Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles (DDGS) co-fermented from wheat and corn in ileal-cannulated grower pigs

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Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles (DDGS) co-fermented from wheat and corn in ileal-cannulated grower pigs[1,2]

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ABSTRACT: Nutrient digestibility in distillers dried grains with solubles (DDGS) is limited by physical constraints such as particle size and by biochemical limitations such as phytate and fiber or non-starch polysaccharides (NSP). To determine the separate effects of these limitations on nutrient digestibility, finely ground DDGS (383 μm) supplemented with phytase (0 or 250 units/kg of feed) and xylanase (0 or 4,000 units/kg of feed) was evaluated in a 2 × 2 factorial arrangement together with unground DDGS (517 μm) and an N-free diet in a 6 × 6 Latin square. Co-fermented wheat and corn DDGS contained 8.6% moisture, 31.0% CP, 1.04% Lys, 8.0% ether extract, 2.0% starch, 40% NDF, and 0.85% P (as-is basis). Diets contained 43.7% DDGS as the sole source of AA; the digesta from pigs fed the N-free diet served to subtract basal endogenous AA losses and as control for energy digestibility. Six ileal-cannulated barrows (37.1 ± 0.8 kg BW) were fed 6 diets at 2.8 x maintenance for DE in six 9-d periods. Feces and ileal digesta were collected for 2 d each. The apparent ileal digestibility (AID) of GE and apparent total tract digestibility (ATTD) of GE and NDF were 2.3, 0.5, and 5.1%-units higher ($P < 0.05$) for the ground than unground DDGS diet, respectively. Consequently, the ATTD of GE was 1.3%-units higher ($P < 0.05$) and the DE content was 0.06 Mcal/kg higher ($P < 0.05$) for ground than unground DDGS, respectively. Grinding of DDGS did not affect the ATTD of crude fiber, ADF, P, and Ca in diets. Grinding of DDGS increased ($P < 0.05$) the AID of most AA in diets including Lys, Met, and Thr by 6.9, 1.1, and 1.7%-unit, respectively. Grinding of DDGS increased ($P < 0.05$) the SID of Lys by 6.2%-unit and SID content of Lys and Thr by 0.06 and 0.02%-unit, respectively. Phytase and xylanase did not interact to affect nutrient digestibility. Phytase increased ($P < 0.001$) the ATTD of P by 10.5%-units, but did not affect AA digestibility. Xylanase did not affect nutrient digestibility. In conclusion, particle size is an important physical characteristic impacting digestibility of energy and AA, but not P in DDGS. Phytate in DDGS

limits digestibility of P, but not energy and AA. The substrate for xylanase in DDGS did not hinder energy and AA digestibility.

Key words: digestibility, distillers dried grains with solubles, phytase, pig, xylanase

INTRODUCTION

Distillers dried grain with solubles (**DDGS**) is a co-product from the ethanol industry (Stein et al., 2006). Because of increased feedstuff prices, DDGS has become an attractive feedstuff for the formulation of swine feeds (Lumpkins et al., 2004). The digestibility of energy and AA in DDGS is lower than in the feedstock (Widyaratne and Zijlstra, 2008), indicating that opportunities for improvement exist. The DDGS contains more fiber or non-starch polysaccharides (**NSP**), especially arabinoxylan, than the feedstock. The phytate-P in DDGS is partially hydrolyzed due to the fermentation process (Widyaratne and Zijlstra, 2007).

The porcine digestive tract does not secrete xylanase and phytase to hydrolyze arabinoxylan and phytate-P, respectively (Pointillart et al., 1984; Golovan et al., 2001). Dietary xylanase increased AA digestibility of wheat grain in pigs (Barrera et al., 2004). Inclusion of high-NSP wheat co-products from flour milling in swine diets decreased energy and AA digestibility that can partially be ameliorated by xylanase (Nortey et al., 2007, 2008). Dietary phytase consistently improved P digestibility in swine diets (Mroz et al., 1994). Reducing particle size of feedstuffs may increase nutrient digestibility in swine (Ivan et al., 1974; Lawrence, 1967) because the surface area to contact with enzymes in gastro-intestinal tract is increased. The impact of grinding DDGS or supplementing DDGS with phytase and xylanase on nutrient digestibility has not been reported previously.

The hypothesis was that nutrient digestibility of DDGS might be limited by physical constraints such as particle size and biochemical limitations such as phytate and NSP. The objectives were to determine: 1) nutrient digestibility in ileal-cannulated grower pigs for ground and unground DDGS, 2) effects of phytase and xylanase and their interaction on nutrient digestibility of ground DDGS, and 3) the digestible nutrient profile of DDGS originating from the co-fermentation of wheat and corn.

MATERIALS AND METHODS

The animal procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee for Livestock, and followed guidelines established by the Canadian Council on Animal Care (1993).

Experimental Diets and Design

The DDGS used for this study was produced at a commercial plant (Husky Energy, Lloydminster, Saskatchewan, Canada). The ethanol plant used residual heat from a co-located oil upgrading plant to dry the DDGS. The DDGS resulted from co-fermentation of wheat and corn in a 1:1 ratio. The DDGS was obtained unground and part of the sample was ground in a hammer mill through a 1.2-mm screen. Mean particle size and log normal standard deviation (s_{gw}) was measured in triplicate using 13 sieves (4.00, 2.26, 1.70, 1.18, 0.85, 0.60, 0.43, 0.30, 0.21, 0.15, 0.11, 0.08, and 0.05 mm) and a pan on a sieve shaker (W. S. Tyler, Mentor, OH) using method S319.3 (American Society of Agricultural Engineers, 2001).

