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Rapid Communication

RAPID COMMUNICATION

Xylanase, and the role of digestibility and hindgut fermentation in pigs on energetic differences among high and low energy corn samples[1]

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Abstract

The experimental objective was to evaluate the digestibility and fermentation differences between high and low energy corn samples and their response to xylanase supplementation. Four corn samples, 2 with higher DE content (**HE-1** and **HE-2**; 3.74 and 3.75 Mcal DE/kg DM, respectively) and 2 with a lower DE content (**LE-1** and **LE-2**; 3.63 and 3.56 Mcal DE/kg DM, respectively) were selected based upon a previous digestibility trial. Sixteen individually housed barrows (PIC 359 × C29; initial BW = 34.8 ± 0.23kg) were surgically fitted with an ileal T-cannula and randomly allotted to treatments in an 8 × 4 Youden square design. Dietary treatments were arranged in a 4 × 2 factorial: HE-1, HE-2, LE-1, and LE-2, with and without xylanase supplementation. Diets were formulated using one of the 4 corn samples, casein, vitamins, minerals, and 0.4% chromic oxide as an indigestible marker. Feed intake was established at approximately 3 times the estimated energy required for maintenance (NRC 2012) based upon the average initial BW of the pigs at the start of each collection period, which consisted of 9 d adaptation, 2 d of fecal, and 3 d of ileal collections. Diets, ileal, and fecal samples were analyzed for DM, GE, and total dietary fiber (**TDF**), to determine apparent total tract **(ATTD**), hindgut fermentation (**HF**), apparent ileal digestibility (**AID**) coefficients. A diet × enzyme interaction was not observed for any of the measured variables ($P > 0.10$). The HE-1 and HE-2 diets had greater ATTD of GE, and HE-2 diet had greater ATTD of DM ($P < 0.001$ and $P = 0.007$, respectively). Xylanase, independent of diet, improved the ATTD of GE and DM (84.8 vs. 83.6% for GE with and without enzyme, respectively, $P = 0.008$; and 84.2 and 83.0% with and without enzyme, respectively, $P = 0.007$). The energetic differences among these corn samples appeared to be driven by fermentability in the hindgut. Supplementing xylanase improves digestibility irrespective of the digestibility energy content of corn.

Key words: apparent ileal digestibility, carbohydrase, dietary fiber, digestibility, swine, total dietary fiber

Introduction

Corn remains the leading carbohydrate source in swine diets in many parts of the world. It has greater energy density compared to other grains, and is relatively uniform in nutrient composition, with the exception to DE; the standard deviation for DE is estimated to be 111 kcal/kg (NRC, 2012), or about 3% of the mean. The energy provided by corn comes primarily from starch, with minor contributions from protein and fat. The portion of energy

coming from nonstarch polysaccharides is very minimal (**NSP**; Newman et al., 2016). Despite the small energy yield from NSPs, the concentration of xylose, a monosaccharide representing about 3% of the grain, can explain about 70% of the variation in energy content among diverse corn coproducts (Gutierrez et al., 2014).

Dietary energy concentration is a significant determinant of pig growth performance (Beaulieu et al., 2009), and is the costliest dietary constituent (Patience, 2017). Even a small variation in the concentration or composition of energy within corn can be problematic, and technologies that can mitigate this variation have value within the pork industry. Pigs cannot digest the NSPs in corn as they lack the proper digestive enzymes (Gutierrez et al., 2013), but NSPs and associated nutrients can be digested via microbial fermentation (Varel and Yen, 1997), and possibly through the addition of exogenous carbohydrases (Bedford, 2000).

Supplementation of exogenous carbohydrases has been shown to aid in the degradation of corn NSP (Ndou et al., 2015); however, the exact mechanism has not been elucidated (Adeola and Cowieson, 2011). If carbohydrases can assist in hydrolyzing NSPs, and if corn varies in fiber content, it can be reasonably hypothesized that the response to exogenous xylanase would be greater in a lower quality (i.e., high fiber), as compared to higher quality (i.e., low fiber) corn source. Therefore, the objectives of this experiment were to evaluate the differences in digestibility and hindgut fermentation between high and low digestible energy corn samples and to determine if the efficacy of xylanase is greater in lower energy, as compared to higher energy, corn.

