Effects of feeding dry or modified wet distillers grains with solubles with or without supplemental calcium oxide on ruminal metabolism and microbial enzymatic activity of beef cattle

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ABSTRACT: The objectives of this study were to determine the interaction of feeding dry (DDGS) or modified wet (MDGS) distillers grains with solubles (DGS) with or without supplemental CaO on in situ DM and NDF disappearance; ruminal pH, VFA, and methane concentration; and cellulase and xylanase activity. Fistulated steers (n = 8; average initial BW = 540 ± 250 kg) were used in a replicated 4 × 4 Latin square design. Treatments were arranged in a 2 × 2 factorial, and steers were randomly allotted to 1 of 4 dietary treatments: 1) 50% DDGS with 0% CaO, 2) 48.8% DDGS supplemented with 1.2% CaO, 3) 50% MDGS with 0% CaO, or 4) 48.8% MDGS supplemented with 1.2% CaO (DM basis). The remainder of the diet was husklage, dry-rolled corn, and vitamin and mineral supplement. There were no interactions (P ≥ 0.12) of DGS type and CaO addition on any parameters measured. Steers fed DDGS had a 17% increase (P < 0.01) in DMI compared to steers fed MDGS; however, CaO supplementation reduced (P = 0.03) DMI by 12%, regardless of DGS type. As expected, addition of CaO increased the pH of the diet by 1.82 pH units. This caused a time by CaO interaction (P = 0.05) for ruminal pH. Regardless of DGS type, steers supplemented with CaO tended to have increased (P = 0.09) ruminal pH at 1.5 h and had increased (P = 0.03) ruminal pH at 3 h postfeeding; however, ruminal pH did not differ (P ≥ 0.24) for the remainder of the day. There was no difference (P = 0.46) in ruminal cellulase activity when comparing type of DGS fed. However, there was a time by CaO interaction (P < 0.01); cattle fed 1.2% CaO diets had 28% greater ruminal cellulase activity only at 0 h postfeeding when compared to cattle fed 0% CaO. Furthermore, feeding supplemental CaO increased (P = 0.04) acetate to propionate ratio (A:P) regardless of type of DGS fed. Increased initial ruminal pH and cellulase activity from supplemental CaO did not increase (P ≥ 0.48) in situ NDF disappearance. No differences (P ≥ 0.48) in ruminal methane concentration were found when comparing DGS type or supplemental CaO. In conclusion, the type of DGS fed had little effect on ruminal metabolism. Even though CaO increased ruminal pH and cellulase activity at some times postfeeding, it was not enough to affect in situ fiber disappearance.

Key words: beef cattle, calcium oxide, distillers grains, rumen metabolism

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INTRODUCTION

At nearly 3 times the fat and protein concentrations of corn, distillers grains with solubles (DGS) are a nutrient-dense, alternative feedstuff for cattle (Lardy, 2007; Loy and Strohbehn, 2007; Klopfenstein et al., 2008). However, including DGS in cattle diets can be challenging due to the sulfuric acid content of DGS (Felix and Loerch, 2011), acidic ruminal pH associated with feeding DGS (Loy et al., 2007; Felix and Loerch, 2011), and performance differences between dry DGS (DDGS) and wet DGS (Ham et al., 1994). Cattle fed 60% DDGS-based diets had decreased ruminal pH from 6.0 prefeeding to 5.1 at 1.5 h postfeeding, and ruminal pH remained below 5.0 from 3 to 12 h postfeeding (Felix and Loerch, 2011). Ruminal pH below 5.5 is indicative of ruminal acidosis characterized by reduced nutrient absorption, variable feed intake, and depressed performance (Owens et al., 1998). Optimum fiber digestion in the rumen occurs between pH 6.0 and 6.7 (Mould et al., 1983).

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As much of the energy in DGS-based diets comes from fiber in DGS, it is imperative to control pH to optimize fiber digestion in the rumen. Treating DDGS with NaOH before feeding improves in situ DM and NDF disappearance; ruminal pH, VFA, and methane concentrations; and cellulase and xylanase activity.

**MATERIALS AND METHODS**

All animal procedures were approved by the University of Illinois Institute of Animal Care and Use Committee and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 2010).