Effects of phytase (0 or 250 units/kg of feed) and xylanase (0 or 4,000 units/kg of feed) were tested in a 2 x 2 factorial arrangement in 4 diets containing ground DDGS, together with a diet containing unground DDGS and a N-free diet, for a total of 6 mash diets in a fractional factorial

arrangement. The 6 diets were fed to 6 pigs in a 6 × 6 Latin square. The phytase was 6-phytase derived from *Escherichia coli* (EC 3.3.26) and the xylanase was endo-1,4-β-xylanase derived from *Trichoderma reseei* (EC 3.2.1.8). Enzymes were from commercial supply and specific activity was confirmed in enzyme premixes. In the prepared diets, the enzyme activity reached the expected level of 250 units of phytase/kg of feed and 4,000 units of xylanase/kg of feed; thus, the possibility of dietary enzyme activity being less than expected can be excluded. One phytase unit was defined as the quantity of phytase that liberated 1 μM of ortho-phosphate per min from 5.1 mM Na-phytate at pH 5.5 and at 37ºC. One unit of xylanase was defined as the quantity of the enzyme that liberated 1 μmol of xylose equivalent per min.

The DDGS was the sole source of CP and AA in the 5 diets containing DDGS (Table 1). Diets also contained 48% cornstarch, sucrose, canola oil, minerals, and vitamins. Most cornstarch is absorbed within the first 25% of the small intestine (Schafer and Drew, 2007), indicating that the selected dietary approach means that the dietary contribution of digesta throughout most of the small intestine was virtually solely originating from DDGS with a concentration of supplemental enzyme that was double their dietary concentration. The N-free diet contained the feedstuffs, cornstarch, canola oil, and sucrose, as unique sources of energy. Quantities for each of these feedstuffs was 57% higher than in the DDGS diets so that the N-free diet could also be used as the control diet for energy digestibility (Stein et al., 2006). Diets were fortified to meet vitamins and minerals requirements (NRC, 1998), except for being marginally limiting in P. Chromic oxide was included in the diets as the indigestible marker.

Experimental Procedures

The experiment was conducted at the Swine Research and Technology Centre at the University of Alberta (Edmonton, Alberta, Canada). Six crossbred barrows (initial BW, 37.1 ±

0.75 kg; Duroc sire × Large White/Landrace F1; Genex Hybrid; Hypor, Regina, Saskatchewan, Canada) were housed in raised individual metabolism pens that allowed freedom of movement (1.2 m wide, 1.2 m long, and 0.9 m high). Pens were equipped with a stainless-steel self-feeder attached to the front of the pen, cup drinker next to the feeder, plastic walls, and slatted flooring in a temperature-controlled room (22.0 ± 2.5ºC). During a 10-d adaptation to pens, barrows had free access to an 18%-CP pre-grower diet. Pigs were then fitted with a simple T-cannula at the distal ileum, approximately 5 cm prior to the ileocecal sphincter. The preparation of the cannulas, surgical procedure, and modifications were described previously (Sauer et al., 1983; De Lange et al., 1989). The pre-and post-operative care was described previously (Li et al., 1993). After surgery, barrows recovered for 7 d with a gradual increase in feed allowance, and were then switched to the experimental diets. Daily feed allowance was adjusted to 2.8 times the maintenance requirement for DE (3 × 110 kcal of DE/kg of $BW^{0.75}$; NRC, 1998), which was fed in 2 equal meals at 0800 and 1500 h. Each 9-d experimental period consisted of a 5-d acclimation to the experimental diets, followed by a 2-d collection of feces and a 2-d collection of ileal digesta. Pigs had free access to water throughout the experiment.

Feces were collected using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). Digesta samples were collected for 2 d using soft plastic tubes (length, 20 cm; i.d., 4 cm) from 0800 to 2000 h containing 15 mL of 5% formic acid that were attached to the opened barrel of the cannula with a rubber band. Tubes were replaced as soon as filled or after 20 min (Li et al., 1993). Collected feces and digesta were pooled for each pig within experimental period and frozen at -20ºC. Prior to analyses, feces and digesta were thawed, homogenized, sub-sampled, and freeze-dried.

Chemical Analyses

Feed and DDGS and the freeze-dried feces and digesta were ground in a centrifugal mill (model ZM 200; Retsch Co., Newtown, PA) over 1.0- and 0.5-mm sieves, respectively. Feed, DDGS, feces, and digesta were analyzed for moisture (method 930.15; AOAC, 1990), ash (method 942.05; AOAC, 1990), ADF (method 973.18; AOAC, 1990), NDF (Van Soest et al., 1991), crude fiber (method 962.09; AOAC, 1990), and ether extract (method 920.39; AOAC, 1990). Feed, DDGS, feces, and digesta were analyzed for CP (method 990.03; AOAC, 1990) and the minerals P and Ca (method 985.01; AOAC, 1990). These analyses were conducted by a commercial laboratory (Norwest Labs, Lethbridge, AB, Canada). The GE of feed, DDGS, feces, and digesta was analyzed by an adiabatic calorimeter (model AC-300, Leco Corp., St. Joseph, MI). Chromic oxide of feed, feces, and digesta was analyzed by spectrophotometry at 450 nm after ashing (Fenton and Fenton, 1979). The AA analysis was performed by HPLC (method 982.30E; AOAC, 2006) at the University of Missouri (Colombia, MO). Dietary starch in DDGS was analyzed using the amyloglucosidase/α-amylase method with a final glucose analysis using a spectrophotometer at 510 nm (method 996.11; AOAC, 1990). In DDGS, total phytate (method 986.11) and available Lys (method 975.44) were analyzed using AOAC (2006) methods. In DDGS, soluble and insoluble NSP and constituent sugars in DDGS were analyzed by gas chromatography (Englyst and Hudson, 1987) and intact phytate, i.e., inositol hexaphosphate , and lower inositol phosphates by HPLC (Newkirk and Classen 1998).

