RESEARCH REPORT
ANIMAL SCIENCE

Title: Applying Enzyme Technology to Optimize the Utilization of Fibrous Feed Ingredients in Swine Diets - Nutrient Digestibility – **NPB #13-191** **revised**

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Industry Summary

The increasing industrial demand for feed grains, partly for biofuel production, has raised the price forecast for feed grains. Co-products are a short-term solution for commercial swine production to control feed cost, but these co-products have substantial amounts of fiber that decrease nutrient digestibility in pigs. Application of fiber degrading enzymes and liquid feeding methods may help to improve the nutritional value of fibrous feed ingredients. The objective of this study was to evaluate the effect of endo-1,4-β-xylanase supplementation (Xyl; with or without), feeding method (dry or liquid) and feedstuff (corn DDGS or wheat middlings) on digestibility of energy and nutrients, intestinal morphology, cecal pH and volatile fatty acids concentrations in growing pigs. Sixty-four pigs (BW 25.9 ± 0.38 kg) were blocked by BW and sex, placed in individual pens and randomly assigned to 8 dietary treatments. Within each feedstuff, diets were fed either liquid or dry, without or with Xyl (24,000 BXU/kg feed). DDGS and wheat middlings-based diets contained 3.32 and 3.19 Mcal/kg ME and 1.03 and 1.07% standardize ileal digestible) lysine, respectively. Pigs were fed equal amounts of ME per day and restricted at 3 times maintenance energy requirements (197 kcal ME/kg $BW^{0.60}$). The daily ration was fed in 2 equal meals. Liquid diets were prepared by steeping DDGS or wheat middlings with water (1:3 weight:volume) with or without Xyl for 24 h, followed by mixing with the respective basal diet and water to achieve a final ratio of 1:2.5 w:v. Diets were fed for 16 days and then pigs were euthanized. Supplementation of Xyl to dry wheat middlings-based diets increased ileal digestibility of GE and NDF as compared to dry wheat middlings-based diets without Xyl (64.50 vs. 54.67%; 52.88 vs. 31.69%, respectively), but this was not the case for pigs fed liquid diets. Supplementation of Xyl in liquid DDGS-based diets improved ileal digestibility of NDF as compared to liquid DDGS-based diets without Xyl, but Xyl did not affect ileal digestibility of NDF in dry DDGS diets. Addition of Xyl to wheat middlings-based diets improved fecal digestibility of GE and N as compared to wheat middlings diets without Xyl (80.37 vs. 78.07%; 80.23 vs. 77.94%, respectively); however, there was no effect of Xyl in DDGS based diets (feedstuff by Xyl interaction, $P < 0.05$). DDGS diets in liquid form reduced fecal digestibility of GE as compared to DDGS diets offered in dry form (81.10 vs. 82.97%); however, no effects were observed when wheat middlings diets were offered, regardless of the feeding method (78.89 vs. 79.55%; feeding method by feedstuff interaction, $P = 0.010$). Pigs fed DDGS diets had greater concentrations of butyrate in the cecum ($P = 0.001$) as compared to pigs fed wheat middlings diets (27.55 vs. 20.44 mmol/L). Pigs fed DDGS-based diets with Xyl had deeper crypts in the jejunum than pigs fed DDGS diets without Xyl (98.20 vs. 86.16 µm), however, there was no effect of Xyl in pigs fed wheat middlings-based

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

Key Findings:

- The results indicate that when DDGS-based diets were fed, neither addition of the enzyme xylanase nor feeding method (dry or liquid) appeared to improve the total tract and ileal digestibility of nutrients. Thus, xylanase did not increase the amount of energy or nutrients available to the pigs, which may have been related to the complex nature of the cell wall structure of DDGS (making it inaccessible to the enzyme).
- Supplementation of xylanase had a clear positive effect on the total tract digestibility of energy and nitrogen and on the ileal digestibility of energy and NDF when supplemented to wheat middlings-based diets. This improves the nutritional value of wheat middlings in pig diets.
- Pre-steeping DDGS or wheat middlings with enzymes (to increase the opportunity for the enzyme to interact with the feedstuffs and promote degradation of fiber) and feeding the diets in liquid form did not improve the nutritional value of DDGS or wheat middlings. Under the conditions of this study, pre-steeping as a processing step or liquid feeding did not prove to be beneficial.

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Scientific Abstract:

The objective of this study was to determine the effect of endo-1,4-β-xylanase supplementation (Xyl; with or without) and feeding method (dry or liquid) on apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of nutrients in growing pigs fed diets containing 30% corn DDGS or 30% wheat middlings. Sixty-four pigs (BW 25.9±0.38 kg) were blocked by BW and sex, placed in individual pens, and randomly assigned to 8 dietary treatments. Within each feedstuff (DDGS or Midds), diets were fed either liquid or dry, without or with Xyl (24,000 BXU/kg feed). Diets contained 3.25 Mcal/kg ME and 1.05% SID lysine. Pigs were fed restricted at 3 times maintenance energy requirements (197 kcal ME/kg BW0.60) in 2 equal meals. Liquid diets were prepared by steeping DDGS or Midds with water (1:3 w:v) with or without Xyl for 24 h, followed by mixing with the respective basal diet and water to achieve a final DM concentration of 25%. Following a 13 d adaptation, fecal samples were collected for 3 d. When DDGS was included, Xyl increased AID of NDF in liquid diets (48.4% vs. 31.6%), but not in dry diets (interaction, P=0.03). Pigs fed liquid diets with DDGS had decreased (P<0.05) ATTD of GE (81.1% vs. 83.0%) and NDF (64.8% vs. 72.1%) compared to pigs fed dry diets. When Midds were included, Xyl increased AID of GE (64.5 vs. 54.7) and NDF (52.9% vs 31.7%) in pigs fed dry diets but not in pigs fed liquid diets (interaction, P=0.09). Pigs fed liquid diets with Midds had greater AID of N (P=0.01) than pigs fed dry diets with Midds (77.7% vs. 72.0%). Pigs fed dry diets with Midds and Xyl had increased ATTD of NDF; however it was reduced when pigs were fed liquid diets with Xyl (interaction, P<0.01). The ATTD of GE (80.4% vs. 78.1%) and N (80.2% vs. 78.0%) was improved (P<0.05) in pigs fed Midds with Xyl compared to diets without Xyl. Pigs fed DDGS based diets had greater concentrations of butyrate in the cecum (P = 0.001) as compared to pigs fed Midds diets (27.55 vs. 20.44 mmol/L). Pigs fed DDGS-based diets with Xyl had deeper crypts in the jejunum than pigs fed DDGS diets without Xyl (98.20 vs. 86.16 µm), however, there was no effect of Xyl in pigs

fed Midds-based diets. This study indicates that Midds based diets with Xyl in liquid feeding did not improve nutrient digestibility. However, addition of Xyl improved ATTD of GE, N and NDF in dry diets with Midds. Furthermore, DDGS based diets with Xyl in liquid feeding improved only AID of NDF. Results suggest that the addition of Xyl appears to be more beneficial in Midds than DDGS based diets.