**Animals and Management**

Eight Angus × Simmental crossbred steers, previously fitted with rumen cannula, were blocked by BW into a large (average initial BW = 635 ± 50 kg; n = 4) and a small (average initial BW = 450 ± 40 kg; n = 4) block and were used in a replicated 4 × 4 Latin square design, such that each BW block represented 1 square. Steers were housed in metabolism stalls at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana. Stalls (2.3 × 1.3 m) are equipped with individual feed bunks and nonsiphoning automatic water bowls. The barn is equipped with a heating, ventilation, and air-conditioning system, providing a controlled environment set at 18.3°C. There was a 2 × 2 factorial arrangement of treatments, and steers were assigned to 1 of 4 dietary treatments: 1) 50% DDGS with 0% CaO, 2) 48.8% DDGS supplemented with 1.2% CaO, 3) 50% MDGS with 0% CaO, or 4) 48.8% MDGS supplemented with 1.2% CaO. The remainder of the diet, on a DM basis, was 20% husklage, 20% or 25% dry rolled corn, and 10% or 5% vitamin and mineral supplement (for 1.2% CaO and 0% CaO diets, respectively; Table 1). To keep Ca inclusion similar across treatments, 2 different supplements were formulated: a high Ca (8.8% analyzed Ca, DM basis) and a low Ca (3.5% analyzed Ca, DM basis). The high-Ca supplement was included in the 0% CaO diets and contained Ca predominantly from limestone. This supplement was formulated to be included at 10% of the diet DM, and this inclusion was necessary to reduce separation of the limestone and other minerals from the ground corn carrier. In comparison, the low-Ca supplement was used in the 1.2% CaO diets and, because it contained less than half of the limestone, had fewer issues with separation; therefore, corn was reduced in the low-Ca supplement, and it was included at 5% of the diet DM. To keep corn similar across diets, the increased inclusion

<table>
<thead>
<tr>
<th>Item</th>
<th>DDGS</th>
<th>MDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>% DM basis</td>
<td>0% CaO</td>
<td>1.2% CaO</td>
</tr>
<tr>
<td>MDGS²</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DDGS³</td>
<td>50.0</td>
<td>48.8</td>
</tr>
<tr>
<td>Cracked corn</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Husklage ⁴</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>HC supplement⁵</td>
<td>10.0</td>
<td>—</td>
</tr>
<tr>
<td>LC supplement⁶</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td>Calcium oxide</td>
<td>—</td>
<td>1.2</td>
</tr>
</tbody>
</table>

1CaO (MicroCal OF200; Mississippi Lime Company, St. Louis, MO) treatment is listed as a percentage of the dietary DM.

2DDGS (ADM West Plant, Decatur, IL) analyzed values: DM, 49.0%; NDF, 28.1%; ADF, 18.8%; CP, 28.2%; EE, 8.1%; Ca, 0.05%; P, 0.73%; S, 0.55%; pH, 3.82.

3MDGS (Aventine Renewable Energy Inc., Pekin, IL) analyzed values: DM, 84.4%; NDF, 35.9%; ADF, 17.4%; CP, 28.6%; EE, 11.4%; Ca, 0.18%; P, 0.83%; S, 0.36%; pH, 4.78.

4Husklage is ensiled corn shucks derived from seed-corn processing; analyzed values: DM, 34.0%; NDF, 27.4%; ADF, 18.4%; CP, 18.32; EE, 8.1%; Ca, 0.05%; P, 0.73%; S, 0.36%; pH, 4.78.

5High-calcium (HC) vitamin and mineral supplement fed to cattle receiving 0% CaO treated diets contained 75.35% ground corn, 22.72% limestone, 0.91% dairy trace mineral salt (included 8.5% Ca as CaCO₃, 5% Mg as MgO and MgSO₄, 7.6% K as KCl, 6.7% Cl as KCl, 10% S as S₈, prilled, 0.5% Ca as CaSO₄ and Availa-4 [Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN], 2% Fe as FeSO₄, 3% Mn as MnSO₄ and Availa-4, 3% Zn as ZnSO₄ and Availa-4, 278 kg/mg Co as Availa-4, 250 ppm I as Ca(IO₃)₂, 150 Se as Na₂SeO₃, 2,205 KIU/kg vitamin A as retinyl acetate, 662.5 KIU/kg vitamin D as cholecalciferol, 22,047.5 IU/kg vitamin E as dl-α-tocopheryl acetate, and less than 1% CP, fat, crude fiber, salt), 0.15% Rumensin 90 (200 g/kg; Elanco, Greenfield, IN), 0.10% Tylosin 40 (88 g/kg; Elanco), and 0.766% fat.

