RESEARCH REPORT
ANIMAL SCIENCE

Title: Applying Enzyme Technology to Optimize the Utilization of Fibrous Feed Ingredients in Swine Diets – Applied Feeding Studies – **NPB #14-001**

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Industry Summary

The long-term goal of this project was to improve the utilization of fibrous ingredients in swine through the strategic application of enzymes. We hypothesized that supplementation of enzymes to feed ingredients (in this case, distillers' dried grains with solubles (DDGS)) in 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. In the first study, we focused on dry diets and aimed to determine if the combination of an enzyme (protease) with a direct-fed microbial could improve performance of pigs. A total of 72 pigs (18 pens with 6 pens per treatment) weighing 25 kg on average were fed diets that were not supplemented, or supplemented with enzymes (xylanase and β-glucanase), or a combination of protease enzyme and a direct fed microbial. No differences were observed for body weight gain, feed intake, feed efficiency, empty gastro-intestinal weights, or cecal and ileal digesta pH, although pigs fed the protease with direct-fed microbial had a numerical improvement of 6.5% in feed efficiency compared to control-fed pigs. In the second experiment, we hypothesized that steeping high fiber ingredients like DDGS with carbohydrase enzymes may improve their feeding value. We investigated growth performance of growing pigs (144 pigs; 24 pens with 6 pigs per pen) fed diets containing DDGS treated with a blend of β-glucanase and xylanase with or without extended steeping. Treating DDGS with a combination of xylanase and β-glucanase with or without steeping resulted in improved feed efficiency compared to the dry control diet without enzymes for the first three weeks suggesting degradation by enzymes of dietary fibrous components that may otherwise limit nutrient utilization in younger pigs. Supplementation with enzymes improved ADG when DDGS were not steeped (water was added to this diet immediately before feeding as a liquid diet), however, steeping appeared to reduce feed intake, resulting in poorer ADG. Pig body weight was 2.9 kg greater for pigs fed non-steeped liquid diets with enzyme compared with pigs fed the steeped liquid diet with enzyme. Liquid steeped diets had concentrations of acetic acid and lactic acid that could be considered suboptimal, indicating poor fermentation. Therefore, the reduction in growth performance and feed intake of pigs fed the steeped liquid diet may have been related to suboptimal fermentation characteristics of DDGS that was added to the feed. Although growth performance of pigs fed DDGS treated with fiber degrading enzymes did not differ from pigs fed non-treated DDGS control, an interesting area of further exploration is the nature and effects of potential metabolites that could be released when DDGS is treated with fiber degrading enzymes under steeped conditions.

Key Findings:

- Supplementation of xylanase and β-glucanase or a combination of protease with direct-fed microbial did not significantly impact growth performance of grower pigs
- Treating DDGS with xylanase and β-glucanase with or without extended steeping improved feed efficiency for the first three weeks suggesting degradation by supplemental enzymes of dietary fibrous components that may otherwise limit nutrient utilization in younger pigs
- Extended steeping of DDGS appeared to reduce feed intake, resulting in poorer ADG, which may have been related to sub-optimal fermentation of the steeped DDGS.

