RESEARCH REPORT
ANIMAL SCIENCE

Title: Improving fiber digestibility in DDGS from ethanol production – **NPB #11-097**

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Industry Summary

The objective of this project was to assess effects of pretreatment methods (chemical, enzymatic, and mechanical) on the digestibility of energy in DDGS. Combinations of different methods were investigated using *in vitro* models. The *in vitro* treatments investigated were: sodium hydroxide, ammonium hydroxide, hydrochloric acid and two types of enzyme, one a cellulase/xylanase mix and the other an enzyme complex containing a wide range of carbohydrases, designed to break down hemicellulose in biomass. The *in vitro* tests consisted of treating the DDGS with the chemical or enzymes and then conducting chemical analyses to determine if the fiber content and digestibility indicators had changed versus a control. The parameters considered for the *in vitro* tests were acid and neutral detergent fiber (ADF, NDF) as well as total dietary fiber (TDF) to get the amount of digestible fiber, cellulose and hemicellulose remaining in the DDGS after treatment, as well as lysine, used as an indicator of digestibility. The most promising methods from the *in vitro* study were then used in an *in vivo* experiment to determine if the improvements observed *in vitro* also resulted in improvements in energy digestibility of DDGS when fed to pigs.

The *in vitro* studies showed that the sodium hydroxide and enzyme treatments were significantly different (lower) in fiber content and the enzyme treatments were also higher in lysine than the control,

showing improved nutritional quality therefore these were selected for the subsequent *in vivo* study. In addition, two more treatments, calcium oxide and mechanical extrusion were tested *in vivo*.

The animal studies showed that the cellulase was effective in improving the ME of DDGS. In contrast, addition of an enzyme mixture, extrusion, or chemical treatments of DDGS did not consistently improve the apparent total tract digestibility (ATTD) of the ingredient.

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Keywords: amino acid digestibility, DDGS, energy digestibility, fiber, pigs

Scientific Abstract:

The *in vitro* and *in vivo* effects of different mechanical, chemical and enzymatic treatments to improve fiber digestibility on energy and nutrient digestibility of pigs were investigated.

These were: sodium hydroxide, ammonium hydroxide, hydrochloric acid and two types of enzyme, one a cellulase/xylanase mix and the other an enzyme complex containing a wide range of carbohydrases, designed to break down hemicellulose in biomass. Of the *in vitro* treatments, the enzymatic tests and the NaOH treatments were selected for animal studies as they showed significantly different values of fiber and digestibility (lysine content).

Extruded DDGS and a CaO treatment were also selected for the *in vivo* study.

Sixty-three barrows (initial BW: 76.1 ± 6.1 kg) were placed individually in metabolism cages and allotted to a randomized complete block design with 7 diets and 9 replicate pigs per diet. After 5 d adaptation

period to the diet, feces and urine samples were collated for 5 d. A corn-based diet was formulated consisting of 97.0% corn and 6 additional diets were formulated by mixing corn with 47.95% DDGS that was untreated or extruded, treated with sodium hydroxide, treated with calcium oxide, treated with cellulase, or treated with an enzyme mixture. The apparent total tract digestibility (ATTD) of GE in corn, untreated DDGS, extruded DDGS, sodium hydroxide DDGS, calcium oxide DDGS, cellulose DDGS, and enzyme-treated DDGS was 86.6, 71.7, 72.8 73.3, 70.4, 75.0, and 73.9%, respectively. The ATTD of GE was greater ($P < 0.01$) in corn than in all other ingredients. The ATTD of GE in cellulose treated DDGS was greater ($P < 0.01$) than in calcium oxide DDGS and untreated DDGS, but the ATTD of GE was not different among extruded DDGS, sodium hydroxide treated DDGS, cellulase treated DDGS, and enzyme treated DDGS. The ATTD of NDF was less ($P < 0.01$) in calcium oxide treated DDGS than in corn, sodium hydroxide treated DDGS, cellulase treated DDGS, and enzyme treated DDGS. The ATTD of ADF was less ($P < 0.01$) in corn and extruded DDGS than all the other diets, but the ATTD of ADF was greater ($P < 0.01$) in extruded DDGS than in corn. The ME was 3,738, 3,442, 3,501, 3,458, 3,318, 3,701, and 3,545 kcal/kg DM in corn, untreated-DDGS, extruded DDGS, sodium hydroxide DDGS, calcium oxide DDGS, cellulose treated DDGS, and enzyme treated DDGS, respectively. The ME was less ($P < 0.01$) in extruded DDGS, sodium hydroxide DDGS, calcium oxide DDGS, enzyme treated DDGS, and untreated-DDGS than in corn and cellulase treated DDGS. In conclusion, in this experiment, no significant improvement in ME or ATTD of GE, OM, NDF, ADF, and cellulose was observed if DDGS was extruded or treated with sodium hydroxide, calcium oxide or an enzyme mixture. However, treatment of DDGS with cellulase resulted in an increase in ATTD of GE and OM, and in ME compared with untreated DDGS.

Introduction:

Distillers dried grains with solubles (DDGS) can be incorporated in swine diets by up to at least 30% (Stein and Shurson, 2009). It has also been documented that although the energy concentration in DDGS is much greater than in corn, the concentration of digestible and metabolizable energy in DDGS is similar to that in corn because the digestibility of energy is much less in DDGS than in corn (Pedersen et al., 2007). The GE in corn originates mainly from starch, CP, and fat, while the GE in DDGS comes from a greater concentration of

fat, crude protein, and fiber, and very little from starch (Stein and Shurson, 2009). Recent research has documented that the fiber in DDGS is fermented by less than 50% (Urriola et al., 2010).