6Low-calcium (LC) vitamin and mineral supplement fed to cattle receiving 1.2% CaO treated diets contained 87.27% ground corn, 8.94% limestone, 1.79% dairy trace mineral salt (composition the same as above), 0.30% Rumensin 90, 0.20% Tylosin 40, and 1.51% fat.
of high-Ca supplement replaced corn. The 1.2% inclusion of CaO was chosen to neutralize the acidity of the DGS. The husklage used in the diet was ensiled corn shucks derived from seed-corn processing, and the analyzed values on a DM basis were as follows: DM, 34.0%; NDF, 28.7%; ADF, 12.5%; CP, 11.4%; ether extractable fat (EE), 3.4%.

Supplementation with CaO occurred daily by mixing into the ration. Dietary treatment sequence was assigned according to procedures outlined by Patterson and Lucas (1962). To maintain dietary Ca across treatments, cattle fed diets supplemented with CaO were fed a reduced Ca supplement, whereas cattle fed diets without CaO received an elevated Ca supplement. Cattle were fed once daily for ad libitum intake.

**Sampling and Analysis**

Sampling periods were 21 d beginning with a 14-d acclimation phase followed by a 7-d collection phase that included a 5-d digestibility collection (Schroeder, 2013), a 1-d rumen fluid collection, and a 1-d in situ incubation phase. To initiate the trial, steers were brought in from pasture, and rumen contents were retained (12 L) to reinoculate the animals before each collection period, negating differences in ruminal microbes. After each sampling period, steers were reinoculated with rumen contents from the initial collection (4 L) before being transitioned to the next experimental diet.

During the digestibility collection (d 1 to 5 of the collection phase) dietary ingredient and feed refusal samples were collected and weighed daily. Individual ingredient samples were then analyzed for DM (24 h at 105°C). Wet dietary ingredient samples were composited within period and freeze-dried (FreeZone, Labconco, Kansas City, MO), then ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Ground dietary ingredient samples were analyzed for ADF and NDF (using Ankom Technology methods 5 and 6, respectively; Ankom 200 Fiber Analyzer, Ankom Technology, Macedon, NY), CP (Leco TruMac, LECO Corporation, St. Joseph, MI), fat (method 2; Ankom Technology), and total ash (500°C for 12 h; HotPack Muffle Oven Model: 770750, HotPack Corp., Philadelphia, PA). The resulting values were used to calculate nutrient composition of the diets. Dietary ingredient composites were subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy analysis of complete minerals (method 975.03; AOAC, 1988). Composited ingredients were also analyzed for pH using an Accumet Basic AB15 pH meter with an Accumet accuCap glass body, gel-filled electrode (Fisher Scientific, Pittsburg, PA). Each dietary ingredient (50 g) was mixed with 200 mL of distilled water for 30 s before the pH electrode was submersed in the mixture and pH was recorded. The solution was then titrated with 1 M NaOH to a final pH of 7.0, and the milliliters of NaOH used were recorded. The resulting values were used to determine total dietary pH and titratable acidity. Feed refusal (10% as is) samples were also collected for 5 d of the 7-d collection phase. Feed refusals were analyzed for DM, NDF, and ADF as described above.

During the rumen fluid collection (d 6 of collection phase), rumen pH was measured by collecting whole, mixed rumen content via rumen canula at 0, 1.5, 3, 6, 9, and 12 h postfeeding. Sampling times were chosen to characterize ruminal pH through peak fermentation and pH recovery to prefeeding level in cattle fed DGS-based diets (Felix et al., 2012). Rumen samples were then filtered through 2 layers of cheesecloth and immediately analyzed for pH using a FiveEasy FiveGo pH meter FE20/FG2 with a LE438 polyoxymethylene body gel-filled electrode with Ag/AgCl reference system and 1.2m BNC/Cinch connection (Mettler Toledo, Columbus, OH).

