RESEARCH REPORT
ANIMAL SCIENCE

Title: Enhancing nutrient utilization of corn DDGS by feed enzymes in the pig intestine
NPB #15-118 Revised

Investigator: Dr Rajesh Jha

Institution: University of Hawaii

Date Submitted: November 25, 2016

Industry Summary:

Price and availability of conventional feedstuffs like corn and wheat used in pig diets are widely variable due to the variation in production and ever-increasing demand. This is also because these grains are used in production of ethanol in large quantity. This situation has led to increase in cost of swine diets, affecting the competitiveness of the swine industry. Thus, there is a need for relatively cheap alternatives to the cereal grains. Distiller's dried grain with solubles (DDGS), a byproduct from the ethanol industry is available in U.S. in large quantities. It has a higher content of crude protein (CP), amino acids (AA), fat, fiber, and minerals than parent cereal grains. Inclusion of DDGS in swine diets has been shown to result in significant reduction in cost of swine feeds. Therefore, DDGS can likely serve as a partial alternative to cereal grains in swine diets. However, utilization of DDGS in formulation of swine diets might be limited due to its low digestibility. Non-starch polysaccharides (NSP) like arabinoxylans and mannans are present in high concentration in DDGS. These dietary fibers are not degraded by endogenous enzymes, increase the digesta viscosity and reduce the digestibility of nutrients. Thus, there is need for determining the reason of the lower digestibility of DDGS in pig intestine and way to enhance its utilization in the swine diets.

The overall objective of the study was to explore why the protein and fibers of DDGS are not well digested and to determine the appropriate strategy to enhance their digestion/utilization in the pig intestine. The specific objectives were to characterize the corn DDGS matrix for their nutritional configuration; to determine in vitro digestion and fermentation of corn DDGS without or with enzymes; and to determine ileal and total tract

nutrient digestibility in weaner pigs fed with or without enzyme-supplemented corn-DDGS-based diets.

A corn DDGS sample was analyzed for its nutrient profile along with individual monomers of NSP. Also, the fiber-starch-protein matrix structure of the DDGS was determined using confocal laser scanning microscopy. In vitro enzymatic (pepsin-pancreatin) digestion followed by in vitro gas production technique was used to simulate the digestion and microbial fermentation occurring in the gastrointestinal tract of pigs and to determine the digestibility and fermentability of DDGS in pig gastrointestinal tract. Also 3 different enzymatic treatments (xylanase, mannanse and a combination of xylanase and mannanse) and a control were used in the in vitro fermentation study to determine the effect of enzymes on DDGS utilization in the pig intestine.

An animal study with weaner pigs was conducted to validate the effect of enzymes on the utilization of DDGS in the pig intestine studied in vitro. For the animal study, four experimental diets were formulated consisting of corn, soybean meal and 15% corn DDGS [control, supplemental xylanase (0.01% of diet), mannanase (0.05% of diet), and xylanase + mannanase]. Thirty-two weaner pigs were used in randomized complete block design for a feeding trial of 20 days. Titanium dioxide was blended into the experimental diets (0.3%) as an indigestible marker on day 14. Fecal samples were collected for terminal 3 days to determine apparent total tract digestibility (ATTD). On day 20, digesta samples from ileum, jejunum and colon were collected to measure apparent ileal digestibility (AID), viscosity and pH.

Protein and starch content of the corn DDGS sample was 27.4% and 9.2%, respectively. The total NSP content of corn DDGS was 31.8%, of that 8.2% was soluble NSP. In vitro apparent ileal digestibility (AID) of DM and gross energy of corn DDGS was found to be 65.3% and 63.4%, respectively. The xylanase supplementation resulted in highest amount of gas production in vitro, which is reflection of fermentability of the fiber components of DDGS. It was also revealed by the matrix structure as seen under confocal laser scanning microscope. In vitro fermentation studies revealed that xylanase affected ($P<0.05$) the production of total short chain fatty acid and propionate, whereas, mannanase affected ($P<0.05$) production of butyrate.

There was no effect of supplemental enzymes on growth performance of piglets as 20 days might not have been sufficient to show an effect. Addition of xylanase reduced ($P<0.05$) the viscosity of jejunal digesta (2.1 to 1.5 centipoise), increased AID of total NSP, arabinoxylan and AID of gross energy. Supplementation of mannanase increased ($P <0.05$) the AID of mannans of DDGS but had no effect on AID of cellulose and protein as well as ATTD of gross energy and protein. In conclusion, supplemental enzymes in diets containing DDGS increases degradation of fibers, increases NSP digestibility, and decreases the viscosity of digesta. However, there is variation in the response of different enzymes; xylanase had the

best effects among tested enzymes. Hence, xylanase enzyme can be used to improve the utilization of corn DDGS in the diet of pigs.

Key Findings:

- The Corn DDGS is rich in both protein and fiber (about 1/4th being soluble fiber). These fibers entrap the nutrients (starch and protein) which limits the access of endogenous enzymes, thereby reduces the digestibility of nutrients.
- Supplemental enzymes degrade fibers and releases the encapsulated nutrients, hence helps in utilizing the fibrous coproducts like DDGS efficiently in swine diets.
- Among tested, xylanase enzyme had the best response in degrading fibers. Thus, xylanse enzyme can be used in corn DDGS based in swine diets.
- Inclusion of higher percentage of fibrous coproducts like corn DDGS with supplemental would reduce the cost of production and increase the return on investment.
- Efficient utilization of fibers in swine diets will also contribute in improving gut health positively.

Keywords: Corn DDGS, Digestibility, Enzymes, In vitro fermentation, Non starch polysaccharides, Pigs

Scientific Abstract:

Price and availability of conventional feedstuffs like corn and wheat used in pig diets are widely variable due to the variation in production and ever-increasing demand. This is also because these grains are used in production of ethanol in large quantity. This situation has led to increase in cost of swine diets, affecting the competitiveness of the swine industry. Thus, there is a need for relatively cheap alternatives to the cereal grains. Distiller's dried grain with solubles (DDGS), a byproduct from the ethanol industry is available in U.S. in large quantities. It has a higher content of crude protein (CP), amino acids (AA), fat, fiber, and minerals than parent cereal grains. Inclusion of DDGS in swine diets has been shown to result in significant reduction in cost of swine feeds. Therefore, DDGS can likely serve as a partial alternative to cereal grains in swine diets. However, utilization of DDGS in formulation of swine diets might be limited due to its low digestibility. Non-starch polysaccharides (NSP) like arabinoxylans and mannans are present in high concentration in DDGS. These dietary fibers are not degraded by endogenous enzymes, increase the digesta viscosity and reduce the digestibility of nutrients. Thus, there is need for determining the reason of the lower digestibility of DDGS in pig intestine and way to enhance its utilization in the swine diets.

The overall objective of the study was to explore why the protein and fibers of DDGS are not well digested and to determine the appropriate strategy to enhance their digestion/utilization in the pig intestine. The specific objectives were to characterize the corn DDGS matrix for their nutritional configuration; to determine in vitro digestion and fermentation of corn DDGS without or with enzymes; and to determine ileal and total tract nutrient digestibility in weaner pigs fed with or without enzyme-supplemented corn-DDGS-based diets.

A corn DDGS sample was analyzed for its nutrient profile along with individual monomers of NSP. Also, the fiber-starch-protein matrix structure of the DDGS was determined using confocal laser scanning microscopy. In vitro enzymatic (pepsin-pancreatin) digestion followed by in vitro gas production technique was used to simulate the digestion and microbial fermentation occurring in the gastrointestinal tract of pigs and to determine the digestibility and fermentability of DDGS in pig gastrointestinal tract. Also 3 different enzymatic treatments (xylanase, mannanse and a combination of xylanase and mannanse) and a control were used in the in vitro fermentation study to determine the effect of enzymes on DDGS utilization in the pig intestine.