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Scientific Abstract:
Fiber degradation may be maximized by the combined usage of enzymes and microbial inoculants. In the first study, the effects of a combination of a protease with a direct fed microbial on pig performance, gastro-intestinal tract weights, and ileal and cecal pH was determined. A total of 72 pigs (initial BW of 25 kg) were blocked by initial weight and randomly assigned within blocks to 18 pens (6 pens per treatment; 2 barrows and 2 gilts per pen). Diets (dry, corn-soybean meal-DDGS based) were fed in a 2-phase feeding program with 2 weeks for each phase. Treatments consisted of a control (not supplemented), a diet supplemented with xylanase and β-glucanase, or a combination of protease and a direct fed microbial. There were no statistical differences for body weight gain, feed intake, feed efficiency, empty gastro-intestinal weights, or cecal and ileal digesta pH, although pigs fed the protease with direct-fed microbial had a numerical improvement of 6.5% in feed efficiency compared to control-fed pigs. In the second experiment, we hypothesized that steeping high fiber ingredients like DDGS with carbohydrases may improve their feeding value. We investigated growth performance of growing pigs fed diets containing DDGS treated with a blend of β-glucanase and xylanase (XB) with or without extended steeping. Treatments were: 1) corn - soybean meal based diet with 30% DDGS fed in dry form (C), 2) C + XB without steeping, fed in liquid form (XBNS), and 3) C + XB with the DDGS steeped with XB (16% DM) for 3 to 10 d at 40°C (XBS). The target activities for XB were 1,050 and 5,500 U/g of DDGS for β-glucanase and xylanase, respectively. A total of 144 pigs (25 kg BW) were assigned to pens (3 barrows and 3 gilts) based on initial weight and allocated to the three treatments in a 2-phase feeding program (3-wk/phase). Diets were delivered by computer controlled liquid feeding system at a feed to water ratio of 1:4, four times per day. Pigs had free access to water. The average pH of steeped DDGS on d 0, 3, 6, 7, and 10 was 4.42, 3.65, 3.86, 3.89 and 3.92, respectively. The pH of diets at feeding time was lower ($P < 0.01$) for XBS (4.7) compared to C and XBNS (5.5). Lactic acid concentrations of the fermented liquid diet increased and then decreased, whereas acetic acid concentrations increased as fermentation progressed. Pigs fed XBNS had higher ($P = 0.02$) ADG than C in phase 1 and in phase 2. Overall ADG was higher ($P < 0.05$) for XBNS than XBS, whilst pigs receiving C were similar to XBS pigs. There were no effects ($P > 0.05$) on ADFI throughout the experiment. However, pigs fed XBS had numerically lower ADFI in phase 1 (-4.0%) and phase 2 (-5.2%) relative to XBNS pigs. Pigs receiving XBNS (1.68) and XBS (1.69) had better feed:gain ($P = 0.001$) than C (1.78) during phase 1. Treatments did not influence ($P > 0.05$) empty gastrointestinal weight. In conclusion, treating DDGS with XB with or without steeping resulted in improved feed efficiency for the first three weeks

suggesting degradation by XB of dietary fibrous components that may limit nutrients utilization in younger pigs. Supplementation with XB improved ADG when DDGS were not steeped, however, steeping appeared to reduce feed intake, resulting in poorer ADG, which may have been related to sub-optimal fermentation of the steeped DDGS.

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.

At a 2012 meeting with the National Pork Board, we identified a list of alternative ingredients that would have direct application to the pork industry. These included byproducts 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). 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). For example, unlike wheat middlings, DDGS has undergone partial fermentation and easily fermentable (soluble) fibers (e.g., small and highly branched arabinoxylans) have already been removed. For DDGS, enzymes should be targeted towards insoluble types of fibers (e.g., highly complex arabinoxylans and cellulose). In addition, some ß-glucanases will have specificity towards cellulose as well and will degrade some of the non-starch polysaccharides (NSP) in corn and wheat products (Bedford, 2000; Paloheimo et al., 2010). We propose that enzyme cocktails can be effective in improving the utilization of byproducts in swine and that these cocktails should be optimized for individual ingredients. Enzymes may be added in a purified form or supplied from inoculations with microbes that have high fiber degrading capacity (de Lange and Zhu, 2012).

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 was to improve the utilization of fibrous ingredients in swine through the strategic application of enzymes. We hypothesized 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.

Materials & Methods

Two independent experiments were conducted to evaluate the impact of exogenous fiber degrading feed enzymes when fed in conventional dry (experiment 1) and liquid diets (experiment 2) containing corn DDGS on growth performance and nutrient digestibility in growing pigs.