Rumen fluid samples for VFA analysis were collected at 0 and 3 h postfeeding. Sample times were chosen to compare dietary effects on ruminal VFA at a basal level (before feeding at 0 h) and VFA during peak fermentation (3 h). Samples were strained through 2 layers of cheesecloth, 50 to 75 mL of rumen fluid were mixed with 10 mL of H₃PO₄, and deionized water was added to achieve a 2:1 dilution (by weight). The mixture was then placed in a refrigerator and remixed by shaking several times per day for 2 d. Three days after collection, rumen fluid samples were removed from the refrigerator, and 40 mL of diluted rumen fluid were centrifuged at 20,000 × g at 25°C for 20 min. Supernatant was filtered through a 0.45-µm filter. The filtered sample was then transferred in 1-mL aliquots to gas chromatography vials with 0.1 mL of 2-ethyl butyrate as an internal standard. Vials were then stored at -20°C until analyzed via gas chromatography (GC; model 5890A, Hewlett-Packard, Palo Alto, CA) for VFA.

Ruminal CH₄ concentration was analyzed by sampling rumen gas via canula puncture with an 18-gauge needle. The gas was collected at 3 h postfeeding to collect at time of peak fermentation, and CH₄ concentration was measured via GC (Gow-Mac 580TCD with a silica gel 60/80 mesh column, Gow-Mac Instrument Co., Bethlehem, PA). Nitrogen was used as a carrier gas with a flow rate of 60 mL/min.

Cellulase and xylanase activity of whole rumen contents was analyzed by collecting whole, mixed rumen content at 0 and 3 h postfeeding. Sample times were chosen to compare dietary effects on enzyme activity at a basal level (before feeding at 0 h) and enzyme activity during peak fermentation (3 h). Whole rumen contents (60 to 70 mL) were placed in a 75-mL conical centrifuge
tube and immediately frozen at –80°C for later analysis. Frozen samples were thawed for 24 h at 4°C, then centrifuged for 20 min at 12,000 × g for 20 min. Supernatant was filtered through a 0.45-µm filter and centrifuged again at 5,000 × g for 15 min in an Amicon Ultra-15 10K centrifugal filter to concentrate the sample. The sample (40 µL) was pipetted into PCR plates containing either 90 µL 1% (wt/vol) carboxymethylcellulose (CMC) in 0.05 M sodium phosphate and 0.15 M sodium chloride buffer (pH 6.0) to determine cellulase activity or 90 µL 1% (wt/vol) wheat arabinoxylan (WAX) in 0.05 M sodium phosphate and 0.15 M sodium chloride buffer (pH 6.0) to determine xylanase activity. Plates were then incubated at 37°C for 0, 7, or 15 min to allow enzymatic breakdown of CMC or WAX into shorter-chain products (oligosaccharides) or their unit sugars, i.e., glucose or xylose and arabinose. After incubation, plates were boiled (100°C) for 10 min to cease further enzymatic degradation of substrate and cooled to 25°C. The concentration of glucose equivalents was subsequently quantified with the para-hydroxybenzoic acid hydrazide (pHBAH) method as described previously (Lever, 1972). The rate of glucose equivalents released was determined for each sample using values from 0-, 7-, and 15-min incubations.

During the in situ collection (d 7 of the collection phase), ruminal fiber degradation was estimated by the NDF disappearance of soybean hulls (SBH) in situ. Four replicate Dacron bags (Ankom Technology, 10 × 20 cm) containing SBH were used for incubation in the rumen. Bags were tied shut with nylon string and then placed in larger mesh sacs with weights. These larger sacs were placed in the rumen on d 7 of the collection phase. After a 24-h incubation, bags were removed, rinsed, and dried at 55°C for 3 d. Following drying, samples were weighed to determine DM and were ground to be analyzed for NDF (using Ankom Technology method 5, referenced above). In addition, 4 bags were used to determine the “washout” (0 h) value of SBH from the in situ bags. These bags were not placed in the rumen but were subjected to the same rinsing and drying procedures as the incubated bags. To determine in situ disappearance the following equation was used (DM basis):

\[
1 - \left( \frac{\text{weight of SBH before incubation}}{\text{weight of SBH after incubation}} \times 100 \right)
\]

To determine NDF disappearance, the weight of SBH NDF was used in the same equation.

