Gut Physiology of Pigs Fed Carbohydrate Degrading Enzymes

National Pork Board Webinar Series Summer 2017


Department of Animal Science
Executive summary

a. Goal of NPB: increase nutrient extraction in low energy feedstuffs
b. Dietary fiber is a complex mix of multiple components that differ among cereal grains
c. Current methods poorly measure the impact of fiber on animal performance
d. Enzymes may increase nutrient extraction, but there are multiple products and multiple cereal co-products
e. Principal findings on the project:
   a. *In vitro* (very small differences among 11 sources of enzymes):
      a. Wheat middlings enzymes ↑ *in vitro* AID of GE, but ↓ fermentability
      b. Corn DDGS with carbohydrases: negligible effects
   b. *In vivo*:
      a. Increase growth and feed intake of pigs fed wheat middlings; no corn DDGS
      b. Still observed differences in digesta viscosity and cytokine profile
   c. Extra-caloric effects of enzymes or oligosaccharides
   d. Future research: chemometric and microbiome (under analysis)

Challenges of feeding grain by-products

• High in fiber content - NDF > 18.7% (Sauvant et al., 2004)
• Variability of NE and nutrient content among sources
• Reduce crude protein and ether extract digestibility (Dégen et al., 2007)
• Increase size and weight of the gastrointestinal tract (Kass et al., 1988)
  – Reduce carcass yield
Alternative feed ingredients have high content of poorly digestible nutrients

- Corn
- DDGS

<table>
<thead>
<tr>
<th>Component</th>
<th>Water</th>
<th>Protein</th>
<th>Fat</th>
<th>Sugars</th>
<th>Starch</th>
<th>Fiber</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
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<tr>
<td>DDGS</td>
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</table>
Analytical vs. physiological characteristics of dietary fiber

Classification based on location of carbohydrates with the plant cell:

- Cell contents
- Cell Wall

Classification based on nutritional and physiological definitions:

- Organic acids
- Sugars
- Starch
- Oligo
- Polyfructan
- B-glucans
- Pectins & gums
- Hemicellulose
- Cellulose
- Lignin Phenol

Classifications of carbohydrates based on analytical methods:

- Non-fiber carbohydrates/neutral detergent soluble carbohydrates
- Neutral detergent fiber (NDF)
- Acid detergent fiber (ADF)
- Crude Fiber
- Non-starch polysaccharides
- Total dietary fiber (TDF)
- Soluble dietary fiber (SDF)
- Insoluble dietary fiber (IDF)

NRC (2007)
What are enzymes, how do they work?

Enzymes modify activation energy to accelerate chemical reactions.

Key-lock specific activity of enzymes.

- Reactant
- Reaction without enzyme
- Activation energy without enzyme
- Activation energy with enzyme
- Reaction with enzyme
- Initial energy level
- Final energy level
- Products

Substrate entering active site of enzyme
Enzyme changes shape slightly as substrate binds
Products leaving active site of enzyme
Xylanases are a group of enzymes that degrade hemicellulose.

GH = glycoside hydrolase

McKee et al. (2012)
Three step *in vitro* digestibility of DM and fiber

Huang et al. (2017); NPB Project # 13-014
Gas increases with *in vitro* fermentation of fiber

\[ G = \frac{A}{1 + (B^C/t^C)} \]

Sources of insoluble fiber with variable fermentable fiber

<table>
<thead>
<tr>
<th>Sources of fiber:</th>
<th>Wheat Straw</th>
<th>Corn dried distillers grains with solubles</th>
<th>Soybean hulls</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDF</td>
<td>85.0%</td>
<td>44.0%</td>
<td>67.0%</td>
</tr>
<tr>
<td>TDF</td>
<td>0.5%</td>
<td>0.7%</td>
<td>8.4%</td>
</tr>
<tr>
<td>IDF</td>
<td>71.0%</td>
<td>42.2%</td>
<td>75.5%</td>
</tr>
<tr>
<td>Fermentability</td>
<td>Non-Fermentable</td>
<td>Moderately fermentable</td>
<td>Fermentable</td>
</tr>
</tbody>
</table>

NDF – Neutral detergent fiber
TDF – Total dietary fiber
SDF – Soluble dietary fiber
IDF – Insoluble dietary fiber

Huang et al. (2016) ASAS Midwest Meeting
In vitro fermentation predicts in vivo apparent total tract digestibility of total dietary fiber

\[ y = 0.1643x + 2.8959 \]
\[ R^2 = 0.819 \]

Huang et al. (2017); NPB Project # 13-014
Three step *in vitro* digestibility adapted to test enzymes

2 Ingredients
+ 11 Enzyme products

Enzymatic hydrolysis - gastric

Enzymatic hydrolysis – small intestine

Filtration and drying

Large intestine fermentation

Filtration and drying

Huang et al. (2017); NPB Project # 13-014
Gas production increases with *in vitro* fermentation of fiber

\[ G = \frac{A}{1 + \left(\frac{B^C}{t^C}\right)} \]

Enzymes break fiber into smaller fractions, but not monosaccharides
Decrease in total gas production in wheat middling with carbohydrate enzymes