An animal study with weaner pigs was conducted to validate the effect of enzymes on the utilization of DDGS in the pig intestine studied in vitro. For the animal study, four experimental diets were formulated consisting of corn, soybean meal and 15% corn DDGS [control, supplemental xylanase (0.01% of diet), mannanase (0.05% of diet), and xylanase + mannanase]. Thirty-two weaner pigs were used in randomized complete block design for a feeding trial of 20 days. Titanium dioxide was blended into the experimental diets (0.3%) as an

indigestible marker on day 14. Fecal samples were collected for terminal 3 days to determine apparent total tract digestibility (ATTD). On day 20, digesta samples from ileum, jejunum and colon were collected to measure apparent ileal digestibility (AID), viscosity and pH.

Protein and starch content of the corn DDGS sample was 27.4% and 9.2%, respectively. The total NSP content of corn DDGS was 31.8%, of that 8.2% was soluble NSP. In vitro apparent ileal digestibility (AID) of DM and gross energy of corn DDGS was found to be 65.3% and 63.4%, respectively. The xylanase supplementation resulted in highest amount of gas production in vitro, which is reflection of fermentability of the fiber components of DDGS. It was also revealed by the matrix structure as seen under confocal laser scanning microscope. In vitro fermentation studies revealed that xylanase affected ($P<0.05$) the production of total short chain fatty acid and propionate, whereas, mannanase affected ($P<0.05$) production of butyrate.

There was no effect of supplemental enzymes on growth performance of piglets as 20 days might not have been sufficient to show an effect. Addition of xylanase reduced ($P<0.05$) the viscosity of jejunal digesta (2.1 to 1.5 centipoise), increased AID of total NSP, arabinoxylan and AID of gross energy. Supplementation of mannanase increased ($P <0.05$) the AID of mannans of DDGS but had no effect on AID of cellulose and protein as well as ATTD of gross energy and protein. In conclusion, supplemental enzymes in diets containing DDGS increases degradation of fibers, increases NSP digestibility, and decreases the viscosity of digesta. However, there is variation in the response of different enzymes; xylanase had the best effects among tested enzymes. Hence, xylanase enzyme can be used to improve the utilization of corn DDGS in the diet of pigs.

Introduction:

Feed constitutes more than 70% of the cost of producing pork. The production of pork in the U.S. is constrained by rising feed costs due to an increased demand for cereal grains by the ethanol industry. For instance, since 2006, the prices of swine feeds have increased by approximately 40%, whereas the price of pork has not increased. On the other hand, co-products from the ethanol industry, such as DDGS, are available in U.S. in large quantities and at relatively low cost. Inclusion of DDGS in swine diets has indeed been shown to result in a significant reduction in cost (Skinner et al., 2012), but their utilization in swine diets has been limited by their poor digestibility. Improving the digestibility of DDGS through the use of supplemental enzymes to effectively degrade it in the pig gut can lead to increased use of corn DDGS in swine diets and a significant reduction in the cost of producing pork. More than 50% of the pork produced in U.S. is exported, and the reduced cost of producing pork will result in improved international competitiveness of the pork industry.

Distiller's dried grains with solubles have higher protein, amino acids, fat, fiber and minerals than parent cereal grains because starch is removed by fermentation, leaving behind concentrated amounts of other nutrients. Crude protein, amino acids and phosphorus are 2 to 3 times higher in corn DDGS than in the parent grains (Stein and Shurson, 2009; Almeida et al., 2011). Utilization of DDGS in formulation of swine diets, however, is limited by its low protein digestibility. The apparent ileal digestibility (AID) of crude protein and lysine for corn DDGS in pigs were 27 and 50% lower, respectively, than for corn (Almeida et al., 2011). In addition to low protein digestibility, utilization of DDGS in swine diets is limited by its high fiber content, which is 2 to 3 times higher than in the parent cereal grains (Stein and Shurson, 2009). Dietary fiber is indigestible by the gastrointestinal (endogenous) enzymes of pigs (Bedford and Schulze, 1998), and reduces nutrient absorption (Schulze et al., 1994) and voluntary feed intake (Nyachoti et al., 2004).

Pork production in the U.S. is also constrained by its environmental footprint due to the excretion of unabsorbed nutrients. Adding supplemental enzyme products to swine feeds containing DDGS will increase the digestibility of nutrients and hence reduce the discharge of these nutrients by pigs to the environment. As well as reducing costs, enzyme supplementation can improve environmental sustainability.

The focus of this study was therefore better understanding and overcoming the limitations to inclusion of corn DDGS in swine diets. Using enzymes to improve digestibility will allow greater inclusion of corn DDGS to replace grains in the diet. In addition, corn DDGS have higher protein content than cereal grains. Increasing their inclusion in swine diets at the expense of cereal grains will reduce the need for inclusion of soybean meal in the diets and further reduce costs. Controlling feed costs is extremely important to the profitability and competitiveness of the swine industry.

Objectives:

The overall objective of the study was to explore why the protein and fibers of DDGS are not well digested and to determine the appropriate strategy to enhance their digestion/utilization in the pig intestine. The specific objectives were:

I. To characterize the corn DDGS matrix for their nutritional configuration.
II. To determine in-vitro digestion and fermentation of corn DDGS without or with enzymes.
III. To determine ileal and total tract nutrient digestibility and fermentability in nursery pigs fed with or without enzyme-supplemented corn-DDGS-based diets.

It was hypothesized that DDGS fibers undergo structural or conformational changes and interact with other components of the grains during ethanol production, resulting in products that are carbohydrase-resistant. It is also hypothesized that DDGS protein undergoes structural or conformational changes during ethanol production, resulting in products that are resistant to gastrointestinal enzyme digestion. Thus, added enzyme would unlock the carbohydrate-protein matrix, thereby enhancing nutrient utilization of corn DDGS in the pig intestine.

Materials & Methods:

Three studies were conducted to achieve the above listed objectives, one study per objective. The first study explored the nutritional content and the matrix characteristics of the corn DDGS. The second study determined the effect of supplementing different feed enzymes (mannanase, xylanase and a combination of xylanase and mannanase) with corn DDGS on in-vitro digestion and fermentation characteristics with a view of identifying the most effective enzyme supplementation strategy, which was used in the animal study (3rd study) to validate the effect of selected enzymes on enhancing nutrient utilization of corn DDGS in the pig intestine.

Study 1. Characterization of nutritional content and matrix structure of the corn DDGS matrix

1.1 Nutrients analysis

A corn DDGS sample was collected (from a Bioethonol plant in U.S.), ground to pass through a 1.0 mm-mesh screen using a laboratory mill. Ground samples was subjected to proximate analysis according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007) with specific methods as follows: DM (135°C for 2 h, AOAC 930.15), ash (AOAC 942.05), CP by determining N using Kjeldahl method (AOAC 976.05, CP = N × 6.25), ether extract (AOAC 920.39; using Soxhlet apparatus and petroleum ether), ADF (AOAC

973.18), and NDF (AOAC 2002.04). Total starch content was determined using test kit (Megazyme International, Ireland). Gross energy was determined using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL). Total and soluble NSP with their constituent sugars were quantified by Gas chromatography, following procedures and calculations as described by Englyst et al. (1994).

1.2 Nutrient matrix characterization

Double Staining of Starch Granules with APTS and Pro-Q Diamond. A double-staining technique was used to label starch molecules and phosphorus-associated molecules within the fiber-starch-protein matrix. Starch molecules in the samples will be stained with APTS (Molecular Probes, Eugene, OR, USA) according to the method described by Blennow et al. (2003).