Introduction

Feed cost is by far the largest cost of pig production (65-75%), and growing-finishing pigs account for approximately 80% of feed consumed. Costs of feed ingredients have increased substantially in recent years, thus there is a great need for developing cost-effective feeding strategies for growing-finishing pigs. Particularly, increases in the price of corn and soybean meal have resulted in losses of approximately $20 to $40 per pig produced. It is, therefore, essential for the economic viability of pork production enterprises to identify viable ingredient alternatives to corn and soybean meal and optimize the nutritional efficiency in which these alternatives can be used by the pig.

Co-products of the ethanol industry (dried distillers grains with solubles), corn processing (corn gluten meal, corn gluten feed, corn germ), wheat processing (wheat middlings), and soybean processing (soy hulls) can be considered as alternative feed ingredients. High levels of fiber in these ingredients cause them to have a lower energetic value to the pig, and generally, growth rate and feed efficiency in pigs are reduced as the dietary fiber level is increased (Kass et al., 1980). Inefficient utilization of fiber may be related in part to lower energetic efficiency (reduced digestibility and fermentability) of utilization of volatile fatty acids (VFA) from microbial fermentation of fiber in the large intestine (Grieshop et al., 2001), reduced nitrogen (N) utilization and increased passage rate (Calvert, 1988), and increased weight and volume of the gastrointestinal tract and visceral organs in response to feeding higher levels of fiber (Coey and Robinson, 1954; Southgate, 1990; Hansen et al., 1992). From a financial perspective, feed efficiency is one of the main drivers of profitability in finishing pigs. Boyd and McCulley (2008) calculated that an improvement in feed efficiency of 0.01 (e.g. changing feed:gain from 2.80 to 2.79) during the finishing period reduced total feed cost by $0.28 to 0.30 per pig. To reduce feed costs, improved conversion efficiency and substitution of lower priced ingredients are pursued and jointly optimized in commercial production. Besides economic consequences, dietary fiber negatively impacts energy and nutrient utilization and will increase waste and nutrient excretion (e.g. Shi and Noblet., 1994; Canh et al., 1998; Davidson and McDonald, 1998; Moeser and van Kempen, 2002.).

It is clear that many feed resources originating from the biofuels and food industries contain high levels of fiber (non-starch polysaccharides, NSP), which cannot be digested by pigs, although partial microbial degradation can occur, primarily in the hindgut. The complex structure of NSP within cell walls of grains limits the ability of enzymes to effectively penetrate and break down nutrients contained within the structures of the grain (Bedford, 1995). In addition, some NSP can have anti-nutritional properties and directly interfere with digestibility of other nutrients (de Vries et al., 2012). Dietary supplementation of enzymes has become of great interest, especially recently because of the high cost of traditional feed ingredients and the potential of enzymes to improve the utilization of high fiber ingredients. The impact of carbohydrate degrading enzyme supplementation to diets of monogastric animals has been subject to various reviews (Chesson, 2001; Bedford, 2000; Choct, 2002; Cowiesen et al., 2006; Zijlstra et al., 2010; NRC, 2012). In poultry, many studies have focused on the supplementation of ß-glucanase and xylanase to reduce the negative effects associated with dietary ß-glucans (barley) and arabinoxylans (wheat and corn), which induce a high viscosity of the content of the intestinal lumen. However, largely because of differences in the digestive physiology between poultry and pigs, pigs are less sensitive to dietary fiber induced viscosity compared to poultry (Dierick and Decuypere, 1994). Indeed, reports on the effects of specific NSP-degrading enzymes in pig diets appear to be inconsistent (NRC, 2012). Clearly, the selection of types and combinations of enzymes and the proper dose, relative to the concentration of substrate present in the ingredients that comprise the diet will have a significant impact on the ultimate effect of enzyme supplementation on nutrient digestibility and growth performance (Zijlstra et al., 2010).

It may be speculated that the inconsistent response to added enzymes in pig diets can be attributed in part to an inefficient use of fiber degradation products by pigs. For example, the degradation of arabinoxylans to its monomer sugars will yield xylose and arabinose. These pentose sugars may be absorbed but cannot be metabolized by pigs and will be excreted in urine (de Lange, 2000). This suggest that fiber degrading enzymes should be chosen that yield oligosaccharides, rather than monomer sugars, and that the use of fiber degradation products for microbial fermentation should be maximized. The latter may be achieved by the combined usage of fiber degrading enzymes and microbial inoculants to treat high fiber ingredients prior to feeding. Recently, de Lange and Zhu (2012) showed synergy between fiber degrading enzymes and microbial inoculants for generating VFA during controlled fermentation of DDGS. On the other hand, enzymes may be used to degrade cellulose to glucose monomers by applying enzymes with cellulose degrading activity. Indeed, the use of feedstocks rich in cellulose have been evaluated as potential substrates for ethanol production (Péron and Partridge, 2010). For example, Kim et al. (2008) reported the hydrolysis of NSP in DDGS (consisting of approximately 20% total glucans, including cellulose and residual starch) to glucose monomers using a combination of cellulase, β-glucosidase, xylanase and feruloyl esterase (which is a hemicellulase accessory enzyme), followed by yeast fermentation. This process resulted in increased ethanol yield and an improved DDGS co-product with higher protein content. Thus, degradation of cellulose in high fiber ingredients by specific use of enzyme cocktails presents a unique opportunity to increase the energy value of these feedstuffs in swine diets by directly providing energy in the form of glucose and by decreasing negative impacts of cellulose on digestive processes.