Calculations

Calculations were made using chromic oxide data. The apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of components in the diet was calculated using the following equation (Adeola, 2001):

AID or ATTD, % = 100 – [100 × (concentration of Cr_2O_3 in feed × concentration of component in feces or digesta / concentration of Cr_2O_3 in feces or digesta × concentration of component in feed)]

The basal ileal endogenous loss (**I_{end}**) of an AA or CP (g/kg of DM intake) was calculated by the equation for the N-free diet (Stein et al., 2007):

I_{end} = [AA or CP in digesta × (Cr_2O_3 in feed/Cr_2O_3 in digesta)]

Standardized ileal digestibility (**SID**) values for each indispensable AA were then calculated by correcting the AID for basal ileal endogenous losses by the equation (Stein et al., 2007):

SID = [AID + (IAA_{end}/AA in feed)]

The DE in the DDGS samples was calculated by subtracting the specific amount of DE in the N-free diet provided by the energy-providing feedstuffs from the DE in each of the DDGS containing diets using the difference method (Adeola, 2001). The content of SID CP and AA was calculated by multiplying SID measured in the digesta sample with total CP and AA content of DDGS.

Statistical Analyses

The N-free diet was solely used for calculations and was excluded from statistical analyses. The data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The main effects of phytase and xylanase and their interaction on the ground DDGS were analyzed using a model included diet as a fixed effect and period and pig as random effects. In addition, in case a significant interaction between the main effects was observed, the means of the 4 ground DDGS treatments were separated using the probability of difference. The unground control DDGS and ground control DDGS were compared using a preplanned contrast. Individual pig was considered the experimental unit. Differences were considered significant if $P < 0.05$.

RESULTS

Pigs remained healthy during the experiment. Orts were not observed; pigs consumed their daily feed allowance throughout the experiment, regardless of the diet offered.

Mean particle size was 517 μm (s_{gw} = 1.93) for unground DDGS and 383 μm (s_{gw} = 1.46) for ground DDGS. Mean particle size was 385 μm (s_{gw} = 1.95) for the unground DDGS diet and 338 μm (s_{gw} = 1.67) for the ground DDGS diet. The GE content of DDGS was 4.71 Mcal/kg (Table 2). The DDGS contained 31.0% CP, 8.0% ether extract, 39.8% NDF, and 0.85% P. In the DDGS, laboratory-defined Lys availability was 94%, arabinoxylans were 53% of the total NSP, and intact inositol hexaphosphate was 26% of phytate. The differences in analyzed chemical and AA composition among the experimental diets were small (Table 3).

The AID and ATTD of GE was 2.3 and 0.5%-units higher ($P < 0.05$; Table 4), respectively, for the ground than the unground control DDGS diet. The ATTD of GE was 1.3%-units higher ($P < 0.05$) for ground than unground DDGS, resulting in a DE content that was 0.06 Mcal/kg higher ($P < 0.05$) for ground than unground DDGS. The ATTD of NDF was 5.1%-units higher ($P < 0.05$) for the diet containing unground than ground DDGS. Grinding of DDGS did not affect the ATTD of crude fiber, NDF, or P in the diets.

Phytase and xylanase did not interact to affect nutrient digestibility of diets (Table 4). Phytase increased ($P < 0.001$) the AID and ATTD of P and did not affect digestibility of other nutrients. Xylanase did not affect nutrient digestibility.

Grinding of DDGS increased ($P < 0.001$) the AID of CP by 2.6%-units and increased ($P < 0.05$) the AID for indispensable and dispensable AA, except for Ile, Trp, Val, and Pro (Table 5). Specifically, grinding of DDGS increased ($P < 0.05$) the AID for Lys, Met, and Thr by 6.9, 1.1,

and 1.7%-units, respectively. Phytase and xylanase did not interact to affect AID of CP or AA. Phytase and xylanase supplementation did not affect the AID of CP or AA.

Grinding of DDGS increased ($P < 0.01$) the SID of CP by 2.2%-units and the SID for the indispensable and dispensable AA, except for Ile, Met, Thr, Trp, Val, Cys, Pro, and Ser (Table 6). Grinding of DDGS increased ($P < 0.01$) SID of Lys by 6.2%-units. Phytase and xylanase did not affect or interact to affect SID of CP or AA.

Grinding in DDGS increased ($P < 0.01$) the SID content of CP by 0.68%-units and increased ($P < 0.05$) the SID content for most indispensable and dispensable AA, except Ile, Met, Trp, Val, Cys, Pro, and Ser (Table 6). Specifically, grinding increased ($P < 0.05$) SID content for Lys and Thr by 0.06 and 0.02%-units, respectively. Phytase and xylanase did not affect the SID content of CP or AA.

DISCUSSION

Nutrient digestibility in DDGS is lower than in the parent grain (Widyaratne and Zijlstra, 2008). The present study reports for the first time that both physical and biochemical constraints limit nutrient digestibility of DDGS. Both of these constraints can be partially mitigated by particle size reduction and phytase supplementation.