Confocal Laser Scanning Microscopy. Stained corn DDGS samples in 50% glycerol (10 μL) will be dropped into a glass bottom culture dish, mixed with 0.1 mL of deionized water, covered with a glass slip, and then observed under a confocal laser scanning microscope (LSM 710, Carl Zeiss MicroImaging GmbH, Germany) equipped with a ×40 1.3 oil objective lens. The excitation was at 488 and 561 nm operating at 1 and 4% of power capacity, respectively, with an emission light interval of 490–560 nm. Images of optical sections of DDGS matrix was recorded with ZEN software (Carl Zeiss MicroImaging GmbH, Germany).

The analyses of the nutritional profile of the corn DDGS provided a detailed nutritional value, while the microscopic analysis provided information on matrix structure.

Study 2. In-vitro digestion and fermentation characteristics of corn DDGS without or with supplemental enzymes

This study was conducted to achieve the second objective. The method is divided in two main steps:

- Enzymatic digestion: treatment of a sample with pepsin and pancreatin (mixture of pancreatic enzymes) to mimic the digestive processes in the stomach and the small intestine and to remove starch, protein and fat.
- Fermentation process: The residue of the pre-digestion is mixed together with inoculum (= bacteria) coming from pig feces, in a buffer solution. The bottle is incubated at 39 °C for 72 h and gas production is measured by means of a pressure transducer. The

results are used to characterize the kinetics of gas production. The residues are analyzed for short-chain fatty acids (SCFA), and other tools as per interest.

The scheme of the in vitro digestion and fermentation technique is presented in Figure 1.

2.1 In vitro digestion

The 2-step in vitro digestion technique (Boisen and Fernandez, 1997) simulates the digestion activities occurring in the upper gastrointestinal tract of the pig and provides information on the apparent ileal digestibility of DM, energy and other nutrients.

The corn DDGS sample was ground and subjected to 2-step in vitro digestion as described by Boisen and Fernandez (1997) with some modifications (Jha et al., 2011). Briefly, 2 g sample was weighed in conical flask. A phosphate buffer solution (100 ml, 0.1 M, pH 6.0) and an HCl solution (40 ml, 0.2 M) was poured into the flasks. Two ml of a chloramphenicol (Sigma C-0378, Sigma-Aldrich Corp., St. Louis, MO) solution (0.5 g/100 ml ethanol) was added to prevent bacterial growth during hydrolysis. Fresh pepsin solution (4 ml, 20 g/L porcine pepsin, Sigma P-0609) was added and the flasks will be placed in a water-bath at 39°C for 2 hr under gentle agitation (50 rpm). Afterwards, 40 ml phosphate buffer (0.2 M, pH 6.8) and 20 ml of 0.6 M NaOH was added into the solution. Fresh pancreatin solution (2 ml, 100 g/l pancreatin; Sigma P-1750) was added and hydrolysis will be continued for 4 hr under the same conditions. After hydrolysis, the residues were collected by filtration on a nylon cloth (42 μm), washed with ethanol (2 × 25 ml 95% ethanol) and acetone (2 × 25 ml 99.5% acetone), dried for 12 hr at 60°C and weighed.

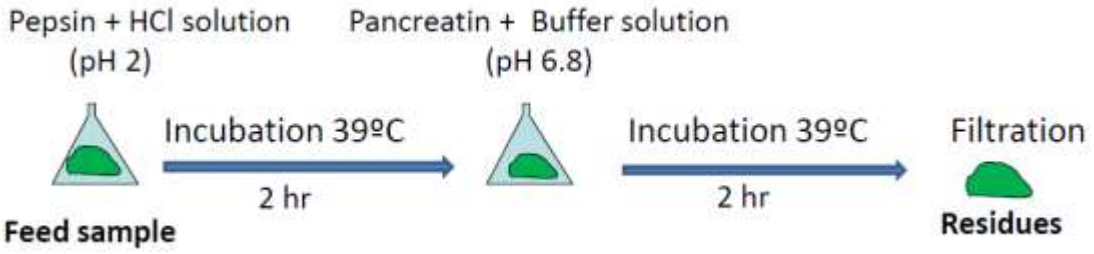
Cycles of the in vitro digestion was repeated until when enough sample residues for the in-vitro fermentation and sample analysis was attained. The residues from the different cycles will be pooled. Residues from the pre-digestion process (mimicking ileal digesta of in vivo) were used as sample for in vitro fermentation study simulating large intestinal activities.

2.2 In vitro fermentation

The in-vitro fermentation technique (Jha et al., 2011) simulates the microbial fermentation occurring in the large intestine of the pigs. It provides information on the total gas and

fermentation metabolites produced by the microbial fermentation, which are directly proportional to the amount of substrate fermented.

Step 1. Pre-digestion with pepsin (simulation of stomach) and pancreatin (simulation of small intestine)



Step 2. In vitro microbial fermentation technique (simulation of large intestine)

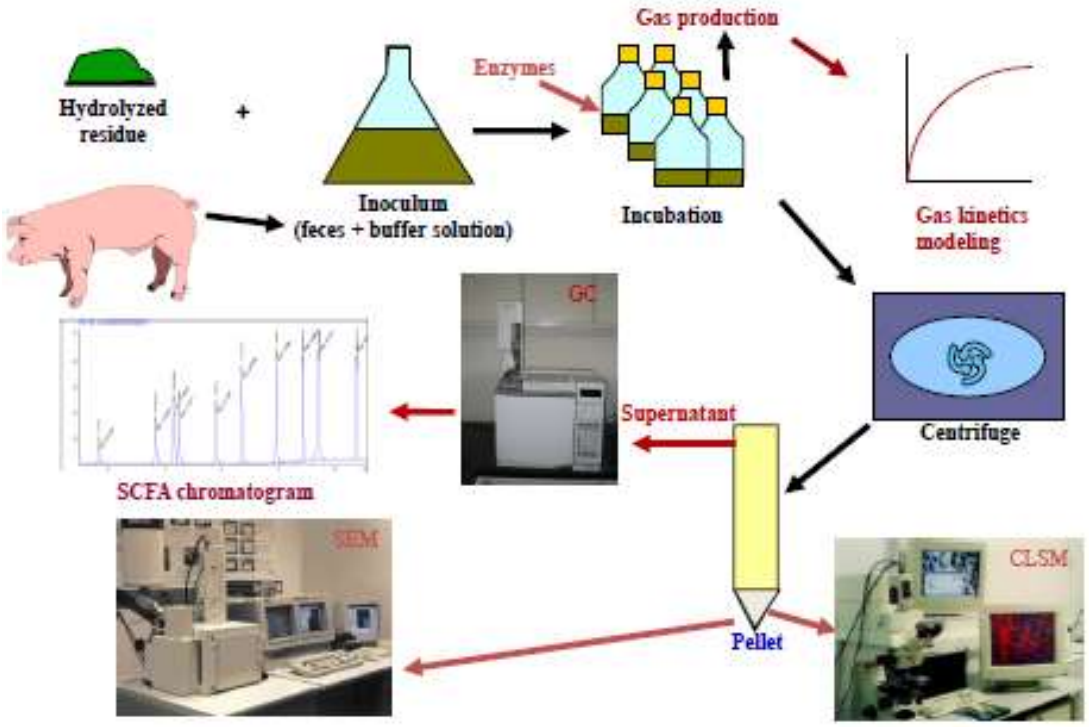


Figure 1. In vitro technique for fiber fermentation study in the pig intestine

The rate of fermentation of the hydrolyzed substrates was assessed in vitro, using a cumulative gas-production technique adapted to the pig by Jha et al. (2011) with minor modification to study the effect of enzymes on degradation and fermentation of substrates as described by Jha et al. (2015). Briefly, 200 mg samples will be incubated at 39°C (in a shaking water-bath with 50 rpm) in a 125 ml glass bottle with 30 ml buffer solution containing macro- and micro-minerals and a fecal inoculum. In addition, a Xylanase (Hostazym X 100, Huvepharma USA, Peachtree City, GA), Mannanase (CTCZYME; CTC Bio Inc., Seoul, Korea) or a combination of Xylanase and Mannanase was added in some of the bottles. Three growing