Experiment 1: This experiment was conducted to determine the effect of a combination of xylanase and β-glucanase or a combination of a protease with a direct fed microbial in conventional dry corn-soybean meal-DDGS based diets on pig performance, gastro-intestinal tract weights, and ileal and cecal pH. Basal phase I and phase II diets (Table 1) were formulated to contain corn, soybean meal and 30% DDGS and met or exceeded nutrient recommendations as suggested by NRC (2012). These basal diets were either not supplemented or supplemented with either xylanase and β-glucanase (XB) or a combination of protease and a direct fed microbial (PDFM). All diets contained phytase enzyme and were fed dry in pellet form. A total of 72 feeder pigs (initial BW of 25 kg) were blocked by initial weight and randomly assigned within blocks to 18 pens (6 pens per treatment; 2 barrows and 2 gilts per pen). Diets were fed in a 2-phase feeding program with 2 weeks for each phase. Pig body weights and feed disappearance were determined weekly. At the end of the experiment, one pig per pen was sacrificed to determine empty gastrointestinal tract (GIT) weight and to determine ileal and cecal digesta pH. Data for growth performance, empty GIT weight and digesta pH were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Data were analyzed as a randomized complete block design with 3 dietary treatments in 6 blocks. Least squares means were reported and differences were considered statistically significant at $P \leq 0.05$, with tendencies at $0.05 < P \leq 0.10$.

Experiment 2: This experiment was conducted to test the impact of supplementation of XB in conventional dry or in liquid (steeped or not) fed diets containing corn DDGS (Table 1). A complete corn-soybean meal based mix (containing phytase at 750 FTU/kg) was prepared to meet nutrient requirements for growing pigs according to NRC (2012). Three experimental treatments were prepared by mixing 70% of the basal mix with 30% DDGS (w/w) as follows: 1) Control, dry diet without XB and no steeping, 2) Liquid diet with XB and no steeping (this was a dry diet that was only mixed with water prior to feeding; and 3) Liquid diet with XB and steeping. The target activities for XB were 1,050 and 5,500 U/g of DDGS for β-glucanase and xylanase, respectively Steeping was accomplished as follows: DDGS and enzymes were mixed with water to achieve approximately 25% DM and left in a fermentation tank for 1 to 7 days. These 'weekly' fermentations were rotated between 2 fermentation tanks, with a thorough cleaning (both base and acid) between batches. On day -2 (e.g. 2 days before start of feeding) water was placed in the receiving tank and allowed to warm to room temperature overnight. The following day, DDGS was added and the mix was moved to one of the 2 fermentation tanks where enzymes were added. Feeding started 2 to 3 d after enzymes had been added and the fermented material was fed over the course of 7 days. This cycle was repeated with subsequent batches of fermented DDGS and enzymes. At feeding, the fermented DDGS with enzymes were mixed with the basal diet and more water to achieve 25% DM and then immediately offered to the pigs. Diets were delivered by a computer controlled liquid feeding system 4 times per day. Pigs had free access to water. A total of 144 feeder pigs (initial BW of 25 kg) were blocked by body weight and randomly assigned within block to pens (24 pens; 3 barrows and 3 gilts per pen). Diets were fed in a 2-phase feeding program (3 weeks per phase). Pig body weights and feed disappearance were determined weekly. Apparent total tract digestibility of ash, organic matter, crude fat, NDF, and ADF were determined using titanium dioxide as indigestible marker (included at 0.2% in the diet). At the end of the experiment, one pig per pen was

sacrificed to determine empty gastrointestinal tract (GIT) weight. Detailed feed sampling was employed upon manufacturing at the feed mill, during DDGS steeping and at liquid feeding for routine nutrient analyses and analyses of pH, lactic acid, and acetic acid concentrations. Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Data were analyzed as a randomized complete block design with 3 dietary treatments in 8 blocks. 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

Experiment 1. There were no statistical differences ($P > 0.05$) between dietary treatments for body weight gain, feed intake and feed conversion efficiency (Table 2). The overall feed conversion efficiency (Feed:gain) was 2.10, 2.12 and 1.97 for the control, XB and PDFM, respectively. It was noteworthy that pigs fed PDFM had 6.5% better feed efficiency than the control-fed pigs, although this was not statistically significant. There were no effects ($P > 0.05$) of treatments on empty GIT weight or cecal and ileal digesta pH.

