue samples were for histology evaluations, and tissue from jejunum were also used to Ki-67 protein staining and expressions of tight junction proteins.

Blood samples were centrifuged at 3,000 × g for 15 min at 4°C to obtain the serum or plasma. Blood and mucosa samples for TNF-α, MDA, or PYY measurements, and tissue samples for tight junction protein expressions were snap frozen in liquid N and stored at -80°C until analysis. Digesta samples for viscosity and pH values needed to be measured immediately after euthanasia. Ileal digesta for digestibility were stored at -20°C until analysis. Tissue samples for histology and Ki-67 protein staining were flushed with saline solution and stored in 10% formalin buffer at room temperature.

Viscosity and pH Measurement

The method of measuring jejunal digesta viscosity was described by Passos et al. (2015) with a viscometer (Brookfield Digital Viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA). The samples were centrifuged at 3,000 × g for 5 min and then the supernatant was pipetted out to a 2 mL-tube and centrifuged at 12,500 × g for 5 min. Viscometer was set at 25°C, and 0.5 mL of digesta supernatant was placed in the viscometer. The final result was calculated as the average of viscosity at 45.0 sec -1 and 22.5 sec -1 shear rates.

The pH value of jejunal, ileal, and colon digesta were measured by AB15 Basic pH meter (Thermo Fisher Scientific Inc., Waltham, MA) immediately after sample collection.

Chemical Analysis

Ileal digesta were freeze dried (24D x 48, Virtis, Gardiner, NY). Diets and freeze-dried ileal digesta samples were finely ground, and analyzed for dry matter (Method 934.01, AOAC, 2006). Titanium concentration was measured at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). The GE was determined using a calorimeter (Model 6200, Parr Instrument Company). Duplicate samples of feed and ileal digesta were analyzed sequentially for NDF and ADF using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp, Fairport, NY). Apparent ileal digestibility of DM, GE, NDF, and ADF were calculated using titanium concentration in the feed and digesta. The digestibility is calculated with the following equation:

$$\text{AID, \%} = \left(1 - \frac{Ti_{feed} \times N_{digesta}}{Ti_{digesta} \times N_{feed}}\right) \times 100\%,$$

where Ti_{feed} is the titanium concentration in the feed, $Ti_{digesta}$ is the titanium concentration in the ileal digesta, N_{feed} is the nutrient concentration in the feed, and $N_{digesta}$ is the nutrient concentration in the ileal digesta.

Histology and Immunohistochemistry for Ki-67

The segments of tissues were dehydrated and embedded in paraffin, cut across the section to 5-mM-thick slides, and mounted on a polylysine-coated slide (Shen et al., 2012). Staining was done using hematoxylin and eosin dyes. Villus height, villus width, and crypt depth were measured under an Infinity 2-2 digital CCD camera attached to an Olympus CX31 microscope (Lumenera Corporation, Ottawa, Canada). The ratio of villus height to crypt depth were also determined. Lengths of 10 well-oriented intact villi and their associated

crypt were measured in each slide. One person executed all the analysis of intestinal morphology.

The segment of jejunum was fixed in the 10% formalin buffer for 3 wks, transferred into 70% ethanol solution and immediately sent to North Carolina State University Histology Lab (Raleigh, NC) for Ki-67 protein staining. The intact crypt was cropped and Image JS software was used for calculating the ratio of Ki-67 positive cells to total cells in the crypt (Almeida et al., 2012).

$$\text{Crypt cell proliferation, \%} = \frac{\text{Ki-67 positive cells}}{\text{Total cells}} \times 100\%$$

ELISA Measurements

Mucosa samples (500 mg) of duodenum, jejunum, ileum, and colon were weighed, and suspended into 1.0 mL PBS solution (MP Biomedicals, LLC. Solon, OH). Samples were homogenized on ice. The homogenate was centrifuged at 14,000 × g for 30°C. The supernatant was divided into three or four aliquot tubes to the following measurements (Shen et al., 2012). Total protein of blood and mucosa samples were analyzed with Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc. Rockford, IL).

As a mediator of inflammatory responses, TNF-α level in blood and mucosa samples was measured by Porcine Immunoassay ELISA Kit (PTA00; R&D System Inc. Minneapolis, MN) as described by Weaver et al. (2014). The detection limit range for TNF-α ELISA was 2.8 to 5.0 pg/mL. Concentrations of TNF-α in mucosa and blood samples were expressed as pg/mg protein and pg/mL, respectively.

As an oxidative stress indicator, MDA was analyzed using Thiobarbituric Acid Reactive Substance Assay Kit (STA-330, Cell Biolabs, San Diego, CA) following the instruction of Weaver et al. (2014). The detection range for this ELISA was 5 to 130 μM. Concentrations of MDA in mucosa and blood samples were expressed as μmol/g protein and μM, respectively.

Concentrations of PYY in plasma samples were analyzed using Porcine PYY ELISA Kit (PP0179, NeoBiolab, Cambridge, MA) following the instruction of Sevarolli Loftus (2015). The detection limit for this kit was 1.0 pg/mL. Concentrations of PYY in plasma samples were expressed pg/mL.

Tight Junction Proteins

Four samples of jejunal tissue in each treatment were used to measure tight junction protein as described by Yang et al. (2015). Tissue samples (100 mg) of jejunum were weighed and suspended into 0.5 mL RIPA lysis and extraction buffer containing 5 μL protease inhibitor cocktail. Tissue samples were homogenized (Tissuemiser; Thermo Fisher Scientific Inc., Rockford, IL) on ice. The homogenate was centrifuged at 10,000 × *g* at 4°C for 10 min to collect supernatant. Protein concentration of the supernatant was adjusted to 8 μg/μL by using a BCA protein assay as mentioned above. The adjusted supernatant was denatured at 100°C for 5 min in the water bath, and was loaded in each well for SDS-PAGE. After SDS-PAGE, the gel was moved on polyvinylidene difluoride (PVDF) membrane for transferring a target protein to membrane. Protein was electrophoretically transferred at 90 mV for 1 hour. These was then blocked in 5% skim milk, and incubated (overnight at 4°C) with primary antibodies against claudin, occludin, zonula occludens (ZO)-1, and β-actin. The

membrane was subsequently washed and incubated (1 h at room temperature) with horseradish-conjugated secondary antibodies. The immunoblot was developed with the DAB substrate kit (34002; Pierce, Rockford, IL). Density of bands was identified by using image analyzer software (LI-COR Biosciences, Lincoln, NE).

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). In all the experiments, the experiment unit was the pen. Initial BW block was considered a random effect. In Exp. 1, mannanase inclusion and sex block were considered fixed effects; in Exp. 2, supplementation of DDGS and xylanase, their interaction, and sex were considered as fixed effects; in Exp. 3, supplementation of xylanase and mannanase, their interaction, and sex were considered fixed effects. Statistical differences were considered significant with $P < 0.05$. Probabilities that is less than 0.10 and equal or greater than 0.05 were considered as tendencies.

RESULTS

Growth Performance

In Exp. 1, supplementation of mannanase did not affect ADG and ADFI during the entire period (Table 4). However, supplementation of mannanase improved ($P < 0.05$) G:F during phase 1 (0.593 to 0.617) and the overall period (0.572 to 0.589).

In Exp. 2, adding DDGS did not affect ADG during either wk or the entire period. Xylanase supplementation tended to increase ($P = 0.061$) ADG during wk 2, and increased ($P < 0.05$) ADG from 616 to 660 g/d during the entire period. Adding DDGS increased ($P <$

0.05) ADFI in wk 3 from 1,141 to 1,267 g/d. An interaction ($P < 0.05$) was observed, indicating that xylanase supplementation might have opposite effect on ADFI of pigs fed diets with 0 or 30% DDGS. Supplemental xylanase or DDGS did not affect G:F during the entire period (Table 5).

In Exp. 3, the initial BW of four treatments were 7.55 ± 0.96 kg at age of wk 4(Table 6). Neither mannanase nor xylanase supplementation affected growth performance, but a tendency ($P = 0.056$) for G:F was observed on the interaction between the two enzyme supplementations.

Viscosity and pH of Digesta

In Exp. 1, supplementation of mannanase reduced ($P < 0.05$) viscosity of jejunal digesta (2.52 to 1.97 cP, respectively). Supplementation of mannanase increased ($P < 0.05$) pH of colon digesta (5.99 to 6.33), but did not change pH of jejunal and ileal digesta (Table 7).

In Exp. 2, supplemental DDGS increased ($P < 0.05$) viscosity of jejunal digesta from 1.86 to 2.38 cP, whereas supplemental xylanase reduced ($P < 0.05$) viscosity from 2.27 to 1.96 cP. There was no interaction between xylanase and DDGS supplementations (Table 8).

Apparent Ileal Digestibility

In Exp. 1, supplementation of mannanase tended to increase AID of DM ($P = 0.093$, 75.4 to 79.1%) and GE ($P = 0.099$, 74.5 to 78.1%). Supplementation of mannanase increased ($P < 0.05$) AID of NDF (31.3 to 49.1%) and ADF (26.8 to 38.7%) (Table 9).

In Exp. 2, supplemental DDGS reduced ($P < 0.05$) AID of DM and GE from 72.3 to 67.8%, and 71.7 to 66.2%, respectively. Supplemental xylanase tended to improve ($P =$

0.072) AID of DM, and increased ($P < 0.05$) AID of GE and NDF from 66.5 to 71.5%, and 45.2 to 52.4%, respectively. No interaction effects between xylanase and DDGS supplementation were observed in AID of nutrients (Table 10).

Histomorphology and Immunohistochemistry for Ki-67

In Exp. 1, supplementation of mannanase tended to increase ($P = 0.094$) the ratio of villus height to crypt depth in duodenum. In jejunum, mannanase tended to increase ($P = 0.097$) villus height, decreased ($P < 0.05$) crypt depth, and improved ($P < 0.05$) the ratio of villus height to crypt depth. In ileum, mannanase increased ($P < 0.05$) villus height and the ratio of villus height to crypt depth (Table 11).

In Exp. 2, supplemental DDGS tended to reduce ($P = 0.063$) villus height, and reduced ($P < 0.05$) the ratio of villus height to crypt depth in duodenum (Table 12). Supplemental xylanase increased *(*$P < 0.05$) crypt depth in duodenum. A tendency ($P = 0.052$) for crypt depth of jejunum was observed on the interaction between DDGS and xylanase supplementation.

In Exp. 3, xylanase increased *(*$P < 0.05$) villus height in duodenum, and proliferation rate in the crypt of jejunum (Table 13).

Immune and Oxidative Status

In Exp. 1, supplementation of mannanase showed no significant influence on MDA concentration in either blood or mucosa samples, and tended to reduce ($P = 0.078$) TNF-α concentration (7.94 to 6.46 pg/mg) in jejunal mucosa (Table 14).

In Exp. 2, supplemental DDGS increased ($P < 0.05$) TNF-α level in colon tissue from 7.08 to 8.06 pg/mg protein (Table 15). Concentrations of TNF-α and PYY in serum were decreased ($P < 0.05$) by supplemental xylanase.

In Exp. 3, supplementation of mannanase tended to decrease ($P = 0.057$) MDA level in jejunum (Table 16).

Tight Junction Proteins

In Exp. 3, supplementation of xylanase increased ($P < 0.05$) expressions of claudin, occludin, and ZO-1, and mannanase increased ($P < 0.05$) expressions of occludin. An interaction for the expression of ZO-1 was observed between xylanase and mannanase supplementations (Figure 1).

DISCUSSION

In Exp. 2, pigs fed diets with and without DDGS were compared, and overall growth performance of pigs with DDGS did not differ from those fed corn and soybean meal based diet. Since the 1970's when the ethanol industry started, numerous studies have been conducted to evaluate the effect of supplementing DDGS to the diet of nursery pigs, showing that increasing dietary DDGS up to 30% did not affect their growth performance (Stein and Shurson, 2009; Jacela et al., 2011). Limited data have focused on the digesta viscosity of nursery pigs fed with DDGS in previous research. In growing pigs, the viscosity for corn-soybean meal was 1.78 cP in ileum and 1.71 cP in cecum, and there was no significant effect on the viscosity of ileal and cecal digesta when supplementing 30% corn/wheat mixed DDGS to the diets (Agyekum et al., 2012). However, in the current study, we observed that

supplementing 30% DDGS to the diet increased jejunal digesta viscosity from 1.97 cP to 2.57 cP. The NSP compositional profiles of DDGS showed that it had greatly increased content of total NSP and soluble NSP, when compared with its corresponding corn. Therefore, the increased soluble NSP in DDGS could cause higher digesta viscosity (Dikeman et al., 2006).

Increased digesta viscosity was associated with decreased AID of DM and GE, but did not affect the AID of NDF and ADF, indicating that 30% DDGS supplementation may decrease the digestibility of other components, such as protein, fat, and starch, by limiting their access to digestive enzymes in the gut. Interestingly, such reduced nutrient digestibility might be balanced by increased ADFI at wk 3, in order to achieve the similar weight gain of pigs fed corn-soybean meal. In contrast, Urriola and Stein (2010) found that the AID of GE was not different between corn and soybean meal diet with and without DDGS. These inconsistences in digesta viscosity and ileal digestibility could be attributed to the age of the animal: older pigs, which are capable of digesting high NSP diets more efficiently, would be expected to be less sensitive to dietary intervention than nursery pigs (Adeola and Cowieson, 2011).