Materials and Methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-13-7511-S).

Animals, Housing, and Experimental Design

Sixteen barrows (PIC 337 × C22/C29; initial BW = 34.8 ± 0.2 kg) were surgically fitted with a T-cannula at the terminal ileum as described by Stein et al. (1998). Pigs recovered for 10 d in individual pens (1.8 × 1.9 m), equipped with a partially slatted concrete floor, a stainless-steel dry feeder, and a nipple drinker, where they had ad libitum access to water and consumed a commercial grower diet to appetite. Following recovery, pigs were randomly allotted to a Youden square design, with 8 dietary treatments in 4 replicate periods each lasting 14 d, providing a total of 8 observations per dietary treatment. Each replicate period consisted of 9 d of dietary adaptation, 2 d of fecal collections, and 3 d of ileal collections. Pigs were weighed and randomly allotted to treatments prior to each replicate period, and no pig was allotted to the same diet across the replicate periods.

Diets and Feeding

Eight corn samples were selected based on their DE values from a previous trial (Newman et al., 2016). Pairs of corn samples with similar DE values were pooled, resulting in the 4 corn samples used in this experiment: 2 dietary treatments with higher DE content (**HE-1** and **HE-2**; 3.74 and 3.75 Mcal DE/kg, respectively) and 2 with a lower DE content (**LE-1** and **LE-2**; 3.63 and 3.56 Mcal DE/kg, respectively). Casein was included as a source of amino acids but not carbohydrate to avoid the negative effects of pigs receiving a protein-deficient diet for an extended period of time. The 4 corn-based diets were arranged in a 4 × 2 factorial arrangement, with the effect of diet (HE-1, HE-2, LE-1, and LE-2) versus enzyme (with or without) as the factors. Xylanase (Econase XT 25P, AB Vista, Marlborough, England) was added at 100 g/tonne providing 16,000 birch xylan units (**BXU**) per kg.

Pigs were fed 3.0 times the estimated energy required for maintenance (NRC, 2012), based upon the average BW at the beginning of each replicate, and were divided into equal rations fed at 0800 and 1600 h. Experimental diets consisted of 1 of 4 corn samples, casein, vitamins and minerals, and chromic oxide (0.4%) as an indigestible marker (Table 1). Diets met or exceeded nutrient requirements for 30 kg pigs (NRC, 2012), and were mixed thoroughly to achieve a uniform final product. Corn was the only source of carbohydrates in each diet.

Sample Collection and Storage

Pens were cleaned of feces on day 9, and fresh fecal samples were collected on day 10 and 11 via grab sampling. Ileal samples were collected for 8 h each day on day 12, 13, and 14 by attaching a 207-mL plastic bag (Whirl-Pak, Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed when filled with digesta or at least every 30 min. All fecal and ileal

Table 1. Ingredient and chemical composition of experimental diets[1]

Item	HE-1	HE-2	LE-1	LE-2
Diet formulation, % as-fed				
Ground corn[2]	83.76	83.76	83.76	83.76
Sodium caseinate	12.70	12.70	12.70	12.70
Monocalcium phosphate	1.30	1.30	1.30	1.30
Limestone	1.20	1.20	1.20	1.20
Salt	0.40	0.40	0.40	0.40
Vitamin premix[3]	0.14	0.14	0.14	0.14
Trace mineral premix[4]	0.10	0.10	0.10	0.10
Chromic oxide	0.40	0.40	0.40	0.40
Nutrients, analyzed, % DM basis				
DE	3.67	3.66	3.57	3.58
ADF	1.80	1.43	1.94	2.18
NDF	6.64	6.42	8.13	8.43
Total dietary fiber	7.20	6.98	8.45	8.79
Nutrients, calculated, % as-fed				
SID[5] Lysine	1.00	1.00	1.00	1.00
SID Methionine	0.44	0.44	0.44	0.44
SID TSAA	0.62	0.62	0.62	0.62
SID Threonine	0.63	0.63	0.63	0.63
SID Tryptophan	0.20	0.20	0.20	0.20
Calcium	0.69	0.69	0.69	0.69
STTD P[6]	0.41	0.41	0.41	0.41
Total P	0.58	0.58	0.58	0.58