The main focus of the proposed project will be to improve the nutritional value of high-fiber co-products from the food and bio-fuel industries (our focus will be on DDGS and wheat middlings because of their availability to the swine industry). Improvement in nutritional values will be pursued through supplementation of exogenous enzymes and application of liquid feeding (Yánez et al., 2011). Previous studies have shown that liquid feeding of barley, wheat and wheat middlings based diets improved feed efficiency (Jensen and Mikkelsen, 1998; MLC, 2005; de Lange and Zhu, 2012). Moreover, enzymes and microbial inoculants generally appear more effective when applied in liquid feeding systems (Scholten et al., 1999), even when enzymes are added to the liquid feed just prior to liquid feed preparation (Columbus et al., 2010). However, responses have not always been consistent (Zhu et al., 2011; de Lange and Zhu, 2012). These observations suggest that short duration soaking or steeping with enzymes improve nutrient digestion and utilization, especially when using high fiber co-products. This process allows the enzymes to interact and penetrate the substrate and it allows for the adjustment of pH and temperature to maximize the efficiency of interaction with the substrate. The application of enzymes may be fine-tuned in liquid feeding systems before exploring application in conventional dry feeding systems. Thus, we expect potential application of enzymes in liquid feeding systems, which have proven to be highly successful in other regions of the world (Brooks et al., 2001; de Lange and Zhu, 2012). However, we also envision the specific application of enzymes to fibrous feedstuffs in a liquid environment before they are dried, or soaking of fiber-rich ingredients before their use in the feed mill. Validation of this technology and its use as a platform for realizing an uplift in the nutritional value of fibrous feedstuffs in the U.S. is essential before application.

Objectives

The long-term goal of this project is to improve the utilization of fibrous ingredients in swine through the strategic application of enzymes. We hypothesize that supplementation of enzymes to feeds in a liquid form will enhance their capacity to degrade fibrous substrates, will increase the energetic utilization of fibrous feedstuffs and will allow for the utilization of non-traditional feedstuffs. Specifically, our objectives are to:

1) Characterize the in vitro degradation by enzymes of various fibrous feedstuffs
2) Determine effects of enzymes on nutrient digestibility of fibrous feedstuffs in swine
3) Determine the economic value of enzymes in improving the nutritional value of fibrous feed ingredients

Materials & Methods

Objective 1) *Determine the in vitro degradation by enzymes of relevant fibrous feed ingredients.*

Bench top in vitro scale steeping studies have been conducted. In these studies, 30 g of DDGS or wheat middlings were mixed with 120 mL (1:4 ratio of DDGS:water) or 180 mL (1:6 ration of midds:water) tap water for DDGS and wheat midds, respectively, and placed into sterile 250 mL Nalgene bottles. Samples were incubated with or without added enzyme at 28°C with continuous shaking (300 rpm). The enzyme used was Viscozyme at 1 mL per gram of feed sample. This enzyme preparation contained a wide range of carbohydrases, including arabinase, cellulase, β-glucanase, hemicellulase, and xylanase. Samples of 5 mL were taken at 0, 3, 6, 12, 24, and 72 h throughout the incubation period. Immediately after collection, each sample was placed in a cooler and the contents were centrifuged at 3,000 rpm for 20 minutes. From each sample, 1.5 ml of supernatant was collected and stored at -20 °C. Samples were sent to a laboratory for total soluble sugar analysis (glucose, fructose and sucrose) using HPLC. Each sample was analyzed in triplicate.
Additional steeping studies were conducted to evaluate the potential of microbial cultures (silage inoculants) to improve fermentation characteristics when supplemented to DDGS in combination with enzymes. DDGS were mixed with water and placed in an incubator at 39°C. Enzymes (β-glucanase and xylanase at 67.2 and 51.4 IU/g of DDGS resp.) were added to the mixture in combination with 1 of 2 silage inoculants (at 3 concentrations). Production of lactic and acetic acid was determined at 0, 24, 48, 120, and 168 hours of steeping.

Objective 2) *Determine the impact of various enzymes on energy and nutrient digestibility of fibrous feed ingredients when fed to swine*

This experiment was conducted using a total of 64 growing pigs, with an average initial BW of 25.9 ± 0.4 kg. Pigs were blocked by body weight and sex, and randomly assigned within blocks to 1 of 8 dietary treatments. Pigs were housed in individual pens (0.91 m by 1.82 m) using 64 pens (8 replicates per treatment). Each pen was equipped with a stainless steel feeder and a nipple drinker. Pigs were limit fed and allowed ad libitum access to water throughout the experiment. Experimental diets were fed for 16 d to evaluate the impact of feedstuffs, feeding methods and xylanase supplementation on growth performance, nutrient digestibility, morphology in the jejunum, pH and concentration of VFA at the ileal and caecal level.
Two feedstuffs (DDGS and wheat middlings), two feeding methods (dry and liquid), two enzyme supplementation treatments (without or with xylanase) were combined to create 8 experimental diets in a 2 x 2 x 2 factorial arrangement. The source of xylanase used was endo-1,4-β-xylanase (Econase XT, ABvista) and was included at 150g/1000 kg of finished feed to reach an activity of 24,000 BXU/kg of feed. This enzyme with primarily xylanase activity was selected for the specific reason that xylans are the primary fiber fraction in corn-soybean meal based diets. In addition, preliminary data using this enzyme product showed positive effects on pig performance.
Feed was manufactured at the North Carolina State University Feed Mill Educational Unit. Diets were manufactured by creating two basal mixes first that contained all ingredients with the exception of the DDGS, wheat middlings, or xylanase. Dry diets were subsequently manufactured from the appropriate basal mix by combining either 30% DDGS or 30% wheat middlings with or without xylanase with 70% of basal mix. Preparation of the liquid diets began twenty-four hours before feeding the animals. The DDGS or wheat middling were steeped separately with water (1:3 weight:volume) in the absence or presence of xylanase for 24 hours and then mixed with the respective basal diet in a 11 kg commercial mixer, which was then fed to the pigs in a final weight:volume ratio of 1:2.5 (de Lange and Zhu, 2012). This method of feed manufacturing ensured that the diets were identical with the exception of fiber source and enzyme addition (Table 3). Based on our preliminary work and the work published by others, we used a steeping time of 24 hours in the present study, which was deemed sufficient to optimize the impact of enzymes on its substrates and is feasible from a practical standpoint.
Diets were representative of current commercial practices and consisted mainly of corn and soybean meal as basal ingredients. Diets met NRC (2012) requirements for all nutrients for 25 kg pigs and they were fed in meal form.