The results from Exp. 2 also showed that, TNF-α level in colon tissue was increased by the inclusion of DDGS, indicating that the intestinal immune systems were stimulated by a higher level of NSP from dietary DDGS, which resulted in a typical inflammation response. However, levels of MDA were not affected by the inclusion of DDGS. Song (2012) also did not observe any difference in serum MDA between nursery pigs fed diets with 0 or 30% highly oxidized DDGS. However, the concentrations of MDA in plasma of finisher pig was

increased by adding 15% DDGS (Li et al., 2012). Such responses could be attributed to the difference in the age of testing pig and the source of DDGS. We also observed that 30% DDGS supplementation decreased the ratio of villus height to crypt depth and tended to decrease villus height in the duodenum. The reduced ratio mainly resulted from shorter villus height in our study. Similarly, 30% corn/wheat DDGS tended to decrease villus height and the ratio of villus height to crypt depth in the ileum (Agyekum et al., 2012). The decreased villus height is usually associated with reduced contact surface area in the gut lumen and inhibited nutrient absorption (Caspary, 1992). Therefore, supplementing DDGS to swine diets could bring negative effect to gut health by increasing TNF-α level and impairing gut morphology.

The type of xylanase used in the current studies was endo-1,4-β-xylanase and its direct involvement in xylan hydrolysis is to cleave the glycosidic bonds between xyloses, and liberate xylooligosaccharides or monosaccharides (Motta et al., 2013). Mannanase used in the present studies is supposed to catalyze the random hydrolysis of β-D-1,4 mannopyranoside linkages, and produce oligosaccharides and small amounts of mannoses (Dhawan and Kaur, 2007). Both enzymes are NSP degrading enzymes. Supplemental NSP enzyme could act on the corn and soybean meal based diets with DDGS, by destroying the plant cell wall, increasing the contact between endogenous enzyme and nutrient substrates, and improving the digestion of energy and DM. In poultry, NSP fractions were reported to increase digesta viscosity, and result in poor nutrient absorption in the small intestine (Smits et al., 1997), while supplemental NSP enzyme reduced digesta viscosity and improved feed

conversion ratio in birds (Chesson, 2001). The mechanism might be the depolymerization of soluble NSP by endo-acting enzymes (Adeola and Cowieson, 2011). In pigs, reduction in digesta viscosity caused by exogenous NSP enzymes has been found, especially in wheat or barley diets (Svihus, 2001). Less evidence has been reported on viscosity of pigs fed diets with DDGS. In current studies, supplemental NSP enzyme reduced viscosity of jejunal digesta.

Reduced jejunal viscosity was also related with increased AID of nutrients. In Exp. 1, the AID of NDF and ADF were improved by mannanase; in Exp.2, the AID of NDF and GE were increased by 7 and 5%, respectively. In grower pigs, supplementation of NSP enzyme increased ATTD of DM and GE in pigs fed diets with mixed grains and co-products (Kiarie et al., 2012). Single supplementation of mannanase positively affected digestibility of CP in grower-finisher pigs fed diets with 10 or 15% DDGS (Yoon et al., 2010). In addition, an increase in nutrient digestibility and growth performance were observed in some studies, but such response was not consistent across all experiments. For example, Pettey et al. (2002) found that supplemental mannanase improved growth performance but had no effect on nutrient digestibility, whereas Kim et al. (2003) found that supplemental NSP enzymes improved both feed efficiency and digestibilities of energy and amino acid.

Supplemental enzymes might also function through improving gut health. Gut health is a comprehensive and complex definition, which has three major components: the diet to provide sufficient nutrients, the mucosa to provide functional digestive system and intact gut barrier, and the microbial community to maintain a balanced and health micro-environment

(Montagne et al., 2003). As part of lumen micro-environment, pH values of digesta from Exp. 1 fell into the normal range, and showed no significant difference except pH value of colon digesta, indicating that supplemental mannanase increased the pH of colon. As we expected, supplemental NSP enzymes might affect the fermentation activity in the large intestine, because the substrates for bacteria are partially hydrolyzed before entering the hindgut. Short-chain fatty acids are produced during bacterial fermentation. Increased SCFA have been related with depletion of pathogens due to decreased pH, but in vitro studies suggested bacterial preference for specific molecular weights of fiber (Stewart and Slavin, 2006). In that case, supplemental mannanase may not alter SCFA production of bacterial fermentation because it would hydrolyze mannans into particles at some particular size not preferred by microbes. Another reason might be that about 90% of these SCFA are rapidly absorbed by the colon, which attributes to a constant pH-microclimate at the epithelial surface (Rechkemmer et al., 1988). So, it is probably unable to detect the changes of fermentation activity only through pH measurements, without considering factors like fluid secretions and microflora variety (Mathew et al., 1993). However, these speculations could not explain the increased pH value of colon digesta observed in the current study.

Supplemental NSP enzymes reduced crypt depth in jejunum in Exp. 1 and 2. In swine, Agyekum et al. (2012) showed no differences in the morphology of duodenum, jejunum, and colon with supplemental NSP enzymes, while Kim et al. (2003) found that feeding NSP enzymes increased villus height. The intestinal structure can partially reveal gut functions. Factors in the lumen content can lead to relatively quick changes in the mucosa because of

the interaction between the mucosal surface and the intestinal digesta. Another interesting finding was that xylanase increased proliferation rate of crypt cells in jejunum in Exp. 3. Ki-67 protein, as a proliferation marker, is present in active phased of the cell cycle, but not in the resting cells (Scholzen and Gerdes, 2000). Mucosal cell proliferation is essential for the maintenance of the integrity of the gastrointestinal barriers (Wong and Wright, 1999). Cell proliferation in the gut can be regulated through luminal nutrients and systemic factors, such as hormones and growth factors, and also be affected under pathological conditions (Wong and Wright, 1999). Without affecting the morphology, increased cell proliferation in the intestine is beneficial to maintain gut health of pigs.

The stimuli of proliferation regulation in the gut could be oxidative stress and inflammation (Assimakopoulos et al., 2004; Reuter et al., 2010). Lipid peroxidation, measured as the concentration of MDA, is an important indicator of oxidative stress. No effect of supplemental mannanase on MDA was found in growing-finishing pigs (Ao et al., 2011; Li et al., 2012). In Exp. 3, MDA concentration in jejunum tended to be decreased by mannanase supplementation, indicating that dietary mannanase seems to have anti-oxidative capability in younger pigs possibly by the hydrolysis of mannans and the production of oligosaccharides (Wang et al., 2008). This could be related with the increased proliferation rate in jejunum.

Tumor necrosis factor-α, known as a pro-inflammatory cytokine, is produced in response to tissue damage, affects intestinal epithelial permeability, and functions as a regulator in growth and differentiation of many immune cells (McKay and Baird, 1999).

Supplemental NSP enzyme suppressed TNF-α concentration in the blood and mucosa. The possible mechanism of NSP enzyme on regulating immune response could be that dietary NSP are hydrolyzed into oligosaccharides. These molecules might inhibit tumor formation, and enhance defense against bacterial challenge (Wang et al., 2010). Another mechanism might be that more efficient assimilation of nutrients were caused by supplemental enzymes. Insufficient nutrition supply is implicated in activation of the immune system (Cook, 1996).

In pigs, peptide YY, as a gastrointestinal peptide, mainly exists in endocrine cells of distal intestine (Wewer Albrechtsen, et al., 2016), and may mediate gastric emptying, inhibit feed intake, and also interfere with other hormones like cholecystokinin and gastrin (Ballantyne, 2006). The decreased PYY level in piglets was also reported by May et al. (2015). However, Singh et al. (2012) observed that supplementation of xylanase increased plasma PYY concentration of broilers. The inconsistent observations suggested that the action of xylanase on PYY regulation is probably different between pigs and broilers. Peptide YY seems not the only determining factor of feed intake, because ADFI in this study was not affected by decreased circulating PYY concentration. However, these hormones could possibly affect gut morphology (Wong and Wright, 1999).

As part of gut health, intestinal permeability is closely related with tight junction proteins. Tight junctions consist mainly of claudins, occludins, and ZO. Expression of these proteins could be mediated by the production of TNF-α (Shen, 2012), indicating that reduced TNF-α was associated with the improved intestinal permeability. One important finding of Exp. 3 was that supplementation of xylanase increased the expression of ZO-1, claudin, and

occludin in jejunum, while mannanase improved the expression of occludin. Similar, xylanase addition increased mRNA expression of occludin in the ileum after *Clostridium perfringens* challenge (Liu et al., 2012). Therefore, dietary NSP enzyme supplementation could maintain the integrity of intestinal barrier, and prevent macromolecular transmission.

In conclusion, supplementation of 30% DDGS in the nursery diets did not decrease growth performance of pigs, but could be potentially harmful to gut health of pigs. Supplementation of NSP enzymes to a DDGS containing diet altered digesta viscosity, increased nutrient digestibility, and improved gut health of nursery pigs. The protective effects of NSP enzyme on gut health might therefore, at least partly, rely on reducing oxidative stress and inflammation, regulating tight junction proteins expression, and improving intestinal proliferation in pigs.

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Table 1. Composition of experimental diets with or without mannanase (Exp. 1)

Item	Phase[1] 1		Phase[1] 2	
Mannanase, U[2]/kg	0	400	0	400
Ingredient, %				
Yellow corn	50.09	50.04	57.48	57.43
DDGS[3]	20.00	20.00	20.00	20.00
Soybean meal	27.20	27.20	20.00	20.00
Supplement[4]	0.00	0.05	0.00	0.05
L-Lys HCl	0.40	0.40	0.30	0.30
DL-Met	0.05	0.05	0.00	0.00
L-Thr	0.06	0.06	0.02	0.02
Salt	0.22	0.22	0.22	0.22
Vitamin premix[5]	0.03	0.03	0.03	0.03
Trace mineral premix[6]	0.15	0.15	0.15	0.15
Dical Phosphate	0.65	0.65	0.70	0.70
Limestone	1.15	1.15	1.10	1.10
Total	100.00	100.00	100.00	100.00
Calculated composition,				
ME, kcal/kg	3,297	3,297	3,302	3,302
Lys[7], %	1.23	1.23	0.98	0.98
Met + Cys[7], %	0.70	0.70	0.58	0.58
Trp[7], %	0.22	0.22	0.18	0.18
Thr[7], %	0.74	0.74	0.60	0.60
Ca, %	0.70	0.70	0.67	0.67
Available P, %	0.34	0.34	0.33	0.33
Analyzed composition,				
DM, %	91.33	91.13	91.09	90.97
GE, kcal/kg	-	-	4,030	4,011
NDF, %	10.9	11.0	11.7	11.5
ADF, %	5.08	5.36	5.04	5.01
Mannanase activity[8], U/kg	0	630	0	790

[1]The experimental period was divided into two parts: phase 1 (1-21 d) and phase 2 (22-40 d).

[2]U: unit

[3]DDGS: distillers dried grains and solubles

[4]Supplement was mannanase (CTCBIO Inc., Seoul, Korea) at 0.05% replacing corn for treatment diets.

Table 1. Continued

[5]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D_3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B_{12}; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

[6]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganese oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[7]Standardized ileal digestible

[8]Mannanase activity: One unit (U) is defined as the amount of enzyme that released one umol mannose from mannan per minute under 0.2 M sodium phosphate (pH 6) at 50 °C.

Table 2. Ingredient composition of experimental diets supplemented with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment			
DDGS[1], %	0		30	
Xylanase, EPU[2]/kg diet	0	1,500	0	1,500
Ingredient, %				
Yellow corn	62.10	62.09	37.41	37.40
DDGS	0.00	0.00	30.00	30.00
Soybean meal	33.00	33.00	28.00	28.00
Xylanase[3]	0.00	0.01	0.00	0.01
L-Lys HCl	0.34	0.34	0.35	0.35
DL-Met	0.12	0.12	0.02	0.02
L-Thr	0.10	0.10	0.02	0.02
Poultry fat	2.00	2.00	2.00	2.00
Salt	0.22	0.22	0.22	0.22
Vitamin premix[4]	0.03	0.03	0.03	0.03
Trace mineral premix[5]	0.15	0.15	0.15	0.15
Dicalcium phosphate	1.10	1.10	0.64	0.64
Limestone	0.84	0.84	1.16	1.16
Total	100.00	100.00	100.00	100.00
Calculated content,				
ME, kcal/kg	3,384	3,384	3,395	3,395
Lys[6], %	1.25	1.25	1.25	1.25
Met + Cys[6], %	0.70	0.70	0.70	0.70
Trp[6], %	0.23	0.23	0.23	0.23
Thr[6], %	0.75	0.75	0.75	0.75

Table 2. Continued

Ca, %	0.70	0.70	0.70	0.70
Available P, %	0.34	0.34	0.34	0.34
Analyzed content,				
DM, %	87.54	87.89	87.35	88.21
GE, kcal/kg	3,878	3,935	4,023	4,091
NDF, %	11.78	11.84	24.00	24.62
ADF, %	3.18	3.23	8.01	7.48
Xylanase activity, EPU/kg diet	270	1,980	310	2,050

[1]DDGS: distillers dried grains with solubles

[2]EPU: endo-pentosanase unit

[3]Xylanase source was Hostazym X 100 (Huvepharma USA, Peachtree City, GA) at 0.01% replacing corn for treatment diets.