[1]Econase XT P was added, at the expense of corn, to 4 of the 8 diets at 100 g/tonne.
[2]Four different samples of corn were used, 2 with higher and 2 with lower DE according to previous data.
[3]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D, 827 IU; vitamin E, 26 IU; vitamin K, 2.6 mg; niacin, 29.8 mg; pantothenic acid, 16.5 mg; riboflavin, 5.0 mg; vitamin B12, 0.023 mg.
[4]Provided per kg of diet: Zn, 165 mg as zinc sulfate; Fe, 165 mg as iron sulfate; Mn, 39 mg as manganese sulfate; Cu, 17 mg as copper sulfate; I, 0.3 mg as calcium iodate; and Se, 0.3 mg as sodium selenite.
[5]SID = Standardized ileal digestible.
[6]STTD P = Standardized total tract digestible phosphorus.

samples were immediately stored at –20 °C for later processing and analysis.

Analytical Methods

Ileal and fecal samples were thawed at room temperature, mixed thoroughly within animal and period, subsampled, and stored at –20 °C for later analysis. Digesta samples were lyophilized (Model 10–100, Virtis Co. Ltd., Gardiner, NY). Diet and fecal samples were dried at 65 °C to a constant weight (Yamato Mechanical Convection Oven DKN810). All digesta, fecal, and diet samples were ground to a particle size of 1.0 mm (Wiley Mill 3379-K35, Thomas Scientific, Swedesboro, NJ) before analysis.

Diets were analyzed in triplicate for NDF using the method of Van Soest and Robertson (1979), and ADF according to Goering and Van Soest (1970). Digesta, diet, and fecal samples were analyzed in duplicate for DM (method 930.15), CrO_3, and GE, and in triplicate for total dietary fiber (**TDF**). Chromic oxide was determined using the method of Fenton and Fenton (1979); absorption was measured at 440 nm using a spectrophotometer (Synergy 4, BioTek, Winooski, VT). Chromic oxide standard samples were assayed to confirm the accuracy of the analytical procedure, and recovery of 100.9 ± 1.7% was attained. Gross energy was determined using a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6,318 kcal/kg; Parr Instrument Co.) was used as the standard for calibration and was determined to contain 6,323 ± 0.65 kcal/kg.

Total dietary fiber was analyzed according to the procedures of a Total Dietary Fiber kit from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland, Megazyme K-RSTCL; Method 985.29; AOAC Int. 2007).

Calculations and Statistical Analysis

The AID and ATTD of dietary components were calculated using equations described by Oresanya et al. (2007). Hindgut fermentation was calculated by difference:

$$\text{Hindgut fermentation, \%.} = \text{ATTD, \%} - \text{AID, \%}$$

The data were analyzed according to the following mixed model:

$$Y_{ijklm} = \mu + \tau_i + \nu_j + \tau_i\nu_j + \rho_k + a_l + e_{ijklm}$$

Where $\boldsymbol{Y_{ijklm}}$ is the observed value for mth experimental unit within the ith level of diet and jth level of enzyme of the lth pig in the kth replicate; μ is the general mean; $\boldsymbol{\tau_i}$ is the fixed effect of the ith diet (i = 1 to 8); $\boldsymbol{\upsilon_j}$ is the fixed effect of the jth enzyme (j = 1 to 2); $\boldsymbol{\tau_i\,\upsilon_j}$ is the interaction term of diet × enzyme; $\boldsymbol{\rho_k}$is the random effect of the kth pig (k = 1 to 16); $\boldsymbol{a_l}$ is the random effect of the lth period (l = 1 to 4); and $\boldsymbol{e_{ijklm}}$ is the associated variance as described by the model for Y_{ijklm} (m = 1 through 8); assuming $\rho_k \sim N\left(0,\ I\sigma_\rho^2\right)$, $a_l \sim N(0,\ \boldsymbol{I}\sigma_a^2)$, and $e_{ijklm} \sim N(0,\ \boldsymbol{I}\sigma_e^2)$, where I is the identity matrix.

The PROC UNIVARIATE procedure in SAS 9.3 (SAS Inst., Cary, NC) was used to verify normality and homogeneity of the residual variance from the reported model. The model was analyzed using PROC MIXED. Least squares means were separated using Fisher's Least Significant Difference test, and treatment differences were considered significant if $P < 0.05$ and trends if $0.05 < P \leq 0.10$.