Experiment 2.
During Phase 1 (week 1 to 3 of the study), ADG was greater ($P < 0.05$) for the liquid-fed diet with enzyme without prior steeping compared to the control dry diet (contained no enzyme), while the liquid-steeped diet (contained enzyme) was intermediate (Table 3). Although ADFI did not differ between treatments, Feed:gain was lower ($P < 0.05$), suggesting better efficiency, for both liquid-fed diets (steeped or not steeped) containing enzyme compared to the dry diet. During Phase 2 (week 4 to 6 of the study) and overall, ADG was lower ($P < 0.05$) for the liquid-steeped diet with enzyme compared to the non-steeped liquid diet with enzyme, with the control dry diet being intermediate. Feed efficiency was not impacted by dietary treatments during Phase 2 or overall. Interestingly, the pigs fed the liquid-steeped diet with enzyme consumed numerically (main effect P values of 0.13, 0.19, and 0.13, for Phase 1, 2, and overall) less feed in Phase 1 (4.6%), Phase 2 (5.9%), and overall (5.3%) compared to non-steeped liquid diets with enzyme. This may partly explain the reduced ADG observed in pigs fed the liquid-steeped diet with enzyme. At the end of the study, pig body weight was greatest ($P < 0.05$) for pigs fed non-steeped liquid diets with enzyme (absolute increase of 2.9 kg) compared with the steeped liquid diet with enzyme, with pig BW being intermediate for the control dry diet without enzyme.

Apparent total tract digestibility of ash, organic matter, and crude fat did not differ between treatments (Table 4). However, apparent total tract digestibility of NDF and ADF were reduced substantially for the liquid-steeped diet with enzyme compared to the non-steeped diet with enzyme or the dry control diet.

Gastro-intestinal weights were measured in one pig per pen. Final body weight of these pigs followed the same trend as observed for the pen data, indicating a tendency for reduced body weight of pigs fed the liquid-steeped diet with enzyme compared to the other two treatments (Table 5). However, no differences were detected in stomach weight, small intestinal weight, or the combined weight of the cecum and colon.

Steeping DDGS reduced pH of the complete diet immediately prior to feeding, as expected (Figure 1). Steeping of DDGS (before it was added to the diet) showed a reduction in pH from 4.42 at the start of fermentation to 3.65 at day 3 of fermentation, after which fermentation seemed to stabilize at a pH of approximately 3.9 (Figure 2). Lactic acid concentrations of fermented DDGS increased from 29 mM on day 0 to 118 mM on day 3, after which concentrations dropped to 53, 52, and 47 mM, on day 6, 7, and 10, respectively (Figure 3). Acetic acid concentrations of fermented DDGS increased from 6.9 mM on day 0 to 14.2, 96.1, 117.5, and 115.5 mM for day 3, 6, 7, and 10, respectively.

Discussion

Two experiments were conducted to evaluate the impact of supplemental enzymes on grower pig performance, gastro-intestinal weights, and nutrient digestibility. In the first experiment, we hypothesized that the use of enzymes (protease) in combination with a direct-fed microbial culture could improve performance of pigs. This was based on recent observations by de Lange and Zhu (2012), who showed synergy between supplemental enzymes and microbial inoculants for generating VFA during controlled fermentation of DDGS. It can be speculated that fiber degradation products for microbial fermentation may be maximized by the combined usage of enzymes and microbial inoculants. In the present study, no effects of the combined use of protease and direct-fed microbial were observed on pig performance and gastro-intestinal weight. In addition, supplementation of fiber-degrading enzymes had no impact on any of the measurements in Exp. 1. Diets in this experiment were fed as dry diets and we proposed that feeding diets in liquid form (either fermented or not fermented) may improve

the efficiency of enzymes in degrading fiber components of the diet, thereby providing an opportunity for improved pig performance. This led us to Exp. 2.