[4]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

[5]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[6]Standardized ileal digestible

Table 3. Composition of the starter diet and experimental diets with mannanase and/or xylanase (Exp. 3)

Item	Diet composition	
	Starter (d 1-5)	Experiment (d 6-20)
Ingredient, %		
Yellow corn	38.34	39.65
DDGS[1]	0.00	15.00
Soybean meal	23.00	23.00
Blood plasma	4.00	2.00
Whey permeate	20.00	10.00
Fish meal	4.00	2.00
Poultry meal	5.00	2.00
L-Lys HCl	0.40	0.44
DL-Met	0.20	0.12
L-Thr	0.12	0.10
Poultry fat	1.50	1.70
Animal and vegetable blend oil	1.50	1.70
Salt	0.22	0.22
Vitamin premix[2]	0.03	0.03
Trace mineral premix[3]	0.15	0.15
Zinc oxide	0.25	0.25
Dicalcium phosphate	0.20	0.46
Limestone	0.71	1.05
Mecadox 10	0.38	0.13
Total	100.00	100.00
Calculated content		
ME, kcal/kg	3,463	3,473
Lys[4], %	1.50	1.35
Met + Cys[4], %	0.82	0.74
Trp[4], %	0.25	0.22
Thr[4], %	0.88	0.80
Ca, %	0.86	0.80
Available P, %	0.46	0.40

[1]DDGS: distillers dried grains and solubles

[2]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

Table 3. Continued

[3]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[4]Standardized ileal digestible

Table 4. Growth performance of pigs fed diets supplemented with mannanase (Exp. 1)

Item	Mannanase, U[1]/kg		SEM	*P*-value
	0	400		
BW, kg				
Initial	17.65	17.62	0.09	0.806
Wk 1	21.68	22.05	0.38	0.359
Wk 2	25.64	25.96	0.57	0.583
Wk 3	30.44	31.68	1.60	0.451
Wk 4	37.31	38.19	0.84	0.311
Wk 5	43.36	44.22	1.39	0.547
ADG, g/day				
Wk 1	600	642	41	0.328
Wk 2	557	605	48	0.330
Wk 3	861	817	35	0.228
Wk 4	914	930	22	0.626
Wk 5	892	862	25	0.406
Wk 1 to 3	672	688	17	0.547
Wk 4 to 5	903	896	16	0.757
Overall	788	792	11	0.802
ADFI, g/day				
Wk 1	943	935	52	0.874
Wk 2	1088	1069	52	0.723
Wk 3	1399	1376	62	0.721
Wk 4	1685	1622	41	0.290
Wk 5	2061	2009	51	0.488
Wk 1 to 3	1143	1127	29	0.698
Wk 4 to 5	1873	1815	35	0.266
Overall	1387	1356	24	0.395
G:F				
Wk 1	0.640	0.697	0.032	0.093
Wk 2	0.509	0.571	0.034	0.088
Wk 3	0.625	0.596	0.024	0.258
Wk 4	0.544	0.584	0.024	0.252
Wk 5	0.434	0.433	0.011	0.981
Wk 1 to 3	0.593	0.617	0.007	0.044
Wk 4 to 5	0.483	0.498	0.008	0.241
Overall	0.572	0.589	0.005	0.037

[1]U: unit

Table 5. Growth performance of pigs fed diets supplemented with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment				SEM	*P*-value[1]		
DDGS, %	0		30					
Xylanase, EPU[2]/kg diet	0	1,500	0	1,500		Ing	Enz	Ing x Enz
BW, kg								
Initial	10.58	10.68	10.64	10.73	0.63	0.630	0.392	0.932
Wk 1	13.07	13.35	13.31	13.44	0.49	0.480	0.376	0.746
Wk 2	18.00	18.87	18.64	19.03	0.57	0.149	0.029	0.385
Wk 3	23.14	24.48	23.93	24.66	0.68	0.288	0.029	0.499
ADG, g/day								
Wk 1	357	381	381	388	31	0.619	0.619	0.767
Wk 2	704	788	762	799	32	0.273	0.061	0.450
Wk 3	734	802	756	804	39	0.762	0.151	0.806
Overall	598	657	633	663	20	0.305	0.032	0.479
ADFI, g/day								
Wk 1	637	673	624	658	41	0.640	0.270	0.974
Wk 2	1,015	1,098	1,066	1,127	60	0.412	0.150	0.827
Wk 3	1,085	1,196	1,315	1,218	51	0.015	0.888	0.041
Overall	913	989	1,002	1,001	37	0.112	0.227	0.220
G:F								
Wk 1	0.562	0.570	0.610	0.571	0.030	0.420	0.604	0.442
Wk 2	0.717	0.720	0.721	0.710	0.030	0.931	0.904	0.813
Wk 3	0.682	0.675	0.579	0.671	0.034	0.128	0.223	0.158
Overall	0.665	0.669	0.633	0.664	0.020	0.380	0.402	0.498

[1]Ing: main effect of DDGS; Enz: main effect of xylanase; Ing x Enz: interaction effect between DDGS and xylanase

[2]EPU: endo-pentosanase unit

Table 6. Growth performance of pigs fed DDGS based diets with mannanase and/or xylanase (Exp. 3)

Item	Treatment[1]				SEM	*P*-value[2]		
	Con	Man	Xyl	Xyl + Man		Xyl	Man	Xyl × Man
BW, kg								
D 0			6.21					
D 6	7.53	7.63	7.52	7.53	0.511	0.729	0.716	0.766
D 13	11.14	10.92	10.96	11.08	0.782	0.974	0.885	0.585
D 20	14.72	14.94	14.91	15.40	0.935	0.503	0.465	0.787
ADG, g/day								
D 6 to 13	515	470	490	507	41.20	0.859	0.676	0.372
D 13 to 20	641	670	660	720	32.44	0.424	0.294	0.709
D 6 to 20	553	563	568	605	34.89	0.356	0.462	0.666
ADFI, g/day								
D 6 to 13	588	516	555	557	40.88	0.908	0.312	0.277
D 13 to 20	842	864	840	982	53.07	0.321	0.167	0.311
Overall	706	676	686	753	53.26	0.511	0.668	0.272
G:F								
D 6 to 13	0.891	0.883	0.890	0.902	0.017	0.729	0.940	0.709
D 13 to 20	0.749	0.774	0.787	0.735	0.026	0.983	0.587	0.149
D 6 to 20	0.779	0.831	0.833	0.803	0.015	0.534	0.607	0.056

[1]Con: control diet; Man: Con with 400 U mannanase/kg feed; Xyl: Con with 1,500 EPU xylanase/kg feed; Xyl + Man: Con with 400 U mannanase and 1,500 EPU xylanase/kg feed

[2]Xyl: main effect of xylanase; Man: main effect of mannanase; Xyl x Man: interaction effect between xylanase and mannanase

Table 7. Viscosity of jejunal digesta (cP[1]), and pH values of digesta from jejunum, ileum, and colon of pigs fed diets with mannanase (Exp. 1)

Item	Mannanase, U[2]/kg		SEM	*P*-value
	0	400		
Viscosity, cP				
Distal jejunum	2.52	1.97	0.24	0.041
pH				
Jejunum	5.69	5.79	0.14	0.610
Ileum	5.64	5.50	0.13	0.387
Colon	5.99	6.33	0.08	0.005

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

[2]U: unit

Table 8. Viscosity of jejunal digesta (cP[1]) in pigs fed diets supplemented with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment				SEM	*P*-value[2]		
DDGS, %	0		30					
Xylanase, EPU[3]/kg diet	0	1,500	0	1,500		Ing	Enz	Ing x Enz
Viscosity, cP	1.97	1.74	2.57	2.18	0.17	0.004	0.023	0.569

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

[2]Ing: main effect of DDGS; Enz: main effect of xylanase; Ing x Enz: interaction effect between DDGS and xylanase

[3]EPU: endo-pentosanase unit

Table 9. Apparent ileal digestibility (AID) of DM, GE, NDF, and ADF in pigs fed mannanase (Exp. 1)

Item	Mannanase, U[1]/kg		SEM	*P*-value
	0	400		
AID, %				
DM	75.4	79.1	1.95	0.093
GE	74.5	78.1	2.00	0.099
NDF	31.3	49.1	4.01	0.001
ADF	26.8	38.7	3.95	0.013

[1]U: unit

Table 10. Apparent ileal digestibility (AID) of DM, GE, NDF, and ADF in pigs fed diets with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment				SEM	*P*-value[1]		
DDGS, %	0		30					
Xylanase, EPU[2]/kg diet	0	1,500	0	1,500		Ing	Enz	Ing x Enz
AID, %								
DM	71.3	75.2	65.8	69.7	2.1	0.013	0.072	0.993
GE	69.3	74.1	63.6	68.8	2.3	0.022	0.035	0.922
NDF	48.6	51.2	41.7	53.6	3.1	0.469	0.025	0.140
ADF	36.5	38.4	38.1	41.0	2.2	0.376	0.315	0.833

[1]Ing: main effect of DDGS; Enz: main effect of xylanase; Ing x Enz: interaction effect between DDGS and xylanase

[2]EPU: endo-pentosanase unit

Table 11. Villus height, villus width, crypt depth, and the ratio of villus height to crypt depth of duodenum, jejunum, and ileum in pigs fed diets with mannanase (Exp. 1)

Item	Mannanase, U[1]/kg		SEM	*P*-value
	0	400		
Duodenum				
Villus height, µm	443	454	19	0.636
Villus width, µm	132	132	5	0.869
Crypt depth, µm	353	327	21	0.207
Villus height/crypt depth	1.26	1.40	0.06	0.094
Jejunum				
Villus height, µm	379	440	32	0.097
Villus width, µm	97	101	4	0.485
Crypt depth, µm	249	212	17	0.024
Villus height/crypt depth	1.58	2.10	0.18	0.035
Ileum				
Villus height, µm	377	432	17	0.026
Villus width, µm	113	109	4	0.532
Crypt depth, µm	233	215	13	0.101
Villus height/crypt depth	1.65	2.02	0.08	0.013

[1]U: unit

Table 12. Villus height, villus width, crypt depth, and the ratio of villus height to crypt depth of duodenum, jejunum, and colon in pigs fed diets with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment				SEM	*P*-value[1]		
DDGS, %	0		30					
Xylanase, EPU[2]/kg diet	0	1,500	0	1,500		Ing	Enz	Ing x Enz
Duodenum								
Villus height, μm	529	523	475	504	38	0.063	0.542	0.366
Villus width, μm	174	172	168	171	11	0.673	0.977	0.776
Crypt depth, μm	359	378	360	430	21	0.168	0.025	0.191
Villus height/crypt depth	1.49	1.42	1.34	1.20	0.08	0.027	0.210	0.685
Jejunum								
Villus height, μm	452	481	454	462	18	0.634	0.318	0.558
Villus width, μm	120	118	120	116	3	0.833	0.397	0.703
Crypt depth, μm	218	231	237	210	11	0.885	0.479	0.052
Villus height/crypt depth	2.11	2.14	1.93	2.24	0.14	0.754	0.222	0.272
Colon								
Crypt depth, μm	422	406	432	418	13	0.430	0.277	0.962

[1]Ing: main effect of DDGS; Enz: main effect of xylanase; Ing x Enz: interaction effect between DDGS and xylanase

[2]EPU: endo-pentosanase unit

Table 13. Villus height, villus width, crypt depth, and the ratio of villus height to crypt depth of duodenum and jejunum, and the proliferation rate of jejunal crypt cells in pigs fed diets with mannanase and/or xylanase (Exp. 3)

Item	Treatment[1]				SEM	*P*-value[2]		
	Con	Man	Xyl	Xyl + Man		Xyl	Man	Xyl × Man
Duodenum								
Villus height, μm	549	535	614	561	21	0.038	0.121	0.364
Villus width, μm	137	141	138	142	4	0.791	0.349	0.884
Crypt depth, μm	337	337	366	332	14	0.382	0.222	0.213
Villus height/crypt depth	1.63	1.61	1.68	1.70	0.06	0.239	0.996	0.763
Jejunum								
Villus height, μm	433	406	435	474	28	0.226	0.841	0.246
Villus width, μm	96	103	101	104	3	0.262	0.102	0.436
Crypt depth, μm	201	191	201	211	11	0.377	0.999	0.399
Villus height/crypt depth	2.18	2.13	2.22	2.31	0.17	0.527	0.896	0.709
Proliferation, %	37.4	41.8	34.8	34.8	1.8	0.015	0.241	0.246

[1]Con: control diet; Man: Con with 400 U mannanase/kg feed; Xyl: Con with 1,500 EPU xylanase/kg feed; Xyl + Man: Con with 400 U mannanase and 1,500 EPU xylanase/kg feed