pigs from the herd of a commercial farm, fed a standard commercial diet devoid of antibiotics, were used as donors for the fecal inoculums. The inoculum prepared from feces was diluted 20 times in the buffer solution and filtered through a 250 μm screen and transferred into the bottle with fermentation substrates. Bottles was sealed with a rubber stopper and placed for incubation. An anaerobic environment was maintained throughout the experiment, from inoculum preparation until the incubation step, by flushing with CO_2 gas. The gas generated by fermentation and CO_2 released by buffering of SCFA produced during the fermentation was measured at 0, 2, 5, 8, 12, 18, 24, 36, 48 and 72 hr by means of a pressure transducer (GP:50 SIN-54978, Grand Island, NY, USA), fitted with digital data tracker (Tracker 211, Intertechnology Inc., Don Mills, ON, Canada). The bottles were vented after every measurement. Fermentation was stopped at 72 hr of incubation by quenching the bottles in ice water, and samples was collected from the bottles and stored frozen for latter analyses.

2.3 Chemical analysis

Samples collected from the bottles at the end of fermentation were centrifuged; and supernatant from each bottle was obtained for analyses, whereas the pellet (residue) from each bottle was obtained, freeze-dried and weighed before analyses. The dried in vitro digestion and fermentation residues and the ground feedstuffs was analyzed for dry matter (method 930.15), crude protein (method 984.13A-D) using AOAC (2007), and starch using test kit (Megazyme International, Ireland). Supernatants from the bottles at the end of the fermentation were analyzed for SCFA by gas chromatography as described by Jha et al. (2010).

2.4 Calculations

In vitro dry matter degradability during pepsin and pancreatin hydrolysis, and in vitro dry matter degradability during fermentation was calculated as described by Jha et al. (2011). The disappearance of the other nutrients was calculated using the degradability of dry matter and the relative content of individual nutrients in the feedstuffs, and in in vitro digestion and fermentation residues.

2.5 Statistical analyses

The in vitro dry matter, crude protein, and starch degradability; total gas production; and SCFA production was analyzed using the MIXED procedure of SAS 9.2 software (SAS Institute Inc., Cary, NC). Enzyme treatment was the fixed factor in the model; and subsample (during both in vitro digestion and fermentation) and batch (only during in vitro digestion) as random factors. Means were separated by the Tukey method using “pdmix” macro of SAS and differences among variables were declared significant at a probability level of 0.05.

Study 3. Ileal and total nutrient digestibility and fermentability in nursery pigs fed enzyme-supplemented corn DDGS-based diets

3.1 Animals and housing

The experiment was conducted at the metabolism educational unit at the North Carolina State University (Raleigh, NC) following the guidelines stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and was approved by the Institutional Animal Care committee of NCSU, Raleigh, NC.

The piglets without creep feed during the lactation period were weaned at day 23. Thirty-two pigs (PIC 337 × Camborough 22), 16 males and 16 females with an average body weight of 7.5kg (after adaptation period of 6 days) was used for the study. They were fed basal diet (Table 1) for an adaptation period of 6 days. All the piglets were housed in an environmentally controlled room where temperature was maintained at 80^{o}F. All the piglets were placed individually in a metabolic cage (5×2.41 feet) without bedding and with elevated plastic covered expanded metal grid flooring. All the cages were equipped with plastic feeder in the front and nipple water drinker. Freedom of movement and nose to nose contact between the pigs in adjacent pens were allowed during the entire experiment.

3.2 Experimental diets

Four experimental diets (1.25% SID Lys, 3370 kcal ME/kg) was formulated (Tables 1 and 2) using corn, corn DDGS (15% in the feed), whey permeate, and soybean meal as major ingredients: diet without enzyme (control) and 3 diets with supplemental feed enzymes. All diets met or exceed the nutrient requirements of the age group of swine (NRC, 2012). Three feed enzymes were used in the study (xylanase, mannanase, and a combination of xylanase and mannanase). The four experimental diets were as follows: Control (without enzyme), control + xylanase, control + mannanase, and control + xylanase + mannanase. The diet was offered in mash form and fed ad libitum for 20 days. Xylanase (Hostazym X 100, Huvepharma USA, Peachtree City, GA), was supplemented at 100 mg/kg of diet. β-Mannanase enzyme (CTCZYME; CTC Bio Inc., Seoul, South Korea) was supplemented at 500 mg/kg of diet.

The basal diet used in the phase-1 of the animal study is presented in Table 1.

Table 1. Ingredient composition of basal (phase-1) diet

Ingredients	**Inclusion, %**
Corn, yellow dent	38.345
Poultry fat	1.50
Soybean meal, dehulled	23.00
Blood plasma	4.00
Fish meal	4.00
L-Lys HCl	0.40
DL-Met	0.20
L-Thr	0.12
Dicalcium phosphate	0.00
Limestone, ground	0.71
Vitamin premix	0.03
Mineral premix	0.15
Salt	0.22
Zinc Oxide	0.25
Whey Permeate	20.00
Poultry Meal	5.00
AV blend oil	1.50
Dicalcium phosphate	0.20
Mecadox 10	0.175
Dehagard 10	0.200

The composition and the nutrient profile of the phase 2 diets along with NSP content are presented in Tables 2 and 3.

Table 2. Ingredient composition and nutrient content of phase-2 diets used in the study

Ingredients %	**control**	**control+ xylanase**	**control+ mannanase**	**Control+ xylanase+ mannanase**
Yellow corn, ground	39.650	39.630	39.550	39.535
Poultry fat	1.70	1.70	1.70	1.70
Soybean meal, dehulled	23.00	23.00	23.00	23.00
Blood plasma	2.00	2.00	2.00	2.00
Fish meal	2.00	2.00	2.00	2.00
L-Lys HCl	0.44	0.44	0.44	0.44
DL-Met	0.12	0.12	0.12	0.12
L-Thr	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.00	0.00	0.00	0.00
Limestone, ground	1.05	1.05	1.05	1.05
Vitamin premix[1]	0.03	0.03	0.03	0.03
Mineral premix[2]	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
Whey Permeate	10.00	10.00	10.00	10.00
Poultry Meal	2.00	2.00	2.00	2.00
AV blend oil	1.70	1.70	1.70	1.70
Dicalcium phosphate	0.46	0.46	0.46	0.46
Corn DDGS[3]	15.00	15.00	15.00	15.00
Mecadox 10	0.125	0.125	0.125	0.125
xylanase[4]	0.000	0.01	0.00	0.01
Mannanase[5]	0.000	0.000	0.05	0.05
Calculated composition				
ME, Kcal/kg	3472.6	3472.6	3472.6	3472.6
Lys[6], %	1.35	1.35	1.35	1.35
Met + Cys6, %	0.744	0.744	0.744	0.744
Trp[6], %	0.224	0.22	0.22	0.224
Thr[6], %	0.80	0.80	0.80	0.80
Ca	0.798	0.798	0.798	0.798
STTD P	0.396	0.40	0.40	0.396
Total P	0.62	0.62	0.62	0.62
Analyzed composition, % DM basis				
Dry matter, %	91.58	91. 56	91.58	91.69
Crude protein	24.97	24.84	25.12	23.88
Ash	5.93	5. 99	5.95	6.1
NDF	9.67	9.53	9.45	9.16
ADF	5.16	4.54	4.3	4.39

Ether extract	7.35	7.19	7.3	7.32

[1]The vitamin premix/kg complete diet provided: 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.