DDGS Co-fermented from Wheat and Corn

Around the world, supply, price, and production efficiency determine the cereal grain that is selected for ethanol production for biofuel. In western Canada and Europe, wheat is used predominantly (Zijlstra and Beltranena, 2008; Cozannet et al., 2010); however, co-fermentation with corn is common for increased production efficiency and risk management for supply and price of grain. The content of ether extract and CP of the DDGS sample in the present study

confirmed that Canada Prairie Spring (**CPS**) wheat and corn were co-fermented in a 1 to 1 ratio. The values for these nutrients were intermediate to the average nutrient profile of corn DDGS (Stein and Shurson, 2009) and wheat DDGS from CPS wheat (Widyaratne et al., 2009).

The specific sample of DDGS used in the present study differed starkly from the sample of DDGS from co-fermented wheat and corn used in previous research (Nyachoti et al., 2005; Widyaratne and Zijlstra, 2007). The nutrient content among samples of DDGS varies because of type and quality of the cereal grain, method and duration of fermentation, duration and drying temperature, and the amount of solubles blended with distillers dried grains (Spiehs et al., 2002; Zijlstra and Beltranena, 2008). In particular, drying conditions and equipment differed between the 2 studies, and drying was much gentler for the DDGS in the present study than the previous study. The Lys per unit of CP is a good indicator of Lys damage during fermentation and drying (Fontaine et al., 2007), and was 3.35 in the present study vs. 1.70 in our previous study (Widyaratne and Zijlstra, 2007). The higher Lys content coincided with a high laboratory-defined Lys availability, more yellow than brown color observed, and a more acceptable odor in the DDGS sample used in the present study. Combined, the chemical and nutritional profile indicated that this DDGS sample is a source of DE and SID AA, contains large quantities of NSP, and contains partially hydrolyzed cereal phytate-P. The digestibility data from the present study indicate that 30% of the energy and therefore OM in DDGS was not digested. Thus opportunities exist to increase digestibility by removing physical constraints such as particle size and biochemical limitations such as phytate and NSP.

Grinding

Grinding grain prior to utilization is common in both the feed and ethanol industries. In ethanol production, grain is ground prior to saccharification to enhance the enzymatic conversion

of starch into glucose to maximize fermentation efficiency and ethanol yield. Following drying, the mean particle size of the unground DDGS in the present study was less than the 600 to 800 μm that is acceptable for complete feeds for pigs (Goodband et al., 2002). The mean particle size of the unground DDGS fell within the range of 434 to 949 μm that was measured previously for corn DDGS from dry grind ethanol plants (Liu, 2008). The DDGS in the present study was ground to a mean particle size of 383 μm to remove a physical limitation for complete digestibility of energy with the gastro-intestinal tract: the size of feed particles. Large particles provide per unit of mass less surface area for digestive enzymes to interact with their substrates in feed particles (Goodband et al., 2002). Larger particles thus require more time for complete digestion; however, time during digesta transit in the intestine is limited. Indeed, grinding to achieve a smaller mean particle size increased in pigs the ATTD of energy in sorghum (Cabrera et al., 1994; Healy et al., 1994), corn (Wondra et al. 1995a, b, c, d), and wheat (Mavromichalis et al., 2000). Therefore, the present study falls in line with previous research, indicating that fine grinding increased energy digestibility. Particle size of DDGS has been mostly ignored in feedstuff tables, but DDGS has a large range in particle size in commercial samples (Liu, 2008) and the data of the present study indicate that the implication of particle size on the ATTD of energy in DDGS cannot be ignored. Specifically, 50% of the commercial samples of corn DDGS had a mean particle size between 660 and 950 μm (Liu, 2008), indicating that reduction of particle size of DDGS with a high mean particle size will have more of an impact on ATTD of energy than measured in the present study. However, the benefit on nutrient digestibility should also be balanced with logistical concerns such as flowability of ground DDGS.

Grinding feedstuffs may also increase CP and AA digestibility. For example, reduced mean particle size increased CP or AA digestibility of protein sources such as soybean meal (Fastinger

and Mahan, 2003) and cereal grains such as sorghum (Owsley et al., 1981) and corn (Wondra et al., 2005a,b,c,d). In previous research, grinding of barley and field pea increased ATTD of CP, but did not reduce total N excretion, indicating that grinding did not increase digestibility of limiting AA in field pea and barley grain (Oryschak et al., 2002). Interestingly in the present study, grinding increased digestibility of the first 3 limiting AA in DDGS, indicating that reducing particle size may also reduce N excretion.

Phytase and Xylanase Supplementation

Effects of phytase and xylanase were studied specifically in ground DDGS so that the physical limitation of mean particle size would interfere less with effects of supplemental enzymes on the chemical constraints phytate and NSP. However, even though surface area was increased by fine grinding, supplementation of phytase or xylanase did not improve digestibility of energy and AA in the present study.

Phytase supplementation improved the ATTD of P in DDGS diets in the present study. The positive response to phytase for the ATTD for P contained in plant-based feedstuffs is one of the most consistent responses among feed additives in swine nutrition. The effectiveness of phytase to hydrolyze phytate-P in pigs has been well documented (e.g., Simons et al., 1990; Lei et al., 1993; Mroz et al., 1994). In wheat-based diets containing wheat millrun, supplementation of phytase increased the ATTD of P (Nortey et al., 2007), indicating that phytate limits the ATTD of P in wheat co-products. The total P content is 2 to 3 times higher in DDGS than in the parent grain stock (Widyaratne and Zijlstra, 2007). Interestingly, the baseline level of ATTD of P is much higher in DDGS than in the feedstock cereal and the phytate-P is partially hydrolyzed during production process of DDGS (Widyaratne and Zijlstra, 2007), indicating that the fermentation process itself increased P digestibility. The DDGS may therefore be an ingredient

to consider in feed formulation to reduce P excretion in pig manure. The data from the present study indicate that residual phytate P remaining in DDGS limits the ATTD of P.