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

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

At the end of the experiment, pigs were fed their last allotted meal, fasted for 6 to 8 h and then euthanized using captive bolt followed by exsanguination. The abdominal cavity was opened and a section of the jejunum was collected, cleaned with deionized water and fixed in 10% formalin solution for measurement of mucosal histology. The digesta content of ileum and caecum were removed immediately and the pH determined. Samples of dry and liquid diets, ileum and feces were prepared for analyses by drying for 3 d at 55ºC. The samples were ground using a kitchen blender. Samples of the ileal content (to determine the apparent ileal digestibility of energy and nutrients, therefore eliminating the impact of hindgut fermentation on nutrient digestibility measurements), cecal samples (to determine pH and volatile fatty acids concentrations), and jejunal samples (to determine intestinal morphology) were collected to better delineate the impact of enzymes and liquid feeding on nutrient availability.

The chemical composition of the experimental diets was analyzed for crude protein, crude fat, crude fiber, ADF, NDF, soluble and insoluble fiber (The University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO) using AOAC (2005) procedures. Concentration of titanium dioxide in the diets, fecal and ileal samples were determined according to Myers et al. (2004). Concentrations were determined relative to a standard curve at 410 nm using a microplate reader (Synergy HT Multi-detection, Bio-Teck Instruments, Inc., Winooski, VT). Gross energy (GE) was determined by dynamic bomb calorimetry (C5000 Calorimetric System, IKA, Wilmington, NC), calibrated using benzoic acid. Nitrogen (N) was measured using the combustion method (LECO, St Joseph, MI). The NDF (Neutral detergent fiber) content was determined according to Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Fairport, NY). The ATTD and AID of GE, N and NDF were obtained using the index ratio procedure (Adeola, 2001). The VFA concentration was measured by gas liquid chromatography (Varian GC model CP-3380, Walnut Creek, CA). Jejunum samples were submitted to the North Carolina State University histology laboratory for hematoxylin and eosin staining. Villus height, villus width and crypt depth were measured using a microscope (Micromaster, Fisher Scientific International Inc., Pittsburgh, PA). Ten villi that were well positioned were randomly selected and measured for each pig and then the data were averaged to provide equal morphological representation per pig.

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

Results

Objective 1) Determine the in vitro degradation by enzymes of relevant fibrous feed ingredients.

In vitro studies showed that pH was not impacted by enzyme supplementation to either DDGS or wheat middlings at any time point following steeping (Table 1). For DDGS, pH was reduced when measured after 72 hr of steeping compared to 0, 3, 6, 12, and 24 hr of steeping, whereas pH was reduced faster (at 24 hr of steeping) for wheat middlings. The reduction in pH indicates fermentation of ingredients, which is expected to not be impacted by enzyme supplementation. This fermentation appeared to be more rapid in wheat middlings, which may be related to the greater concentrations of soluble (ie. fermentable) fiber in wheat midds compared to DDGS. To avoid fermentation of substrate and specifically determine the impact of enzyme supplementation on energy and nutrient digestibility in pigs, we implemented a 24 hr steeping time for the in vivo work.

Total soluble sugar concentrations were greater in DDGS and wheat midds samples that were steeped with enzyme (Table 2). Interestingly, the total soluble sugar concentrations were already greater at the time point identified as 0 hr of steeping. Concentrations of total soluble sugar increased slightly over time. The samples at time 0 were collected immediately following the addition of enzyme to the liquid mixture of the ingredient and water. Samples were placed on ice and then frozen. These results indicate that the release of total soluble sugar was extremely fast and nearly complete right after the enzyme was added. The amount of enzyme used may have been excessive causing very efficient degradation of substrate.

Incubation of DDGS with enzyme and silage inoculants increased the concentration of lactic acid and acetic acid over time (Figures 1 and 2). Lactic acid concentration increased from a low of 22.7 mM on day 0 to a high of 135.4 mM at 168 hours of fermentation and appeared to be greater when silage inoculants were included with the enzyme in the fermentation. Acetic acid concentrations increased from 11.9 mM on day 0 to 45.2 mM at 168 hours of fermentation. The acetic acid concentration was lowest when silage inoculant was included at 3 times the recommended rate. According to van Winsen et al. (2001), good quality fermented feed should have a lactic acid concentration of more than 150 mM and an acetic acid concentration of less than 40 mM.

Objective 2) Determine the impact of various enzymes on energy and nutrient digestibility of fibrous feed ingredients when fed to swine

Growth performance

Feeding method (dry or liquid), enzyme supplementation (with or without Xyl), and feedstuff addition (DDGS or wheat middlings) did not produce statistical differences ($P > 0.30$) in final BW, ADG and G:F ratio (Table 4). A main feeding method effect ($P = 0.001$) was observed for ADFI, indicating that pigs fed liquid diets had lower ADFI than pigs fed dry diets (1.29 vs. 1.34 kg/d). Also, ADFI depended on the feedstuff used ($P = 0.0001$). Pigs fed DDGS-based diets had lower feed intake than pigs fed wheat middlings-based diets (1.29 vs. 1.34 kg/d).

Nutrient digestibility

A tendency for an interactive effect between feeding method, Xyl supplementation and feedstuff ($P = 0.056$) was observed for AID of GE (Table 5). Xylanase supplementation to dry wheat middlings-based diets tended to improve AID of GE compared to dry wheat middlings-based diets without Xyl (64.50 vs. 54.67%); however, Xyl supplementation to liquid wheat middlings-based diets did not affect AID of GE (62.88 vs. 59.94%). Xylanase supplementation to DDGS-based diets did not improve AID of GE, regardless of feeding method.

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

(46.87 vs. 49.76%), but the addition of Xyl in liquid DDGS-based diets enhanced AID of NDF as compared to liquid DDGS-based diets without Xyl (46.54 vs. 31.58%).

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

An interaction between feedstuff and Xyl supplementation was observed (P = 0.001) for ATTD of GE. Addition of Xyl to the wheat middlings-based diets improved ATTD of GE as compared to the wheat middlings-based diets without Xyl (80.37 vs. 78.07%). However, addition of Xyl to the DDGS-based diets did not impact ATTD of GE (81.55 vs. 82.51%).