[2]The trace mineral premix/kg complete diet provided: 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.

[3]DDGS: distillers dried grains with solubles.

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

[5]Mannanase (CTCBIO Inc., Seoul, Korea) was used at 0.05% (replacing corn for treatment diets).

[6]Standardized ileal digestibility.

Table 3. Non starch polysaccharide profile of phase-2 diets used in the study

NSP		**control**	**control+ xylanase**	**control+ mannanase**	**Control+ xylanase+ mannanase**
Arabinose	Total	1.79	1.50	1.51	1.45
	Ins	1.19	0.86	0.91	0.90
	Sol	0.60	0.65	0.61	0.55
Xylose	Total	2.85	2.69	2.65	2.73
	Ins	2.63	2.38	2.38	2.47
	Sol	0.23	0.31	0.27	0.26
Mannonose	Total	0.65	0.65	0.66	0.66
	Ins	0.47	0.41	0.44	0.44
	Sol	0.18	0.24	0.21	0.22
NCP Glucose	Total	2.66	2.74	2.69	2.81
	Ins	1.23	1.31	1.25	1.35
	Sol	1.43	1.43	1.45	1.46
Galactose	Total	1.86	2.08	2.19	1.99
	Ins	0.81	1.95	0.75	0.76
	Sol	1.05	0.14	1.44	1.23
Cellulose		1.53	1.55	1.55	1.64
Total NSP		11.34	11.25	11.21	11.27
Total Soluble NSP		3.48	3.97	2.77	3.71
Arabinose: Xylose (A:X) ratio		0.63	0.57	0.56	0.53

Total NSP in the diet offered was around 11% out of which 3% were soluble i.e. one fourth of them were soluble. So, the larger proportion of NSP in the diet were insoluble. The A:X ratio was around 0.6 which is high. Higher A:X ratio is indication of higher substitution of arabinose on the xylan backbone, higher branching and more cross linkage which makes difficult for the xylanase to act on.

3.3 Experimental design and sampling

Thirty-two piglets were used in a randomized complete block design, where individual pigs were treated as experimental unit. Blocks were sex (fixed effect) and initial body weight (random effect).

The piglets without creep feed during the lactation period were weaned at day 23. They were fed basal diet (table 1) for an adaptation period of 6 days. From day 7-13 all the piglets were fed experimental diet. From day 14 titanium dioxide was mixed to all diets and fed to pigs. Fecal samples were collected over 3 consecutive days from day 17-19. On day 19, pigs were fasted overnight and exactly 4 hours after refeeding on day 20 in the morning, pigs were killed by penetration of captive bolt followed by exsanguination.

After killing, the abdomen was opened and the GIT was removed. Digesta samples from the ileum (30 cm immediately before ileo-cecal junction) and the colon (medial colon, 30 cm) was collected and homogenized on ice and subsampled. Digesta from jejunum was collected for viscosity determination and from jejunum, ileum and colon for pH determination.

3.4 Chemical analysis

Digesta and fecal samples was thawed, homogenized and freeze-dried. Diets, lyophilized digesta and feces were further ground using Wiley mill (Thomas Model 2 Wiley® Mill – Thomas scientific) to pass through 1 mm screen to get a uniform particle size. Proximate analysis of the samples was conducted according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007). Other analyses were conducted using specific procedure, as mentioned in corresponding sections below.

Dry matter was determined according to AOAC method 930.15 (135°C for 2 h), ash (AOAC 942.05), crude protein by determining N using Kjeldahl method (AOAC 976.05, CP = N × 6.25), ether extract (AOAC 920.39; using Soxhlet apparatus and petroleum ether). Acid detergent fiber (AOAC 973.18) and Neutral detergent fiber (AOAC 2002.04) was determined using Ankom^{200} Fiber Analyzer (Ankom Technology, NY). Heat stable amylase was used for NDF determination. Total starch content (AOAC 996.11) was determined using test kit (Megazyme International, Ireland). Gross energy was determined using a Parr Isoperibol oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL) with benzoic acid as calibration standard. NSP was analyzed using GC as described by Jha et al (2010).

Apparent ileal digestibility (AID) of DM, GE, NSP, arabinoxylan, mannan were calculated using the concentration of titanium dioxide in the feed as well as in ileal digesta. Equation below was used to calculate the digestibility:

$$\text{AID}, \% = \left(1 - \frac{\text{Titanium dioxide}_{\text{feed}} \times \text{Nutrient}_{\text{digesta}}}{\text{Titanium dioxide}_{\text{digesta}} \times \text{Nutrient}_{\text{feed}}}\right) \times 100\%,$$

where Titanium dioxide $_{\text{feed}}$ is the concentration of titanium dioxide in the feed, Titanium dioxide $_{\text{digesta}}$ is the concentration of titanium dioxide in the ileal digesta, Nutrient$_{\text{feed}}$ is the

nutrient concentration in the feed, and $Nutrient_{digesta}$ is the nutrient concentration in the ileal digesta.

3.5 Non starch polysaccharide determination

Total and soluble NSP of with their constituent sugars were quantified by Gas chromatography, following procedures and calculations as described by Englyst et al. (1994). Chromatographic analysis was done using a GC system (TRACE™ 1300 gas chromatograph, Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector and a fused silica capillary column (DB-17HT, Agilent Technologies, Wilminton, DE, USA), using 2-Deoxy-D-Glucose as an internal standard. Additional NSP-related calculations were done as follows:

Cellulose = NSP glucose – non cellulosic Polysaccharide (NCP) glucose

Total NSP = rhamnose + fucose + arabinose + xylose + mannose + NCP-glucose + cellulose

Soluble NSP = total NSP – insoluble NSP

Arabinoxylan = arabinose + xylose

3.6 Viscosity determination

Viscometer (Brookfield Digital Viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with C-40 cone and plate was used to measure the viscosity of jejunal digesta. 15 ml of digesta was taken in a tube and centrifuged at 3000 × g for 5 minutes, then 2 ml of supernatant was transferred in 5ml tube and centrifuged at 12,500 × g for 5 min. Finally, 0.5 ml aliquot obtained from the supernatant solution was used in the viscometer to measure the viscosity. The viscometer was set at 25^{o}C. Viscosity of each samples was measured 4 times at shear rates 45.0 sec -1 and 22.5 sec -1. The final result was calculated as average between the viscosities measured at 45.0 sec -1 and 22.5 sec -1 shear rates.

3.7 pH determination

pH of digesta (ileulm, jejunum, colon) was measured immediately after collection using a digital pH meter (Accumet, Fisher Scientific, US).

3.8 Statistical analysis

Data were analyzed using MIXED procedure of SAS v9.2 (SAS Institute Inc., Cary, NC). In this experiment, pigs were allotted to randomized complete block design using initial BW and sex as blocking factors. The experimental unit was the individual pig. Initial BW was a random effect, whereas enzyme supplement and DDGS inclusion were considered fixed effects.

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

Results:

1. Nutrient profile and matrix corn DDGS

1.1 Nutrient profile of corn DDGS

Protein and starch content of the corn DDGS sample was 27.4% and 9.2%, respectively. The total NSP content of corn DDGS was 31.8%, of that 8.2% was soluble NSP.