The effect of phytase supplementation on digestibility of AA and energy is not consistent among studies (Adeola and Sands, 2003). Phytase increased the AID of AA in pigs fed a diet based on corn, tapioca, and soybean meal (Mroz et al., 1994) and increased the ATTD of energy in wheat-based diets (Johnston et al., 2004). Similarly, phytase increased the AID of Lys, Thr, Val, Leu, and Ile in diets containing wheat and wheat millrun (Nortey et al., 2007). In contrast, phytase did not improve the digestibility of AA or energy in the present study, similar to the lack of response in AA digestibility observed in pigs fed oat-based diets (Bruce and Sundstol, 1995), pigs fed dehulled soybean meal (Traylor et al., 2001), or pigs fed diets containing various combinations of corn, wheat, barley, field pea, soybean meal, and canola meal (Liao et al., 2005), although phytase increased the AID of Lys in the diet containing wheat, soybean meal, and canola meal. Clearly, residual phytate did not limit the digestibility of AA or energy of DDGS in the present study.

Supplementation of NSP-degrading enzymes to diets containing co-products is of considerable interest due to the increased use of high fiber co-products in swine diets (Zijlstra et al., 2010). Xylanase supplementation did not improve the digestibility of energy, AA, or P in the present study or growth performance in nursery pigs fed diets containing 30% corn DDGS (Jones et al., 2010). The results contrast the positive effects of xylanase on nutrient digestibility of wheat (Barrera et al., 2004; Diebold et al., 2004; Woyengo et al., 2008) and wheat co-products from flour milling (Yin et al., 2000; Nortey et al., 2007, 2008) that were observed in previous studies. Furthermore, supplementation of a multi-enzyme complex to diets containing wheat DDGS improved growth performance and ATTD of nutrients in finisher pigs (Emiola et al.,

2009), although the barley and corn contained in these diets might have also interacted with the multi-enzyme to provide the positive response. Similar to our data for a sample of DDGS from co-fermented wheat and corn that was not overheated, xylanase did not improve nutrient digestibility in overheated wheat DDGS (Widyaratne et al., 2009). The lack of xylanase effect on nutrient digestibility in feedstuffs that are high in xylans is puzzling and requires further investigation.

The current paradigm is that high levels of NSP in co-products such as DDGS reduce its nutritional value (Souffrant, 2001). Pigs do not secrete enzymes to hydrolyze NSP, which are normally a substrate for enzymes such as xylanase and β-glucanase. Whether such supplemental enzymes improve nutrient digestibility depends on 2 factors: 1) a match must exist between the supplemental enzyme and substrate (Bach Knudsen and Hansen, 1991), and 2) the substrate must indeed be the factor that foremost limits nutrient digestibility. Xylanase may improve nutrient digestibility through disruption or solubilization of cell wall NSP, thereby reducing or eliminating the encapsulating effects of the cell wall (Dierick and Decuypere, 1994). We know that the specific xylanase used in the present study matches with wheat arabinoxylans (Nortey et al., 2007, 2008) and that the DDGS used was co-fermented from wheat and corn and thus contained a substantial amount of wheat arabinoxylans. However, the composition or tertiary structure of the arabinoxylans might have been altered during fermentation and drying (Cyran et al., 2003) so that the supplemented xylanase could not hydrolyze the wheat arabinoxylans or the high content of wheat protein prevented xylanase activity (McLauchlan et al., 1999).

A synergy between the 2 supplemental enzymes has been suggested, i.e., xylanase breaks down the NSP structure and provides subsequently better access for phytase to hydrolyze phytate-P (Parkkonen et al., 1997). The interaction did not exist in the present study; however,

this synergy existed occasionally in previous studies with pigs. For example, the synergy was apparent in diets based on barley and field pea to improve energy digestibility in pigs (Oryschak et al., 2002), in diets containing wheat and wheat millrun to improve P digestibility in pigs (Nortey et al., 2007), and in diets based on wheat to improve ADG, AA digestibility, and apparent metabolizable energy in poultry (Selle et al., 2003; Wu et al., 2004). However, the effect of the combination of xylanase and phytase to improve ADG and P digestibility of pigs fed diets containing wheat middlings appeared mostly due to phytase (Olukosi et al., 2007). In contrast, supplemental phytase and xylanase decreased the ATTD of energy in pigs fed a wheat-based diet (Kim et al., 2005). In the present study, the lack of effect of xylanase likely prevented a synergy to occur.

Results of the present study indicate that DDGS has a potential to replace energy and protein-yielding feedstuffs for grower pigs and that physical and biochemical constraints limit nutrient digestibility of DDGS. In conclusion, phytate in DDGS limits digestibility of P, but not energy and AA. The substrate for xylanase in DDGS did not hinder energy and AA digestibility. Mean particle size is an important physical characteristic impacting digestibility of energy and AA, but not P in DDGS. Particle size reduction should be part of a feed processing package to enhance the nutritional value of DDGS.