[2]Xyl: main effect of xylanase; Man: main effect of mannanase; Xyl x Man: interaction effect between xylanase and mannanase

Table 14. Malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α) levels of serum, duodenum, jejunum, and ileum samples in pigs fed diets with mannanase (Exp. 1)

Item	Mannanase, U[1]/kg		SEM	*P*-value
	0	400		
MDA				
Serum, uM	3.73	3.70	0.39	0.957
Duodenum, umol/g protein	0.87	0.75	0.09	0.354
Jejunum, umol/g protein	0.99	1.31	0.17	0.101
Ileum, umol/g protein	0.79	0.87	0.08	0.459
TNF-α				
Serum, pg/mL	88.49	77.80	23.29	0.656
Duodenum, pg/mg protein	6.70	7.59	0.88	0.329
Jejunum, pg/mg protein	7.94	6.46	0.74	0.078
Ileum, pg/mg protein	5.28	4.67	0.56	0.262

[1]U: unit

Table 15. Tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), and peptide YY (PYY) levels in pigs fed diets with distillers dried grains with solubles (DDGS) and xylanase (Exp. 2)

	Treatment				SEM	*P*-value[1]		
DDGS, %	0		30					
Xylanase, EPU[2]/kg diet	0	1,500	0	1,500		Ing	Enz	Ing x Enz
Plasma								
TNF-α, pg/mL	107.58	66.63	109.31	73.11	22.72	0.809	0.028	0.889
MDA, µM	13.79	12.40	12.21	11.95	2.89	0.727	0.777	0.847
PYY, pg/mL	56.43	33.24	49.71	45.74	6.07	0.642	0.036	0.131
Jejunum								
TNF-α, pg/mg	7.05	6.36	6.12	6.79	0.56	0.666	0.989	0.233
MDA, µmol/g protein	1.84	1.87	2.17	2.10	0.36	0.449	0.967	0.890
Colon								
TNF-α, pg/mg	6.54	7.61	8.05	8.07	0.53	0.041	0.247	0.266
MDA, µmol/g protein	2.24	2.93	3.38	2.97	0.52	0.255	0.781	0.291

[1]Ing: main effect of DDGS; Enz: main effect of xylanase; Ing x Enz: interaction effect between DDGS and xylanase

[2]EPU: endo-pentosanase unit

Table 16. Malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α) levels of plasma, duodenum, and jejunum samples in pigs fed diets with mannanase and/or xylanase (Exp. 3)

Item	Treatment[1]				SEM	*P*-value[2]		
	Con	Man	Xyl	Xyl + Man		Xyl	Man	Xyl × Man
MDA								
Plasma, uM	12.91	11.14	11.51	13.93	2.06	0.739	0.876	0.317
Duodenum, umol/g protein	0.51	0.55	0.50	0.46	0.05	0.307	0.914	0.418
Jejunum, umol/g protein	0.57	0.37	0.40	0.32	0.07	0.110	0.057	0.388
TNF-α								
Plasma, pg/ml	138.29	103.60	151.25	65.43	36.00	0.733	0.111	0.490
Duodenum, pg/mg protein	2.88	3.23	3.30	3.34	0.20	0.238	0.373	0.479
Jejunum, pg/mg protein	4.37	3.61	4.18	4.25	0.35	0.537	0.335	0.250

[1]Con: control diet; Man: Con with 400 U mannanase/kg feed; Xyl: Con with 1,500 EPU xylanase/kg feed; Xyl + Man: Con with 400 U mannanase and 1,500 EPU xylanase/kg feed

[2]Xyl: main effect of xylanase; Man: main effect of mannanase; Xyl x Man: interaction effect between xylanase and mannanase

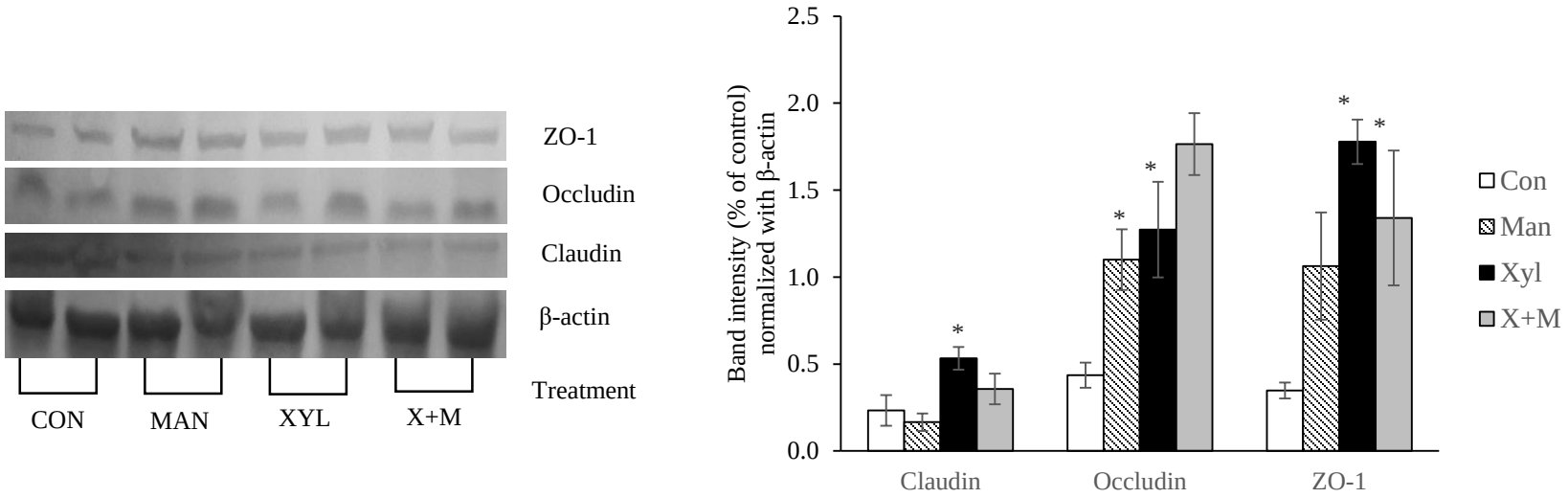

Figure 1. Expression of tight junction proteins in piglets supplemented with mannanase and/or xylanase. 3-wk-old piglets were fed with mannanase and/or xylanase for 20 days. Subsequently, proteins were isolated from the intestinal tissues of piglets and then the expression of claudin, occludin, and zonula occludens (ZO) -1 was measured using immunoblotting. Data are represented as mean ± SEM, n = 4. *$P < 0.05$

CHAPTER 4

EFFECTS OF SUPPLEMENTAL CORN EXPRESSING GLUCANASE AND CORN DISTILLER DRIED GRAINS WITH SOLUBLES ON GROWTH AND GUT HEALTH IN NURSERY PIGS

Abstract: Sixty pigs (10.2 ± 1.3 kg initial BW at 5 wk of age) were used in a 21-d trial to evaluate the effects of supplemental corn expressing β-glucanase (Agrivida, Medford, MA) on growth performance, digesta viscosity, nutrient digestibility, and gut health of nursery pigs fed diets with corn distillers dried grains with solubles (DDGS). Pigs were randomly allotted to 6 dietary treatments with a 2 × 3 factorial arrangement based on sex and initial BW. The first factor was DDGS inclusion (15 or 30%), and the second factor was glucanase supplementation (0, 150, or 450 U/kg feed). Feed intake and BW were recorded weekly. On d 14, titanium dioxide (0.3%) was blended into experimental diet as an indigestible marker. Plasma samples were collected on d 17 to measure tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), and protein carbonyl. On d 21, all pigs were euthanized to collect tissue samples (for morphology and tight junction proteins) and mucosa samples (for concentrations of TNF-α, MDA, and protein carbonyl) from duodenum and jejunum. Distal jejunal digesta was also collected to measure viscosity. Ileum digesta was collected to measure apparent ileal digestibility (AID) of nutrients. Increasing DDGS from 15% to 30% had no influence on overall growth performance. Glucanase tended to decrease ($P = 0.092$) viscosity of jejunal digesta regardless of DDGS inclusion in the diet. Increasing DDGS in the diet increased ($P < 0.05$) AID of crude fat, and tended to decrease ($P < 0.10$) AID of NDF and ADF. Glucanase tended to increase ($P = 0.076$) AID of NDF. Increasing dietary DDGS increased ($P < 0.05$) MDA level, tended ($P = 0.067$) to increase TNF-α level in duodenum, and inhibited ($P < 0.05$) proliferation rate in the jejunal crypt. Supplemental glucanase tended to decrease ($P = 0.062$) MDA concentration in duodenum. Neither DDGS nor glucanase

supplementation influenced the expression of tight junction proteins. In conclusion, increasing dietary DDGS from 15% to 30% did not affect the overall performance during 21 d, but resulted in higher oxidative stress and inflammatory response, and lower crypt proliferation in the small intestine. Supplemental glucanase could decrease digesta viscosity and oxidative stress, and improve nutrient digestibility. This suggested that supplemental glucanase has the potential to improve growth and gut health of pigs when fed with corn, soybean meal, and DDGS based diets.

Key words: β-glucanase, distillers dried grains with solubles, growth performance, gut health, nursery pigs

INTRODUCTION

Cereal β-glucans have the main chain composed of glucose residues, which are linked by β-(1→4) and (1→3) glycosidic bonds (Burton and Fincher, 2014). It is potentially harmful because it has negative effects to decrease nutrient digestibility and growth performance of pigs. Its gel-forming characteristic after water absorption can alter gastrointestinal viscosity (Brennan and Cleary, 2005). Increasing β-glucan in the diet decreased apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of CP and energy (Metzler-Zebeli and Zebeli, 2013). Dietary β-glucan intake could also modulate immune functions of animals, and regulate cytokine or free radical production (Volman et al., 2008).

Non-starch polysaccharide (NSP) degrading enzymes, like xylanase and β-glucanase, are recognized to be capable of hydrolyzing dietary NSP, and might succeed in dealing with the problems caused by NSP-rich feedstuffs in the swine diets (Adeola and Cowieson, 2011). Previous research of NSP degrading enzymes have focused on the use of specific ingredients, for example β-glucanase in barley based diets (Zijlstra et al., 2010). However, there is little information on single supplementation of glucanase used in corn distiller dried grains with solubles (DDGS) containing diets. Usually it has been combined with xylanase, phytase, or protease, and some studies showed positive effects of these dietary enzyme combinations on pigs (Kiarie et al., 2012; Passos and Kim, 2014), but such effect was not always consistent in other studies (Jones et al., 2010; Kerr et al., 2013).

There is about 2.2 to 2.9% of β-glucans in DDGS, 0.6 to 0.8% in corn, and 0.6 to 0.7% in soybean meal (Choct et al., 2010; Pedersen et al., 2014). This indicated that supplementation of DDGS in corn and soybean meal based diets could increase the content of β-glucans in the diet. Besides, as the development of transgenic technology, glucanase could be expressed in seeds of the gene-modified corn, and this kind of corn can be directly used in the animal feed to provide nutrients and glucanase without purification or premixing (Zhang et al., 2013). With such approach, the used of glucanase might be largely expanded in the feed industry. We hypothesized that supplementing this corn expressing glucanase to DDGS containing diets will be beneficial, and the inclusion of DDGS could interact with the supplementation of glucanase. The objective of this study is to determine the effect of corn expressing glucanase on growth performance, digesta viscosity, nutrient digestibility, and gut health in nursery pigs when fed with two levels of DDGS.

MATERIALS AND METHODS

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee (Raleigh, NC).

Animal and Experimental Design

The experiment was conducted at the Metabolism Educational Unit at North Carolina State University (Raleigh, NC). Sixty pigs (10.2 ± 1.3 kg) at 5 wk of age were randomly allotted to 6 dietary treatments based on a 2 × 3 factorial arrangement. The first factor was DDGS inclusion (15 or 30%), and the second factor was glucanase supplementation (0, 150, or 450 U/kg feed). The source of glucanase was ground GraINzyme corn (Agrivida,

Medford, MA) with the actual glucanase activity at 50 U/g product. Two different basal diets (99.1%) were mixed depending on the inclusion of DDGS, and then GraINzyme corn were added at the ration of 0%, 0.3%, and 0.9% with the normal corn added at 0.9%, 0.6%, and 0%, to reach the target glucanase activity 0, 150, and 450 U/kg feed in each dietary treatment. The diet composition was summarized in Table 1, and nutrient levels of all diets met the requirement suggested by NRC (2012). So, there were 6 dietary treatments, and each treatment has 5 male pens and 5 female pens (1 pig per pen). The experimental period was 21 d. Pens (1.50 × 0.74 m) with slatted floor were equipped with 1 nipple drinker and 1 self-feeder. Pigs had free access to water and feed. Body weight and feed intake were recorded weekly. Feed efficiency was calculated as G:F. Fecal score was recorded on d 0, 3, 7, 10, 14, 17, and 21, with five categories from 1 (extremely dry) to 5 (watery). On d 14, titanium dioxide (0.3%) was blended into experimental diet as an indigestible marker for measuring AID.