The primary focus of Exp. 2 was to improve the nutritional value of DDGS through supplementation of exogenous enzymes and application of liquid feeding. 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). Enzymes and microbial inoculants generally appear more effective when applied in liquid feeding systems (Scholten et al., 1999) and this is the case even when enzymes are added to the liquid feed just prior to feeding (Columbus et al., 2010). We hypothesized that short duration soaking or steeping with enzymes will improve nutrient digestion and utilization, especially when using high fiber co-products, such as DDGS. We used a combination of xylanase and β-glucanase as supplemental enzymes. Treating DDGS with a combination of xylanase and β-glucanase with or without steeping resulted in improved feed efficiency for the first three weeks suggesting degradation by enzymes of dietary fibrous components that may limit nutrients utilization in younger pigs. Supplementation with enzymes improved ADG when DDGS were not steeped, however, steeping appeared to reduce feed intake, resulting in poorer ADG. Pig body weight was 2.9 kg greater pigs fed non-steeped liquid diets with enzyme compared with pigs fed the steeped liquid diet with enzyme. Liquid steeped diets had concentrations of acetic acid and lactic acid that could be considered suboptimal, indicating poor fermentation. Ideally, liquid feed should contain a concentration of at least 100 mM of lactic acid (Brooks, 2003) and less than 40 mM of acetic acid (van Winsen et al., 2001), with a pH of less than 4.5 (van Winsen et al., 2000). These conditions are desirable in order to prevent growth of pathogens (Missotten et al., 2015) and prevent excessive growth of yeasts (Plumed-Ferrer et al., 2005). In the present study, the assumption was made, based on our previous work, that fermentation is stable and reaches a steady state from 3 days onwards. This was not the case in the current study, in which DDGS was steeped for up to 10 days. Whether this is related specifically to DDGS (poor fermentation characteristics) or contamination of the fermentation is not clear. It is therefore possible that poorer performance of pigs fed the steeped liquid diet was caused by the suboptimal fermentation characteristics of the feed. Although lactic acid and acetic acid concentrations were within desirable parameters for the first few days of fermentation, concentrations of acetic acid increased after day 6 to concentrations greater than 100 mM. Liquid diets were fed until 10 days of fermentation and pig response was likely negatively influenced, in part, because of the suboptimal fermentation characteristics. In the current study, we only treated and fermented the DDGS component of the diet (with enzymes added) and when mixing this with the basal diet, it would reduce the concentration of acetic acid in the final liquid feed. Therefore, the negative impact of acetic acid from the fermentation on palatability (Canibe et al., 2007a) should be less, although it seemed to still be detectable in the present study, based on reduced feed intake in pigs fed fermented liquid diet. The limited impact of enzyme supplementation in liquid diets is consistent with our earlier work, funded by the Pork Board, in which we were not able to demonstrate a positive effect of xylanase on the digestibility and feeding value of DDGS in growing pigs (Moran et al., 2016). Interestingly, digestibility of ADF and NDF in the current study was reduced when pigs were fed the fermented liquid diet, compared to the diet that was not steeped and the control, dry diet. This is similar to the reduction in NDF digestibility in pigs fed liquid DDGS-containing diets compared to dry diets reported by Moran et al. (2016). The reason for reduced apparent total tract ADF and NDF digestibility in liquid, steeped diets is not clear. Although growth performance of pigs fed DDGS treated with fiber degrading enzymes did not differ with pigs fed non-treated DDGS control, an interesting area of further exploration is the nature and effects of potential metabolites that could be released when DDGS is treated with fiber degrading enzymes in steeped conditions (e.g., Wiseman et al., 2016ab).

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Table 1. Composition of experimental diets, as-fed basis[1]

	Grower Phase I	Grower Phase II
Ingredient, %		
Corn, yellow dent	33.47	39.42
Wheat, hard red winter	10	10
Corn DDGS	30	30
Soybean meal, 47.5% CP	22.63	16.65
Animal-vegetable blend	1.00	1.00
L-lysine HCl	0.29	0.31
Salt	0.50	0.50
Limestone	1.48	1.41
Monocalcium phosphate, 21% P	0.12	0.00
Vitamin-mineral premix	0.50	0.50
Phytase	0.01	0.01
Titanium dioxide	0.00	0.20
Calculated Nutrient Composition		
Dry Matter, %	89.20	89.15
ME, kcal/kg	3,355	3,354
NE, kcal/kg	2,380	2,416
Crude Protein, %	23.37	21.04
ADF, %	6.19	6.03
NDF, %	15.72	15.75
Crude Fiber, %	4.11	4.04
Crude Fat, %	6.15	6.20
Phytase units/kg	750	750
SID Amino Acids:		
Lys, %	1.08	0.95
Thr, %	0.68	0.60
Met, %	0.35	0.32
Met+Cys, %	0.66	0.60
Trp, %	0.20	0.17
Total Lysine, %	1.26	1.12
Ca, %	0.70	0.63
Total P, %	0.52	0.47
Available P, %	0.35	0.32