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Table 1. Ingredient composition (as-fed basis) of the experimental diets

	DDGS[1]					
	Ground				Unground	
Ingredient, %	CON	PHY	XYL	PHY + XYL	CON	N-free
Cornstarch[2]	48.27	48.44	48.17	48.34	48.28	85.25
DDGS, ground[3]	43.70	43.70	43.70	43.70	-	-
DDGS, unground[4]	-	-	-	-	43.70	-
Phytase[5]	-	0.10	-	0.10	-	-
Xylanase[6]	-	-	0.10	0.10	-	-
Sugar	3.00	3.00	3.00	3.00	3.00	5.25
Solka floc[7]	-	-	-	-	-	3.00
Canola oil	1.00	1.00	1.00	1.00	1.00	1.75
Limestone	1.15	0.88	1.15	0.88	1.15	0.16
Mono-/dicalcium phosphate	-	-	-	-	-	1.13
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[8]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[9]	0.50	0.50	0.50	0.50	0.50	0.50
KCO_3	-	-	-	-	-	0.50
M_gO	-	-	-	-	-	0.10
Celite[10]	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide	0.37	0.37	0.37	0.37	0.37	0.37

[1]CON = control, without enzyme; XYL = xylanase; PHY = phytase; DDGS = distillers dried grains with solubles.

[2]Melojel (National Starch and Chemical Co., Bridgewater, NJ).

[3]DDGS was co-fermented from wheat and corn and was ground through a 1.2-mm hammermill screen.

[4]DDGS was not ground.

[5]Phytase was included at 100 g/1,000 kg of feed to provide 250 phytase unit/kg of feed.

[6]Xylanase was included at 167 g/1,000 kg of feed to provide 4,000 U/kg of feed.

[7]International Fiber Corp., North Tonawanda, NY.

[8]Provided the following per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantotenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin 0.2 mg; and vitamin B_{12}, 0.025 mg.

[9]Provided the following per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[10]World Minerals Inc., Santa Barbara, CA.

Table 2. Analyzed energy and nutrient composition (as-is basis) of the DDGS[1]

Item	DDGS
Moisture, %	8.6
CP, %	31.0
GE, Mcal/kg	4.71
Ether extract, %	8.0
Ash, %	4.9
Crude fiber, %	7.1
ADF, %	11.1
NDF, %	39.8
Total NSP,[2] %	26.1
Soluble	3.9
Insoluble	22.2
Starch, %	2.0
P, %	0.85
Total phytate,[3] mg/g	10.5
Ca, %	0.11
Indispensable AA, %	
Arg	1.53
His	0.79
Ile	1.31
Leu	2.76
Lys	1.04

Met	0.54
Phe	1.86
Thr	1.15
Trp	0.27
Val	1.50
Dispensable AA, %	
Ala	1.53
Asp	1.80
Cys	0.64
Glu	6.61
Gly	1.34
Pro	2.56
Ser	1.37
Tyr	1.39
Available Lys	0.98

[1]DDGS = distillers dried grains with solubles. The DDGS was co-fermented from wheat and corn.

[2]Content of individual sugars was (%; total, soluble, and insoluble, respectively): arabinose, 5.7, 0.4, and 5.3; xylose, 8.1, 0.4, and 7.6; mannose, 1.6, 0.4, and 1.2; glucose, 9.0, 1.7, and 7.3; and galactose, 1.7, 0.9, and 0.8.

[3]Content of individual inositol phosphates was (mg/g): inositol triphosphate, 0.58; inositol quadraphosphate, 1.23; inositol pentaphosphate, 1.65; and inositol hexaphosphate, 2.77.

Table 3. Analyzed energy and nutrient composition (as-fed basis) of the experimental diets

	DDGS[1]					
	Ground				Unground	
Item	CON	PHY	XYL	PHY + XYL	CON	N-free
Moisture, %	8.7	8.5	8.4	8.4	8.7	6.3
CP, %	16.22	16.18	15.29	15.40	15.20	0.64
GE, Mcal/kg	4.12	4.12	4.12	4.12	4.12	3.86
Ether extract, %	3.84	4.02	3.98	3.94	3.53	1.83
ADF, %	8.20	8.20	8.20	8.20	8.20	1.90
NDF, %	13.00	13.00	13.00	13.00	13.00	5.40
Crude fiber, %	3.20	3.20	3.20	3.20	3.20	1.30
Starch, %	41.40	41.32	40.89	41.35	41.00	74.83
Ash, %	5.55	5.28	5.39	5.29	5.53	3.51
P, %	0.39	0.39	0.38	0.40	0.40	0.21
Ca, %	0.67	0.56	0.69	0.59	0.68	0.33
Indispensable AA, %						
Arg	0.77	0.77	0.73	0.74	0.72	0.01
His	0.38	0.38	0.37	0.37	0.36	0.00
Ile	0.82	0.79	0.78	0.80	0.77	0.19
Leu	1.22	1.25	1.20	1.19	1.13	0.04
Lys	0.52	0.52	0.49	0.49	0.48	0.02
Met	0.26	0.26	0.26	0.25	0.25	0.01

Phe	0.80	0.80	0.76	0.76	0.74	0.02
Thr	0.53	0.54	0.51	0.51	0.50	0.01
Trp	0.18	0.18	0.18	0.17	0.19	0.04
Val	0.80	0.80	0.76	0.77	0.75	0.00
Dispensable AA, %						
Ala	0.70	0.72	0.69	0.68	0.65	0.02
Asp	0.90	0.91	0.87	0.86	0.84	0.02
Cys	0.32	0.33	0.30	0.30	0.29	0.02
Glu	4.39	4.30	4.04	4.12	4.15	0.15
Gly	0.73	0.72	0.68	0.68	0.68	0.01
Pro	1.52	1.52	1.40	1.43	1.45	0.00
Ser	0.70	0.71	0.66	0.66	0.66	0.02
Tyr	0.50	0.51	0.48	0.48	0.46	0.01

[1]CON = control, without enzyme; DDGS = distillers dried grains with solubles; PHY = phytase; XYL = xylanase. The DDGS was co-fermented from wheat and corn.