Sample Collection and Preparation

On d 17, blood samples of all pigs were collected from jugular vein with BD sterile vacutainers (BD, Franklin Lakes, NJ) for plasma. Blood samples were centrifuged at 3,000 × g for 15 min at 4°C. Plasma samples were stored at -80°C until analysis. Plasma samples were used to measure tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), and protein carbonyl.

On d 21, all pigs were euthanized to collect mucosa samples from duodenum and jejunum for determining concentrations of TNF-α, MDA, and protein carbonyl. Tissue

samples from jejunum were collected for western blot of tight junction proteins. These samples were stored at -80°C. Tissue samples from duodenum and jejunum were stored in 10% formalin buffer at room temperature for morphology and Ki-67 protein staining. Distal jejunal digesta (40 mL) was collected to measure viscosity immediately after euthanasia. Ileal digesta (100 mL) was collected and frozen at -20°C.

Viscosity of Digesta

The method to measure digesta viscosity was described by Passos et al. (2015) with a viscometer (Brookfield Digital Viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA). The digesta samples were centrifuged at 3,000 × g for 5 min and then the supernatant was pipetted out to a 2 mL tube and centrifuged at 12,500 × g for 5 min. Viscometer was set at 25°C, and 0.5 mL of digesta supernatant was placed in the viscometer. The final result was calculated as the average of viscosity at 45.0 sec^{-1} and 22.5 sec^{-1} shear rates.

Chemical Analysis

Before analysis, ileal digesta was freeze-dried (24D x 48, Virtis, Gardiner, NY). Feed and ileal digesta samples were ground and analyzed for DM (Method 934.01, AOAC, 2006). Titanium concentration was measured according to the modified protocol (Myers et al., 2004). The GE was determined using a calorimeter (Model 6200, Parr Instrument Company). Crude fat was quantified using a modified ether extract method (AOAC, 2006). Nitrogen in the feed and digesta samples was quantified using TruSpec N Nitrogen Determinator (LECO Corp., St. Joseph, MI) to calculate CP (6.25 × N). Samples of feed and ileal digesta were

analyzed sequentially for NDF and ADF using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp, Fairport, NY). Apparent ileal digestibility of DM, GE, Crude fat, CP, NDF, and ADF were calculated using titanium concentration of feed and digesta.

Apparent ileal digestibility of DM, GE, CP, NDF, and ADF were calculated using titanium concentration in the feed and digesta. The digestibility was calculated with the following equation:

$$\text{AID, \%} = \left(1 - \frac{Ti_{feed} \times N_{digesta}}{Ti_{digesta} \times N_{feed}}\right) \times 100\%,$$

where Ti_{feed} represents the titanium concentration in the feed, $Ti_{digesta}$ is the titanium concentration in the ileal digesta, N_{feed} represents the nutrient concentration in the feed, and $N_{digesta}$ is the nutrient concentration in the ileal digesta.

ELISA Measurement

Mocusa samples (500 mg) of duodenum and jejunum were weighed, and suspended into 1.0 mL PBS solution (MP Biomedicals, LLC. Solon, OH). Samples were homogenized on ice. The homogenate was centrifuged at 14,000 × g for 30°C. The supernatant was divided into four aliquot tubes to determine concentrations of total protein, TNF-α, MDA, and protein carbonyl (Shen et al., 2012). Total protein of plasma and mucosa samples were analyzed with Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc. Rockford, IL).

As a mediator of inflammatory responses, TNF-α level in plasma and mucosa samples was measured by Porcine Immunoassay ELISA Kit (PTA00; R&D System Inc. Minneapolis,

MN) as described by Weaver et al. (2014). The detection limit range for TNF-α ELISA was 2.8 to 5.0 pg/mL. Concentrations of TNF-α in mucosa and plasma samples were expressed as pg/mg protein and pg/mL, respectively.

As an oxidative stress indicator (lipid perioxidation), MDA was analyzed using Thiobarbituric Acid Reactive Substance Assay Kit (STA-330, Cell Biolabs, San Diego, CA) following the instruction of Weaver et al. (2014). The detection range for this ELISA was 5 to 130 μM. Concentrations of MDA in mucosa and plasma samples were expressed as μmol/g protein and μM, respectively.

As another biomarker of oxidative stress (protein oxidation), protein carbonyl content was measured using the ELISA kit (STA-310, Cell Biolabs, San Diego, CA). Concentrations of protein carbonyl in mucosa and plasma were expressed as umol/g protein.

Morphology and Immunohistochemistry for Ki-67

The segments of duodenum were embedded in paraffin, cut cross the section to a 5-m thick slides, and mounted on a polylysine-coated slide. Then slides were stained (hematoxylin and eosin) and examined under an Infinity 2-2 digital CCD camera attached to an Olympus CX31 microscope (Lumenera Corporation, Ottawa, Canada). Villus height (from the tip of the villi to the villous-crypt junction), villus width (width of the villus at the half of villus height), and crypt depth (from this junction to the base of the crypt) were determined. The ratio of villus height to crypt depth was also calculated. Lengths of 10 well-oriented intact villi and their associated crypt were measured in each slide. The same person executed all the analysis of intestinal morphology.

The segment of jejunum was fixed in the 10% formalin buffer for 3 wks, transferred into 70% ethanol solution and immediately sent to North Carolina State University Histology Lab (Raleigh, NC) for Ki-67 protein labeling. The intact crypt was cropped and Image JS software was used for calculating the ratio of Ki-67 positive cells to total cells in jejunal crypt (Almeida et al., 2012).

$$\text{Crypt cell proliferation, \%} = \frac{\text{Ki-67 positive cells}}{\text{Total cells}} \times 100\%$$

Tight Junction Proteins

Four samples of jejunal tissue in each treatment were used to measure tight junction proteins as described by Yang et al. (2015). Tissue samples (100 mg) of jejunum were weighed and suspended into 0.5 mL RIPA lysis and extraction buffer containing 5 µL protease inhibitor cocktail. Tissue samples were homogenized (Tissuemiser; Thermo Fisher Scientific Inc., Rockford, IL) on ice. The homogenate was centrifuged at 10,000 × *g* at 4ºC for 10 min to collect supernatant. Protein concentration of the supernatant was adjusted to 8 µg/µL by using a BCA protein assay as mentioned above. The adjusted supernatant was denatured at 100ºC for 5 min in the water bath, and was loaded in each well for SDS-PAGE. After SDS-PAGE, the gel was moved on polyvinylidene difluoride (PVDF) membrane for transferring a target protein to membrane. Protein was electrophoretically transferred at 90 mV for 1 hour. These was then blocked in 5% skim milk, and incubated (overnight at 4ºC) with primary antibodies against claudin, occludin, zonula occludens (ZO) -1, and β-actin. The membrane was subsequently washed and incubated (1 h at room temperature) with horseradish-conjugated secondary antibodies. The immunoblot was developed with the DAB

substrate kit (34002; Pierce, Rockford, IL). Density of bands was identified by using image analyzer software (LI-COR Biosciences, Lincoln, NE).

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). This was a randomized complete block design, and the experimental unit was the individual pen. Replicate effect based on initial BW was considered as a random effect. The supplementation of DDGS and glucanase, their interaction, and sex were considered fixed effects. Statistical differences were considered significant with $P < 0.05$. Probabilities that is less than 0.10 and equal to or greater than 0.05 were considered as tendencies.

RESULTS

Growth Performance

There was no significant difference on initial BW among treatments (Table 2). During wk 3, increasing DDGS in the diet tended to reduce ($P = 0.051$) ADG. An interaction ($P < 0.05$) was observed on the overall ADG between DDGS and glucanase supplementations. Increasing DDGS in the diet did not affect ADFI in either week or the overall period. A tendency ($P = 0.058$) for the overall ADFI was observed on the interaction between DDGS and glucanase supplementations. Increasing DDGS from 15% to 30% resulted in a higher ($P < 0.05$) G: F of pig during wk 1.

Fecal score ranged between 2.4 and 3 (Table 3). An interaction ($P < 0.05$) were observed on d 7, showing that supplemental glucanase decreased fecal score of pigs fed diets with 15% DDGS.

Viscosity and AID

Glucanase supplementation tended to decrease ($P = 0.092$) viscosity of jejunal digesta regardless of DDGS inclusion in the diet (Table 4). Increasing DDGS in the diet increased ($P < 0.05$) AID of crude fat, and tended to decrease ($P < 0.10$) AID of NDF and ADF. Supplemental glucanase tended to increase ($P = 0.076$) AID of NDF. No interaction was observed.

Immune and Oxidative Status

Increasing the inclusion of DDGS increased ($P < 0.05$) MDA level, and tended ($P = 0.067$) to increase TNF-α level in duodenum. Supplemental glucanase tended to decrease ($P = 0.062$) MDA concentration in duodenum. Protein carbonyl concentrations in plasma or mucosa samples were not affected by DDGS or glucanase. No interactions were observed.

Morphology and Immunohistochemistry for Ki-67

Increasing DDGS from 15% to 30% in the diet decreased ($P < 0.05$) proliferation rate in the jejunal crypt, and had no effect on the morphology of pigs (Table 6). Supplemental glucanase tended to reduce ($P = 0.072$) villus width in jejunum. No interactions were observed.

Tight junction proteins

Supplementation of DDGS and glucanase had no significant influence on the expression of ZO-1, occludin, and claudin (Figure 1).

DISCUSSION

In the present study, increasing dietary DDGS from 15 to 30% did not affect the overall growth performance. Similarly, Jones et al. (2010) reported that increasing dietary DDGS from 15% to 30% did not affect growth performance of nursery pigs, while Linneen et al. (2008) observed that a great reduction of ADG and ADFI occurred in growing and finishing pigs when dietary DDGS increased from 15 to 20%, and no difference was observed on G:F. This could be due to the difference on age of pigs used in these studies. For example, Jacela et al. (2011) also found the similar ADG, ADFI, and G:F of nursery pigs, but observed the decreased overall ADG and ADFI, and improved G:F of grower pigs, when dietary DDGS increased from 0 to 30% in the diet.

This similar growth performance between pigs fed diets with 15 % and 30 % DDGS, could also be supported by the result of nutrient digestibility in our study. Although AID of NDF and ADF were reduced by 4.36% and 5.62%, respectively, when dietary DDGS increased from 15% to 30%. The AID of DM, GE, and CP were not influenced by the increased inclusion level of DDGS. However, the AID of crude fat was higher when pigs fed diets with 30% DDGS. Increased fat digestibility in pigs could be attributed to increased dietary fat (Kil et al., 2010). Analyzed content of crude fat in corn and DDGS used in this study were 3.4 and 9.3% of DM, respectively. Replacement of corn with DDGS in the diet caused the increase in crude fat concentration and fat digestion in the present study.

In the current study, inhibited proliferation in jejunal crypt and increased MDA level in duodenum of pigs were observed, when dietary DDGS increased from 15% to 30%. During

the proliferation of small intestine, stem cells located at the bottom of crypt differentiate, migrate along the crypt-villus axis, and reach the top of villi, while the Paneth cell still resides at the crypt bottom (Wong and Wright, 1999). The impaired proliferation in jejunum would cause villus atrophy, and affect intestinal health. It has been recognized that the components of feedstuffs could contribute to the modulation of intestinal epithelial growth in the small intestine of pigs (Kitt et al., 2001). The mechanism how dietary DDGS changed the proliferation of crypt cells might be that polyunsaturated unsaturated fatty acid from DDGS significantly increased MDA content and lipid peroxidation, which inhibited cell proliferation (Victor et al., 1981).

It is well recognized that feed enzymes hydrolyze cereal NSP, and produce oligosaccharides and monosaccharides in the intestine. This could improve feed efficiency and nutrient digestibility by increasing the access of nutrients to digestive enzymes (Kerr and Shurson, 2013). In the current study, supplemental glucanase in the diet increased ADG, and tended to increase ADFI of pigs fed diets with 15% DDGS, but such effect seemed not to occur in pig fed diets with 30% DDGS. This was different from our expectation. This reason might be that the amount of glucanase (up to 450 U/kg feed) used in this study was not sufficient to hydrolyze β-glucan in the 30% DDGS diet. Glucanase tended to decrease viscosity of jejunal digesta and improve AID of NDF in this study. High gut viscosity inhibits the rate of diffusion of nutrients and digestive enzymes and decreases nutrient digestion and absorption (Wellock et al., 2008). Similarly, supplementing a blend of β-glucanase and protease to a corn and soybean meal based diet, increased AID of NDF in pigs

(Ji et al., 2008). Such increase in ileal digestibility of NDF indicated that supplemental enzymes shifted part of the digestion from large intestine to small intestine, and potentially increased the energetic efficiency of fiber use (Ji et al., 2008).

The changes in hindgut fermentation could affect gut health of pigs (Lindberg, 2014). Gut health is associated with health mucosa, which can be evaluated by gut morphology, levels of cytokines, and intestinal permeability. Currently, glucanase supplementation tended to decrease MDA level and villus width in the small intestine, suggesting less oxidative stress and higher capability of nutrient absorption (Schieber and Chandel, 2014; Huygelen et al., 2014). Generally, the mode of action of dietary enzyme on the gastrointestinal tract could be explained by the hydrolysis of dietary NSP, which could release nutrients, and affect microbial fermentation in the lumen (Ravindran, 2013). These might stimulate the regulation of gut morphology and the production of functional proteins. Production of TNF-α led to some changes in the presence of tight junction proteins through myosin light chain kinase (MLCK)-mediated pathway (Shen, 2012). In that case, transmembrane proteins like occludin and claudin, and their scaffolding proteins like ZO-1 could be regulated. However, in the present study, supplementation of glucanase did not affect the levels of TNF-α level in tissue and blood samples, therefore did not cause any change on the expressions of tight junction proteins, such occludin, ZO-1, and claudin.

