Effects of acid extrusion on the degradability of maize distillers dried grain with solubles in pigs\textsuperscript{1,2}

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ABSTRACT: Commonly used feed processing technologies are not sufficient to affect recalcitrant nonstarch polysaccharides (NSP) such as arabinoxylans present in maize distillers dried grain with solubles (DDGS). Instead, hydrothermal treatments combined with acid catalysts might be more effective to modify these NSP. The objective of this experiment was to investigate the effects of hydrothermal maleic acid treatment (acid extrusion) on the degradability of maize DDGS in growing pigs. It was hypothesized that acid extrusion modifies DDGS cell wall architecture and thereby increases fermentability of NSP. Two diets, containing either 40\% (wt/wt) unprocessed or acid-extruded DDGS, were restrictedly fed to groups of gilts (n = 11, with 4 pigs per group; initial mean BW: 20.8 ± 0.2 kg) for 18 d and performance and digestibility were analyzed. Acid extrusion tended to decrease apparent ileal digestibility (AID) of CP (approximately 3 percentage units [% units]; P = 0.063) and starch (approximately 1% unit; P = 0.096). Apparent digestibility of CP and starch measured at the mid colon (2% units, P = 0.030, for CP and 0.3% units, P < 0.01, for starch) and apparent total tract digestibility (ATTD; 3% units, P < 0.01, for CP and 0.2% units, P = 0.024, for starch) were lower for the acid-extruded diet compared with the control diet. Hindgut disappearance was, however, not different between diets, indicating that reduced CP and starch digestibility were mainly due to decreased AID. Acid extrusion tended to increase AID of NSP (6% units; P = 0.092) and increased digestibility of NSP measured at the mid colon (6% units; P < 0.01), whereas hindgut disappearance and ATTD of NSP did not differ between diets. Greater NSP digestibility was mainly due to greater digestibility of arabinosyl, xylosyl, and glucosyl residues, indicating that both arabinoxylan and cellulose degradability were affected by acid extrusion. In conclusion, these results show that acid extrusion did not improve degradation of DDGS for growing pigs. Although acid extrusion seemed to facilitate more rapid degradation of NSP and shifted fermentation to more proximal gastrointestinal segments, total extent of NSP degradation was not affected. More than 35\% of the NSP from DDGS remained undegraded, independent of technological processing. Enzyme technologies that specifically target ester-linked acetyl, feroloyl, or coumaroyl groups were identified to be of interest for future research.

Key words: corn, distillers dried grain with solubles, feed processing, fermentation, fiber, nonstarch polysaccharides

Acid extrusion of fibrous feedstuffs for pigs

INTRODUCTION

Currently, the use of maize distillers dried grain with solubles (DDGS) as a protein source in animal feed is limited because of the inferior protein quality and high level (approximately 30%) of nonstarch polysaccharides (NSP; Shurson et al., 2012b) and of complex, highly substituted glucuronoarabinoxylans (GAX; Huisman et al., 2000; Appeldoorn et al., 2010; de Vries et al., 2013). Although NSP can be fermented by microflora in the gastrointestinal tract of the pig, degradability of NSP from DDGS is typically only approximately 50% (Urriola et al., 2010). In addition, especially in young pigs, NSP may affect digestion of other nutrients, both directly due to physical hindrance and indirectly due to physiological changes in the gut (Bach Knudsen et al., 1993; Le Gall et al., 2009).

Processing technologies can be used to modify plant cell wall architecture and improve NSP degradability, but commonly used technologies in feed production—for example, hammer milling and pelleting—are insufficient to affect recalcitrant NSP structures (de Vries et al., 2012). Hydrothermal pretreatments using acid catalysts are established methods to degrade lignocellulosic material (Sun and Cheng, 2002). For animal feed applications, however, potential protein damage and high residual acid or mineral concentrations limit the use of extreme processing temperatures and high acid concentrations (van den Borne et al., 2012). Instead, milder acid treatments using dicarboxylic organic acids, such as maleic acid, at lower temperatures could be of interest. In a previous in vitro study (de Vries et al., 2013), hydrothermal treatment (autoclaving) with maleic acid effectively increased solubilization of NSP from maize DDGS. The objective of the current experiment was to study the effects of hydrothermal acid treatment (acid extrusion) on the degradability of maize DDGS, in particular its fiber fraction, in pigs. It was hypothesized that acid extrusion modifies DDGS cell wall structure and thereby increases fermentability of NSP.

MATERIALS AND METHODS

The experiment was conducted at research farm “De Haar” of Wageningen University, Wageningen, The Netherlands. All experimental procedures were approved by the Animal Care and Use Committee of Wageningen University.

Materials and Experimental Diets

Pelleted maize DDGS was obtained from a commercial bioethanol plant (Abengoa Bioenergy, Rotterdam, The Netherlands) and crushed using a roller mill (major flaking mill; E R & F Turner Ltd., Ipswich, UK).

Two diets, containing 40% (wt/wt, as-fed basis) unprocessed (control diet) or acid-extruded DDGS (described below), were formulated to meet or exceed nutrient requirements of growing pigs (CVB, 2007a; Table 1 and Table 2). Additional sodium and potassium bicarbonate were added to the acid-extruded diet to neutralize the diet (pH > 4). Resulting differences in nutrient density between the control and acid-extruded diet were compensated for by adjusting the feeding level for the acid-extruded diet. Chromium oxide was included in the diets as an indigestible marker.