Table 4. Digestibility of energy, fiber, and P and the DE content of the experimental diets and DDGS supplemented with phytase and xylanase

	DDGS[1]									
	Ground				Unground	Pooled	*P*-values[2]			
Item	CON	PHY	XYL	PHY + XYL	CON	SEM	PHY	XYL	PHY × XYL	Grinding
AID of diet, %										
GE	76.4	76.0	76.5	76.7	74.1	0.2	0.935	0.300	0.412	< 0.001
P	48.9	55.7	47.6	54.5	52.2	1.3	0.020	0.663	0.971	0.418
Ca	49.4	55.9	56.3	61.3	60.4	1.6	0.101	0.082	0.827	0.031
ATTD of diet, %										
GE	84.9	84.6	84.8	85.1	84.4	0.1	0.892	0.328	0.059	0.034
DE, Mcal/kg (as fed)	3.50	3.48	3.49	3.50	3.48	0.01	0.728	0.376	0.054	0.027
P	50.8	57.5	45.6	59.8	54.4	1.4	< 0.001	0.571	0.163	0.350
Ca	49.2	52.1	49.3	59.8	60.5	1.9	0.119	0.359	0.379	0.069
Crude fiber	24.3	24.6	25.7	24.1	23.7	0.5	0.592	0.704	0.472	0.750
ADF	57.2	56.1	57.9	58.2	55.4	0.5	0.779	0.235	0.557	0.306

NDF	45.3	43.6	41.3	45.4	40.2	0.7	0.391	0.439	0.051	0.016
ATTD of DDGS, %										
GE	70.9	70.1	70.5	71.3	69.6	0.2	0.892	0.329	0.058	0.033
DE, Mcal/kg (as-fed)	3.34	3.30	3.32	3.36	3.28	0.01	0.877	0.380	0.061	0.033

[1]AID = apparent ileal digestibility; ATTD = apparent total tract digestibility; CON = control, without enzyme; DDGS = distillers dried grains with solubles; PHY = phytase; XYL = xylanase. The DDGS was co-fermented from wheat and corn. Least-squares means are based on 6 pig observations per diet.

[2]*P*-values for main factors PHY and XYL and their interaction. A pre-planned contrast compared CON ground vs. CON unground DDGS.

Table 5. Apparent ileal digestibility of CP and AA in diets containing DDGS

	DDGS[1]									
	Ground				Unground	Pooled	*P*-values[2]			
Item, %	CON	PHY	XYL	PHY + XYL	CON	SEM	PHY	XYL	PHY × XYL	Grinding
CP	80.8	80.5	80.3	80.6	78.2	0.3	0.939	0.661	0.556	0.001
Indispensable AA										
Arg	83.4	83.3	83.1	83.6	80.5	0.3	0.750	0.994	0.622	< 0.001
His	80.3	79.7	80.2	80.2	78.3	0.2	0.479	0.625	0.491	0.003
Ile	83.2	82.4	82.9	83.3	82.1	0.2	0.595	0.458	0.150	0.061
Leu	84.2	84.2	84.2	84.3	82.5	0.2	0.885	0.866	0.948	0.003
Lys	62.2	62.1	60.1	60.6	55.3	0.7	0.867	0.169	0.813	< 0.001
Met	83.2	83.4	83.5	82.6	82.1	0.2	0.275	0.504	0.142	0.041
Phe	86.4	86.1	85.9	86.1	84.7	0.2	0.680	0.456	0.396	< 0.001
Thr	76.8	77.2	75.9	76.7	75.1	0.3	0.717	0.055	0.690	0.012
Trp	85.3	85.3	85.8	84.8	86.1	0.2	0.251	0.995	0.204	0.170
Val	79.5	78.9	78.6	78.9	77.9	0.2	0.806	0.457	0.390	0.058

Dispensable AA										
Ala	74.2	74.7	74.5	74.5	71.4	0.3	0.610	0.859	0.682	0.001
Asp	62.5	62.4	62.1	61.6	58.4	0.4	0.721	0.374	0.835	< 0.001
Cys	77.1	77.2	76.6	76.4	75.3	0.3	0.891	0.281	0.754	0.028
Glu	89.9	89.7	89.6	89.8	89.1	0.1	0.998	0.624	0.269	0.016
Gly	68.6	67.4	67.4	68.3	62.8	0.6	0.872	0.893	0.395	0.002
Pro	78.7	78.9	78.8	80.1	71.1	1.5	0.818	0.847	0.863	0.112
Ser	76.9	77.2	75.9	76.7	75.1	0.2	0.294	0.216	0.631	0.025
Tyr	84.4	84.3	83.6	83.7	82.4	0.2	0.887	0.067	0.826	< 0.001

[1]CON = control, without enzyme; DDGS = distillers dried grains with solubles; PHY = phytase; XYL = xylanase. The DDGS was co-fermented from wheat and corn. Least-squares means are based on 6 pig observations per diet.

[2]*P*-values for main factors PHY and XYL and their interaction. A pre-planned contrast compared CON ground vs. CON unground DDGS.