Gas production, mL/g DM

Fermentation hours

Con, E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11
Negligible impact of enzymes on *in vitro* fermentation of fiber in corn DDGS
Fiber in cereal co-products is heterogenous, enzymes act on readily degradable fiber

Un-digestible Hind gut fermentation AID

Corn DDGS Corn basal diet Wheat basal diet Wheat grain Wheat millrun diet

Readily degradable fiber: susceptible to acid and mastication (mainly hemicellulose) 8-12 h

Fermentable fiber: breakdown by microbial activity 24-36 h

Recalcitrant fiber: resistant to attacks from exogenous enzyme or bacteria (mainly cellulose and entrapped hemicellulose)

Urriola et al., 2012; Shrestha, 2012, and Ndou et al., 2015
Animals: pigs (n = 54, 25kg BW) were individually fed experimental diets for 28-d

Feed and water: ad libitum and free access

Factorial design: 2 × 3 arrangement

Basal diets: corn-soybean (CSB), CSB + 40% corn DDGS, CSB + 30% wheat middlings (WM)

Carbohydrases: with or without 100 mg/kg cocktail (1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanase, 35 U/g galactosidase)
Growth performance

Diet = **0.02**
Enzyme = **0.051**
Diet × Enzyme = **0.42**

Diet < **0.01**
Enzyme = **0.08**
Diet × Enzyme = **0.43**

Means within a row without same superscript are different (*P* < 0.05)
Digestibility (AID and ATTD) of DM

Diet < 0.01
Enzyme = 0.44
Diet × Enzyme = 0.04

Diet < 0.01
Enzyme = 0.53
Diet × Enzyme = 0.50

Means within a row without same superscript are different (P < 0.05)
Digestibility (AID and ATTD) of GE

Diet < 0.01
Enzyme = 0.44
Diet × Enzyme = 0.04

Diet < 0.01
Enzyme = 0.26
Diet × Enzyme = 0.24

AID of GE

ATTD of GE

abc Means within a row without same superscript are different (P < 0.05)
Peak shear stress (whole digesta)

Diet = 0.111; Enzyme =0.01
Diet $\times$ Enzyme = 0.22
Digesta liquid $\times$ 0.01

Diet = 0.04; Enzyme =0.72
Diet $\times$ Enzyme = 0.86
Digesta liquid < 0.01

Diet < 0.01; Enzyme =0.89
Diet $\times$ Enzyme = 0.89
Digesta liquid = 0.09

Means within a row without same superscript are different ($P < 0.05$)
Feeding enzymes increases expression of interleukin-1 β in ileum
Fiber diets promote anti-inflammatory, while enzymes promote pro-inflammatory response

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ileal tissue</th>
<th>Colonic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Th1</td>
<td>Th2</td>
</tr>
<tr>
<td>CSB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDGS</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>WM</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>CSB+E</td>
<td>=</td>
<td>↓</td>
</tr>
<tr>
<td>DDGS+E</td>
<td>=</td>
<td>↓</td>
</tr>
<tr>
<td>WM+E</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>
Enzymes decrease MUC 2 expression and may decrease fermentable losses of Thr

Ferrandis-Villa et al. (2017) and NPB report # 014-045
Overview of LC-MS based chemometrics

Basal diet → Enzyme

Digesta samples

Jejunum → Ileum → Cecum

Sample preparation

Extraction Derivatization

LC-MS analysis

LC column → MS system

Multivariate data analysis

Scores plot Modelng → Correlation → Loadings plot → Marker identification

Marker analysis

Clustering analysis

Structural analysis

Quantitation

Bioinformatics Pathway analysis
Chemometric (LC/MS) composition of ileal digesta differs by diet, but not by enzyme addition.
Chemometric (LC/MS) composition of cecal digesta differs by diet, but not by enzyme addition.
Carbohydrases modify fiber in wheat middlings and corn DDGS, but do not increase digestibility.

Decrease fermentation

Increase in AID, but not ATTD of DM

Change in cytokines

Decrease in mucin

Increase in viscosity

Microbes
Increase degradation of recalcitrant fiber and implications of different portions on gut physiology

- Recalcitrant or indigestible fiber
- Slow fermentable fiber
- Rapidly fermentable fiber

Next NPB project - composition and pretreatment to increase degradation

Fiber degradation, %

Fermentation time

T1, T2
Conclusions

• *In vitro* experiments demonstrate that carbohydrase enzymes
  – Little effect on digestibility of corn fiber and marginal effects on wheat fiber
  – Marginal differences among products
• *In vivo* experiments demonstrate that carbohydrase enzymes degrade wheat fiber and to less extend corn fiber
  – Products of fiber degradation (oligosaccharides) impact
    • Viscosity
    • Immunity
  – Data supports hypothesis of extra-caloric effects, but can you measure it?
Funding and collaborations

• Funding:
  – National Pork Board (NPB)

• Collaborations:
  – Minnesota Discovery, Research, and InnoVation Economy (MnDRIVE)
  – Danisco Animal Nutrition (DuPont)
  – Archer Daniels Midland (ADM)
  – USDA – ARS
We are looking forward to seeing you in September

September 16-19, 2017 @ St Paul RiverCentre

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