The low rate of fermentability of fiber in DDGS is the main reason for the low digestibility of energy in DDGS compared with corn. It was, however, also shown that the soluble fibers in DDGS are fermented by more than 90%, whereas the insoluble fibers in DDGS are fermented only by around 40% (Table 2; Urriola et al., 2010). More than 90% of all the fiber in DDGS are insoluble fiber, but because the fermentability, and therefore also the energy contribution, of soluble fiber is much greater than from insoluble fiber, any treatment that can solubilize some of the insoluble fibers will result in increased energy contribution from the fibers in DDGS. It is, therefore, apparent that to improve the digestibility of energy in DDGS, procedures that can solubilize some of the dietary fibers in DDGS are needed.

There are several processes that can be used to improve the solubility and fermentability of dietary fiber in fibrous feedstuffs, which may consequently increase the energy value of the ingredient. These processes include physical processes (e.g., grinding, heating, extrusion) and chemical processes such as hydrolytic and oxidative agents (Fahey et al., 1993). Treatment with sodium hydroxide increase rumen digestibility of OM in barley straw from 52% to 76% and the digestibility of DM by 22% in other crop residues (Fahey et al., 1993), but there are no data on the use of NaOH in DDGS fed to pigs. Anhydrous NH_3, NH_4OH, thermo-ammoniation, and urea have also been used to treat fibrous materials. A combination of ammonia and high pressure may improve solubilization and fermentability of fiber (Realf and Abbas, 2004; Bals et al., 2006), but there is no information about the effects of these procedures on the fermentability of fiber in DDGS. It is, however, possible that these procedures may be used to increase the energy value of DDGS, but no research has been conducted to test this hypothesis.

The effect of dietary exogenous enzymes (cellulases and xylanases) on digestibility of corn and wheat DDGS has been studied (Emiola et al., 2009; Yanez et al, 2011), but positive effects of enzymes on the solubility and fermentability of dietary fiber have not been reported. It is, however, possible, that if enzymes are used for pretreatment of DDGS rather than for inclusion in diets containing DDGS, positive responses may be

obtained because the enzymes can then be allowed to exert their effect without restrictions on time, temperature or pH as is the case when enzymes are included in diets fed to pigs. It is also possible that enzymes can be used in combinations with physical or chemical treatments to improve effects on digestibility, but no data have been reported to on this possibility until this study.

Objectives

It was the objective of the current work to first measure the *in vitro* and then test *in vivo* the effects of different treatments to improve fiber digestibility on energy and nutrient digestibility of pigs. The treatments consisted of chemical, mechanical and enzymatic actions on DDGS.

MATERIALS AND METHODS:

***In Vitro* Study**

Low-fat DDGS was obtained from Center Ethanol Company LLC (Sauget, IL). To prevent the DDGS from degrading due to heat or contamination , composite samples were kept in multiple 5-gallon buckets and stored in a -20°C freezer. For each pretreatment, approximately 240g (~400ml) of DDGS were put in into each of 3-1L beakers.

Three major types of pretreatments were evaluated separately on DDGS: chemical (dilute acid or base), mechanical (grinding and extrusion) and enzymatic (enzyme provided by Novozymes, Bagsværd, Denmark). With the exception of the ammonia pretreatment, performed at 60^{o}C to compare the results to previous biomass work, all other chemical and enzymatic tests were performed at room temperature, so as not to damage the DDGS. All samples were ground in the laboratory to 200 micrometers average particle size with a Wiley Mill (Thomas Scientific, NJ). Each type of pretreatment was compared to a control. For each pretreatment type, either time or concentration were changed, as shown in Table 1. The total number of experimental units was 54, including the control.

Dilute hydrochloric acid: For this project we used dilute hydrochloric acid to digest the DDGS in a manner closer to what is encountered in animal digestion. Two concentrations of acid were used at room temperature: 0.1 and 0.05 M. The acid was mixed in the DDGS sample at a 1:1 volume ratio and left stirring for either 4 or 24 hours after which time the sample was centrifuged, to separate liquid from solid.

Dilute ammonia and dilute sodium hydroxide: The procedure for dilute ammonia was the same as for the dilute hydrochloric acid, with the exception that it was conducted at 60^{o}C and for only 1 or 4 hours residence time. The concentrations of ammonia was 0.05 and 0.1 M and the volume mixing ratio also 1:1.

Enzymatic pretreatment: This pretreatment was performed on dry substrate, by mixing either of two different concentration of commercial cellulosic enzymes with DDGS: the manufacturer's recommended dose (per kg dry matter) and twice the manufacturer's recommended dose. Those were labeled as "high" (the max dose) and "low" (the recommended dose). After stirring to achieve sample and enzyme mixing, the residence time was 24 and 48 hours. The enzymes used were experimental biomass enzymes by Novozymes (Bagsværd, Denmark): enzyme complex NS22119 containing a wide range of carbohydrases designed to break down hemicellulose in biomass, and cellulase complex NS22086 which is a mixture of cellulase and xylanase. No other chemical or biological materials nor water were added to the DDGS at any time before or after the introduction of the enzymes.