Table 6. Standardized ileal digestibility of CP and AA in DDGS[1]

	DDGS									
	Ground				Unground	Pooled	*P*-values[2]			
Item, %	CON	PHY	XYL	PHY + XYL	CON	SEM	PHY	XYL	PHY × XYL	Grinding
CP	87.2	86.9	87.1	87.4	85.0	0.3	0.965	0.803	0.596	0.004
Indispensable AA										
Arg	89.8	89.7	89.9	90.3	87.3	0.3	0.820	0.540	0.700	0.004
His	84.8	84.2	84.9	84.8	83.1	0.2	0.476	0.416	0.502	0.010
Ile	87.7	87.1	87.6	87.9	86.9	0.2	0.593	0.351	0.264	0.143
Leu	88.1	88.0	88.1	88.3	86.7	0.2	0.954	0.626	0.809	0.013
Lys	70.5	70.4	68.9	69.5	64.3	0.6	0.867	0.348	0.821	0.002
Met	86.1	86.3	86.4	85.6	85.1	0.2	0.353	0.638	0.188	0.066
Phe	89.9	89.5	89.6	89.8	88.5	0.2	0.685	0.925	0.402	0.004
Thr	80.0	79.8	78.9	79.4	78.1	0.3	0.826	0.248	0.604	0.052
Trp	91.2	91.2	91.7	91.1	92.0	0.2	0.490	0.610	0.404	0.176
Val	84.3	83.7	83.7	84.0	83.1	0.2	0.753	0.781	0.432	0.135

Dispensable AA										
Ala	81.1	81.5	81.5	81.7	78.8	0.3	0.676	0.614	0.887	0.006
Asp	70.1	69.8	69.9	69.6	66.5	0.3	0.728	0.758	0.933	0.001
Cys	82.2	82.1	82.0	81.7	80.8	0.2	0.773	0.570	0.861	0.078
Glu	91.9	91.7	91.7	91.9	91.2	0.1	0.993	0.941	0.359	0.037
Gly	85.2	84.2	85.1	85.9	80.6	0.6	0.952	0.514	0.464	0.014
Pro	98.0	98.2	99.8	99.9	91.3	0.5	0.875	0.522	0.928	0.164
Ser	83.3	83.5	82.7	83.5	81.8	0.2	0.336	0.594	0.583	0.061
Tyr	88.6	88.5	88.1	88.2	87.1	0.2	0.986	0.243	0.742	0.005

[1]CON = control, without enzyme; DDGS = distillers dried grains with solubles; PHY = phytase; XYL = xylanase. Least-squares means are based on 6 pig observations per diet. The DDGS was co-fermented from wheat and corn.

[2]*P*-values for main factors PHY and XYL and their interaction. A pre-planned contrast compared CON ground vs. CON unground DDGS.

Table 7. Content of standardized ileal digestible CP and AA in DDGS[1]

	DDGS									
	Ground				Unground	Pooled	*P*-values[2]			
Item, % of DM	CON	PHY	XYL	PHY + XYL	CON	SEM	PHY	XYL	PHY × XYL	Grinding
CP	27.00	26.92	26.95	27.04	26.32	0.08	0.974	0.809	0.594	0.004
Indispensable AA										
Arg	1.37	1.37	1.38	1.38	1.33	0.01	0.894	0.528	0.702	0.003
His	0.67	0.67	0.67	0.67	0.66	0.01	0.545	0.782	0.782	0.014
Ile	1.15	1.14	1.15	1.15	1.14	0.01	0.547	0.203	0.203	0.139
Leu	2.43	2.43	2.43	2.43	2.39	0.01	0.889	0.717	0.889	0.015
Lys	0.73	0.73	0.72	0.72	0.67	0.01	0.894	0.398	0.830	0.002
Met	0.46[ab]	0.46[ab]	0.47[a]	0.46[b]	0.46	0.01	0.163	0.777	0.014	0.110
Phe	1.67	1.66	1.67	1.67	1.65	0.01	0.599	1.000	0.383	0.004
Thr	0.92	0.92	0.91	0.91	0.90	0.01	0.926	0.220	0.592	0.033
Trp	0.25	0.25	0.25	0.25	0.25	0.01	0.390	0.555	0.390	0.345
Val	1.26	1.25	1.25	1.26	1.25	0.01	0.721	0.721	0.451	0.145

Dispensable AA										
Ala	1.24	1.25	1.25	1.25	1.21	0.01	0.687	0.592	0.893	0.005
Asp	1.26	1.26	1.26	1.25	1.20	0.01	0.691	0.691	0.897	< 0.001
Cys	0.53	0.53	0.52	0.52	0.52	0.01	0.686	0.336	0.686	0.072
Glu	6.07	6.06	6.06	6.08	6.03	0.01	0.904	0.926	0.348	0.043
Gly	1.14	1.13	1.14	1.15	1.08	0.01	0.996	0.504	0.504	0.018
Pro	2.51	2.52	2.56	2.58	2.34	0.04	0.860	0.525	0.933	0.162
Ser	1.14	1.14	1.13	1.14	1.12	0.01	0.287	0.474	0.634	0.069
Tyr	1.23	1.23	1.22	1.22	1.21	0.01	0.867	0.168	0.807	0.010

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CON = control, without enzyme; DDGS = distillers dried grains with solubles; PHY = phytase; XYL = xylanase. The DDGS was co-fermented from wheat and corn. Least-squares means are based on 6 pig observations per diet.

[2]*P*-values for main factors PHY and XYL and their interaction. A pre-planned contrast compared CON ground vs. CON unground DDGS.