Results and Discussion

Pigs were successfully cannulated at the distal ileum, recovered, and remained healthy throughout the experiment. The mean initial BW of periods 1, 2, 3, and 4 was 34.8 ± 0.2, 43.8 ± 0.4, 54.5 ± 0.5, and 66.5 ± 0.5 kg, respectively.

Corn is considered a relatively uniform grain, but the dietary fiber content may vary by up to 70% (NRC, 2012; Newman et al., 2016). Corn sources in this study were selected to have differing levels of DE. The HE diets had approximately 150 kcals more DE per kg than the LE diets (Table 1). Diets with less DE, ME, and NE, all other dietary constituents being equal, have a higher fiber content (Bach Knudsen, 1997; Noblet and van Milgen, 2004). Consequently, the energy densities among these diets were reflective of the dietary fiber content: HE samples had an average of 6.5% NDF and 7.1% TDF, while the LE samples averaged 8.3% NDF and 8.6% TDF, on a DM basis (Table 1).

Even though the corn sources differed in energy and fiber content, there was no effect of diet on the AID of GE and DM ($P = 0.744$ and $P = 0.686$, respectively; Table 2). The HE-1 and HE-2 diets had greater ATTD of GE, and HE-2 diet had greater ATTD of DM ($P < 0.001$ and $P = 0.007$, respectively; Table 2), but diets did not differ in ATTD of TDF ($P > 0.05$; Table 2). The products of hindgut fermentation in pigs fed the HE-2 diet may have been

Table 2. Comparison of apparent ileal (AID) and total tract digestibility (ATTD), and hindgut fermentation (HF) in 2 higher energy (HE) and 2 lower energy (LE) corn samples with and without xylanase[1]

	Diet				Enzyme			P-value[2]	
Item, %	HE-1	HE-2	LE-1	LE-2	+	-	Pooled SEM	Diet	Enzyme
AID									
GE	79.1	80.2	80.2	79.7	80.2	79.4	0.95	0.744	0.311
DM	77.5	78.2	78.9	78.5	78.5	78.0	0.92	0.686	0.522
TDF	22.0	23.6	27.3	23.8	25.8	22.6	1.40	0.100	0.043
HF[3]									
GE	5.3	5.5	3.0	3.9	4.6	4.2	1.43	0.092	0.737
DM	6.0	6.6	3.9	4.8	5.7	5.0	1.63	0.113	0.678
TDF	10.4	10.3	7.0	6.7	8.8	8.4	1.45	0.059	0.946
ATTD									
GE	84.4[b]	85.7[b]	83.2[a]	83.6[a]	84.8	83.6	0.61	<0.001	0.008
DM	83.5[a]	84.8[b]	82.8[a]	83.3[a]	84.2	83.0	0.63	0.007	0.011
TDF	32.4	33.9	34.3	30.5	34.6	31.0	1.58	0.400	0.048

[1]Econase XT 25P was added at 100 g/tonne.
[2]No interactions between diet and enzyme were observed, $P > 0.10$.
[3]HF = Hindgut fermentation = (total tract digestibility – apparent ileal digestibility).

more efficiently retained by the animal or microbiome, resulting in a greater ATTD of GE, but not ATTD of DM.

When averaged, the HE diets have 27% less NDF and 22% less TDF, yet the release of GE and TDF in the hindgut tended to be approximately 55 and 50% greater (respectively) in HE diets, as compared to LE ($P < 0.10$). These data suggest that HE samples were fermented to a greater extent than the LE samples; this is an agreement with Jones et al. (2015), where a corn source subjected to drought conditions had lower ATTD of crude fiber. It has been shown that when dietary fiber is increased, there is more microbial hindgut fermentation due to the increased substrate (Urriola and Stein, 2010; Abelilla and Stein, 2019), yet the data herein showed the inverse. The concentration, physiochemical properties, and composition of fiber can alter digestion and fermentation (Wenk, 2001; Anguita et al., 2006; Jha and Berrocoso, 2016); as such, the dietary fiber or energy level alone may not be sufficient to explain the influence of corn source on fermentation. This improved HF observed with the HE diets may be attributed to the lower amount of insoluble dietary fiber, which is poorly fermented and is composed of polysaccharides that are partially resistant to microbial degradation (Bach Knudsen, 1997; Gutierrez et al., 2014). It is possible the digesta entering the hindgut from pigs fed the HE diets may have been richer in another dietary constituent resulting in the greater hindgut disappearance of GE (Table 2). Likewise, the increased NDF in the LE diets could have reduced retention time in the large intestine resulting in reduced microbial fermentation (Bindelle et al., 2008). It has been shown that increasing insoluble corn fiber linearly decreases GE and TDF digestibility (Acosta and Patience, 2018).