Table 4. Basic nutrients and non-starch polysaccharide content of the DDGS samples used in both in vitro and in vivo study

Items, %, DM basis		Value
DM, %		92.4
CP		27.4
GE, Kcal/kg		4892
Ash		3.6
Starch		9.2
Ether extract		1.28
Total NSP		
	Total	31.84
	Soluble	8.27
	Cellulose	6.00
Arabinose		
	Total	7.90
	Soluble	2.35
Xylose		
	Total	10.22
	Soluble	2.63
Mannose		
	Total	1.26
	Soluble	0.06
Glucose		
	Total	4.81
	Soluble	3.05
Galactose		
	Total	1.65
	Soluble	0.18
A:X ratio		0.77

The structure of corn DDGS is more complex represented by higher A:X ratio. Total NSP content in DDGS is high (31.84%), out of which only 8% is soluble and rest are insoluble NSP

1.2 Matrix characterization of DDGS using confocal laser scanning microscope

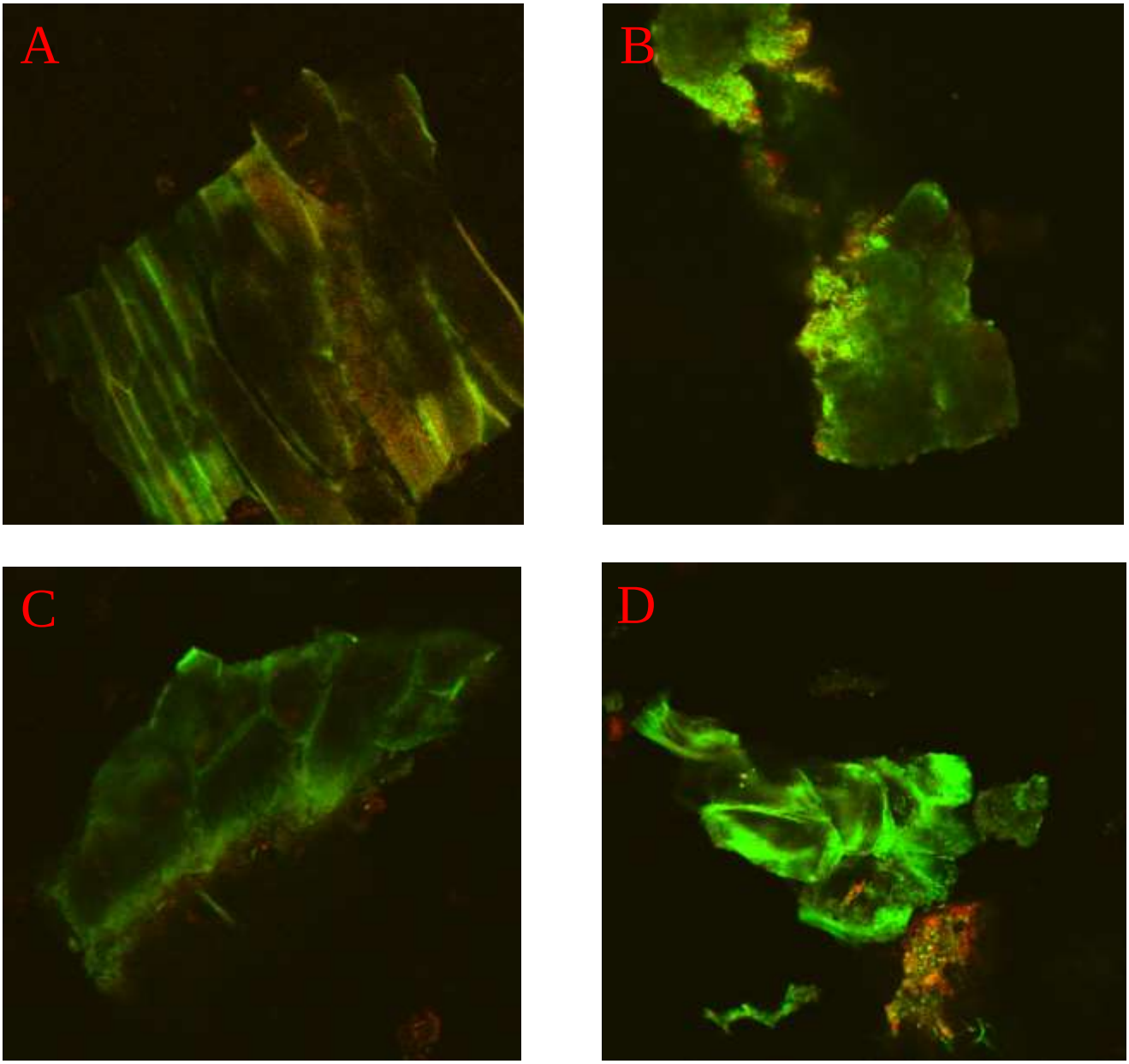


Figure 2. Confocal laser scanning microscopic images (40×) of the corn distillers dried grains with solubles (DDGS), A- corn DDGS; B- corn DDGS + xylanase; C- corn DDGS+ mannanase; and D- corn DDGS + xylanase + mannanase

Aminofluorophore 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) is one of the specific dyes that reacts with the reducing ends of starch molecules. Starch and starch residues turn green as they are stained with florescent dye APTS. The green color gets brighter with increasing concentration of reducing sugars in the starch. If image shows starch having greater flourescent intensity, it means starch remaining in the sample contains higher amount of reducing sugar. i.e. higher fluorescent intensity (green) = Presence of large amount of reducing sugar = higher amylose content of starch.

Pro-Q Diamond phosphoprotein stain allows sensitive detection of phosphorylation levels of protein in gels. The P-associated components such as protein, lipids, and nucleic acids that are stained with Pro-Q Diamond have colors that range from yellowish green to red depending on the concentration of reducing sugar and P in the matrix.

2. In-vitro digestion and fermentation characteristics of corn DDGS without or with enzymes

2.1 In vitro digestibility of corn DDGS

In vitro AID of dry matter and gross energy of corn DDGS was found to be 65.3% and 63.4%, respectively.

2.2 In vitro fermentation characteristics of corn DDGS

Addition of Xylanase produced highest amount of gas followed by combination of xylanase and mannanse. The DDGS without enzyme had the lowest gas production suggesting that addition of enzymes supported in degradation of fibers from the corn DDGS.

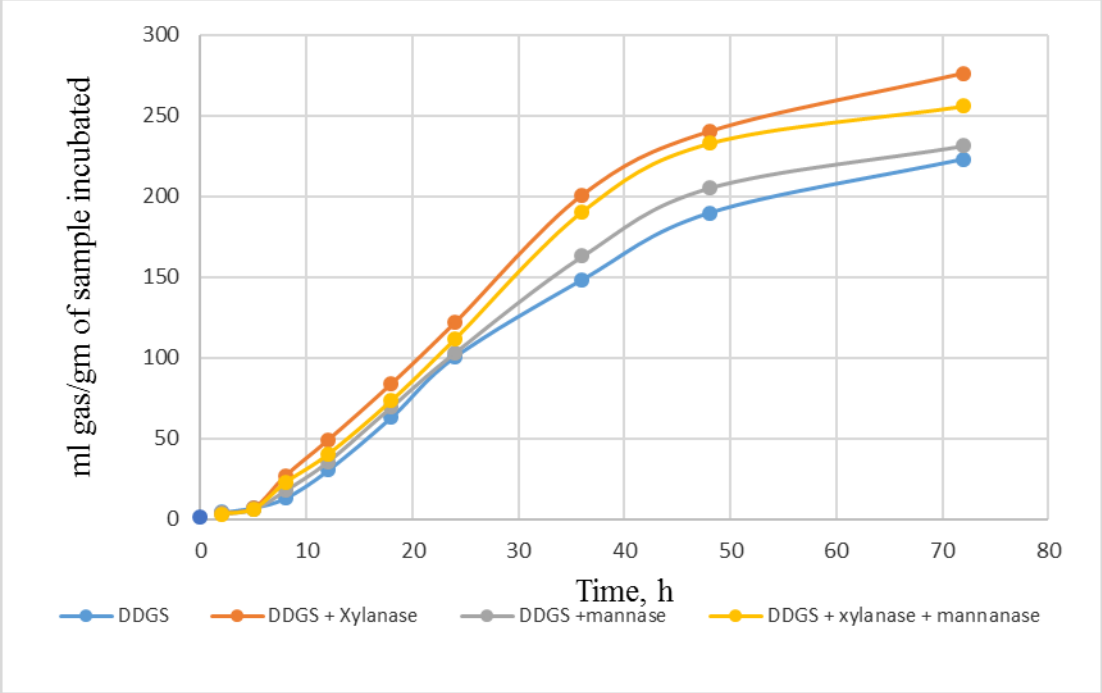


Figure 3. Kinetics of gas production of digested residue of corn DDGS during 72 hours incubation with pig feces as microbial inoculum.