Hydrothermal Acid Treatment

Distillers dried grain with solubles was mixed with maleic acid (5%, wt/wt, DM basis) and water to reach a DM content of 40% (wt/wt), using a paddle mixer (Type F60; Halvor Forberg, Bygland, Norway). After soaking overnight, the DDGS mixture was extruded using a co-rotating double screw extruder (M.P.F. 50; Baker Perkins, Peterborough, UK), without additional steam conditioning. The extruder had a length:diameter ratio of 25. The screw configuration was as follows: four 1.5D feed screw elements, one 1D single lead element, three 1D feed screw elements, one 1D single lead element, two 1D feed screw elements, two 4D 90-degree forwarding block paddles, one 1.5D feed screw elements, one 4D 90-degree forwarding block paddles, one 1.5D feed screw element, two 4D 90-degree forwarding block paddles, and two 1.5D single lead elements. A die with 2 orifices (6 mm) was used; no die face cutter was used. Feeding rate was 54 kg/h and screw speed was 250 rpm. Barrel temperatures in the 9 subsequent segments of the extruder were set at 30, 50, 72, 82, 90, 105, 115, 120, and 120°C. Product was collected after the process reached a steady state and cooled to room temperature. Pressure and product temperature at the die were 862 +/- 68.9 kPa and 104 ± 1.4°C, respectively, and DM content of the extruded DDGS was 72% (wt/wt). The acid treatment conditions were based on preliminary results of our group, indicating that 5% (wt/wt, DM basis) maleic acid added 1 h before extrusion resulted in an 5% solubilization of NSP compared with unprocessed DDGS (40% DM, wt/wt).

Animals and Experimental Procedures

A total of 48 gilts (initial BW: 20.8 ± 0.2 kg; Topigs 20 × Talent; Topigs Vught, The Netherlands) were allocated to 1 of the 2 treatments based on BW and housed in groups of 4 pigs per pen. The experiment was performed in 2 rooms of 6 pens each. Dietary treatments were balanced across both rooms. Diets were fed as mash and mixed into a slurry with water (1:3) in the feed trough, which provided enough space such that all pigs could eat simultaneously. The daily feed allowance was 2.4 times energy require-
ments for maintenance (100 kcal ME/kg of BW$^{0.75}$; CVB, 2007b) in 2 equal meals at 0700 and 1600 h. Feed refusals were collected 30 min after feeding. Pigs had free access to water. After a 5-d gradual transition from a commercial starter diet to the experimental diets, pigs were allowed to adapt to the experimental diets for 14 d followed by 4 d collection of feces. At d 24 (room 1) or 25 (room 2), animals were anesthetized, the abdominal cavity was opened, and the gastrointestinal tract from stomach to anus was removed from the abdominal cavity, after which animals were euthanized. Before removal, the different segments of the gastrointestinal tract were isolated using tie wraps, to prevent mixing of digesta from different segments. The gastrointestinal tract was segmented and digesta samples were collected by gentle finger stripping. Pigs were fed approximately 4 h before dissection to ensure presence of fresh digesta in the terminal ileum. Feed intake per pen was recorded throughout the experiment. Pigs were weighed at the start of the experiment (d 0), adaption period (d 5), feces-collection period (d 19), and the end of the experiment (d 24 or 25) after consuming their morning meal. Feces were collected directly from the rectum, 2 times daily after feeding. Digesta from the last 100 cm of the small intestine (terminal ileum) and middle 50 cm of the colon (mid colon) were collected by gentle finger stripping immediately after dissection. Digesta and feces were pooled per pen by weight, immediately frozen (–20°C), and freeze-dried.

**Chemical Analyses**

Before chemical analyses, individual freeze-dried samples were ground in a mixer mill (MM 2000; Retsch GmbH, Haan, Germany) at an amplitude of 80, during 1 min. All chemical analyses were performed in duplicate using standard laboratory methods (AOAC, 2005; ISO, 2013). Feed refusals were analyzed for content of air-dry matter (AOAC, 2005). Diets, digesta, and feces were analyzed for content of DM (AOAC, 2005), ash (AOAC, 2005), chromium (Williams et al., 1962), nitrogen (diets by the Kjeldahl method; digesta and feces by the Dumas method; AOAC, 2005; using a Thermoquest NA 2100 Nitrogen and Protein Analyzer; Interscience B.V., Breda, The Netherlands), total starch (AOAC, 2005; using a commercial test kit, Megazyme International Ltd., Bray, Ireland), and total NSP measured as neutral sugars and uronic acids minus glucosyl originating from remaining starch in the NSP extract from diets or in digesta and feces (measured enzymatically as described above). Nitrogen content of diets was corrected for differences between analytical methods (Kjeldahl vs. Dumas method) using $N_{\text{Dumas}} = N_{\text{Kjeldahl}} \times 0.9885 + 0.0103$ (Etheridge et al., 1998). Crude protein content was calculated as N × 6.25 for diets (ISO, 2009) or N × 5.90 for DDGS (Kim et al., 2008).

Diets, DDGS, and ileal digesta were analyzed for content of AA (Hendriks et al., 1996) and DDGS samples were analyzed for reactive Lys (Moughan and Rutherford, 1996). Before AA and reactive Lys analysis, diets were defatted using a Soxhlet apparatus and petroleum ether and subsequently ground in a mixer mill (MM 2000; Retsch GmbH) at an amplitude of 80 for 2 min. Amino acids were analyzed using a 5-mg sample that was hydrolyzed using 1 mL of 6 M HCl for 23 h at 110°C in glass tubes, sealed under vacuum. The tubes were opened, norleucine was added to each tube as an internal standard, and the tubes were then dried under vacuum (Savant SpeedVac Plus, SC210A; Thermo Scientific). Amino acids were dissolved in 2 mL of loading buffer (sodium acetate; pH 2.2). Amino acids were separated by ion exchange chromatography using a Biochrom 20 AA analyzer (Biochrom, Cambridge, UK) and analyzed by postcolumn derivatization with ninhydrin, using photometric detection at 570 or 440 nm (Pro). Reactive Lys was analyzed using 5-mg samples that were incubated during 7 d with 1 mL O-methylisourea (OMIU) to convert all Lys molecules with a free ε-amino group into homoarginine (Moughan and Rutherford, 1996). Homoarginine was measured in the dried sample according to the AA analysis procedure previously described. The amount of OMIU-reactive Lys was calculated from the amount of homoarginine using the molecular weights of homoarginine and Lys.