Consequently, increasing dietary DDGS from 15% to 30% did not affect the overall performance during 21 d, but resulted in higher oxidative stress and inflammatory response, and lower crypt proliferation in the small intestine. Supplemental glucanase seemed to

increase ADG and ADFI of pigs fed diets with 15% DDGS. It could also decrease digesta viscosity and oxidative stress, and improve nutrient digestibility. This suggested that supplemental glucanase has the potential to improve growth and gut health of pigs when fed with corn, soybean meal, and DDGS based diets.

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Table 1. Composition of experimental diets supplemented with glucanase and distillers dried grains and solubles

DDGS[1], %	15			30		
Glucanase, U/kg	0	150	450	0	150	450
Ingredient, %						
Yellow corn	49.76	49.46	48.86	37.95	37.65	37.05
Poultry fat	2.00	2.00	2.00	2.00	2.00	2.00
DDGS	15.00	15.00	15.00	30.00	30.00	30.00
Soybean meal	23.00	23.00	23.00	20.00	20.00	20.00
Whey permeate	5.00	5.00	5.00	5.00	5.00	5.00
Blood plasma	2.00	2.00	2.00	2.00	2.00	2.00
Supplement[2]	0.00	0.30	0.90	0.00	0.30	0.90
L-Lys HCl	0.43	0.43	0.43	0.45	0.45	0.45
DL-Met	0.10	0.10	0.10	0.05	0.05	0.05
L-Thr	0.08	0.08	0.08	0.05	0.05	0.05
Salt	0.22	0.22	0.22	0.22	0.22	0.22
Vitamin premix[3]	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15
Dical Phosphate	0.55	0.55	0.55	0.32	0.32	0.32
Limestone	1.30	1.30	1.30	1.40	1.40	1.40
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
Mecadox 10	0.13	0.13	0.13	0.13	0.13	0.13
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition,						
ME, kcal/kg	3,386	3,386	3,386	3,394	3,394	3,394
Lys[5], %	1.24	1.24	1.24	1.24	1.24	1.24
Met + Cys[5], %	0.70	0.70	0.70	0.70	0.70	0.70
Trp[5], %	0.21	0.21	0.21	0.21	0.21	0.21

Table 1. Continued

Thr[5], %	0.73	0.73	0.73	0.73	0.73	0.73
Ca, %	0.71	0.71	0.71	0.70	0.70	0.70
Available P, %	0.33	0.33	0.33	0.33	0.33	0.33
Analyzed composition,						
DM, %	88.98	89.05	89.31	88.88	89.20	89.00
GE, kcal/kg	4,082	4,085	4,117	4,179	4,186	4,162
NDF, %	15.68	15.64	14.03	20.93	19.79	21.53
ADF, %	4.43	4.36	4.15	5.92	5.67	5.91
Glucanase activity[6], U/kg	0	192	428	0	160	406

[1]DDGS: distillers dried grains and solubles

[2]Supplement was corn-expressed glucanase from Agrivida (Medford, MA), with 50 U glucanase/g product.

[3]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D_3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B_{12}; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

[4]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganese oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[5]Standardized ileal digestible

[6]The actual activity of glucanase in the diet was determined by the manufacturer (Agrivida, Medford, MA).

Table 2. Growth performance of pigs supplemented with glucanase and distillers dried grains and solubles

DDGS[1], %	15			30			SEM	*P*-value[2]		
Glucanase, U/kg	0	150	450	0	150	450		Ing	Enz	Ing×Enz
BW, kg										
Initial	10.20	10.20	10.20	10.22	10.20	10.20	0.61	0.934	0.994	0.999
Wk 1	14.30	14.59	14.61	15.06	14.67	14.40	0.77	0.315	0.770	0.138
Wk 2	19.44	19.58	20.23	20.57	20.16	19.51	0.95	0.317	0.930	0.070
Wk 3	24.91	24.91	25.69	26.33	25.21	24.67	1.15	0.655	0.646	0.162
ADG, g/day										
Wk 1	585	628	631	692	638	599	33	0.169	0.617	0.021
Wk 2	760	776	802	787	786	730	28	0.573	0.848	0.122
Wk 3	782	819	839	823	720	738	32	0.051	0.596	0.051
Overall	700	746	738	767	715	689	27	0.806	0.631	0.028
ADFI, g/day										
Wk 1	820	900	864	898	867	810	46	0.906	0.297	0.064
Wk 2	1128	1173	1163	1188	1158	1067	43	0.571	0.321	0.106
Wk 3	1356	1423	1430	1407	1332	1275	61	0.182	0.869	0.206
Overall	1091	1173	1131	1165	1119	1051	43	0.461	0.261	0.058
G:F										
Wk 1	0.712	0.702	0.731	0.772	0.739	0.742	0.019	0.022	0.477	0.433
Wk 2	0.675	0.664	0.697	0.662	0.678	0.687	0.020	0.854	0.430	0.746
Wk 3	0.578	0.579	0.587	0.586	0.542	0.581	0.013	0.257	0.112	0.184
Overall	0.642	0.637	0.653	0.659	0.639	0.658	0.012	0.416	0.369	0.822

[1]DDGS: distillers dried grains and solubles

[2]Ing: main effect of DDGS; Enz: main effect of glucanase; Ing x Enz: interaction effect between DDGS and glucanase

Table 3. Fecal score[1] of pigs supplemented with glucanase and distillers dried grains and solubles, on d 0, d 3, d 7, d 10, d 14, d 17, and d 21

DDGS[2], %	15			30			SEM	*P*-value[3]		
Glucanase, U/kg	0	150	450	0	150	450		Ing	Enz	Ing×Enz
Fecal Score										
D 0	2.7	2.9	2.8	2.8	3.0	3.0	0.12	0.188	0.245	0.895
D 3	2.5	2.9	2.8	3.0	3.0	2.8	0.18	0.169	0.500	0.330
D 7	2.8	2.4	2.8	2.9	2.8	2.6	0.14	0.379	0.546	0.041
D 10	2.7	2.9	2.9	3.0	2.8	3.0	0.12	0.302	0.619	0.244
D 14	2.9	3.0	3.0	2.8	2.8	2.8	0.10	0.054	0.857	0.857
D 17	2.8	2.5	2.9	2.9	2.9	2.6	0.14	0.572	0.572	0.060
D 21	2.8	2.9	2.7	2.5	2.9	2.8	0.13	0.547	0.185	0.311

[1]Fecal Score with 5 categories: 1-extreme dry, 2-firm and shaped, 3-soft and shaped, 4-loose, and 5-watery

[2]DDGS: distillers dried grains and solubles

[3]Ing: main effect of DDGS; Enz: main effect of glucanase; Ing x Enz: interaction effect between DDGS and glucanase

Table 4. Viscosity of distal jejunal digesta (cP[1]) and apparent ileal of digestibility (AID) of DM, GE, crude fat, CP, NDF, and ADF in pigs supplemented with glucanase and distillers dried grains and solubles

DDGS[2], %	15			30			SEM	*P*-value[3]		
Glucanase, U/kg	0	150	450	0	150	450		Ing	Enz	Ing×Enz
Viscosity, cP	2.64	2.53	2.43	2.57	2.49	2.02	0.17	0.233	0.092	0.527
AID, %										
DM	73.67	74.36	75.45	73.21	74.14	75.16	1.13	0.730	0.266	0.994
GE	78.48	77.70	79.08	79.79	77.68	78.67	0.97	0.710	0.288	0.651
Crude fat	91.37	91.71	92.70	93.86	93.71	93.78	0.58	0.002	0.490	0.442
CP	84.27	83.33	85.14	85.39	84.86	85.63	0.93	0.176	0.387	0.854
NDF	44.17	52.43	52.61	43.30	46.47	46.66	2.87	0.075	0.076	0.597
ADF	42.87	33.38	39.94	32.98	30.91	35.43	3.46	0.054	0.181	0.546

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

[2]DDGS: distillers dried grains and solubles

[3]Ing: main effect of DDGS; Enz: main effect of glucanase; Ing x Enz: interaction effect between DDGS and glucanase

Table 5. Malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), and protein carbonyl levels of plasma, duodenum, and jejunum samples in pigs supplemented with glucanase and distillers dried grains and solubles

DDGS[1], %	15			30			SEM	*P*-value[2]		
Glucanase, U/kg	0	150	450	0	150	450		Ing	Enz	Ing×Enz
MDA										
Plasma, uM	10.17	9.59	11.76	9.81	10.19	8.37	1.42	0.356	0.992	0.330
Duodenum, umol/g protein	0.76	0.64	0.63	0.76	0.78	0.70	0.05	0.022	0.062	0.233
Jejunum, umol/g protein	0.38	0.33	0.40	0.39	0.32	0.41	0.05	0.891	0.355	0.963
TNF-α										
Plasma, pg/ml	52.12	58.68	52.05	61.00	62.93	85.61	15.89	0.222	0.723	0.596
Duodenum, pg/mg protein	3.94	3.50	3.96	4.27	4.17	4.30	0.31	0.067	0.535	0.807
Jejunum, pg/mg protein	3.28	3.04	3.27	3.37	2.96	3.18	0.30	0.907	0.540	0.941
Protein carbonyl										
Plasma, umol/g protein	5.22	4.38	5.40	4.84	5.30	5.56	0.35	0.355	0.104	0.496
Duodenum, umol/g protein	11.19	10.55	11.16	12.00	11.71	9.91	0.76	0.703	0.382	0.245
Jejunum, umol/g protein	4.17	4.00	3.91	4.31	4.43	4.40	0.48	0.268	0.971	0.887

[1]DDGS: distillers dried grains and solubles

[2]Ing: main effect of DDGS; Enz: main effect of glucanase; Ing x Enz: interaction effect between DDGS and glucanase

Table 6. Proliferation rate of jejunal crypt cells, as well as villus height, villus width, crypt depth, and the ratio of villus height to crypt depth (VH/CD) of duodenum and jejunum in pigs supplemented with glucanase and distillers dried grains and solubles

DDGS[1], %	15			30			SEM	*P*-value[2]		
Glucanase, U/kg	0	150	450	0	150	450		Ing	Enz	Ing×Enz
Proliferation, %	45.1	46.2	44.6	41.7	43.1	39.7	1.9	0.008	0.335	0.854
Duodenum										
Villus height	564	513	548	547	523	525	26	0.629	0.351	0.803
Villus width	161	158	159	157	169	166	6	0.309	0.698	0.418
Crypt depth	326	317	350	345	335	326	14	0.663	0.619	0.158
VH/CD	1.76	1.63	0.59	1.59	1.57	1.62	0.10	0.403	0.668	0.581
Jejunum										
Villus height	420	408	425	437	417	453	20	0.262	0.388	0.897
Villus width	143	141	126	140	138	139	4	0.495	0.072	0.049
Crypt depth	157	158	168	155	162	157	7	0.586	0.594	0.482
VH/CD	2.74	2.62	2.55	2.89	2.69	2.94	0.18	0.130	0.613	0.591

[1]DDGS: distillers dried grains and solubles

[2]Ing: main effect of DDGS; Enz: main effect of glucanase; Ing x Enz: interaction effect between DDGS and glucanase

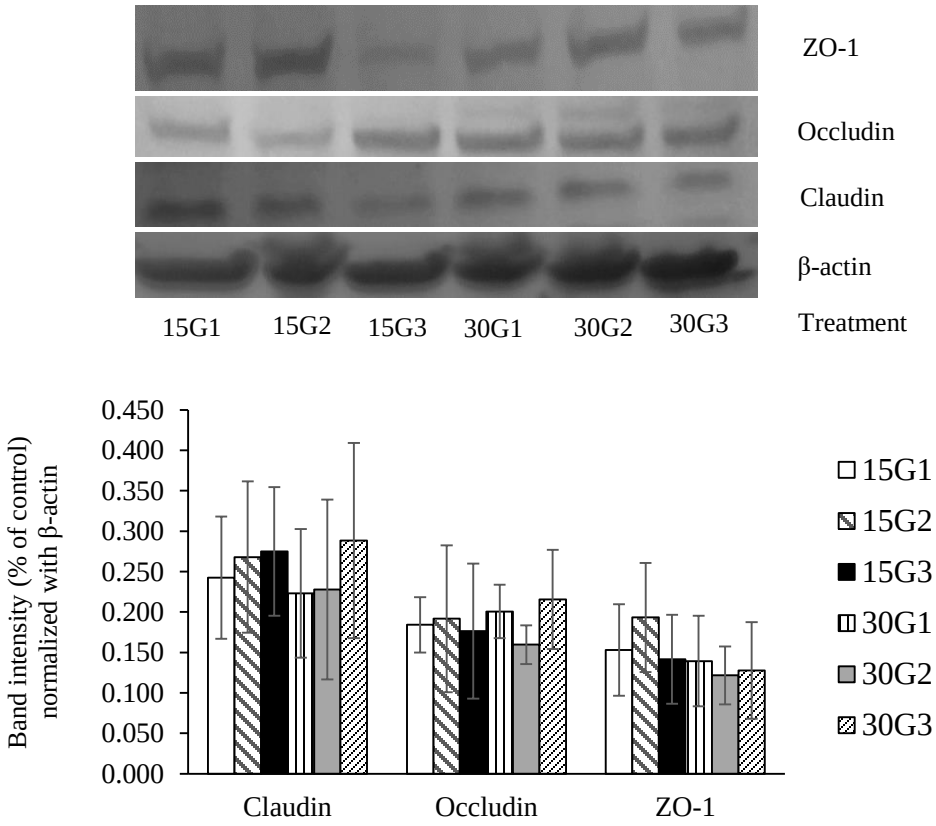

Figure 1. Expression of tight junction proteins in piglets supplemented with glucanase and distillers dried grains and solubles (DDGS). 5-wk-old piglets were fed with glucanase and DDGS for 21 days. The expression of claudin, occludin, and zonula occludens (ZO) -1 was measured using immunoblotting. Data are represented as mean ± SEM, n = 4. *$P < 0.05$