2.3 Short-chain fatty acid production after fermentation of Corn DDGS without or with enzymes

Supplementation of enzyme had no effect (P>0.05) on acetate production. However, supplementation of xylanase significantly increased the production of propionate as well as total SCFA. However, significant effect of mannanase (P<0.05) was found in production of butyrate.

Table 5. Concentration of short chain fatty acids in the solution after fermentation of undigested residue of corn DDGS with pig fecal inoculum, µmol/ gm of sample incubated

Item	Treatment				SEM	*P-value*		
	Control	Xyl1	Man2	Xyl + Man		Xyl	Man	Xyl*Man
Acetate	1275	1406	1338	1263	67.45	0.677	0.555	0.130
Propionate	655	774	619	884	28.21	<.0001	0.197	0.012
Butyrate	78	92	107	112	5.14	0.077	<.0001	0.406
Total SCFA	2008	2273	2065	2259	74.09	0.002	0.768	0.638

3. Ileal and total tract nutrient digestibility in nursery pigs fed enzyme-supplemented corn DDGS-based diets

Growth performance:

Growth performance of nursery pigs fed corn DDGS diets supplemented with or without enzymes are presented in Table 6. There was no effect (P>0.05) of enzyme supplementation on either of the performance parameters recorded.**Table 6.** Growth performance of nursery pigs fed with corn DDGS based diet supplemented with or without enzymes

Item	Treatment				SEM	*P*-value		
	Control	Xyl[1]	Man[2]	Xyl + Man		Xyl	Man	Xyl×Man
BW, kg								
Initial	6.210	6.210	6.210	6.210				
Wk 1	7.530	7.520	7.630	7.530	0.511	0.729	0.716	0.766
Wk 2	11.140	10.960	10.920	11.080	0.782	0.974	0.885	0.585
Wk 3	14.720	14.910	14.940	15.400	0.935	0.503	0.465	0.787
ADG, g								
Wk 2	515	490	470	507	41.2	0.859	0.676	0.372
Wk 3	641	660	670	720	32.44	0.424	0.294	0.709
Overall	553	568	563	605	34.89	0.356	0.462	0.666
ADFI, g								
Wk 2	588	555	516	557	40.88	0.908	0.312	0.277
Wk 3	842	840	864	982	53.07	0.321	0.167	0.311
Overall	706	686	676	753	53.26	0.511	0.668	0.272
Gain: feed								
Wk 2	0.891	0.89	0.883	0.902	0.017	0.729	0.940	0.709
Wk 3	0.749	0.787	0.774	0.735	0.026	0.983	0.587	0.149
Overall	0.779	0.833	0.831	0.803	0.015	0.534	0.607	0.056

[1]Xylanase, [2]Mannanase

Nutrient digestibility

The AID of nutrients and NSP including some individual monomers are presented in Table 7. There was significantly higher (P<0.05) AID of total NSP in the pig supplemented with xylanase while lowest in the control diet. Similar trend was found for AID of Arabinoxylan. However, there was higher AID of Mannan in the Mannanase supplemented diet with significant interaction (P<0.05) between the treatment with a combination of xylanase and mannanase. There was no effect of enzymes (P>0.05) on protein digestion.

Table 7. Apparent ileal digestibility of nutrients and non-starch polysaccharides of nursery pigs fed with corn DDGS based diet supplemented with or without enzymes

Item	Treatment				SEM	*P*-value		
	Control	Xyl[1]	Man[2]	Xyl + Man		Xyl	Man	Xyl*Man
Total NSP Dig	33.61	40.06	35.40	40.24	1.98	0.006	0.620	0.685
Arabinoxylan Dig	21.02	33.58	27.81	30.41	2.17	0.001	0.407	0.025
Mannan Digestibililty	46.97	45.54	50.55	52.80	1.68	0.504	0.007	0.507
Cellulose Digestibility	14.01	13.81	14.33	13.97	0.53	0.601	0.648	0.880
Energy	55.09	58.38	59.44	59.93	0.68	0.009	0.0002	0.048
Protein	60.52	64.25	63.04	65.17	1.78	0.111	0.343	0.655

[1]Xylanase, [2]Mannanase

The ATTD of nutrients is presented in Table 8. There was no effect of enzymatic treatments (P>0.05) on the DM, GE and CP digestibility. However, there was significant effect of Xylanase (P<0.05) on ATTD of NDF and ADF in pigs fed corn DDGS diets.

Table 8. Total tract nutrient digestibility of nursery pigs fed with corn DDGS based diet supplemented with or without enzymes

Digestibility	Treatment				SEM	*P*-value		
	Control	Xyl[1]	Man[2]	Xyl+Man		Xyl	Man	Xyl*Man
DM Digestibility	83.3	84.3	83.3	83.8	0.714	0.291	0.762	0.709
GE Digestibility	80.0	81.1	79.9	80.6	0.887	0.336	0.691	0.851
CP Digestibiity	79.0	79.7	79.7	78.0	1.438	0.740	0.740	0.414
NDF Digestibility	35.1	41.2	40.4	45.8	2.359	0.033	0.060	0.890
ADF Digestibility	18.1	26.9	19.7	31.4	2.296	<.0001	0.158	0.492

[1]Xylanase, [2]Mannanase

Physico-chemical properties of digesta

The viscosity of jejunal digesta and pH value of jejunal, ileal and colonic digesta of nursery pigs fed corn DDGS diets supplemented with or without enzymes are presented in Table 9. Xylanase supplementation decreased (P<0.05) the viscosity of jejunal digesta significantly. There was no effect (P>0.05) of enzyme supplementation on the pH value of jejunal, ileal and colon digesta.

Table 9. Viscosity of jejunal digesta and pH value of jejunal, ileal and colonic digesta of nursery pigs fed with corn DDGS based diet supplemented with or without enzymes

Items	Treatment				SEM	*P*-value		
	Control	Xyl[1]	Man[2]	Xyl + Man		Xyl	Man	Xyl*Man
Viscosity	2.1	1.5	2.1	1.7	0.178	0.013	0.760	0.687
pH								
Jejunum	6.8	6.9	7.1	6.8	0.145	0.561	0.578	0.342
Ileum	7.0	7.0	6.9	6.9	0.121	0.657	0.486	0.907
Colon	6.0	6.2	6.2	6.2	0.110	0.694	0.453	0.467

[1]Xylanase, [2]Mannanase

Discussion

The Corn DDGS is rich in both protein and fiber as compared to parent grain corn. Also, the NSP content is very high in DDGS with almost 1/4th being soluble NSP. The NSP are found to reduce the digestibility of nutrients and energy in pig intestine (Jha et al., 2010). However, the NSP are fermented, primarily in the large intestine and may contribute to improved gut health (Jha and Berrocoso, 2015).

The total gas produced and SCFA production results of in vitro fermentation study suggest that the complex matrix of the fiber-protein-starch was broken effectively by use of enzymes. However, the effect varies based on the enzyme used (Jha et al., 2015). This was also supported by the matrix structure as revealed by confocal laser scanning microscopy. Moreover, the production of more SFCA by the use of enzyme can be useful strategy to gain more energy value from the DDGS and improve gut health of swine as the SCFA decreases the gut pH and are found to support the growth of beneficial bacteria in gut favorably (Jha and Berrocoso, 2015).