Diets and feces were analyzed for content of ether extract (crude fat) using a Soxhlet apparatus and petroleum ether after hydrochloric acid hydrolysis (ISO, 2005). Diets were additionally analyzed for content of NDF (AOAC, 2005) and total and phytate bound P (enzymatic–colorimetric; using a commercial test kit, K-PHYT 07/11; Megazyme International Ltd.).

The control diet and pooled samples of digesta and feces from pigs fed the control diet (n = 1; 6 pens with 4 pigs per pen) were analyzed for content of esterified
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Calculations and Statistical Analysis

Apparent ileal and total tract digestibility were calculated according to the marker method with Cr₂O₃ as a marker (Kotb and Luckey, 1972). The unprocessed control diet was used as the reference to calculate digestibility of the control and acid-extruded DDGS diet. Body weight, ADG, and ADFI were analyzed using a GLM (PROC GLM, SAS version 9.2; SAS Inst. Inc., Cary, NC). All other data were analyzed using a generalized linear model with β-distributed error for the response variable and a logit link function (PROC GLIMMIX, SAS version 9.2). Diet and the blocking factor room were included as fixed effects. The interaction between the blocking factor room and dietary treatment was tested but found to be not significant and excluded from the model. Pen was the experimental unit. Distributions of the means and residuals were examined to verify model assumptions. Significance of differences was tested using type III likelihood ratio statistics. Data are presented as back-transformed means and pooled SEM unless otherwise stated. Differences among means with \( P < 0.05 \) were considered statistically significant; differences among means with \( 0.05 < P \leq 0.10 \) were considered a statistical trend.

RESULTS AND DISCUSSION

Diets and Animal Performance

In 1 pen (acid-extruded diet), 2 pigs showed signs of diarrhea during the experiment. Laboratory analyses of feces by the Dutch animal health service (GD, Deventer, The Netherlands) confirmed that these pigs suffered from *Escherichia coli* infection, and this pen was excluded from analyses. The pigs in all other pens had firm feces.

The acid-extruded diet had a slightly lower nutrient content and greater ash content compared to the control diet (Table 3), due to the inclusion of acid and additional bicarbonate. By adjusting the feeding level for the acid-extruded diet (103% of the control diet), we endeavored to equalize nutrient intake of both treatment groups. As pigs receiving the acid-extruded diet did not always consume their total daily allowance (Table 4), this pen was excluded from analyses. The pigs in all other pens had firm feces.

The acid-extruded diet had a slightly lower nutrient content and greater ash content compared to the control diet (Table 3), due to the inclusion of acid and additional bicarbonate. By adjusting the feeding level for the acid-extruded diet (103% of the control diet), we endeavored to equalize nutrient intake of both treatment groups. As pigs receiving the acid-extruded diet did not always consume their total daily allowance (Table 4), this was, however, not fully accomplished. Crude protein and Lys content of the DDGS (Table 1) were within ranges reported previously for maize DDGS (Stein et al., 2006; Pahm et al., 2008; Shurson et al., 2012b). The total and reactive Lys:CP ratios indicate that the DDGS was of average quality, with moderate protein damage (Shurson et al., 2012b). Hydrothermal treatments may reduce protein quality due to specific interactions between protein and AA with other components, such as the Maillard reaction (Fontaine et al., 2007). The total and reactive Lys:CP ratios in unprocessed and acid-extruded DDGS were, however, similar (Table 1), indicating that little additional protein damage occurred during hydrothermal acid treatment (Fontaine et al., 2007). Extrusion conditions used in the current study were moderate, while the content of potentially reducing sugars in DDGS is relatively low (<10%; Kim et al., 2008), reducing the risk of protein damage (Björck and

Table 1. Analyzed chemical composition of unprocessed and acid-extruded distillers dried grain with solubles (DDGS; %, DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Unprocessed DDGS</th>
<th>Acid-extruded DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % as-fed basis</td>
<td>92</td>
<td>72</td>
</tr>
<tr>
<td>CP²</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>Indispensable AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>1.10</td>
<td>1.11</td>
</tr>
<tr>
<td>His</td>
<td>1.11</td>
<td>1.01</td>
</tr>
<tr>
<td>Ile</td>
<td>1.16</td>
<td>1.11</td>
</tr>
<tr>
<td>Leu</td>
<td>3.53</td>
<td>3.42</td>
</tr>
<tr>
<td>Lys</td>
<td>0.86</td>
<td>0.90</td>
</tr>
<tr>
<td>Reactive Lys³</td>
<td>0.84</td>
<td>0.88</td>
</tr>
<tr>
<td>Phe</td>
<td>1.50</td>
<td>1.46</td>
</tr>
<tr>
<td>Thr</td>
<td>1.20</td>
<td>1.15</td>
</tr>
<tr>
<td>Val</td>
<td>1.56</td>
<td>1.49</td>
</tr>
<tr>
<td>Dispensable AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>2.28</td>
<td>2.21</td>
</tr>
<tr>
<td>Asp</td>
<td>2.00</td>
<td>1.98</td>
</tr>
<tr>
<td>Glu</td>
<td>4.07</td>
<td>4.13</td>
</tr>
<tr>
<td>Gly</td>
<td>1.19</td>
<td>1.16</td>
</tr>
<tr>
<td>Pro</td>
<td>2.81</td>
<td>2.76</td>
</tr>
<tr>
<td>Ser</td>
<td>1.48</td>
<td>1.43</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.34</td>
<td>1.30</td>
</tr>
<tr>
<td>Total AA³</td>
<td>2.72</td>
<td>2.66</td>
</tr>
<tr>
<td>Total Lys:CP</td>
<td>0.029</td>
<td>0.027</td>
</tr>
<tr>
<td>Reactive Lys:CP</td>
<td>0.028</td>
<td>0.027</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Molar composition of carbohydrates⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhamnosyl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Mannosyl</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Galactosyl</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Uronyl</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Ara:Xyl⁶</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>UA:Xyl⁷</td>
<td>0.35</td>
<td>0.32</td>
</tr>
</tbody>
</table>