### Statistical Analysis

The experimental design was a replicated 4 × 4 Latin square. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Repeated measures were used to analyze ruminal pH and VFA concentrations and cellulase and xylanase activity. The model was

\[
Y_{ijklmno} = \mu + S_i + c_{j(i)} + p_k + D_l + C_m + (DC)_{lm} + T_{n} + (TD)_{ln} + (TC)_{mn} + (TDC)_{lmn} + e_{ijklmno}
\]

where \( Y_{ijklmno} \) = response variable, \( \mu \) = mean, \( S_i \) = the fixed effect of square, \( c_{j(i)} \) = the random effect of calf nested within square, \( p_k \) = the random effect of period, \( D_l \) = the fixed effect of DGS type (dry or modified wet), \( C_m \) = the fixed effect of CaO addition (0% or 1.2%), \( (DC)_{lm} \) = the fixed effect of the interaction of the DGS type and CaO addition, \( T_{n} \) = the fixed effect of repeated time of collection, \( (TD)_{ln} \) = the fixed effect of the interaction of time of collection and DGS type, \( (TC)_{mn} \) = the fixed effect of the interaction of time of collection and DGS type and CaO addition, and \( e_{ijklmno} \) = the experimental error.

Ruminal methane concentration and in situ DM and NDF disappearance were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.). The model was

\[
Y_{ijklmn} = \mu + S_i + c_{j(i)} + p_k + D_l + C_m + (DC)_{lm} + e_{ijklmn}
\]

where \( Y_{ijklmn} \) = response variable, \( \mu \) = mean, \( S_i \) = the fixed effect of square, \( c_{j(i)} \) = the random effect of calf nested within square, \( p_k \) = the random effect of period, \( D_l \) = the fixed effect of DGS type (dry or modified wet), \( C_m \) = the fixed effect of CaO addition (0% or 1.2%), \( (DC)_{lm} \) = the fixed effect of the interaction of the DGS type and CaO addition, and \( e_{ijklmn} \) = the experimental error.

For both models, individual animal was the experimental unit. There were no interactions of DGS type and CaO addition (\( P \geq 0.12 \)), therefore, only main effects will be discussed. Significance is declared at \( P \leq 0.05 \). Trends are discussed at 0.05 < \( P < 0.10 \).

### RESULTS AND DISCUSSION

Despite the fact that diets containing MDGS were 0.45 pH units more acidic than diets containing DDGS (Table 2), there was no effect (\( P = 0.21 \)) of DGS type on ruminal pH (Fig. 1). There was a time by CaO interaction (\( P = 0.05 \)) for ruminal pH. Steers fed CaO tended to have elevated (\( P = 0.09 \)) ruminal pH at 1.5 h (6.10 compared to 5.92 for 1.2% and 0% CaO, respectively) and had elevated (\( P = 0.03 \)) ruminal pH at 3 h postfeeding (5.99 compared to 5.75 for 1.2% and 0% CaO, respectively). However, steers fed 1.2% CaO had a similar (\( P \geq 0.24 \))
Table 2. Effects of supplementing 0% CaO or 1.2% CaO (DM basis) to steers fed dry (DDGS) or modified wet (MDGS) distillers grains with solubles based diets on DM intake and in situ digestibility