Table 2. Effects of fiber degrading enzymes and protease with direct fed microbial on growth performance, gastrointestinal weight and digesta pH of growing pigs fed corn DDGS, Exp. 1

		Enzyme			
	Control	**XB[1]**	**Protease + DFM[1]**	**SEM**	***P*-value**
Phase 1, week 1 to 2					
Initial BW, kg	27.78	28.53	27.91	1.433	0.858
ADG, kg/d	1.161	1.149	1.175	0.056	0.897
ADFI, kg/d	1.975	1.916	1.925	0.044	0.370
Feed:gain, kg/kg	1.703	1.684	1.640	0.071	0.671
Final BW, kg	43.16	43.01	43.34	0.726	0.898
Phase 2, week 3 to 4					
ADG, kg/d	1.022	1.005	1.057	0.043	0.476
ADFI, kg/d	2.596	2.640	2.443	0.145	0.385
Feed:gain, kg/kg	2.555	2.623	2.332	0.180	0.269
Final BW, kg	57.46	57.08	58.14	0.911	0.510
Overall, week 1 to 4					
ADG, kg/d	1.089	1.074	1.114	0.034	0.514
ADFI, kg/d	2.286	2.278	2.184	0.083	0.414
Feed:gain, kg/kg	2.102	2.124	1.966	0.100	0.264
Gastro-intestinal tract weight g/kg BW					
Stomach	6.00	5.72	5.83	0.423	0.799
Small intestine	28.97	27.97	29.93	1.524	0.459
Caecum	1.82	1.80	1.86	0.181	0.952
Large intestine	15.47	15.05	14.59	1.350	0.812
Digesta pH					
Ileum	6.99	7.02	7.09	0.144	0.799
Caecum	5.73	5.62	5.73	0.093	0.425

[1]XB, xylanase and β-glucanase; DFM, direct fed microbial

Table 3. Effects of adding fiber degrading enzymes (xylanase + β-glucanase) in diets with non-steeped or steeped DDGS fed to growing pigs, Exp. 2

Item	Enzyme[1]			SEM	P-value
	Control Dry	Liquid Not steeped	Liquid Steeped		
Phase I, week 1 to 3	25.0	25.0	25.3	0.445	0.847
Initial BW, kg					
ADG, kg/d	0.902[b]	0.959[a]	0.918[ab]	0.021	0.043
ADFI, kg/d	1.601	1.614	1.540	0.037	0.132
Feed:gain, kg/kg	1.779[a]	1.680[b]	1.681[b]	0.026	0.001
Final BW	44.0[b]	45.3[a]	44.4[b]	0.448	0.037
Phase II, week 4 to 6					
ADG, kg/d	1.146[ab]	1.184[a]	1.087[b]	0.029	0.010
ADFI, kg/d	2.322	2.359	2.219	0.076	0.187
Feed:gain, kg/kg	2.031	1.997	2.042	0.049	0.634
Final BW, kg	68.1[ab]	70.1[a]	67.2[b]	0.831	0.008
Overall, week 1 to 6					
ADG, kg/d	1.024[ab]	1.072[a]	1.003[b]	0.020	0.009
ADFI, kg/d	1.964	1.986	1.881	0.051	0.130
Feed:gain, kg/kg	1.918	1.857	1.875	0.033	0.185

[1]DDGS with enzymes were steeped for 3 to 10 d at 40°C, mixed with a basal diet and fed as a complete liquid diet (25% DM). The non-steeped diet was a complete diet with DGGS and enzymes and was mixed with water (25% DM) immediately prior to feeding.