Sample Analysis (in-vitro)

After the chemical/enzymatic tests, the samples were analyzed as follows: total dietary fiber (TDF), according to AOAC testing method 32.1.17, acid detergent fiber (ADF), AOAC 973.18, Neutral Detergent Fiber (NDF), AOAC 2002.4 and Lysine by hydrolysis method AOAC 994.12, Alternative III, followed by analysis by LCMSMS.

Because some of the treatments had extreme pH conditions (too high or too low) for handling purposes, the sample was washed with deionized water before analyzing. Mass loss was monitored and recorded, in order to quantify the loss of solubilized materials and taken into account in the final measurements of the parameters.

Data were analyzed by analysis of variance (ANOVA) and post-hoc paired difference tests based on least-squares analysis (two-sided Dunnett vs. control) with SYSTAT (v.13 for Windows). The purpose of data analysis for the *in vitro* portion of the study was to aid in selecting the treatments that performed the best *in vitro*, so as to be using them in the animal nutrition study, therefore the experimental unit was one 50 mg sample of well-mixed treated DDGS. All treatments were conducted and analyzed in triplicate.

In Vivo Study Methods

The Institutional Animal Care and Use Committee at the University of Illinois (Urbana, IL) reviewed and approved the protocol for this experiment.

Diets, Animals, and Experimental Design

Pigs used in this experiment were the offspring of G-performer boars mated to F-25 gilts (Genetiporc, Alexandria, MN). One batch of low-fat DDGS (**DDGS-CV**) was obtained from the Center Ethanol (Sauget, IL). This batch was divided into 6 sub-batches. One sub-batch was used without any treatment and one batch was extruded prior to diet mixing (**DDGS-EX**) using a Model 2500 INSTA-PRO single screw extruder. One batch was treated with sodium hydroxide (**DDGS-Na**) and one batch was treated with calcium oxide (**DDGS-Ca**) as described by Felix et al. (2012). The last 2 batches were treated with cellulase (**DDGS-Ce)** or with an enzyme mixture (mainly xylanases and hemicellulases; **DDGS-NZ**). Both the cellulase and the enzyme mixture were sourced from Novozyme (Bagsværd, Denmark) and were used at the maximum dose (5 wt. % of dry solids, for 48 hours). Yellow dent corn was grown locally and obtained from the University of Illinois Feed Mill (Champaign, IL).

Sixty-three growing barrows (initial BW: 76.1 ± 6.1 kg) were placed in metabolism cages and allotted to a randomized complete block design with 7 diets and 9 replicate pigs per diet. Each metabolism cage was equipped with a feeder and a nipple drinker. Seven corn-based diets were formulated (Table 2). The basal diet contained 97.0% corn (as-fed basis) and the remaining 6 diets contained 47.95% corn and 50.0% of each of the 6 sources of DDGS (as fed basis). Vitamins and minerals were included in the diets to meet or exceed the requirements for weanling pigs (NRC, 2012). The only sources of energy in the diets were corn and the DDGS.

Feeding and Sample Collection

Pigs were fed in a daily amount of 3 times the maintenance energy requirement (i.e., 197 kcal of ME/kg of $BW^{0.60}$; NRC, 2012) of the smallest pig in each replicate. The total amount of feed was divided into 2 equal meals that were fed at 0800 and 1700 h. Water was available at all times.

Pigs were fed experimental diets for 12 d. The initial 5 d were considered an adaptation period to the diet. Chromic oxide and ferric oxide were added to the diet as indigestible markers in the morning meals on d 6 and on d 11, respectively. The fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at -20ºC immediately after collection. Urine was also collected and urine collections started on d 7 at 0800 h and ceased on d 12 at 0800 h. Urine buckets were placed under the metabolism cages to permit total collection. They were emptied in the morning and afternoon and a preservative of 50 mL of 6 *N* HCL was added to each bucket when they were emptied. The collected urine was weighed and a 20% subsample was stored at -20ºC.

Sample Analyses.

After completing sample collections, fecal samples were dried at 65ºC in a forced-air oven and ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) before analyses and urine samples were thawed and mixed within animal and diet, and a subsample was collected for chemical analysis. Urine samples were lyophilized before energy analysis (Kim et al., 2009). Diets and ingredient samples were analyzed for CP by combustion (Method 999.03; AOAC Int., 2007) using a Rapid N cube (Elementar Americas

Inc, Mt. Laurel, NJ) and acid hydrolyzed ether extraction (**AEE**), which was determined by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Diet and ingredients samples were also analyzed for P and Ca by the inductively coupled plasma spectroscopy procedure (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007].

Diets, ingredients, and fecal samples were analyzed for ash (Method 942.05; AOAC Int., 2007), DM (Method 930.15; AOAC Int., 2007), ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Diets, ingredients, fecal, and urine samples were also analyzed for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL) and ingredients were analyzed for AA [Method 982.30 E (a, b, c); AOAC Int., 2007].

Calculations and Statistical Analysis

Energy values that were determined from the excretion of GE in the feces and urine were subtracted from the intake of GE to calculate DE and ME for each diet (Adeola, 2001). The DE and ME in the corn diet were divided by 0.970 to calculate the DE and ME in corn. The contributions of DE and ME from corn to the diets containing untreated and treated DDGS were then calculated and subtracted from the total DE and ME of these diets, and the concentrations of DE and ME in untreated and treated DDGS were calculated by difference (Adeola, 2001). Calculations of the DE and ME in all ingredients were completed on an as-fed basis as well as on a DM basis. Cellulose concentration was calculated in all ingredients as the difference between ADF and ADL. Hemicellulose, was calculated in all ingredients as the difference between NDF and ADF. The ATTD of GE, OM, ADF, NDF, cellulose, and hemicellulose was also calculated for all diets and for each untreated and treated DDGS (Adeola, 2001).