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

There was an interaction (P < 0.001) between feeding method, Xyl supplementation and feedstuff for the ATTD of NDF. The addition of Xyl in the dry wheat middlings-based diets increased the ATTD of NDF as compared to the dry wheat middlings-based diets without Xyl (67.55 vs. 62.28%). In contrast, supplementation of Xyl in liquid wheat middlings-based diets decreased ATTD of NDF as compared to liquid wheat middlings-based diets without Xyl (57.24 vs. 65.08%). Supplementation of Xyl in the dry DDGS-based diets did not impact ATTD of NDF as compared to dry the DDGS-based diets without Xyl (70.40 vs. 73.70%). Likewise, addition of Xyl in the liquid DDGS-based diets did affect ATTD of NDF as compared to liquid DDGS-based diets without Xyl (65.44 vs. 64.17%).

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

pH and VFA

No significant differences in ileal pH were found among treatments (Table 6). However, a main feedstuff effect (P = 0.004) was observed for cecum pH, indicating that pigs fed diets containing wheat middlings had greater cecum pH than pigs fed the diets containing DDGS (5.76 vs. 5.62).

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

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

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

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

Morphology

An interaction was observed between feeding method, Xyl supplementation and feedstuff for villi height (P = 0.018; Table 7). Addition of Xyl in liquid DDGS-based diets increased villi height as compared to the liquid DDGS-based diets without Xyl (319.56 vs 276.29 µm). But, no statistical differences were observed in villi height when Xyl was added to the dry DDGS-based diets as compared to the dry DDGS-based diets without Xyl (322.96 vs 350.42 µm). Supplementation of Xyl in the liquid wheat middlings-based diets did not affect villi height as compared to liquid wheat middlings-based diets without Xyl (295.45 vs. 327.46 µm). Likewise, no differences were observed in villi height when Xyl was added to the dry wheat middlings-based diets as compared to the wheat middlings-based diets without Xyl (289.65 vs. 305.21 µm).

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

An interaction between feedstuff and Xyl supplementation (P = 0.007) was detected for crypt depth. Pigs fed DDGS-based diets with Xyl had greater crypt depth than pigs fed DDGS-based diets without Xyl (98.20 vs. 86.16 µm). However, the crypt depth in pigs fed wheat middlings-based diets with Xyl was not statistically different from pigs fed wheat middlings-based diets without Xyl (91.62 vs. 101.92 µm).

Discussion

Based on in vitro data and data from published work, we implemented a 24 hour steeping time for the in vivo work to specifically determine the impact of enzyme supplementation on energy and nutrient digestibility in pigs. An enzyme with primarily xylanase activity was selected for the specific reason that xylans are the primary fiber fraction in corn-soybean meal based diets. In addition, preliminary data using this enzyme product showed positive effects on pig performance.

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

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

The total NSP content in corn DDGS comprises 23.1% of dry matter, and the water insoluble portion represents about 88% of this total (Ward, 2008). A large proportion of insoluble NSP in corn DDGS is expected because the soluble portion is degraded during fermentation and ethanol production. The arabinose and xylose

portion provide an estimate of the arabinoxylan fraction, which is 11.7% of dry matter (Ward, 2008). Similarly, the NSP in wheat co-products are mainly arabinoxylans and cellulose (Zijlstra et al., 1999); total NSP and arabinoxylan content in wheat middlings range from 19% to 31.8% and from 11.5% to 15.9% of the dry matter, respectively. The NSP entrap nutrients and act as a physical barrier to effective nutrient hydrolysis and absorption. It has been proposed that supplementation of xylanase may improve the nutritional value of NSP diets by partially hydrolyzing soluble and insoluble NSP, breaking NSP-containing cell walls and thereby liberating their contents for enzymatic hydrolysis (Dierick and Decuypere, 1996; Diebold et al., 2005). Xylanase randomly breaks the arabinoxylan backbone into smaller chains and reduces their molecular weight (Tapingkae et al., 2008). Therefore, feedstuffs with greater arabinoxylan content will have more encapsulated nutrients and thus derive greater benefit from xylanase supplementation (Nortey et al., 2008).

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

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

In the present study, liquid feeding as pretreatments did not appear to be an advantageous strategy to enhance digestibility of nutrients in wheat middlings-based diets. However, supplementation of Xyl had a clear positive effect on ATTD of GE and N and on AID of NDF when supplemented to wheat middlings-based diets. This positive response is supported by several studies. Diebold et al. (2004) reported a positive effect on the AID of GE and NDF with Xyl supplementation in weanling pigs fed wheat based diets. Similarly, Nortey et al. (2007) found that inclusion of Xyl in wheat based diets containing wheat millrun improved AID and ATTD of GE in grower pigs. The increase in nutrient digestibility due the supplementation of Xyl could be due the arabinoxylan hydrolysis by Xyl, exposing the enclosed intracellular nutrients to digestive enzymes within the gut lumen permitting more complete digestion. The lack of effects of Xyl in liquid wheat middlings-based diets may be associated with the presence of natural xylanase inhibitors. Cereal grains such as wheat, rye, and barley contain proteins that can inhibit xylanase activity (Bonnin et al., 2005; Debyser et al., 1999 and Paloheimo et al., 2011). Perhaps the steeping process of wheat middlings with water and supplemental Xyl, not only stimulates Xyl activity as more substrate is accessed (improved mobility of Xyl), but it also could stimulate the activity of xylanase inhibitors (increased mobility of xylanase inhibitors). Enzyme inhibition is a natural phenomenon that occurs in plant seeds to act as defense mechanism and regulate plant metabolic processes (Nortey et al., 2008). The presence of inhibitors can therefore negate the effects that can be achieved by adding xylanase to swine diets that contain wheat. The effects of endogenous xylanase inhibitors and xylanase have been studied more extensively in the food industry, especially in bread making (Debyser et al., 1999).

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

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

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

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

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

Under the conditions of this experiment, the combination of liquid feeding and the application of Xyl appeared to be unable to disrupt the cell wall structures of DDGS because no improvement in apparent ileal and apparent total tract digestibility of GE, N and NDF was observed in growing pigs fed diets containing 30% corn DDGS combined with Xyl and in liquid form. Likewise, liquid feeding did not improve nutrient digestibility in pigs fed wheat middlings-based diets, but the addition of Xyl enhanced the AID of NDF and the ATTD of GE and N in growing pigs fed dry diets containing 30% of wheat middlings.