¹Unless otherwise indicated.
²Crude protein content was calculated from the N content using a protein conversion factor of 5.90 (Kim et al., 2008).
³Measured as O-methylisourea–reactive Lys (Moughan and Rutherfurd, 1996).
⁴Calculated as the sum of Arg, His, Ile, Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Gly, Pro, Ser, and Tyr.
⁵Molar percent; presented as anhydrous sugar moieties.
⁶Ara:Xyl = molar ratio of arabinosyl:xylosyl.
⁷UA:Xyl = molar ratio of uronyl:xylosyl.
Asp, 1983; Singh et al., 2007). In addition, the Maillard reaction occurs at a slower rate under acidic conditions (Ajandouz et al., 2001). As discussed above, a considerable part of the DDGS protein was heat damaged before acid extrusion, presumably as a result of exposure to the severe pretreatment conditions (Bothast and Schlicher, 2005; Saunders and Rosentrater, 2009; Shurson et al., 2012a) during the dry-grind ethanol production process. This may have reduced the chance for protein damage during hydrothermal acid treatment.

Table 2. Composition of experimental diets (%*, as-fed basis)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>Acid-extruded diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed DDGS (89% DM)</td>
<td>40.00</td>
<td>49.40</td>
</tr>
<tr>
<td>Acid-extruded DDGS (72% DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maleic acid</td>
<td>–</td>
<td>1.80</td>
</tr>
<tr>
<td>Water</td>
<td>9.40</td>
<td>–</td>
</tr>
<tr>
<td>Maize starch</td>
<td>25.65</td>
<td>25.65</td>
</tr>
<tr>
<td>Casein</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Soy protein isolate</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Soy oil</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Mineral and vitamin premix</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Potassium bicarbonate</td>
<td>0.70</td>
<td>1.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.15</td>
<td>0.50</td>
</tr>
<tr>
<td>t-Lys HCL</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>t-Thr</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>t-Trp</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Cr$_2$O$_3$</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>Calculated nutrient composition</td>
<td>4,228</td>
<td>4,105</td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>0.40</td>
<td>0.39</td>
</tr>
<tr>
<td>Digestible P</td>
<td>0.31</td>
<td>0.30</td>
</tr>
<tr>
<td>Digestible Lys</td>
<td>1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>Digestible Met + Cys</td>
<td>0.57</td>
<td>0.55</td>
</tr>
<tr>
<td>Digestible Thr</td>
<td>0.63</td>
<td>0.61</td>
</tr>
<tr>
<td>Digestible Trp</td>
<td>0.17</td>
<td>0.16</td>
</tr>
</tbody>
</table>

1 Maleic acid (5%, wt/wt, DM basis) was mixed with distillers dried grain with solubles (DDGS) and soaked overnight before extrusion. The acid-extruded diet was fed at 103% of the control diet to compensate for differences in nutrient density between the diets.
2 DDGS = distillers dried grain with solubles.
3 Provided per kilogram of diet: 6,000 IU vitamin A (retinyl acetate), 1,200 IU vitamin D (cholecalciferol), 40 mg vitamin E (DL-α-tocopherol), 1.0 mg vitamin B$_1$ (thiamin), 3 mg vitamin B$_2$ (riboflavin), 1 mg vitamin B$_6$ (pyridoxine HCl), 1.5 mg vitamin K$_1$ (menadione), 15 μg vitamin B$_12$ (cyanocobalamin), 150 mg choline chloride, 20 mg niacin, 10 mg pantothenic acid (d-calcium pantothenate), 0.2 mg folic acid, 0.2 mg Co as CoSO$_4$·7H$_2$O, 15 mg Cu as CuSO$_4$·H$_2$O, 80 mg Fe as FeSO$_4$·H$_2$O, 0.7 mg I as KI, 30 mg Mn as MnO, 0.2 mg Se as Na$_2$SeO$_3$, and 50 mg Zn as ZnSO$_4$·H$_2$O.
4 Calculated based on data from Centraal Veevoederbureau (2007a), Stein and Shurson (2009), and Cervantes-Pahn and Stein (2010). Digestible P and AA contents indicate apparent ileal digestible contents.

Table 3. Analyzed chemical composition of experimental diets (%*, DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control diet</th>
<th>Acid-extruded diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % as-fed basis</td>
<td>87.55</td>
<td>86.89</td>
</tr>
<tr>
<td>CP</td>
<td>22.83</td>
<td>22.54</td>
</tr>
<tr>
<td>Starch</td>
<td>27.79</td>
<td>25.87</td>
</tr>
<tr>
<td>Crude fat</td>
<td>10.77</td>
<td>10.73</td>
</tr>
<tr>
<td>Ash</td>
<td>6.28</td>
<td>7.09</td>
</tr>
<tr>
<td>NDF</td>
<td>19.63</td>
<td>13.46</td>
</tr>
<tr>
<td>P</td>
<td>0.74</td>
<td>0.76</td>
</tr>
<tr>
<td>Phytate-bound P</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>Nonstarch polysaccharides (NSP)</td>
<td>18.78</td>
<td>15.73</td>
</tr>
<tr>
<td>Molar composition of NSP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhamnosyl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>Mannosyl</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Galactosyl</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Uronyl</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Ara: Xyl</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td>UA: Xyl</td>
<td>0.26</td>
<td>0.29</td>
</tr>
<tr>
<td>Indispensable AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td>His</td>
<td>0.75</td>
<td>0.73</td>
</tr>
<tr>
<td>Ile</td>
<td>0.89</td>
<td>0.91</td>
</tr>
<tr>
<td>Leu</td>
<td>2.05</td>
<td>2.11</td>
</tr>
<tr>
<td>Lys</td>
<td>1.40</td>
<td>1.45</td>
</tr>
<tr>
<td>Phe</td>
<td>1.02</td>
<td>1.04</td>
</tr>
<tr>
<td>Thr</td>
<td>0.85</td>
<td>0.90</td>
</tr>
<tr>
<td>Val</td>
<td>1.11</td>
<td>1.13</td>
</tr>
<tr>
<td>Dispensable AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>1.15</td>
<td>1.21</td>
</tr>
<tr>
<td>Asp</td>
<td>1.69</td>
<td>1.82</td>
</tr>
<tr>
<td>Glu</td>
<td>3.15</td>
<td>3.25</td>
</tr>
<tr>
<td>Gly</td>
<td>0.87</td>
<td>0.88</td>
</tr>
<tr>
<td>Pro</td>
<td>1.52</td>
<td>1.65</td>
</tr>
<tr>
<td>Ser</td>
<td>0.90</td>
<td>0.92</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.81</td>
<td>0.82</td>
</tr>
<tr>
<td>Total AA</td>
<td>19.04</td>
<td>19.75</td>
</tr>
</tbody>
</table>