Data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). Homogeneity of the variances among treatments was confirmed using the HOVTEST = BF procedure of SAS. The UNIVARIATE procedure of SAS was used to identify outliers, but no outliers were identified. Diet was the fixed effect and replicate was the random effect. The Least Significant Means statement was used to calculate

treatment means and the PDIFF option was used to separate means if differences were detected. The pig was the experimental unit for all analyses and an alpha level of 0.05 was used to assess significance among means.

RESULTS:

In vitro

The average values of ADF, NDF and TDF as well as Lysine are shown in Table 6 for the enzyme treatments. Both enzyme treatments, cellulase and enzyme complex showed significant differences from control in ADF, TDF and Lysine and no statistical differences in NDF.

ADF values for most of the chemical treatments were statistically different ($P<0.01$) than the control but they were higher.. Exceptions were the 0.05 M HCl treatments and the cellulase treatments. ADF values were higher than control for all the chemical treatments except NaOH.

NDF values of all chemical treatments were statistically different ($P<0.01$) than control except the 0.1 M NaOH, which was the same.. The NDF values were also higher than control in all the chemical treatments, except the 0.1 M NaOH.

TDF values for all chemical treatments were all statistically different ($P<0.01$) than control except the 0.1 M NaOH. The TDF values were also higher than control in all the chemical treatments except the 0.1 M NaOH .

The Lysine data shows significant differences ($P<0.01$) from control for all enzyme treatments and for the 0.05 and 0.1 M Ammonia and 0.1 M NaOH treatments. Lysine values are higher than control in the enzyme treatments but not in the chemical treatments.

In vivo

Gross energy intake was less ($P < 0.01$) for pigs fed the DDGS-CV diet than for pigs fed the DDGS-EX, DDGS-Ce, or DDGS-NZ diets, but no difference in GE intake were observed among pigs fed DDGS-CV, DDGS-Na, and DDGS-Ca diets (Table 4). Fecal excretion of GE was less ($P < 0.01$) for pigs fed the DDGS-Ce

diet than for pigs fed the DDGS-Ca diet, but greater ($P < 0.01$) than for pigs fed the corn diet. Fecal excretion of GE among DDGS-Ex, DDGS-Na, DDGS-Nz, and DDGS-CV diets were not different. Pigs fed the DDGS-Ce, DDGS-NZ, DDGS-CV diets had greater ($P < 0.01$) urine excretion of GE than pigs fed the corn diet, but not different than pigs fed the DDGS-Ex, DDGS-Na, or the DDGS-Ca diets. The concentration of DE (as-fed basis) was greater ($P < 0.01$) in DDGS-NZ and DDGS-EX diets than in the corn, DDGS-Na, and DDGS-Ca diets, but not different from the DDGS-CV and the DDGS-Ce diets. The ME (as-fed basis) concentration was greater ($P < 0.01$) in the corn, DDGS-Ex, and DDGS-NZ diets than in the DDGS-Na and DDGS-Ca diets, but the ME concentration was less ($P < 0.01$) in the DDGS-CV diet than in the DDGS-Ce diet.

The DE concentration (as-fed basis) was greater ($P < 0.01$) in DDGS-Ce than in corn, DDGS-Na, DDGS-Ca, and DDGS-CV, but the DE concentration was not different among DDGS-EX, DDGS-Ce, and DDGS-NZ. However, on a DM basis, DE in DDGS-Ce was greater ($P < 0.01$) than in all other ingredients. Likewise, the DE (DM basis) in DDGS-CV was less ($P < 0.01$) than in corn, DDGS-EX, and DDGS-NZ. The ME (as-fed basis) was greater ($P < 0.01$) in corn and DDGS-Ce than in DDGS-EX, DDGS-Na, DDGS-Ca, and DDGS-CV, but the ME of DDGS-NZ was not different from that of corn and DDGS-Ce. On a DM basis, ME in DDGS-EX, DDGS-Na, DDGS-Ca, DDGS-NZ, and DDGS-CV was less ($P < 0.01$) than in corn and DDGS-Ce.

The ATTD of GE was greater ($P < 0.01$) in the DDGS-Ce diet than in the DDGS-Ca and DDGS-CV diets, but the corn diet had greater ($P < 0.01$) ATTD of GE than in all other diets (Table 3). The ATTD of OM was greater ($P < 0.01$) in the corn diet than in all other diets and the ATTD of OM was greater ($P < 0.01$) in the DDGS-Na and DDGS-NZ diets than in the DDGS-Ca diet. The ATTD of NDF was greater ($P < 0.01$) in the DDGS-Na diet than in all other diets, but the ATTD of NDF was less ($P < 0.01$) in the DDGS-EX and DDGS-Ca diets than in the corn and DDGS-NZ diets. The ATTD of ADF was greater ($P < 0.01$) in the DDGS-CV, DDGS-Na, DDGS-Ca, DDGS-Ce, and DDGS-NZ diets than than in the corn and DDGS-EX diets, but the DDGS-EX diet had greater ($P < 0.01$) ATTD of ADF than corn. The ATTD of cellulose was less ($P < 0.01$) in the DDGS-EX diet than in the DDGS-CV, DDGS-Na, DDGS-Ce, and DDGS-NZ diets, but not different from the ATTD of cellulose in the corn and DDGS-Ca diets. The ATTD of hemicellulose was less ($P <$

0.01) in the DDGS-Ca diet than in all other diets, but the ATTD of hemicellulose was greater ($P < 0.01$) in the DDGS-Na diet than in the DDGS-CV, DDGS-EX, DDGS-Ce, and DDGS-NZ diets.