In conclusion, liquid feeding and the application of Xyl demonstrated a limited potential to enhance degradation and feeding value of the fiber fraction in corn DDGS-based diets. On the other hand, the addition of

Xyl improved the nutritional value of wheat middlings-based diets but liquid feeding as a pretreatment did not enhance further the nutritional value of wheat middlings based diets. Overall, the apparent total tract digestibility of GE in diets containing wheat middlings was increased by 2.9% with the supplementation of xylanase.

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Table 1. Effect of enzyme supplementation (Viscozyme) and steeping time of DDGS and wheat midds on pH

	Time, hrs					
Treatment	**0**	**3**	**6**	**12**	**24**	**72**
DDGS						
Control	4.48	4.47	4.48	4.60	4.49	3.77
Enzyme	4.60	4.50	4.46	4.63	4.65	3.55
Wheat midds						
Control	5.64	6.01	5.98	6.02	4.64	4.28
Enzyme	5.37	5.62	5.61	5.82	5.52	4.33

Table 2. Effect of enzyme supplementation (Viscozyme) and steeping time of DDGS and wheat midds on total sugar (mg/mL) concentrations

	Time, hrs					
Treatment	**0**	**3**	**6**	**12**	**24**	**72**
DDGS						
Control	0.62	1.24	1.20	1.22	1.26	
Enzyme	38.31	38.88	38.00	40.25	49.24	45.25
Wheat midds						
Control	2.55	4.90	5.81	6.35	0.89	2.74
Enzyme	26.28	28.56	31.32	34.43	28.22	31.40

Table 3. Composition of the experimental diets, as feed basis[1]

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

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

Table 4. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on growth performance in growing pigs.

			Initial BW[2], kg	Final BW, kg	ADG, kg	ADFI, kg	G:F
Feeding method × Feedstuff × Xyl Interaction							
Dry	DDGS	-	26.30	35.38	0.61	1.30	0.47
Dry	DDGS	+	25.67	35.96	0.66	1.32	0.50
Dry	Midds	-	26.48	35.80	0.64	1.37	0.47
Dry	Midds	+	25.61	35.34	0.62	1.36	0.46
Liquid	DDGS	-	26.32	35.26	0.61	1.27	0.48
Liquid	DDGS	+	25.65	35.53	0.63	1.26	0.50
Liquid	Midds	-	25.95	35.33	0.62	1.34	0.46
Liquid	Midds	+	25.25	35.63	0.64	1.29	0.50
	SEM		0.389	0.429	0.028	0.020	0.019
Feeding method × Xyl Interaction							
Dry		-	26.39	35.59	0.63	1.34	0.47
Dry		+	25.64	35.65	0.64	1.34	0.48
Liquid		-	26.13	35.30	0.61	1.31	0.47
Liquid		+	25.45	35.58	0.64	1.27	0.50
	SEM		0.275	0.306	0.020	0.014	0.014
Feedstuff × Xyl Interaction							
DDGS		-	26.31	35.32	0.61	1.29	0.48
DDGS		+	25.66	35.75	0.65	1.29	0.50
Midds		-	26.22	35.57	0.63	1.36	0.46
Midds		+	25.43	35.48	0.63	1.33	0.48
	SEM		0.275	0.305	0.020	0.014	0.014
Feeding method × Feedstuff Interaction							
Dry	DDGS		25.99	35.67	0.64	1.31	0.49
Dry	Midds		26.04	35.57	0.63	1.37	0.46
Liquid	DDGS		25.98	35.39	0.62	1.26	0.49
Liquid	Midds		25.60	35.48	0.63	1.32	0.48
	SEM		0.275	0.301	0.020	0.014	0.014
Main Effect of Feeding Method							
Dry			26.01	35.62	0.63	1.34	0.47
Liquid			25.79	35.44	0.62	1.29	0.48
	SEM		0.19	0.213	0.014	0.010	0.010
Main Effect of Feedstuffs							
DDGS			25.98	35.53	0.63	1.29	0.49
Midds			25.82	35.53	0.63	1.34	0.47
	SEM		0.19	0.212	0.014	0.010	0.010
Main Effect of Xyl							
-			26.26	35.44	0.62	1.32	0.47
+			25.54	35.61	0.64	1.31	0.49

SEM	0.19	0.219	0.014	0.010	0.010
P-values					
FM × FS × Xyl	0.849	0.375	0.400	0.807	0.302
FM × Xyl	0.905	0.708	0.731	0.224	0.429
FS × Xyl	0.804	0.399	0.400	0.278	0.544
FM × FS	0.434	0.757	0.731	0.982	0.689
FM	0.420	0.545	0.639	0.001	0.475
FS	0.559	0.981	0.975	0.0001	0.161
Xyl	0.012	0.596	0.334	0.319	0.148

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

Table 5. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of nutrients.