Table 4. Effects of adding fiber degrading enzymes (xylanase + β-glucanase) in diets with non-steeped or steeped DDGS on apparent total tract digestibility in growing pigs, Exp. 2

Item	Enzyme[1]			SEM	P value
	Control Dry	Liquid Not steeped	Liquid Steeped		
Ash	57.62	55.74	59.38	1.27	0.15
Organic matter	81.38	80.42	81.96	0.49	0.11
NDF	54.62[a]	50.83[a]	33.85[b]	1.68	<0.001
ADF	58.94[a]	55.65[a]	25.90[b]	4.09	<0.001
Crude Fat	74.77	72.16	74.47	2.74	0.76

[1]DDGS with enzymes were steeped for 3 to 10 d at 40°C, mixed with a basal diet and fed as a complete liquid diet (25% DM). The non-steeped diet was a complete diet with DGGS and enzymes and was mixed with water (25% DM) immediately prior to feeding.

Table 5. Effects of adding fiber degrading enzymes (xylanase + β-glucanase) in diets with non-steeped or steeped DDGS on gastro-intestinal tract weight of growing pigs, Exp. 2

Item	Control Dry	Enzyme[1] Liquid Not steeped	Enzyme[1] Liquid Steeped	SEM	P value
Final body weight, kg	74.86	73.50	69.68	2.12	0.06
stomach weight, kg	0.48	0.52	0.48	0.03	0.27
small intestine weight, kg	2.32	2.33	2.20	0.12	0.54
cecum + colon weight, kg	1.30	1.21	1.40	0.09	0.12

[1]DDGS with enzymes were steeped for 3 to 10 d at 40°C, mixed with a basal diet and fed as a complete liquid diet (25% DM). The non-steeped diet was a complete diet with DGGS and enzymes and was mixed with water (25% DM) immediately prior to feeding.

Figure 1. Impact of steeping on pH of the complete diet measured immediately prior to feeding