The ATTD of GE was greater ($P < 0.01$) in corn than in all other ingredients, but the ATTD of GE in DDGS-Ce was greater ($P < 0.01$) than in DDGS-Ca and DDGS-CV. The ATTD of OM was greater ($P < 0.01$) in DDGS-Ce than in DDGS-CV and DDGS-Ca, but the ATTD of OM was not different among DDGS-EX, DDGS-Na, DDGS-Ce, and DDGS-NZ. The ATTD of OM was greater ($P < 0.01$) in corn than in all other ingredients. The ATTD of NDF was less ($P < 0.01$) in DDGS-Ca than in corn, DDGS-Na, DDGS-Ce, and DDGS-NZ, but no differences on ATTD of NDF were observed among DDGS-CV, DDGS-EX, and DDGS-Ca. The ATTD of ADF was less ($P < 0.01$) in corn and DDGS-EX than in all the other ingredients, but the ATTD of ADF was greater ($P < 0.01$) in DDGS-EX than in corn. The ATTD of cellulose was greater ($P < 0.01$) in DDGS-CV, DDGS-Na, DDGS-Ce, and DDGS-NZ than in corn and DDGS-EX, but no differences were observed in the ATTD of cellulose between DDGS-EX and DDGS-Ca. The ATTD of hemicellulose was less ($P < 0.01$) in DDGS-CV and DDGS-Ce than in corn, DDGS-EX, DDGS-Na, and DDGS-NZ, but greater ($P < 0.01$) than the ATTD of hemicellulose in DDGS-Ca. **Discussion:**

In vitro

Both chemical and enzymatic treatments showed significant differences from control. However, in most of the chemical treatments' cases it was an increase in the values of the fiber, rather than a decrease versus the control, so these results are omitted. While there may be a few reasons why the results for the chemical treatments are higher than control, our aim for the *in vitro* portion of the project was to identify treatments that reduced the amount of measured fiber, targeting specifically ADF and NDF to be lower, so that they could be used in the animal studies. The emphasis on ADF and NDF reductions is due to evidence in the biomass pretreatment literature of a solubilization into more readily fermentable oligosaccharides from cellulose (ADF) and hemicellulose (NDF-ADF) due to acid or base applications (Bals B.,et al. 2006). Therefore, we were looking not simply for a change, but a reduction of ADF and NDF in the treated DDGS, which is typically due to at least partial breakdown of the cellulose and hemicellulose.

Therefore, in addition to looking at statistical significance for the differences from the control samples, we also looked at the type of change in the significant treatments to be tested *in vivo*. More specifically, we

looked at negative changes in the differences from control in the fiber readings (with emphasis on ADF and NDF for the reasons stated above) and for positive differences in the Lysine values. According to this criterion, we selected all the enzymatic treatments as promising for improving the digestibility of fiber in DDGS (Table 6). We also selected the NaOH treatment, which showed significant differences from ADF control at 0.1 M concentration and negative differences in fiber, as well as significantly higher Lysine content.

In vivo

The concentration of energy and nutrients in the samples of corn and DDGS-CV that were used in this experiment are close to expected values (Stein et al., 2006; Stein and Shurson, 2009; NRC, 2012),

The fact that the ATTD of GE increased as cellulase was added to the DDGS indicates that the cellulase enzyme contribute to an increased digestibility or fermentability of cellulose in DDGS. It is believed that DDGS contains approximately 23% cellulose (Back Knudsen, 2011) and because cellulose consists only of glucose, any digestion of cellulose in the small intestine will result in increased absorption of glucose, which will contribute to an increase in the energy digestibility of the diet. Likewise, if the cellulase enzyme contributes to greater fermentability of cellulose, increased quantities of VFA will be absorbed from the hindgut of the pigs, which will also result in an increase in the energy contribution from DDGS. The current data do not allow us to establish the mechanism for the increase in energy digestibility that was observed when DDGS was treated with cellulase. However, the fact that the ATTD of GE was improved when cellulase was added to the diet indicates that either digestibility or fermentability of the cellulose was improved.

It is possible that some of the positive effects of enzymes on energy digestibility is a result of hydrolysis of the fiber that may be attached to nutrients in fibrous ingredients and thereby hinder digestion (Bedford, 1995). However, in the present experiment we did not attempt to determine digestibility or fermentability of nutrients other than ADF and NDF and we do not know if absorption of CP, fat, or glucose from starch contributed to the increase in energy digestibility that we observed as a result of treatment with the celullase enzymes.

In contrast to the cellulase treatment, inclusion of the enzyme mixture did not improve the ATTD of GE or the ME of DDGS. This indicates that it is more challenging to develop enzyme mixtures that can improve the ATTD of the arabinoxylans and other hemicelluloses in DDGS than it is to improve the ATTD of cellulose. This observation is in

agreement with previous research that has indicated that xylanase enzymes do not always improve the ATTD of GE in corn DDGS (Yáñez et al, 2011). In contrast, pigs fed a wheat-DDGS based diet that was supplemented with carbohydrase enzymes (xylanase, β-glucanase, and celullase) had a greater GE digestibility compared with diets that were not supplemented with the enzymes (Emiola et al., 2009).