			AID, %				ATTD, %			
			GE		NDF	N	GE		NDF	N
Feeding method × Feedstuff × Xyl Interaction										
Dry	DDGS	-	65.68	x	49.76 a	72.37	83.85		73.70 a	83.17
Dry	DDGS	+	62.19	xy	46.87 ab	71.76	82.08		70.40 ab	81.12
Dry	Midds	-	54.67	y	31.70 b	69.85	77.49		62.28 d	77.44
Dry	Midds	+	64.50	x	52.89 a	73.85	80.30		67.55 bc	79.59
Liquid	DDGS	-	58.72	xy	31.58 b	72.96	81.18		64.17 cd	81.20
Liquid	DDGS	+	67.19	x	46.54 ab	76.52	81.01		65.44 cd	80.96
Liquid	Midds	-	59.94	xy	33.34 b	78.29	78.65		65.08 cd	78.44
Liquid	Midds	+	62.88	xy	29.99 b	77.03	80.44		57.24 e	80.86
	SEM		3.40		5.59	2.31	0.67		1.22	1.07
Feeding method × Xyl Interaction										
Dry	-		60.17		40.73	71.11	80.67		67.99	80.31
Dry	+		63.34		49.88	72.80	81.19		68.98	80.35
Liquid	-		59.33		32.46	75.63	79.92		64.63	79.82
Liquid	+		65.04		38.26	76.77	80.73		61.34	80.91
	SEM		2.40		3.96	1.63	0.47		0.86	0.75
Feedstuff × Xyl Interaction										
DDGS	-		62.20		40.67	72.66	82.51	a	68.94	82.19 a
DDGS	+		64.69		46.70	74.14	81.55	ab	67.92	81.04 a
Midds	-		57.30		32.52	74.07	78.07	c	63.68	77.94 b
Midds	+		63.69		41.44	75.44	80.37	b	62.39	80.23 a
	SEM		2.40		3.95	1.63	0.47		0.86	0.75
Feeding method × Feedstuff Interaction										
Dry	DDGS		63.93		48.32	72.06	82.97	a	72.05	82.15
Dry	Midds		59.58		42.29	71.85	78.89	c	64.91	78.51
Liquid	DDGS		62.95		39.06	74.74	81.10	b	64.81	81.08
Liquid	Midds		61.41		31.67	77.66	79.55	c	61.16	79.65
	SEM		2.40		3.96	1.63	0.47		0.86	0.75
Main Effect of Feeding Method										
Dry			61.76		45.30	71.96	80.93		68.48	80.33
Liquid			62.18		35.36	76.20	80.32		62.98	80.37
	SEM		1.70		2.81	1.15	0.33		0.61	0.53
Main Effect of Feedstuffs										
DDGS			63.44		43.69	73.40	82.03		68.43	81.61
Midds			60.50		36.98	74.75	79.22		63.04	79.08
	SEM		1.70		2.80	1.15	0.33		0.61	0.53
Main Effect of Xyl										
-			59.75		36.60	73.37	80.29		66.31	80.06
+			64.19		44.07	74.79	80.96		65.16	80.63
	SEM		1.70		2.79	1.15	0.33		0.61	0.53
P-values										
FM × FS × Xyl			0.056		0.010	0.155	0.172		<.0001	0.614
FM × Xyl			0.601		0.676	0.868	0.762		0.016	0.491
FS × Xyl			0.421		0.717	0.974	0.001		0.876	0.027

FM × FS	0.563	0.863	0.343	0.010	0.048	0.150
FM	0.861	0.017	0.013	0.205	<.0001	0.962
FS	0.226	0.097	0.412	<.0001	<.0001	0.002
Xyl	0.071	0.065	0.387	0.167	0.187	0.452

[a - g] Values within a column with the same letter are not different ($P > 0.05$)

[x - y] Values within a column with the same letter show tendency ($0.05 < P \leq 0.10$)

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

Table 6. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on pH and VFA in ileal and caecal contents at slaughter.

			Ileal	Caecum							
					VFA, mmol/L[2]				VFA, molar proportions		
			pH	pH	C_2	C_3	C_4	Total VFA	C_2/Total VFA	C_3/Total VFA	C_4/Total VFA
Feeding method × Feedstuff × Xyl Interaction											
Dry	DDGS	-	6.38	5.54	89.44	61.46	25.85	176.74	0.51	0.34	0.15
Dry	DDGS	+	6.55	5.66	100.51	67.24	24.09	191.85	0.52	0.35	0.13
Dry	Midd	-	6.41	5.71	94.85	66.17	22.29	183.31	0.52	0.35	0.13
Dry	Midd	+	6.42	5.80	92.51	66.23	18.38	177.12	0.53	0.36	0.11
Liquid	DDGS	-	6.47	5.64	89.65	64.68	28.84	183.18	0.48	0.35	0.17
Liquid	DDGS	+	6.48	5.63	97.44	54.03	31.43	182.89	0.52	0.29	0.18
Liquid	Midd	-	6.43	5.75	86.99	70.75	21.47	179.21	0.48	0.39	0.13
Liquid	Midd	+	6.52	5.77	88.14	63.10	19.63	170.87	0.52	0.36	0.12
	SEM		0.12	0.07	9.40	7.07	2.83	16.07	0.02	0.02	0.02
Feeding method × Xyl Interaction											
Dry	-		6.40	5.62	92.14	63.82	24.07	180.03	0.52	0.35 ab	0.14
Dry	+		6.48	5.73	96.51	66.74	21.24	184.48	0.52	0.36 ab	0.12
Liquid	-		6.45	5.70	88.32	67.72	25.16	181.19	0.48	0.37 a	0.15
Liquid	+		6.50	5.70	92.79	58.56	25.53	176.88	0.52	0.33 b	0.15
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01	0.01
Feedstuff × Xyl Interaction											
DDGS	-		6.43	5.59	89.55	63.07	27.35	179.96	0.49	0.35	0.16
DDGS	+		6.51	5.64	98.97	60.63	27.76	187.37	0.52	0.32	0.15
Midd	-		6.42	5.73	90.92	68.46	21.88	181.26	0.50	0.37	0.13
Midd	+		6.47	5.78	90.33	64.66	19.00	174.00	0.52	0.36	0.11
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01	0.01
Feeding method × Feedstuff Interaction											
Dry	DDGS		6.46	5.60	94.97	64.35	24.97	184.29	0.51	0.35	0.14
Dry	Midd		6.41	5.75	93.68	66.20	20.33	180.22	0.52	0.36	0.12
Liquid	DDGS		6.48	5.64	93.54	59.35	30.14	183.03	0.50	0.32	0.18
Liquid	Midd		6.48	5.76	87.57	66.92	20.55	175.04	0.50	0.38	0.12
	SEM		0.09	0.05	6.64	5.00	2.00	11.36	0.01	0.01	0.01
Main Effect of Feeding Method											
Dry			6.44	5.67	94.33	65.28	22.65	182.26	0.52	0.35	0.13
Liquid			6.48	5.70	90.56	63.14	25.34	179.04	0.50	0.35	0.15
	SEM		0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01
Main Effect of Feedstuffs											
DDGS			6.47	5.62	94.26	61.85	27.55	183.66	0.51	0.33	0.16
Midd			6.45	5.76	90.62	66.56	20.44	177.63	0.51	0.37	0.12
	SEM		0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01
Main Effect of Xyl											
-			6.42	5.66	90.23	65.77	24.61	180.61	0.50	0.36	0.15
+			6.49	5.71	94.65	62.65	23.38	180.68	0.52	0.34	0.13
	SEM		0.06	0.03	4.70	3.54	1.41	8.03	0.01	0.01	0.01

P-values									
FM × FS × Xyl	0.483	0.710	0.799	0.664	0.776	0.772	0.775	0.667	0.620
FM × Xyl	0.837	0.278	0.994	0.233	0.427	0.701	0.203	0.031	0.393
FS × Xyl	0.826	0.979	0.454	0.892	0.415	0.521	0.812	0.596	0.752
FM × FS	0.770	0.740	0.726	0.570	0.222	0.864	0.669	0.069	0.210
FM	0.653	0.623	0.573	0.671	0.185	0.778	0.187	0.781	0.153
FS	0.776	0.004	0.586	0.350	0.001	0.597	0.703	0.013	0.011
Xyl	0.431	0.245	0.509	0.536	0.541	0.995	0.061	0.201	0.393

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

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

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

Table 7. Effects of feeding method (FM), feedstuff (FS) and xylanase supplementation[1] (Xyl) on intestinal morphology.