Xylanase is increasingly supplemented in poultry and swine diets to attenuate the antinutritive factors of fiber, improve performance, and increase energy and nutrient digestibility by hydrolyzing the β 1–4 arabinoxylan glyosidic bonds of arabinoxylans (Kiarie et al., 2016). The efficacy of xylanase in pig studies, unlike poultry studies, has been inconsistent in improving nutrient and energy digestibility, particularly in corn-based diets (Jones et al., 2015; Weiland, 2017; Abelilla and Stein, 2019). Enzyme supplementation has been shown to reduce variation in ingredient quality (Bedford, 2000), and is effective when supplemented in poor quality cereal grains, at least in poultry (Choct et al., 1995; Bedford et al., 1998). However, a diet by enzyme interaction was not observed in this study. Thus, the effect of xylanase reported herein was independent of the corn source.

One intention of xylanase supplementation is the hydrolysis of dietary arabinoxylans in the small intestine to promote intestinal absorption of monosaccharides, rather than microbial fermentation, and consequently increase the quantity of metabolically useful energy available from the diet (Wilfart et al., 2007; Conzannet et al., 2012; Bach Knudsen et al., 2013). At the ileal level, xylanase increased TDF digestibility before the terminal ileum by approximately 15% ($P < 0.05$, Table 2), but did not improve the AID of DM or GE ($P > 0.05$, Table 2). Xylanase supplementation has been reported to improve the AID of GE and DM in young pigs fed corn-based diets (Myers and Patience, 2014; Passo et al., 2015), improve the AID of energy and fiber in diets containing more viscous cereal grains (Nortey et al., 2007; Widyaratne et al., 2009), and improve digestibility in diets with greater content of insoluble corn fiber (Yáñez et al., 2011; Ndou et al., 2015). In this study, the inefficacy of xylanase in the ileum to improve GE or DM digestibility could be contributed to the concentration and type of fiber, length of supplementation, or minimal absorption of pentose sugars in the small intestine.

Xylanase increased the ATTD of GE, DM, and TDF (83.6 vs. 84.8% for GE, $P < 0.001$; 83.0 vs. 84.2% for DM, $P = 0.007$; 34.6% vs. 31.0% for TDF, $P = 0.048$; Table 2). The use of carbohydrases in diets formulated with corn or corn coproducts has been shown to improve energy or nutrient digestibility at the total tract level (Myers et al., 2014; Ndou et al., 2015), but contrasting data has also been reported (Willamil et al., 2012; Moran et al., 2016). Xylanase did not improve hindgut fermentation of GE, DM, or TDF ($P > 0.05$). The effect of xylanase in this study was presumably additive and only identifiable across the total tract. The data reported herein suggest xylanase promoted additional energy and nutrient release in the large intestine by providing a more favorable substrate to the microbiome as a result of altering the fiber structure in the small intestine. This is supported by the increased AID of TDF followed by improvements in ATTD of GE, DM, and TDF, and supports one proposed mode of action for xylanase (Partridge and Bedford, 2001).

In conclusion, the difference in energy content between the HE and LE corn samples appeared to be related to differences in fermentation in the lower gut, rather than to digestibility in the upper gut, a truly unexpected finding. The hypothesis that xylanase would have greater efficacy in diets containing lower energy corn sources was not supported. However, xylanase was effective in improving the ATTD of GE, DM, and TDF regardless of corn source. In totality, these data suggest that differences observed in the energetic value of corn with varying quality and nutrient composition are driven by their ability to be fermented, while the magnitude of the benefits of supplementing xylanase in corn-based diets is not impacted by their energy density.

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