Extrusion is a technology that uses high temperature and high pressure during the processing of cereal grains, which may improve energy and nutrient digestibility in cereal grains (Hancock and Behnke, 2001). Extrusion denatures protein and gelatinizes the starch in cereal grains, which may make these nutrients more digestible (Hancock and Behnke, 2001; Zijlstra et al., 2009). Extrusion increases the digestibility of energy in field peas (Stein and Bohlke, 2007), but results of the present experiment indicate that this is not the case for DDGS. Possible reasons for this observation include that DDGS has already been fermented which may prevent any further improvements from extrusion. The main reason for the improved ATTD of GE in field peas is an improved ileal digestibility of starch (Stein and Bohlke, 2007), but the concentration of starch in DDGS is much less than in field peas (Stein and Shurson, 2009; NRC, 2012), which may be the reason extrusion is not effective in improving the ATTD of GE in DDGS.

To our knowledge, no previous data have been published on the effects of treatments with sodium hydroxide or calcium oxide of DDGS, but we hypothesized that these treatments might help solubilize some of the fibers in DDGS, which in turn could have resulted in improvements in the ATTD of GE. However, results indicate that the procedures we used in the present research were ineffective in obtaining this effect. Future research will need to be conducted to determine if other chemical treatments or increased dosages of sodium hydroxide or calcium oxide may be used to improve the ATTD of GE in DDGS by solubilizing the lignin, which may encapsulate several constituents of the plant cell wall such as amorphous hemicellulose and crystalline cellulose (Mansfield, et al., 1999).

Sodium hydroxide is considered a hydrolytic agent that solubilizes the hemicellulose, lignin, and silica constituents of the plant cell wall. The solubilization is mainly due to changes in the lignin-hemicellulose matrix that take places when the cell wall is in contact with sodium hydroxide (Fahey et al., 1993). Thus, alkaline treatments breaks down the hydrogen bonds in hemicellulose, cellulose, and lignin fractions of the plant cell wall (Kahar, 2013), which results in changes in the structure of the plant cell wall, which may improve the access of microbial enzymes to the constituents of the plant (Fahey et al., 1993). This may be the reason of the improvement in the ATTD of NDF that was observed in the DDGS that was treated with sodium hydroxide. A similar observation was reported when sodium hydroxide treated

sorghum grain was fed to cows (Miron et al., 1997). However, the increased solubility of NDF that was observed in this experiment was not sufficient to improve the ATTD of GE in DDGS treated with sodium hydroxide.

Calcium oxide may also be used to solubilize fiber, and although it is a less common than sodium hydroxide and enzymes it offers an inexpensive way to treat fed ingredinets. The reduction in ATTD of NDF that was observed when DDGS was treated with calcium oxide was not expected because feedlot cattle fed diets containing Brix sugar cane treated with calcium oxide had increased digestibility of NDF compared with cattle fed untreated Brix sugar cane (Magalhaes et al., 2012b). However, there is less substrate to solubilize in DDGS compared with the concentration of fiber in Brix sugar cane (Magalhaes et al., 2012a). However, growth performance was not improved in feedlot cattle fed corn stover and modified wet distillers grains with solubles that were treated with calcium oxide compared with feedlot cattle fed untreated corn stover and modified wet distillers grains with solubles even though calcium oxide increased digestibility of DM and fiber compared with the untreated ingredients (Duckworth, 2013). Thus, additional research is needed to investigate if calcium oxide can be used to improve the feeding value of high-fiber ingredients fed to pigs.

Conclusions:

Chemical, mechanical and enzymatic treatments for DDGS were investigated for improving the fiber digestibility for swine. These were: sodium hydroxide, ammonium hydroxide, hydrochloric acid and two types of enzyme, one a cellulase/xylanase mix and the other an enzyme complex containing a wide range of carbohydrases, designed to break down hemicellulose in biomass. Of the *in vitro* treatments, the enzymatic and the NaOH treatments were selected for animal studies. Extruded DDGS and a CaO treatment were also selected for the *in vivo* study but were not tested *in vitro*.

Of the treatments selected by *in vitro* tests and investigated in this research by animal studies, only the cellulase enzyme was shown to be effective in improving the ME of DDGS. In contrast, addition of an enzyme mixture, extrusion, or chemical treatments of DDGS did not consistently improve the ATTD of the ingredient.

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Tables:

Table 1. Experimental design of the *in vitro* study

Treatment type	Pretreatment	Variables	# of treatments	Replicates	Exp. units
Control			1	6	6
Chemical	HCl	Time and concentration	4	3	12
Chemical	Sodium hydroxide	Time and concentration	4	3	12
Chemical	Ammonia	Time and concentration	4	3	12
Enzymatic	Enzyme mix	Time and concentration	4	3	12
Total	-	-	-	-	54

Table 2. Analyzed composition of corn, pretreated distillers dried grains with solubles (DDGS) and untreated DDGS, as-fed basis