1 Unless otherwise indicated.
2 The acid-extruded diet was fed at 103% of the control diet to compensate for differences in nutrient density between the diets.
3 Crude protein content was calculated from the N content using a protein conversion factor of 6.25 (ISO, 2009).
4 Molar percent; presented as anhydrous sugar moieties.
5 Ara: Xyl = molar ratio of arabinosyl: xylosyl.
6 UA: Xyl = molar ratio of uronoyl: xylosyl.
7 Calculated as sum of Arg, His, Ile, Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Gly, Pro, Ser, and Tyr.

The sugar composition of the DDGS indicated the presence of cellulose and GAX, as expected (de Vries et al., 2013). The slightly lower starch (approximately 1 percentage unit; % unit), NSP (approximately 3% units), nonglucosyl polysaccharide (NSP – glucosyl; approximately 2% units), and NDF (approximately 6%
Acid extrusion of fibrous feedstuffs for pigs

Table 4. Growth performance of growing pigs fed diets containing 40% (wt/wt) unprocessed (control) or acid-extruded distillers dried grain with solubles as the only nonstarch polysaccharide source1

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Acid extruded</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations2</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Initial mean BW, kg</td>
<td>20.8</td>
<td>20.7</td>
<td>0.10</td>
<td>0.247</td>
</tr>
<tr>
<td>Final mean BW, kg</td>
<td>32.8</td>
<td>31.0</td>
<td>0.42</td>
<td>0.003</td>
</tr>
<tr>
<td>ADG, g/pig</td>
<td>530</td>
<td>475</td>
<td>7.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADFI, g/pig</td>
<td>860</td>
<td>815</td>
<td>7.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G:F, g/g</td>
<td>0.62</td>
<td>0.58</td>
<td>0.006</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1Recorded between start of adaptation period and end of the experiment (19 or 20 d), with the exception of initial mean BW, which was recorded at start of the experiment before feeding the experimental diets.

2Number of replicate pens of 4 pigs each.

Feed intake and weight gain were lower for pigs fed the acid-extruded diet compared with pigs fed the unprocessed control diet (P < 0.05; Table 4). As feed allowance was restricted to 2.4 times the energy requirements for maintenance, the reduced feed intake of the pigs fed the acid-extruded diet was related to orts in this group. This might have resulted from altered physicochemical properties of this diet such as increased water binding capacity (Kyriazakis and Emmans, 1995; de Vries et al., 2013) and reduced pH (Ravindran and Kornegay, 1993) or from reduced palatability of the diet due its acidity (Ravindran and Kornegay, 1993). Gain-to-feed ratio was reduced for pigs fed the acid-extruded diet (P < 0.05). Overall, G:F was high, which was probably related to an increased relative weight of the gastrointestinal tract and its contents in response to the high fiber diets fed in this experiment (Jensen and Jørgensen, 1994; Jørgensen et al., 1996).

Dry Matter and Nutrient Digestibility

Ileal and total tract digestibility of DM and nutrients are presented in Tables 5 and 6. Apparent ileal digestibility (AID) of CP from the DDGS was calculated to be approximately 60% (calculated by the difference method; AID for protein sources other than DDGS as reported in literature; CVB, 2007a), which is in the range previously reported for moderately heat-damaged maize DDGS (Stein et al., 2006; Urriola et al., 2009; Almeida et al., 2013). Similarly, calculated AID of Lys (approximately 50%), Arg (approximately 60%), and Pro (approximately 65%), AA that can be involved in the Maillard reaction, are in the range expected for moderately heat-damaged DDGS (Stein et al., 2006; Urriola et al., 2009; Almeida et al., 2013).

Acid extrusion tended to decrease AID of CP (approximately 3% units; P = 0.063), AA (approximately 3% units; P = 0.067), and starch (approximately 1% unit; P = 0.096). Digestibility of CP and starch measured at the mid colon and ATTD were lower for the acid-extruded diet compared with the control diet, but hindgut disappearance did not differ between diets. Therefore, the reduced CP and starch digestibility in pigs fed the acid-extruded diet were mainly due to decreased AID. The trend for reduced AID of AA was mainly explained by reduced AID of Ile, Leu, Val, Asp, Glu, and Pro. Apparent total tract digestibility of crude fat tended to be lower (0.4% units; P = 0.104) for the acid-extruded diet compared with the control diet. Little protein damage of DDGS was found after acid extrusion and also AID of Lys seemed not to be affected by acid extrusion. Moreover, the majority (>90%) of starch originated from maize starch, which was not exposed to acid extrusion. Hence, reduced digestibility of the acid-extruded diet presumably resulted indirectly from changes in digestive or absorptive processes rather than from chemical modification of the nutrients present in DDGS. The acid-extruded diet contained approximately

Table 5. Apparent digestibility (%) measured at the distal ileum (ileum), middle of the colon (colon), or in feces (total tract) as well as hindgut disappearance (%) of DM and nutrients in growing pigs fed diets containing 40% (wt/wt) unprocessed (control) or acid-extruded distillers dried grain with solubles as the only nonstarch polysaccharide source