CHAPTER 5

EFFECTS OF SUPPLEMENTAL NON-STARCH POLYSACCHARIDE DEGRADING ENZYMES ON GROWTH PERFORMANCE IN NURSERY AND GROWER PIGS FED LOW NUTRIENT DIETS

Abstract: The objective was to investigate the effect of supplementing a blend of non-starch polysaccharide (NSP) degrading enzymes on growth performance in nursery and grower pigs when fed with low nutrient diet. In Exp. 1, a total of 180 piglets (BW of 5.7 ± 0.7 kg) at 3-wk age were assigned to 3 treatments (20 pens/trt, and 3 pigs per pen) in a 42-d trial based on the initial BW and sex as blocks. Three dietary treatments were a positive control diet (PC) formulated to meet or exceed NRC (2012) nutrient requirements, a low nutrient diet (LN) with NE and standardized ileal digestible (SID) Lys reduced by 8% and 35% respectively, and LN supplemented with NSP degrading enzymes (LNE). In Exp. 2, a total of 180 pigs (BW of 26.6 ± 4.1 kg) at age of wk 10 were assigned to 3 treatments (20 pens/trt, and 3 pigs per pen) in a 42-d trial based on the initial BW and sex as blocks. Three dietary treatments were PC formulated to meet or exceed NRC (2012) nutrient requirements, a low AA diet (LA) with SID Lys reduced by 35%, and LA supplemented with NSP degrading enzymes (LAE). In both experiments, pigs fed low nutrient diet had much poorer ($P < 0.05$) growth performance than those fed with PC. Supplementing NSP enzymes did not significantly improve growth performance of nursery or grower pigs when fed with low nutrient diets.
Key words: grower, growth performance, non-starch polysaccharide degrading enzymes, nursery pigs

INTRODUCTION

The use of co-products from milling, bio-fuel, or oil-extracting industry as raw ingredients is an effective way to reduce feed cost, and facilitate sustained development of animal production. The mainly used co-products in swine diets include distillers dried grains with solubles (DDGS) and soybean meal. However, most of these feedstuffs are rich in non-starch polysaccharides (NSP), i.e. DDGS contains 25 to 30% NSP, and soybean meal contains about 20% NSP (Choct et al., 2010; Pedersen et al., 2014). Arabinoxylan is the major NSP in DDGS and wheat bran, as galactomannan in soybean meal (Hsiao et al., 2006; Rosenfelder et al., 2013; Pedersen et al., 2014). Non-starch polysaccharides are unable to be digested in the gastrointestinal tract of pigs because they do not produce specific endogenous digestive enzymes. Therefore, supplemental NSP degrading enzymes can be beneficial to improve the nutritive value of feed, and decrease the cost of animal products (Zijlstra et al., 2010). However, the research on the application of these enzymes showed variable results (Adeola and Cowieson, 2011; Kiarie et al., 2016). The effect of enzymes depends on the substrates and characteristics of the diet, physiology of pigs, source of enzymes, and formulating methods (Pedersen et al., 2012; Woyengo et al., 2014; Kiarie et al., 2016).

Based on our previous research, NSP degrading enzymes did not significantly increase growth performance of pigs when the nutrient levels of diets met or exceeded NRC (2012) requirement. That could be possible if the digestible nutrients in the diet were strictly controlled, supplemental enzyme to that diet would liberate nutrients by hydrolyzing substrates, and result in a greater improvement of growth performance in pigs (Bedford,

2002). Moreover, results of a meta-analysis showed that effects of NSP enzymes were correlated with levels of NE and standardized ileal digestible (SID) Lys in the diet, depending on the age of pigs. It was proposed that NE ≤ 2390 kcal/kg and SID Lys ≤0.90% for piglets, and NE ≥ 2440 kcal/kg and SID Lys ≤ 0.65% for growing pigs, were the conditions when NSP enzymes could have a maximum effect on feed efficiency. Therefore, we speculate that compared with normal diets, adding NSP enzymes may be more promising, when the nutrient levels in the diet, such as energy or Lys, are deficient. The objective of these two experiments was to evaluate the effect of NSP degrading enzymes on growth performance of nursery and grower pig fed low nutrient diets, which were formulated based on NE and SID AA values.

MATERIALS AND METHODS

The experimental protocols were approved by North Carolina State University Animal Care and Use Committee (Raleigh, NC).

Animal and Experimental Design

Both studies were conducted at the North Carolina Swine Evaluation Station (Clayton, NC). A commercial enzyme preparation (ADISSEO, France) containing endo-1,4-β-xylanase, β-glucanase, mannanase, and other NSP degrading enzymes was used in these two experiments.

In Exp. 1, one hundred and eighty barrow and gilts (5.7 ± 0.7 kg) at age of wk 3 were allotted to 3 treatments in a randomized complete block design based on the initial BW and sex as blocks. Three dietary treatments included a positive control diet (PC), a low nutrient

diet (LN), and LN supplemented with NSP degrading enzymes (LNE). Each treatment had 10 male pens and 10 female replicate pens, with 3 pigs per pen. The nutrient levels of PC met or exceed the NRC (2012) requirement. Compared with PC, the LE contained 8% lower NE and 35% less SID Lys, with other SID AA lower to varying extent (Table 1), mainly by adding wheat bran and removing crystal AA supplements in the diet. The experimental period was 42 d, and divided into phase 1 (wk 1 to 2) and phase 2 (wk 3 to 6).

In Exp. 2, one hundred and eighty barrow and gilts (26.6 ± 4.1 kg) at age of wk 10 were allotted to 3 treatments in a randomized complete block design based on the initial BW and sex as blocks. Three dietary treatment included a positive control diet (PC), a low AA diet (LA), and LA supplemented with NSP degrading enzymes (LAE). Each treatment had 10 male pens and 10 female replicate pens, with 3 pigs per pen. The positive control diet met or exceeded the NRC (2012) requirement. Compared with PC, the LA had normal energy level, but contained 35% less SID Lys, with other SID AA lower to varying extent (Table 2) mainly by removing crystal AA supplements in the diet. The experimental period was 42 d, and divided into phase 1 (wk 1 to 4) and phase 2 (wk 5 to 6).

The analyzed enzyme activity was 5,189 unit xylanase/g supplement, measured by the manufacturer (ADISSEO, France). The unit is defined as the release of 1μmol of xylose or glucose equivalent per minute from a substrate (birchwood xylan) at 50°C and pH 4. In each experiment, two basal diets for each phase were mixed separately: PC and low nutrient diet, and then enzyme supplementation were premixed and hand-added to the low nutrient diet to make the enzyme containing diet.

Pens (4.0 × 1.4 m) with solid concrete floor were equipped with a nipple drinker and a 1-hole self-feeder. Pigs had free access to feed and water. All the diets were pelleted, and the temperatures of conditioning and pelleting were no more than 80°C. Body weight and feed intake were recorded every two weeks. Mortality and morbidity were also recorded. Feed efficiency was calculated as G:F. Fecal score in Exp. 1 were recorded every two weeks.

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). In this experiment, pigs were allotted based on a randomized complete block design. The experimental unit was the pen. Initial BW block was considered as a random effect. Treatment and sex were considered as fixed effects. Tukey method was used to do multiple comparisons. Adjusted *P*-value of PC vs LN, LN vs LNE, PC vs LA, and LA vs LAE were obtained. Statistical differences were considered significant with $P < 0.05$. Probabilities that is less than 0.10 and equal to or greater than 0.05 were considered as tendencies.

RESULTS

Exp. 1

There was no significant difference on initial BW among treatments (Table 3). Compared with PC, pigs fed LN had much lower ($P < 0.05$) ADG, ADFI, and G:F in either wk or the overall period. The overall reduction in ADG of nursery pigs fed LN was 34.8%, and the greater reduction occurred during wk 3 and 4, was 38.1%. The overall decrease in ADFI of pigs fed LN was 15.4%, and the greatest decrease also occurred during wk 3 and 4,

was 16.2%. The overall decrease in G:F of pigs fed LN was 22.9%, and the greatest decrease also occurred during wk 3 and 4, was 34.0%.

There was no significant difference between nursery pigs fed LN and LNE, showing enzyme supplementation did not improve growth performance of nursery pigs fed diets with 8% lower NE and 35% less SID Lys. Fecal score fell into the range of 1 to 2, and showed no difference among treatments. One pig in PC group died from suppurative meningoencephalitis on d 12.

Exp. 2

There was no significant difference on initial BW among treatments (Table 4). Compared with PC, grower pigs fed LA had lower ($P < 0.05$) ADG, ADFI, and G:F in either wk or the overall period.. The overall reduction in ADG of grower pigs fed LA was 30.8%, and the reduction occurring every two weeks ranged between 30.2% and 31.3 %. The overall decrease in ADFI of pigs fed LA was 15.0%, and the decrease occurring every two weeks ranged between 13.4% and 17.0%. The overall decrease in G:F of pigs fed LA was 18.9%, and the decrease occurring every two weeks ranged between 16.7 and 20.0%.

There was also no significant difference on growth performance between grower pigs fed LA and LAE, showing supplementing NSP enzyme did not improve growth performance of grower pigs when the SID Lys in the diet was 35% lower than that of PC. One pig in PC group died from broken legs on d 40.

DISCUSSION

Low nutrient diet in Exp. 1 contained 8% lower NE and 35% less SID Lys than that of NRC (2012) requirement, and in Exp. 2 was only restricted on SID Lys level. The amount of soybean meal in the low nutrient diet was utilized to reach the targeted Lys level without supplementing crystalline AA. Therefore, sulfur-containing AA, Trp, and Thr in low nutrient diet were deficient to different extents. More than 20% DDGS were supplemented to the diets of Exp. 1 and 2. Wheat bran was also used in the Exp. 1 to reduce the energy level. Significant differences between pigs fed with control and low nutrient diets were observed in these two studies. This could be explained by the great reduction of NE and SID AA concentrations in the diets. Zhou et al. (2016) reported that NE value could be reduced by 3.8% while maintaining the SID AA/NE ratio, which did not affect growth performance in weaned pigs. Kerr et al. (1995) found that if CP level and total Lys in the diet were reduced by 4% and 35% respectively, ADG and G:F of grower pigs fed such diet were decreased by 17% and 14%, respectively.

Supplementation of NSP degrading enzymes to diets was observed to improve G:F as well as the nutrient digestibility in pigs (Bedford, 2000; Patridge, 2001). These enzymes were largely used in the diets usually composed of feedstuffs with high NSP content. These feedstuffs have a great proportion of NSP, such as arabinoxylans and β-(1→3, 1→4)-glucans (Bach Knudsen, 1997). Soybean meal contains relatively high level of β-galactomannans and α-1,6-galactosides (Choct et al., 2010). Non-starch polysaccharide degrading enzymes such as xylanase, β-glucanase, mannanase, and α-galactosidase, assist in cleaving β-1,4-glycosidic

bonds that the monogastric animal is unable to break down (Patridge, 2001). Kim et al. (2006) found that NSP enzyme supplementation improved nutrient digestibility and allowed at least 3% reduction of ME with constant SID Lys level in the diets without adverse effects on growth performance of grower pigs. Similarly, dietary NSP enzymes compensated 5% reduction in DE and 12% reduction in total Lys, and showed similar growth performance with pigs fed normal diets (Wang et al., 2009). In the current study, supplemental NSP enzymes showed no significant effect on growth performance of pigs fed low nutrient diets. One speculation would be the unbalanced SID Lys/ NE in the diets. The ratio of Lys to NE may affect the ability of utilizing energy for protein synthesis in pigs. The released AA and energy by enzymatic hydrolysis did not increase proportionately. Therefore, with inadequate Lys/NE ratio, the rate of protein deposition was not increased significantly in this study. Although dietary NSP enzymes did not significantly affect growth performance, numeric improvements were observed between pigs fed low nutrient diets with and without NSP enzymes in this study. When nursery pigs were fed diets with low levels of energy and AA, dietary NSP enzymes numerically increased ADG, ADFI, and G:F by 5.6%, 4.9%, and 0.53%, respectively. When grower pigs were fed diets with low AA, dietary NSP enzyme also numerically improved ADG, ADFI, and G:F by 2.6%, 1.1%, and 1.7%, respectively. Greatest improvement occurred during the first two weeks in either nursery or grower study. The overall improvements were too small to be detected, suggesting that the reduction in NE and SID Lys content in this study were so great that the amounts of released nutrients in the diet might still be insufficient to contribute to the growth of pigs.