Item	Corn	Pretreated DDGS					Untreated DDGS
		Extrusion	Sodium hydroxide	Calcium oxide	Cellulase	Enzyme mix	
GE, kcal/kg	3,820	4,464	4,222	4,253	4,446	4,485	4,519
DM, %	85.65	87.91	84.31	85.38	86.30	87.68	88.40
CP, %	7.68	28.86	28.52	28.07	29.86	29.93	29.31
Ash, %	1.18	5.18	9.13	8.24	5.45	5.29	5.17
OM[1], %	84.45	82.73	76.78	78.88	80.85	82.39	83.23
AEE[2], %	3.04	6.52	7.44	6.64	6.52	7.18	6.66
NDF, %	8.59	25.07	25.85	26.06	24.84	27.28	26.44
ADF, %	3.15	11.92	14.53	14.76	14.64	14.87	14.90
ADL, %	0.33	2.44	4.22	3.73	4.00	4.75	5.05
Cellulose[3], %	2.81	9.48	10.31	11.03	10.64	10.12	9.85

Hemicellulose[4], %	5.45	13.16	11.32	11.31	10.20	12.41	11.54
Indispensable, AA %							
Arg	0.39	1.44	1.31	1.43	1.45	1.46	1.43
His	0.22	0.76	0.71	0.77	0.78	0.78	0.77
Ile	0.26	1.10	1.06	1.13	1.13	1.15	1.13
Leu	0.96	3.33	3.23	3.42	3.40	3.49	3.37
Lys	0.26	0.93	0.82	0.91	0.95	0.95	0.94
Met	0.17	0.60	0.57	0.61	0.62	0.63	0.61
Phe	0.39	1.42	1.36	1.44	1.45	1.46	1.41
Thr	0.29	1.10	1.04	1.09	1.14	1.11	1.11
Trp	0.06	0.22	0.23	0.20	0.23	0.23	0.22
Val	0.36	1.49	1.42	1.52	1.54	1.55	1.50
Dispensable, AA %							
Ala	0.60	2.02	1.95	2.06	2.08	2.08	2.02

Asp	0.54	1.86	1.76	1.83	1.91	1.86	1.81
Cys	0.17	0.55	0.50	0.53	0.58	0.56	0.54
Glu	1.44	3.91	3.80	3.98	3.98	3.97	3.78
Gly	0.32	1.19	1.14	1.20	1.23	1.21	1.20
Pro	0.66	2.00	1.94	2.03	2.04	2.11	2.01
Ser	0.39	1.24	1.18	1.23	1.26	1.24	1.29
Tyr	0.22	1.02	0.99	1.02	1.02	1.07	1.06
Total AA	7.66	26.15	24.98	26.37	26.77	26.87	26.15

[1]OM was calculated as the difference between DM and ash.

[2]AEE = acid hydrolyzed ether extract.

[3]Cellulose was calculated as the difference between ADF and ADL.

[4]Hemicellulose was calculated as the difference between NDF and ADF.

Table 3. Ingredient composition of experimental diets containing corn, pretreated distillers dried grains with solubles (DDGS), and untreated DDGS, as-fed basis

	Diet

Item	Corn	Pretreated DDGS					Untreated DDGS
		Extrusion	Sodium hydroxide	Calcium oxide	Cellulase	Enzyme mix	
Ingredients, %							
Ground corn	97.00	47.95	47.95	47.95	47.95	47.95	47.95
DDGS	-	50.00	50.00	50.00	50.00	50.00	50.00
Dicalcium phosphate	1.50	-	-	-	-	-	-
Ground limestone	0.80	1.35	1.35	1.35	1.35	1.35	1.35
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition							
GE, kcal/kg	3,689	4,120	3,943	3,976	4,098	4,101	4,078
DM, %	86.26	88.40	86.25	86.23	87.35	87.63	87.66

CP, %	7.69	18.86	17.77	18.17	18.32	18.56	18.16
Ash, %	4.27	5.14	6.88	6.35	4.85	4.78	4.79
OM[2], %	81.99	83.26	79.37	79.88	82.50	82.85	82.87
NDF, %	8.12	16.43	16.57	15.94	16.55	16.74	17.15
ADF, %	2.97	8.14	8.78	9.12	9.34	9.18	9.45
ADL, %	0.71	1.94	1.86	2.34	3.46	1.88	2.92
Cellulose[3], %	2.26	6.20	6.92	6.77	5.88	7.30	6.53
Hemicellulose[4], %	5.16	8.29	7.79	6.82	7.22	7.56	7.70
P, %	0.51	0.52	0.49	0.50	0.53	0.52	0.50
Ca, %	0.88	0.73	0.83	1.33	0.63	0.61	0.75
					0.00.063	0.000.61	

[1]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10

mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[2]OM was calculated as the difference between DM and ash.

[3]Cellulose was calculated as the difference between ADF and ADL.

[4]Hemicellulose was calculated as the difference between NDF and ADF.

Table 4. Concentration of digestible and metabolizable energy in corn, untreated distillers dried grains with solubles (DDGS), and treated DDGS, as-fed basis[1]

		Pretreated DDGS							
Item	Corn	Extrusion	Sodium hydroxide	Calcium oxide	Cellulase	Enzyme mix	Untreated DDGS	SEM	*P*-value
Diet									
GE intake, kcal	8,661[c]	9,576 [a]	9,268[ab]	9,188[ab]	9,506[a]	9,411[a]	9,005[bc]	150.3	< 0.01
GE in feces, kcal	1,161[c]	1,950[ab]	1,904[ab]	2,027[a]	1,824[b]	1,910[ab]	1,896[ab]	54.83	< 0.01