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Acid extruded</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations1</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>68.6</td>
<td>65.3</td>
<td>1.71</td>
<td>0.090</td>
</tr>
<tr>
<td>CP</td>
<td>68.1</td>
<td>64.8</td>
<td>1.59</td>
<td>0.063</td>
</tr>
<tr>
<td>Starch</td>
<td>97.5</td>
<td>96.4</td>
<td>0.56</td>
<td>0.096</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>79.7</td>
<td>79.4</td>
<td>0.38</td>
<td>0.375</td>
</tr>
<tr>
<td>CP</td>
<td>76.4</td>
<td>74.3</td>
<td>0.58</td>
<td>0.030</td>
</tr>
<tr>
<td>Starch</td>
<td>99.6</td>
<td>99.3</td>
<td>0.06</td>
<td>0.006</td>
</tr>
<tr>
<td>Total tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>80.6</td>
<td>79.8</td>
<td>0.36</td>
<td>0.065</td>
</tr>
<tr>
<td>CP</td>
<td>78.0</td>
<td>75.4</td>
<td>0.49</td>
<td>0.006</td>
</tr>
<tr>
<td>Crude fat</td>
<td>97.9</td>
<td>97.5</td>
<td>0.10</td>
<td>0.104</td>
</tr>
<tr>
<td>NDF</td>
<td>59.9</td>
<td>63.7</td>
<td>1.43</td>
<td>0.030</td>
</tr>
<tr>
<td>Hindgut disappearance2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>12.0</td>
<td>14.5</td>
<td>1.62</td>
<td>0.152</td>
</tr>
<tr>
<td>CP</td>
<td>10.0</td>
<td>10.3</td>
<td>1.59</td>
<td>0.820</td>
</tr>
<tr>
<td>Starch</td>
<td>2.2</td>
<td>3.0</td>
<td>0.52</td>
<td>0.144</td>
</tr>
</tbody>
</table>

1Number of replicate pens of 4 pigs each.

2Calculated by the difference between ileal and total tract digestibility.
3% low molecular weight sugars that were formed during the acid-extrusion process (discussed above) and the remaining NSP in the acid-extruded diet were more soluble (18% of hemicellulose was water soluble in the acid-extruded DDGS vs. 15% in unprocessed DDGS; no further data presented). Literature indicates that the presence of high levels of nonabsorbed water-soluble low molecular weight sugars can increase osmolality and water content of the chyme (Wiggins, 1984). This may increase passage rate and reduce absorption of nutrients, possibly explaining the observed decreased AID and G:F. Indeed, observed DM contents of digesta and feces were lower in pigs fed the acid-extruded diet compared with those fed the control diet (8 vs. 11% [wt/wt; \( P < 0.01 \) in ileum, 19 vs. 24% \([P < 0.01]\) in colon, and 25 vs. 28% \([P < 0.01]\) in feces). Alternatively, increased microbial activity in the distal ileum and high concentrations of low molecular weight arabinosyl (\(\text{Ara} \)) and xylosyl (\(\text{Xyl} \)) sugars (Schutte et al., 1991, 1992) may have influenced apparent digestibility values by increasing the production of microbial biomass and endogenous losses and reducing hydrolysis and absorption of nutrients (reviewed by Montagne et al., 2003; Richards et al., 2005). Furthermore, although the amount of acid added to the acid-extruded diet was within the range of concentrations of organic acids applied more often in pig diets, this bivalent acid is stronger (\(pK_a = 1.93 \) and \(pK_a = 6.14 \); Kertes and King, 1986) and effects of maleic acid on digestive and metabolic processes cannot be excluded (Rosenberg and Segal, 1964; Ravindran and Kornegay, 1993; Dibner and Buttin, 2002).

**Table 6.** Apparent digestibility (%) of AA in growing pigs fed diets containing 40% (wt/wt) unprocessed (control) or acid-extruded distillers dried grain with solubles as the only nonstarch polysaccharide source, measured at the distal ileum

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Acid extruded</th>
<th>Pooled SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations(^1)</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Indispensable AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>78.2</td>
<td>77.1</td>
<td>0.68</td>
<td>0.267</td>
</tr>
<tr>
<td>His</td>
<td>70.9</td>
<td>71.5</td>
<td>0.74</td>
<td>0.587</td>
</tr>
<tr>
<td>Ile</td>
<td>68.9</td>
<td>66.4</td>
<td>1.26</td>
<td>0.085</td>
</tr>
<tr>
<td>Leu</td>
<td>76.0</td>
<td>74.3</td>
<td>0.62</td>
<td>0.087</td>
</tr>
<tr>
<td>Lys</td>
<td>79.5</td>
<td>79.5</td>
<td>0.64</td>
<td>0.959</td>
</tr>
<tr>
<td>Phe</td>
<td>76.7</td>
<td>75.7</td>
<td>0.57</td>
<td>0.290</td>
</tr>
<tr>
<td>Thr</td>
<td>60.6</td>
<td>57.5</td>
<td>2.04</td>
<td>0.173</td>
</tr>
<tr>
<td>Val</td>
<td>66.9</td>
<td>64.5</td>
<td>1.17</td>
<td>0.072</td>
</tr>
<tr>
<td>Dispensable AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>67.5</td>
<td>66.0</td>
<td>1.34</td>
<td>0.269</td>
</tr>
<tr>
<td>Asp</td>
<td>60.3</td>
<td>56.2</td>
<td>2.14</td>
<td>0.093</td>
</tr>
<tr>
<td>Glu</td>
<td>74.3</td>
<td>70.4</td>
<td>1.44</td>
<td>0.026</td>
</tr>
<tr>
<td>Gly</td>
<td>52.0</td>
<td>48.5</td>
<td>2.70</td>
<td>0.231</td>
</tr>
<tr>
<td>Pro</td>
<td>68.8</td>
<td>63.1</td>
<td>1.90</td>
<td>0.018</td>
</tr>
<tr>
<td>Ser</td>
<td>65.6</td>
<td>64.2</td>
<td>1.99</td>
<td>0.499</td>
</tr>
<tr>
<td>Tyr</td>
<td>76.6</td>
<td>75.1</td>
<td>0.57</td>
<td>0.104</td>
</tr>
<tr>
<td>Total AA(^2)</td>
<td>70.3</td>
<td>67.8</td>
<td>1.19</td>
<td>0.067</td>
</tr>
</tbody>
</table>

1Number of replicate pens of 4 pigs each.  
2Calculated as sum of Arg, His, Ile, Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Gly, Pro, Ser, and Tyr.