It is recognized that the improvement in growth performance of pigs caused by NSP enzyme supplementation can be due to the enzymatic hydrolysis of NSP for energy (Kim et al., 2003). In our previous studies, supplementation of NSP enzyme could also improve gut health by reducing inflammatory response and oxidative stress, and improving gut morphology and permeability. Moreover, dietary NSP enzymes could change the microbial profile in the intestinal lumen, such as increasing the growth of *lactobacilli* by releasing more sugar from the diet (Kiarie et al., 2007). Supplementation of NSP enzymes to the high-NSP diets resulted in an increase in acetic rather than lactic acids (Höberg and Lindberg, 2004). Therefore, the improvement in gut health could also contribute to the improved growth of pigs when NSP enzymes are utilized in swine diets.

The results of the current study indicated that supplementation of NSP enzymes did not significantly improve growth performance of pigs when fed with low nutrient diets. However, 8% and 35% of reduction on energy and SID Lys would cause an extreme adverse situation, in which the declined growth performance of pigs could not be alleviated by exogenous supplementation of NSP degrading enzymes.

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Table 1. Composition of experimental diets for nursery pigs[1]

Item, %	Phase 1 (wk 1 to 2)		Phase 2 (wk 2 to 6)	
	PC	LN	PC	LN
Ingredient,				
Corn	32.13	22.17	47.74	31.52
Soybean meal	25.00	25.00	27.00	20.50
Blood plasma	2.00	0.00	0.00	0.00
Poultry meal	2.00	0.00	0.00	0.00
Whey permeate	12.00	12.00	0.00	0.00
DDGS[2]	20.00	20.00	20.00	20.00
Wheat bran	0.00	16.00	0.00	23.50
Soy oil	3.40	2.00	2.00	2.00
L-Lys HCl	0.45	0.00	0.41	0.00
DL-Met	0.11	0.00	0.06	0.00
L-Thr	0.08	0.00	0.06	0.00
Limestone	1.40	1.60	1.15	1.52
Dicalcium phosphate	0.40	0.20	0.80	0.18
Vitamin premix[3]	0.03	0.03	0.03	0.03
Mineral premix[4]	0.15	0.15	0.15	0.15
Salt	0.22	0.22	0.22	0.22
Zinc oxide	0.25	0.25	0.25	0.25
Antibiotics	0.38	0.38	0.13	0.13
Total	100.00	100.00	100.00	100.00
Calculated composition				
DM, %	90.49	89.89	89.44	89.03
ME, kcal/kg	3,437	3,173	3,380	3,137
NE, kcal/kg	2,448	2,263	2,428	2,233
Lys[5], %	1.35	0.88	1.23	0.80
Met + Cys[5], %	0.74	0.64	0.68	0.67
Trp[5], %	0.23	0.22	0.21	0.20
Thr[5], %[4]	0.79	0.65	0.73	0.62
Ca, %	0.81	0.80	0.70	0.70
STTD P[6], %	0.40	0.40	0.33	0.33
Total P, %	0.62	0.65	0.58	0.61

[1] PC: control diet; LN: low energy and AA diet; LNE: LN supplemented with non-starch polysaccharide (NSP) degrading enzymes

[2]DDGS: distillers dried grains and solubles

[3]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

Table 1. Continued

[4]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

[5]Standardized ileal digestible

[6]Standardized total tract digestible

Table 2. Composition of experimental diets for grower pigs[1]

Item, %	Phase 1 (wk 1 to 4)		Phase 2 (wk 5 to 6)	
	PC	LA	PC	LA
Ingredient,				
Corn	57.01	57.60	65.67	66.22
Soybean meal	15.50	15.50	9.50	9.50
DDGS[2]	22.50	22.50	20.00	20.00
Soy oil	2.00	2.00	2.00	2.00
L-Lys HCl	0.45	0.00	0.48	0.08
DL-Met	0.03	0.00	0.02	0.00
L-Thr	0.08	0.00	0.09	0.00
L-Trp	0.03	0.00	0.04	0.00
Limestone	1.15	1.15	1.05	1.05
Dicalcium phosphate	0.80	0.80	0.70	0.70
Vitamin premix[3]	0.03	0.03	0.03	0.03
Mineral premix[4]	0.15	0.15	0.15	0.15
Salt	0.22	0.22	0.22	0.22
Antibiotics	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Calculated composition,				
DM, %	89.32	89.26	89.17	89.12
ME, kcal/kg	3,404	3,398	3,417	3,412
NE, kcal/kg	2,496	2,497	2,545	2,546
Lys[5], %	0.99	0.64	0.86	0.55
Met + Cys[5], %	0.56	0.54	0.49	0.47
Trp[5], %	0.18	0.15	0.16	0.12
Thr[5], %	0.60	0.53	0.52	0.43
Ca, %	0.67	0.67	0.59	0.59
STTD P[6], %	0.31	0.31	0.27	0.27
Total P, %	0.54	0.54	0.49	0.49

[1]PC: control diet; LA: low AA diet; LAE: LA supplemented with non-starch polysaccharide (NSP) degrading enzymes

[2]DDGS: distillers dried grains and solubles

[3]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.

[4]The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

Table 2. Continued

[5]Standardized ileal digestible

[6]Standardized total tract digestible

Table 3. Growth performance and fecal score of nursery pigs fed low NE and AA diets with non-starch polysaccharide (NSP) degrading enzymes[1]

Item	PC	LN	LNE	SEM	*P*-value	
					PC vs. LN	LN vs. LNE
BW, kg						
Initial	5.72	5.72	5.73	0.22	0.978	0.884
Wk 2	7.98	7.18	7.41	0.29	<.0001	0.173
Wk 4	15.04	11.55	11.87	0.58	<.0001	0.615
Wk 6	25.14	18.38	19.09	0.84	<.0001	0.388
ADG, g/day						
Wk 1-2	161	104	120	7.27	<.0001	0.186
Wk 3-4	504	312	318	21.95	<.0001	0.936
Wk 5-6	722	488	516	22.56	<.0001	0.408
Wk 1-6	462	301	318	15.82	<.0001	0.400
ADFI, g/day						
Wk 1-2	194	170	177	9.97	0.071	0.794
Wk 3-4	611	512	548	28.22	0.008	0.491
Wk 5-6	1080	910	944	42.06	<.001	0.647
Wk 1-6	628	531	557	24.24	<.0001	0.451
G:F						
Wk 1-2	0.830	0.623	0.681	0.023	<.0001	0.195
Wk 3-4	0.920	0.607	0.580	0.076	0.01	0.963
Wk 5-6	0.671	0.539	0.548	0.008	<.0001	0.722
Wk 1-6	0.738	0.569	0.572	0.009	<.0001	0.960
Fecal score[2]						
Wk 1-2	1.65	1.45	1.50	0.075	0.151	0.884
Wk 3-4	1.60	1.40	1.45	0.074	0.130	0.875
Wk 5-6	1.15	1.05	1.10	0.050	0.342	0.761

[1]PC: control diet; LN: low energy and AA diet; LNE: LN supplemented with non-starch polysaccharide (NSP) degrading enzymes

[2]Fecal Score category: 1=form and shaped, normal; 2=soft and shaped, normal; 3=watery, diarrhea

Table 4. Growth performance of grower pigs fed low AA diets with non-starch polysaccharide (NSP) degrading enzymes[1]

Item	PC	LA	LAE	SEM	*P*-value	
					PC vs. LA	LA vs. LAE
BW, kg						
Initial	26.63	26.64	26.64	1.36	0.999	0.999
Wk2	38.93	35.19	35.83	1.64	<0.0001	0.292
Wk4	52.85	44.91	45.72	1.95	<0.0001	0.419
Wk6	68.09	55.35	56.09	2.48	<0.0001	0.740
ADG, g/day						
Wk 1-2	878	611	657	25.93	<0.0001	0.219
Wk 2-4	994	694	706	25.01	<0.0001	0.859
Wk 4-6	1085	745	741	41.12	<0.0001	0.990
Wk 1-6	987	683	701	29.26	<0.0001	0.729
ADFI, g/day						
Wk 1-2	1597	1383	1449	55.44	<0.0001	0.293
Wk 2-4	1967	1698	1656	73.25	0.0001	0.771
Wk 4-6	2295	1904	1936	89.45	<0.0001	0.899
Wk 1-6	1953	1661	1680	69.73	<0.0001	0.922
G:F						
Wk 1-2	0.551	0.441	0.454	0.006	<0.0001	0.245
Wk 2-4	0.512	0.411	0.429	0.010	<0.0001	0.390
Wk 4-6	0.472	0.393	0.382	0.008	<0.0001	0.585
Wk 1-6	0.507	0.411	0.418	0.004	<0.0001	0.587

[1]PC: control diet; LA: low AA diet; LAE: LA supplemented with non-starch polysaccharides (NSP) degrading enzymes

CHAPTER 6

GENERAL CONCLUSION

The literature review has introduced several ingredients that could substitute corn or soybean meal in swine diets. There are some problems existing in the application of these ingredients. For example, considering the cross-linked structure and hydrophobicity of kafirins in sorghum grains, we hypothesized that it would have lower nutrient digestibility in pigs. However, sorghum turned out to be a good replacement for corn. Pigs fed with sorghum based diets had increased daily gain without affecting nutrient digestibility. Additionally, supplemental sorghum reduced oxidative stress in both blood and jejunum tissue, and improved jejunal morphology in nursery pigs.

Another issue is high content of non-starch polysaccharides (NSP) in these plant-based feedstuffs, especially after oil, protein, or starch being extracted from cereal grains or oil seeds. For example, corn distiller dried grains with solubles (DDGS) contains about 30% of NSP. In the present studies, high NSP diets (up to 30% DDGS in the diet) did not affect the overall growth performance, but it had lower apparent ileal digestibility (AID) of DM and GE, increased viscosity of jejunal digesta, decreased villus height and the ratio of villus height to crypt depth in duodenum, increased inflammatory response in colon of nursery pigs. These results suggested feeding high NSP diets can affect gut health and potentially the growth of nursery pigs.

Exogenous enzyme supplementation is an effective method to expand the use of these ingredients by hydrolyzing the corresponding substrates. The enzymes we used in these experiments fall into two groups: proteases and NSP degrading enzymes. Keratinases, a kind of proteases, are targeting kafirins in sorghum grains by cleaving disulfide bonds. The results

showed that supplemental keratinase did not improve growth performance of nursery pigs during 6-wk feeding. However, it increased nutrient digestibility, reduced oxidative stress, and improved the ratio of villus height to crypt depth in duodenum. These effects were irrespective of corn or sorghum based diets.

Another three enzymes utilized in our studies are mannanase, xylanase, and glucanase. They are targeting NSP fractions in the diets: mannans, xylans, and β-glucans, respectively. They are endo-acting, so the products of their enzymatic hydrolysis are mainly oligosaccharides and small amounts of monosaccharides. Arabinoxylans and galactomannans are the major NSP in DDGS and soybean meal, respectively. So, supplementing xylanase and mannanase to a diet containing corn, soybean meal, and DDGS seems promising. In the current studies, supplemental mannanase and xylanase increased growth performance and nutrient digestibility, reduced digesta viscosity, decreased oxidative stress and inflammatory response, and improved gut morphology and permeability. However, these effects varied among studies with pigs at different ages and diets with different inclusion levels of NSP-rich ingredients.

β-glucans are relatively high in the DDGS containing diets, compared with corn based diets. In the present study, increasing dietary DDGS from 15% to 30% increased AID of crude fat, and did not affect the overall growth performance. But it decreased the proliferate rate of jejunal crypt, and increased oxidative stress and inflammatory response in duodenum. Supplemental glucanase decreased digesta viscosity and oxidative stress without any improvement on growth performance. It seemed that the effect of glucanase did not depend

on the inclusion level of DDGS in the diets, maybe because β-glucans are not the dominant NSP in DDGS.

Considering that adding NSP degrading enzymes did not show any great improvement on growth performance when the nutrient levels of these diets met or exceeded the NRC requirement, we hypothesized that they might have more potential when the diet is deficient in NE or standardized ileal digestible (SID) AA. However, it showed that 8% and 35% of reduction on NE and SID Lys would result in an extreme adverse situation, in which declined growth performance of pigs could not be improved by supplementing a blend of NSP enzymes.

Overall, exogenous supplementation of enzymes could improve nutrient digestibility and gut health of nursery pigs, and potentially the growth of pigs. With the development of fermentation and transgenic technologies, the decreasing cost of feed enzymes might greatly expand their utilization in the feed industry.