The results from the animal study show that large fraction of arabinoxylan and mannan was depolymerized into shorter oligosaccharides by use of supplemental xylanase and mannanase, indicating the ability that xylanase and mannanase has in degrading fibers present in DDGS and diet offered. Xylanase and mannanase alone increased the digestibility of arabinoxylan and mannan respectively but significant effect of mannanase on digestibility of total NSP was not seen. Though amount of mannan in the diet was less, mannans are better utilized in the body of swine than arabinoxylan. The amount of mannan in other feedstuff has been presented, however amount of mannan in corn DDGS was not explained.

Supplementation of xylanase significantly increased the digestibility of total NSP from 33% in the control diet to 40%, whereas arabinoxylan digestibility was increased from 20% in the control diet to 33% in the diet included with xylanase. There was only 7% increase in the digestibility of total NSP and 10-12% increase in the digestibility of arabinoxylan. This lower

amount of increase in digestibility margin can be because of complex structure of heteroxylan in the diet offered as well as in the DDGS. Indicators of complex arabinoxylan structure is A:X ratio and amount of insoluble arabinoxylan. Higher the A:X ratio, higher is the degree of substitution, higher is crosslinking between arabinoxylan and lower is the enzymatic degradation. Insoluble dietary fraction of corn contains higher amount of diferulates i.e. approximately 5-7 times more than that is present in wheat DDGS (Pedersen et al., 2014). Hence greater amount of ferulic acid can be expected in the diet offered.

Mannanase supplementation significantly increased the digestibility of mannan from 46% in the control diet to 50 in the diet supplemented with mannanase and 52% when xylanase was also added along with mannanase. There was about 4-7% increase in the digestibility of mannan when supplemented with mannanase. However, significant effect of mannanase on the digestibility of total NSP was not seen. It can be because amount of mannan in DDGS itself is very less, also amount of mannan in the diet offered was less than 1%, which indicates that mannanase did not get enough substrate to act on. The effect of mannanase can be elucidated more clearly when supplemented in the diet containing coproducts high in mannans like copra meal and palm kernel meal (Sundu et al., 2003).

Soluble NSP have the ability to form gel when they come in contact with liquid. Soluble β-glucan and arabinoxylan are generally involved in development of viscosity (Zijlstra et al., 1999). Amount of β-glucan is high in wheat whereas amount of arabinoxylan in high in corn DDGS (Zijlstra et al., 1999). Amount of soluble arabinoxylan in the diet offered was less than 1%, therefore the viscosity of jejunal digesta in the control group was lower (2.1). However, supplementation of xylanase reduced the viscosity of jejunal digesta from 2.1 in the control group to 1.5 in xylanase and 1.7 in xylanase and mannanase. Xylanase cleaves the glycosidic bond of xylose back bone irrespective of their solubility but varies in their specificity (Pedersen et al., 2014). That means, it depends upon the degree of branching and substitution. If there is higher degree of substitution of arabinose in soluble NSP then enzymatic degradation would be minimal and vice versa. Xylanase solubilizes insoluble arabinoxylan to soluble fragments and soluble arabinoxylan to low molecular weight arabinoxylan.

Supplementation of xylanase significantly increased the production of propionate as well as total SCFA. i.e. excess propionate formed would provide energy to animal via gluconeogenesis. Whereas, effect of mannnase was found in the production of butyrate, signifying its role in providing energy to colonocytes and supporting gut health.

Dissemination of results

1. A workshop with pig farmers and stakeholders was organized in Hilo, HI to share the finding of the study in conjunction with other alternative feedstuffs feeding program to pigs.
2. An oral presentation entitled "Effect of supplemental enzymes on growth performance, digesta viscosity, nutrient and fiber digestibility of nursery pigs" was made in the 28th Annual CTAHR/COE Student Research Symposium (April 8-9, 2016), Honolulu, HI, USA.
3. An oral presentation entitled "Effect of supplemental enzyme on growth performance, digesta viscosity, apparent total tract digestibility of nutrients in nursery pigs" was made in ASAS/ADSA/CSAS/WSASAS Joint Annual Meeting (July 19-23, 2016) in Salt Lake City, UT, USA.
4. An abstract is published in the conference proceeding of Joint Annual Meeting (July 19-23, 2016) as: U. P. Tiwari, H. Chen, S. W. Kim, and R. Jha. 2016. Effect of supplemental enzyme on growth performance, digesta viscosity, apparent total tract digestibility of nutrients in nursery pigs. Journal of Animal Science, 94 (Suppl. 5):441 (abstract # 933).
5. A manuscript to submit in Journal of Animal Science is in progress.

References:

Almeida, F. N., G. I. Petersen, and H. H. Stein. 2011. Digestibility of amino acids in corn, corn coproducts, and bakery meal fed to growing pigs. J. Anim. Sci. 89:4109-4115.

Bedford, M. R., and H. Schuzle. 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11:91-114.

Blennow, A., M. Hansen, A. Schulz, K. Jorgensen, A. M. Donald, and J. Sanderson. 2003. The molecular deposition of transgenically modified starch in the starch granule as imaged by functional microscopy. J Structural Biol. 143:229–241.

Boisen, S., and J. A. Fernandez. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed. Sci. Technol. 68:277–286.

Englyst H. N., M. E. Quigley and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst. 119:1497–1509.

Jha, R., B. Rossnagel, R. Pieper, A. Van Kessel, and P. Leterme. 2010. Barley and oat cultivars with diverse carbohydrate composition alter ileal and total tract nutrient digestibility and fermentation metabolites in weaned piglets. ANIMAL. 4:724–731.

Jha, R., J. Bindelle, A. Van Kessel and P. Leterme. 2011. In vitro fibre fermentation of feed ingredients with varying fermentable carbohydrate and protein levels and protein synthesis by colonic bacteria isolated from pigs. Anim. Feed. Sci. Technol., 165:191–200.

Jha, R. and J. D. Berrocoso. 2015. Review: Dietary fiber utilization and its effects on physiological functions and gut health of swine. ANIMAL. 9:1441-1452.

Jha, R., T. A. Woyengo, J. Li, M. R. Bedford, T. Vasanthan and R. T. Zijlstra. 2015. Enzymes enhance degradation of the fiber-starch-protein matrix of distillers dried grains with solubles as revealed by a porcine in vitro fermentation model and microscopy. J. Anim. Sc. 93:1039–1051.

Nyachoti, C. M., R. T. Zijlstra, C. F. M. de Lange, and J. F. Patience. 2004. Voluntary feed intake in growing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 84: 549-566.

Pedersen, M. B., S. Dalsgaard, K. E. B. Knudsen, S. Yu, and H. N. Lærke. 2014. Compositional profile and variation of Distillers Dried Grains with Solubles from various origins with focus on non-starch polysaccharides. Anim. Feed Sci. Technol. 197:130-141.

Schulze, H., P. van Leeuwen, M. W. Verstegen, J. Huisman, W. B. Souffrant, and F. Ahrens. 1994. Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. J. Anim. Sci. 72: 2362-2368.

Skinner, S., A. Weersink, and C. F. deLange. 2012. Impact of dried distillers grains with solubles (DDGS) on ration and fertilizer costs of swine farmers. Can. J. Agric. Econ. 60:335-356.

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

Sundu B. and J. Dingle. 2003. Use of enzymes to improve the nutritional value of palm kernel meal and copra meal. Queensland Poult Sci Symp.11:1–15.

Zijlstra, R.T., Lange, C.F.M. De, Patience, J.F., 1999. Nutritional value of wheat for growing pigs: chemical composition and digestible energy content. Can. J. Anim. Sci. 79:187–194.