**Table 7.** Apparent digestibility (%) measured at the distal ileum (ileum), middle of the colon (colon), or in feces (total tract) as well as hindgut disappearance (%) of nonstarch polysaccharides (NSP) and its constituent sugars in growing pigs fed containing 40% (wt/wt) unprocessed (control) or acid-extruded distillers dried grain with solubles as the only NSP source

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Acid extruded</th>
<th>Pooled SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations(^1)</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSP(^2)</td>
<td>20.3</td>
<td>26.6</td>
<td>3.27</td>
<td>0.092</td>
</tr>
<tr>
<td>NGP(^3)</td>
<td>19.2</td>
<td>25.6</td>
<td>4.66</td>
<td>0.208</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>12.1</td>
<td>23.0</td>
<td>4.55</td>
<td>0.047</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>33.2</td>
<td>37.2</td>
<td>4.62</td>
<td>0.424</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>23.4</td>
<td>27.7</td>
<td>5.45</td>
<td>0.447</td>
</tr>
<tr>
<td>Uronyl</td>
<td>39.8</td>
<td>40.8</td>
<td>3.91</td>
<td>0.817</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSP(^2)</td>
<td>59.2</td>
<td>65.5</td>
<td>1.59</td>
<td>0.004</td>
</tr>
<tr>
<td>NGP(^3)</td>
<td>57.6</td>
<td>64.0</td>
<td>1.61</td>
<td>0.004</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>56.8</td>
<td>65.1</td>
<td>1.75</td>
<td>0.001</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>50.5</td>
<td>59.2</td>
<td>2.00</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>62.7</td>
<td>69.1</td>
<td>1.82</td>
<td>0.008</td>
</tr>
<tr>
<td>Uronyl</td>
<td>77.7</td>
<td>78.7</td>
<td>1.46</td>
<td>0.509</td>
</tr>
<tr>
<td>Total tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSP(^2)</td>
<td>63.8</td>
<td>63.0</td>
<td>1.28</td>
<td>0.551</td>
</tr>
<tr>
<td>NGP(^3)</td>
<td>61.8</td>
<td>61.4</td>
<td>0.73</td>
<td>0.694</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>60.6</td>
<td>61.0</td>
<td>0.08</td>
<td>0.757</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>55.3</td>
<td>56.1</td>
<td>1.38</td>
<td>0.566</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>68.6</td>
<td>66.8</td>
<td>2.35</td>
<td>0.479</td>
</tr>
<tr>
<td>Uronyl</td>
<td>79.8</td>
<td>78.0</td>
<td>0.59</td>
<td>0.055</td>
</tr>
<tr>
<td>Hindgut disappearance(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSP(^2)</td>
<td>43.0</td>
<td>36.8</td>
<td>4.48</td>
<td>0.203</td>
</tr>
<tr>
<td>NGP(^3)</td>
<td>42.1</td>
<td>36.0</td>
<td>5.44</td>
<td>0.299</td>
</tr>
<tr>
<td>Arabinosyl</td>
<td>48.0</td>
<td>38.5</td>
<td>5.68</td>
<td>0.133</td>
</tr>
<tr>
<td>Xylosyl</td>
<td>20.1</td>
<td>20.5</td>
<td>7.04</td>
<td>0.955</td>
</tr>
<tr>
<td>Glucosyl</td>
<td>45.3</td>
<td>38.3</td>
<td>6.55</td>
<td>0.320</td>
</tr>
<tr>
<td>Uronyl</td>
<td>40.0</td>
<td>37.2</td>
<td>3.54</td>
<td>0.457</td>
</tr>
</tbody>
</table>

1Number of replicate pens of 4 pigs each.  
2Monosaccharides represent anhydrous sugar moieties.  
3NGP = nonglucosyl polysaccharides; monosaccharides represent anhydrous sugar moieties.  
4Calculated by the difference between ileal and total tract digestibility.

**Nonstarch Polysaccharide Degradation**

Apparent total tract digestibility of NSP was approximately 63%, and 20 (control diet) to 27% (acid-extruded diet) was degraded before the end of the ileum (Table 7). Although detailed structural characterization of the NSP in DDGS is lacking, it is expected, based on the composition of maize and maize fiber fractions, that complex, highly
Acid extrusion of fibrous feedstuffs for pigs

Acid extrusion of fibrous feedstuffs for pigs were dominated (Huisman et al., 2000; Appeldoorn et al., 2010; de Vries et al., 2013). These heteroxylans originate from a complex cell wall matrix where hemicellulose, cellulose, and lignin are intertwined. They consist of a xylan backbone substituted with monomeric arabino-furanosyl and glucuronic acid residues and acetyl and feruloyl esters as well as oligomer side chains containing Ara, Xyl, galactosyl, and feruloyl residues and are highly cross-linked by diferulic acid bridges (Saulnier and Thibault, 1999; Huisman et al., 2000; Appeldoorn et al., 2010). The ratio of Ara and uronyl (UA) to Xyl residues is indicative for the degree of substitution and therefore related to the structure of the GAX present in the feed or of the undegraded GAX remaining in digesta and feces. The greater Ara:Xyl in ileal digesta compared with the feed together with a higher relative degradation of Xyl compared to Ara (Table 7; Fig. 1) indicates that mainly linear, lowly-substituted xylan fragments were degraded in the upper gastrointestinal tract. This was followed by a reduction in Ara:Xyl and UA:Xyl in the mid colon, indicating that more complex, highly-substituted xylan fragments were degraded mainly in the cecum and proximal colon.