			µm					
			Villi Height		**Villi width**		**Crypt depth**	
Feeding method × Feedstuff × Xyl Interaction								
Dry	DDGS	-	350.42	a	124.38		84.85	
Dry	DDGS	+	322.96	ac	152.06		94.63	
Dry	Midd	-	305.21	bcd	116.61		91.06	
Dry	Midd	+	289.65	cd	129.22		84.35	
Liquid	DDGS	-	276.29	d	131.54		87.47	
Liquid	DDGS	+	319.56	abc	135.22		101.77	
Liquid	Midd	-	327.46	ab	148.82		112.78	
Liquid	Midd	+	295.45	bcd	135.00		98.90	
	SEM		12.56		7.65		5.59	
Feeding method × Xyl Interaction								
Dry	-		327.81		130.49		87.96	
Dry	+		306.30		140.64		89.49	
Liquid	-		301.87		140.18		100.13	
Liquid	+		307.51		135.11		100.33	
	SEM		8.88		5.41		3.96	
Feedstuff × Xyl Interaction								
DDGS	-		313.36		127.96		86.16	b
DDGS	+		321.26		143.64		98.20	a
Midd	-		316.33		132.71		101.92	a
Midd	+		292.55		132.11		91.62	ab
	SEM		8.88		5.41		3.96	
Feeding method × Feedstuff Interaction								
Dry	DDGS		336.69		138.22	a	89.74	
Dry	Midd		297.43		122.91	b	87.71	
Liquid	DDGS		297.93		133.38	ab	94.62	
Liquid	Midd		311.46		141.91	a	105.84	
	SEM		8.88		5.41		3.96	
Main Effect of Feeding Method								
Dry			317.06		130.56		88.72	
Liquid			304.69		137.64		100.23	
		SEM	6.28		3.82		2.80	
Main Effect of Feedstuffs								
DDGS			317.31		135.80		92.18	
Midd			304.44		132.41		96.77	
		SEM	6.28		3.82		2.80	
Main Effect of Xyl								
	-		314.84		130.33		94.04	
	+		306.90		137.87		94.91	
		SEM	6.28		3.82		2.80	
P-values								
FM × FS × Xyl			0.018		0.911		0.463	
FM × Xyl			0.133		0.240		0.867	
FS × Xyl			0.081		0.139		0.007	

FM × FS	0.005	0.032	0.100
FM	0.170	0.197	0.005
FS	0.154	0.534	0.251
Xyl	0.376	0.170	0.827

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

Figure 1. Impact of steeping of DDGS with enzymes with or without silage inoculants on concentrations of lactic acid

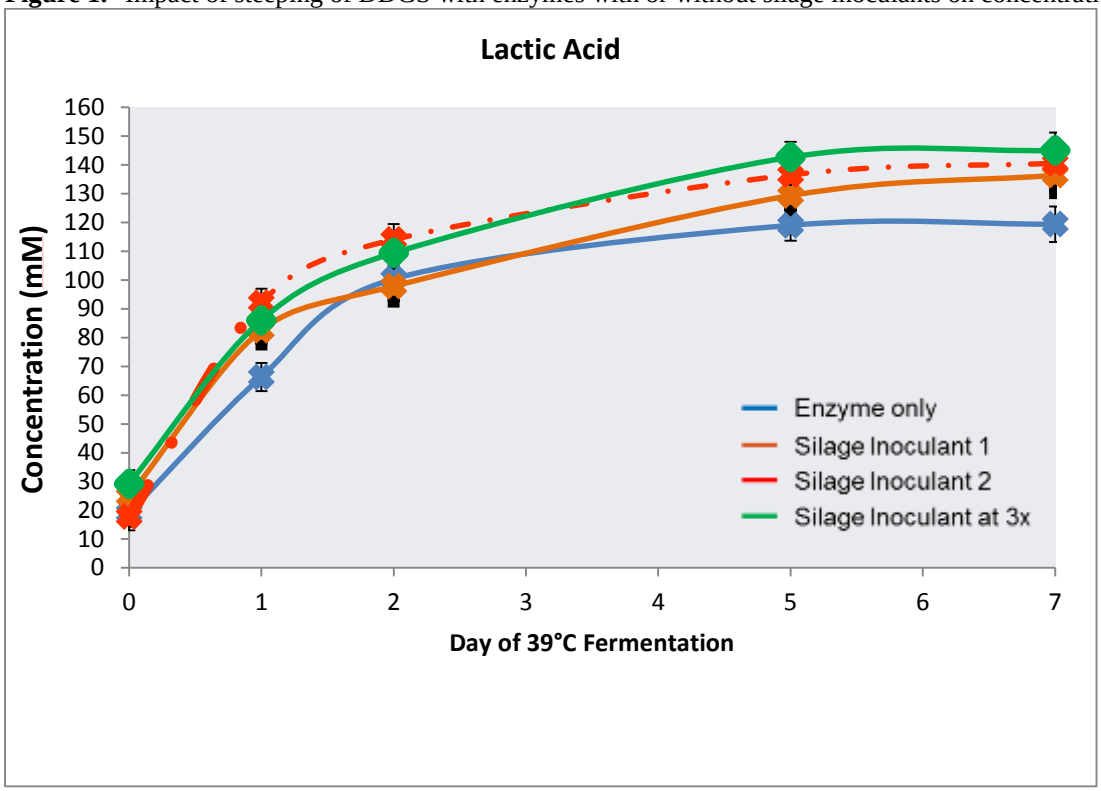

Figure 1. Impact of steeping of DDGS with enzymes with or without silage inoculants on concentrations of acetic acid