GE in urine, kcal	206.0[c]	424.1[a]	330.7[b]	341.0[b]	370.0[ab]	410.0[ab]	356.2[ab]	31.42	< 0.01
DE, kcal/kg	3,193[c]	3262[ab]	3133[d]	3099[d]	3295[a]	3267[ab]	3,218[bc]	19.31	< 0.01
ME, kcal/kg	3,105[ab]	3,074[ab]	2,993[c]	2,951[c]	3,132[a]	3,089[ab]	3,057[b]	21.02	< 0.01
Ingredient									
DE, kcal/kg	3,292[bc]	3,367[abc]	3,109[d]	3,041[d]	3,432[a]	3,378[ab]	3,279[c]	34.18	< 0.01
DE, kcal/kg DM	3,844[b]	3,830[b]	3,688[c]	3,561[d]	3,977[a]	3,852[b]	3,709[c]	39.36	< 0.01
ME, kcal/kg	3,201[a]	3,078[b]	2,916[c]	2,833[d]	3,194[a]	3,108[ab]	3,043[b]	37.90	< 0.01
ME, kcal/kg DM	3,738[a]	3,501[b]	3,458[b]	3,318[c]	3,701[a]	3,545[b]	3,442[b]	43.62	< 0.01

[a-d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Data are means of 9 observations per treatment.

Table 5. Apparent total tract digestibility (ATTD) of GE, OM, NDF, ADF, cellulose and hemicellulose in corn, untreated distillers dried grains with solubles (DDGS), and treated DDGS, as-fed basis[1]

			Pretreated DDGS						
Item	Corn	Untreated DDGS	Extrusion	Sodium hydroxide	Calcium oxide	Cellulase	Enzyme mix	SEM	*P*-value
Diets									
ATTD of GE, %	86.6^{a}	78.9^{cd}	79.2^{bcd}	79.5^{bc}	77.9^{d}	80.4^{b}	79.7^{bc}	0.49	< 0.01
ATTD of OM, %	89.74^{a}	80.93^{bcd}	80.75^{cd}	81.20^{bc}	79.96^{d}	81.95^{b}	81.30^{bc}	0.42	< 0.01
ATTD of NDF, %	56.77^{ab}	53.59^{bc}	51.41^{c}	59.18^{a}	51.33^{c}	54.10^{bc}	55.28^{b}	1.23	< 0.01
ATTD of ADF, %	57.59^{c}	71.48^{a}	66.58^{b}	72.44^{a}	71.73^{a}	71.61^{a}	71.58^{a}	1.43	< 0.01
ATTD of Cellulose[2], %	59.03^{c}	70.56^{a}	64.12^{bc}	74.49^{a}	68.98^{ab}	70.57^{a}	74.51^{a}	2.48	< 0.01
ATTD of Hemicellulose[3], %	56.95^{a}	31.64^{d}	36.74^{c}	44.22^{b}	26.17^{e}	32.16^{cd}	37.35^{c}	2.04	< 0.01
Ingredients									
ATTD of GE, %	86.60^{a}	71.7^{cd}	72.8^{bcd}	73.3^{bc}	70.4^{d}	75.0^{b}	73.9^{bc}	0.86	< 0.01

ATTD of OM, %	89.74[a]	71.07[cd]	71.38[bcd]	72.24[bc]	69.49[d]	73.66[b]	72.75[bc]	0.87	< 0.01
ATTD of NDF, %	56.77[ab]	52.46[cde]	49.54[de]	59.87[a]	49.36[e]	53.11[bcd]	55.73[bc]	1.48	< 0.01
ATTD of ADF, %	57.59[c]	74.35[a]	68.71[b]	75.53[a]	74.57[a]	74.42[a]	73.64[a]	1.55	< 0.01
ATTD of Cellulose[2], %	59.03[c]	73.02[a]	65.27[bc]	77.43[a]	71.05[ab]	73.33[a]	77.33[a]	2.83	< 0.01
ATTD of Hemicellulose[3], %	56.95[a]	17.68[d]	28.75[c]	37.97[b]	9.65[e]	17.57[d]	25.30[c]	2.69	< 0.01

[a-e]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Data are means of 9 observations per treatment.

[2]Cellulose was calculated as the difference between ADF and ADL.

[3]Hemicellulose was calculated as the difference between NDF and ADF.

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Table 6: Average and Standard deviation of *in vitro* test treatments (n= 3); superscript indicates treatments statistically different from the control

	ADF		NDF		TDF		Lysine	
	%	+/-	%	+/-	%	+/-	%	+/-
Control	12.3	0.3	22.8	0.5	29.5	0.5	0.9	0.0
Cellulase Low 24 hours	9.6[a]	0.7	14.9	1.4	10.9[a]	0.5	1.2[a]	0.0
Cellulase Low 48 hours	8.9[a]	0.4	20.3	1.3	12.2[a]	1.7	1.2[a]	0.0
Cellulase High 24 hours	9.3[a]	0.4	18.5	1.3	11.5[a]	1.7	1.2[a]	0
Cellulase High 48 hours	9.6[a]	0.3	21.0	0.4	11.5[a]	0.6	1.1[a]	0.1

Enzyme Complex Low 24 hours	12.2	0.2	26.5	1.7	14.4[a]	0.9	1.1[a]	0.0
Enzyme Complex Low 48 hours	11.1	0.6	17.9	1.0	13.7[a]	0.5	1.1[a]	0.0
Enzyme Complex High 24 hours[b]	15.6	0.5	22.7	0.6	13.7[a]	0.4	1.2[a]	0.0
Enzyme Complex High 48 hours	12.9	0.5	16.8	1.3	12.5[a]	0.6	1.1[a]	0.0