More than 35% of the NSP from DDGS remained undegraded. Based on the sugar composition of the unfermented NSP found in feces and previous observations on degradation of maize GAX, it is expected that the unfermented residue contained dense substituted xylans with large oligomeric side chains. These are firmly anchored in the cell wall through ester linkages (Appeldoorn et al., 2010). To further identify limiting structures in degradation of NSP from DDGS, the content of esterified coumaric and ferulic acid in feed and pooled samples from ileum and colon contents and feces were analyzed (Fig. 2). Those phenolic acids cross-link polysaccharides as well as lignin within the cell wall (Ralph et al., 1994; Saulnier and Thibault, 1999; Appeldoorn et al., 2010, 2013) and have been shown in maize GAX to be resistant to hydrothermal and enzymatic treatment. Hence, they may contribute to the recalcitrance of maize GAX structures. To our knowledge, this is the first time that degradation of phenolic compounds from DDGS in pigs are studied and we believe these data provide interesting novel insights in the degradation of DDGS fiber that can be used in future research. The data are indicative rather than statistically analyzed and presented for the control diet only, due to limited analytical capacity. Contents in feed were 3.6 g/kg (wt/wt, DM basis) for monoferulic acid, 0.2 g/kg for diferulic acid, and 0.3 g/kg for coumaric acid and increased two- to four-fold in digesta and feces. It was calculated that the DDGS cell wall fraction contained approximately 2 to 3% of phenolic acids. In particular, the content of diferulic acids (approximately 0.1%) is lower than reported for maize bran (Saulnier and Thibault, 1999). Monoferulic acid was degraded more easily than diferulic acid, whereas coumaric acid was degraded to the least extent (Fig. 2C). These preliminary data indicated that phenolic acid associated linkages within the cell wall were partly degraded by the pigs’ microbiota. The fact that coumaric acid and, to a smaller extent, diferulic acid disappearance was substantially lower than NSP disappearance indicated, however, that these components were indeed associated with a more resistant part of the cell wall. Possibly, technologies that specifically target phenolic acid associated (ester) linkages, such as alkali treatments or enzymes having coumaroyl or feruloyl esterase activity, can facilitate more extensive degradation of DDGS NSP. Furthermore, acetyl esterases may be useful because of the high substitution of recalcitrant maize GAX with acetyl groups (Appeldoorn et al., 2013).

Acid extrusion tended to increase AID of NSP (6% units; $P = 0.092$), only reaching statistical significance for AID of Ara residues (11% units; $P = 0.047$). At the mid
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colon, digestibility of NSP was greater (6% units; \( P < 0.01 \)) for the acid-extruded diet. Greater digestibility was mainly due to greater disappearance of Ara, Xyl, and glucosyl residues (Table 7), indicating that both arabinoxylan and cellulose degradability were affected by acid extrusion. In vitro hydrothermal acid treatment did not affect cellulose solubilization (de Vries et al., 2013), but apparently, acid extrusion opened the cell wall architecture facilitating accessibility of cellulose to microbial enzymes. Hindgut disappearance and ATTD of NSP and its constituent sugars did not differ between diets, indicating that although acid extrusion shifted fermentation of NSP to more proximal segments of the gastrointestinal tract, total extent of NSP degradation was not affected. Apparently, acid extrusion affected mainly NSP structures that are not resistant to degradation by microbial enzymes in the pigs’ gastrointestinal tract. Although opening of the cell wall matrix facilitated more easy degradation of those structures, the most recalcitrant NSP structures (approximately 35% of total) were still not affected. An increased passage rate in the upper gastrointestinal tract, caused by high concentrations of nonabsorbed low molecular weight sugars or increased microbial activity, could have contributed to this observation. Furthermore, factors such as physicochemical properties and postileal nutrient flow may have affected digesta passage rate in the cecum and colon and thus time available for fermentation in the large intestine (Drochner, 1993). To prevent formation of high quantities of small saccharides, future technologies to increase degradation of NSP from DDGS should be aimed at targeted degradation of only recalcitrant structures without affecting relatively easy degradable NSP to prevent counteracting effects on passage rate and nutrient digestion and absorption. Enzyme technologies would, therefore, be preferred over chemical degradation, due to their specific activities. The fact that ATTD of NDF was greater in the acid-extruded diet (\( P < 0.03 \); Table 7) seems to be somewhat contradictory to the unchanged ATTD of NSP but might be explained by a shift of fiber fractions recovered in NDF and differences in particle size between the diets (de Vries et al., 2012, 2013). As NDF is analyzed by gravimetric methods, including filtration, processing will affect the quantity and type of polysaccharides that are recovered in NDF, due to differences in physicochemical properties, in particular particle size, and solubility of NSP. Hence, evaluation of degradability of NDF fractions does not allow full evaluation of NSP degradation and seems rather futile when diets that fairly differ in type of NSP or in physicochemical properties, such as particle size, are compared.

In conclusion, acid extrusion did not improve degradation of DDGS in growing pigs. Apparent ileal and total tract digestibility of CP and starch tended to be lower, resulting in reduced feed efficiency. Although acid extrusion seemed to facilitate more rapid degradation of NSP and shifted fer-

Figure 2. Content and degradability of coumaric acid, monoferulic acid, and diferulic acid from the unprocessed distillers dried grain with solubles (control) diet observed in growing pigs. (A) Esterified coumaric acid, monoferulic acid, and diferulic acid content (g/100 g DM) in feed, digesta, and feces. (B) Coumaric acid:xylosyl and ferulic acid:xylosyl ratio (mol/100 mol xylosyl) in feed, digesta, and feces. (C) Apparent digestibility of coumaric acid, monoferulic acid, and diferulic acid (%) measured at the distal ileum (ileum) or middle of the colon (colon) or in feces (total tract). Digesta or feces samples from 6 pens, with 4 pigs each, were pooled by weight before analyses (\( n = 1 \)).
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