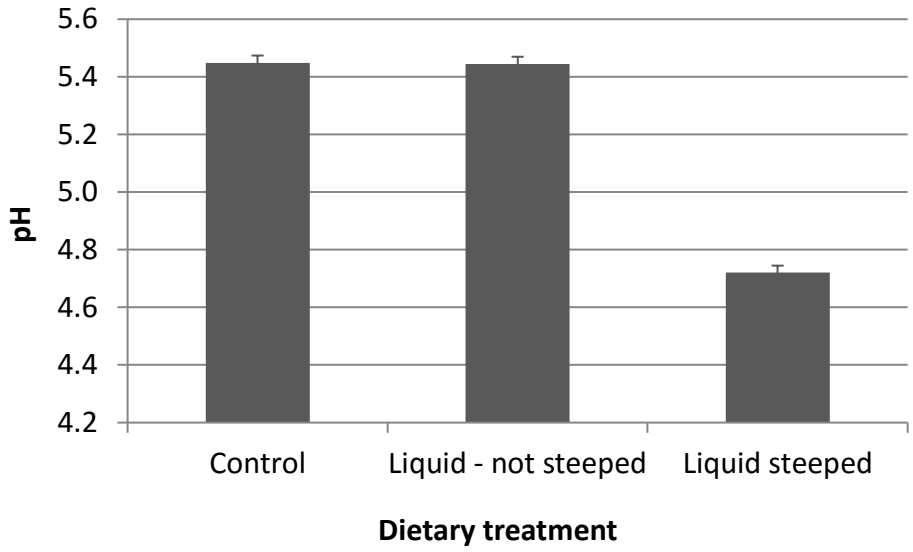


Figure 2. Impact of steeping DDGS over time on daily pH

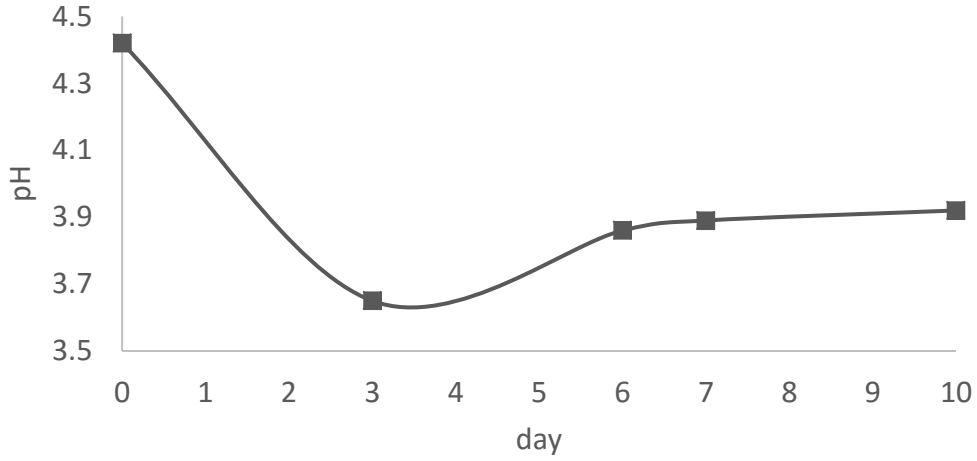
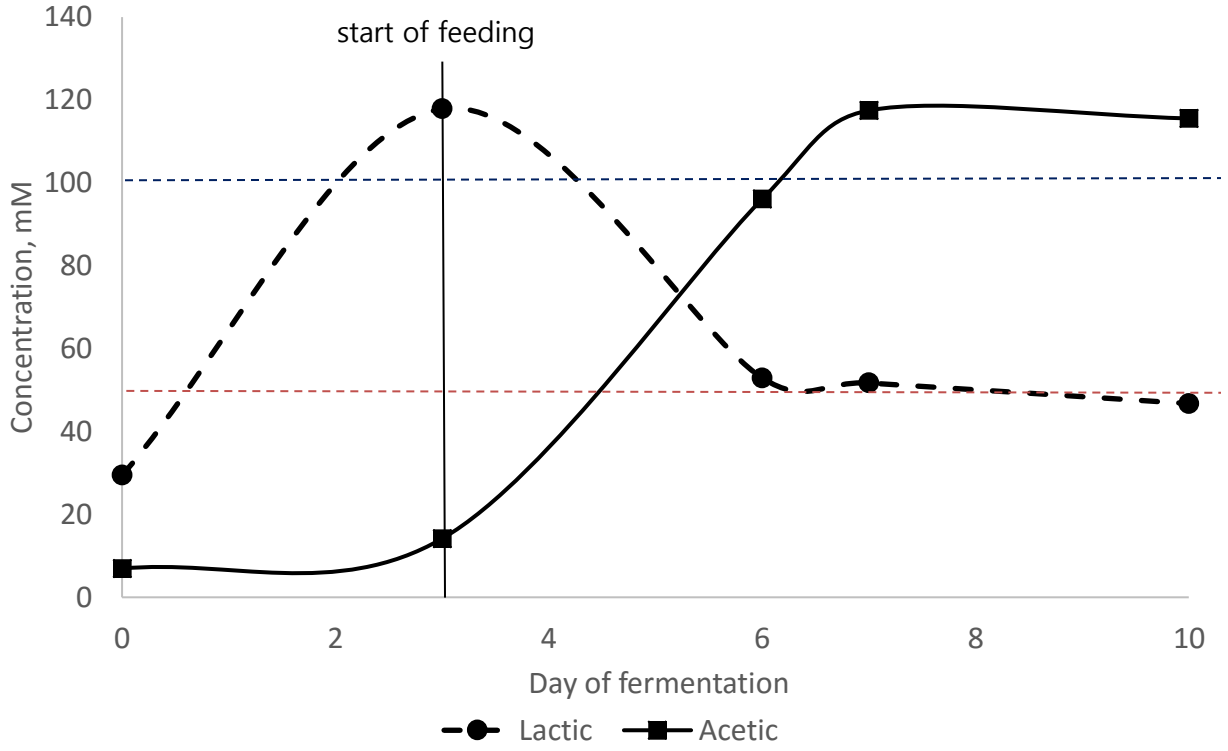


Figure 3. Daily lactic acid and acetic acid concentrations of fermented DDGS averaged over the weekly fermentations



The optimal concentration of lactic acid during fermentation is 100 mM or greater (Blue dotted line). The optimal concentration of acetic acid during fermentation is below 50 mM (Red dotted line). Enzymes (xylanase and β-glucanase) were added on day 0 and feeding of the diet with fermented DDGS with enzyme was started on day 3.