<table>
<thead>
<tr>
<th>Item</th>
<th>DDGS</th>
<th>MDGS</th>
<th>0% CaO</th>
<th>1.2% CaO</th>
<th>SEM</th>
<th>P-value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg</td>
<td>9.82</td>
<td>8.13</td>
<td>9.57</td>
<td>8.38</td>
<td>0.37</td>
<td>&lt;0.01 &lt;0.03</td>
</tr>
<tr>
<td>Dietary pH</td>
<td>5.95</td>
<td>5.50</td>
<td>4.81</td>
<td>6.63</td>
<td>0.08</td>
<td>&lt;0.01 &lt;0.01</td>
</tr>
<tr>
<td>NaOH to buffer dietary acidity&lt;sup&gt;2&lt;/sup&gt; mL/g</td>
<td>0.17</td>
<td>0.21</td>
<td>0.29</td>
<td>0.08</td>
<td>0.01</td>
<td>&lt;0.01 &lt;0.01</td>
</tr>
<tr>
<td>NaOH to buffer dietary acidity&lt;sup&gt;3&lt;/sup&gt; L/d</td>
<td>1.62</td>
<td>1.77</td>
<td>2.77</td>
<td>0.62</td>
<td>0.08</td>
<td>0.21 &lt;0.01</td>
</tr>
<tr>
<td>SBH DMD,&lt;sup&gt;4&lt;/sup&gt; %</td>
<td>27.0</td>
<td>26.6</td>
<td>28.7</td>
<td>25.0</td>
<td>1.24</td>
<td>0.83 0.05</td>
</tr>
<tr>
<td>NDF disappearance of SBH, %</td>
<td>22.2</td>
<td>22.6</td>
<td>23.1</td>
<td>21.7</td>
<td>1.39</td>
<td>0.86 0.48</td>
</tr>
</tbody>
</table>

<sup>1</sup>D = the main effect of distiller grains (DGS) type, C = main effect of CaO treatment. There was no interaction (P > 0.12) of D × C.

<sup>2</sup>Milliliters of 1 M NaOH needed to titrate 1 g of the diet to pH 7.00.

<sup>3</sup>Liters of 1 M NaOH needed to titrate daily DMI to pH 7.00.

<sup>4</sup>SBH DMD = in situ dry matter disappearance of soybean hulls (SBH).

ruminal pH compared to cattle fed 0% CaO at 0, 6, 9, and 12 h postfeeding. This could be explained in part by dietary pH. Diets containing supplemental CaO had 1.82 units greater (P < 0.01) pH than diets that did not contain CaO. The increase in pH of feed entering the rumen may have buffered initial ruminal pH in steers fed CaO supplemented diets. Despite the time differences, CaO addition did not affect mean (P = 0.21) ruminal pH (6.02 for 1.2% CaO and 5.91 for 0% CaO). Felix et al. (2012) compared 25% and 60% DDGS treated with 0% or 2% NaOH and found that neutralizing the acid in DDGS with NaOH before feeding increased mean ruminal pH.

Although we had hypothesized that cellulase and xylanase activity would be affected by changes in ruminal pH, there were no differences (P ≥ 0.13) when comparing DGS type or CaO addition for xylanase activity (Table 3). However, there was a time postfeeding by CaO addition interaction (P < 0.01) for cellulase activity. At 0 h postfeeding, steers fed 1.2% CaO diets had greater (P = 0.02) cellulase activity in rumen contents than cattle fed 0% CaO, but by 3 h postfeeding cellulase activity was not different (P = 0.59) between CaO inclusions. Despite increasing dietary pH as well as ruminal pH and cellulase activity at certain times, in the present study, the addition of CaO decreased (P = 0.05) DM disappearance (DMD) of SBH in situ by approximately 13% (Table 2). The DMD of SBH in situ in the present experiment was greater than reported by Felix et al. (2012) when they fed 60% DDGS-based diets with or without NaOH; however, they reported a 51% increase in 24-h in situ NDF disappearance when cattle were fed 2% NaOH compared to those fed 0% NaOH. In this trial, although CaO decreased DMD of SBH in situ, NDF disappearance of SBH was not affected (P ≥ 0.48) by treatment.

One reason for the conflicting results in the current study when compared to those of Felix et al. (2012) could be the different alkaline agents used in each experiment. Felix et al. (2012) used 1 M NaOH, a stronger base (pK<sub>a</sub> = 13.2) compared to CaO (pK<sub>a</sub> = 12.4) used in the present study. However, although Felix et al. (2012) also used a greater concentration of alkaline agent in the diet than was used in this experiment (2% of diet DM compared to 1.2%, respectively), CaO supplemented diets in the current trial had a greater pH than NaOH-treated 60% DDGS diets in the Felix et al. (2012) trial (6.63 vs. 5.96, respectively), likely a result of the greater initial starting pH of the DGS used in the current trial. Mould et al. (1983) described that for optimum cellulose metabolism in the rumen, a pH range of 6.0 to 6.7 should be maintained because bacterial species that contribute to fiber digestion in the rumen are greatly impeded at ruminal pH below 6.0. In this experiment, no cattle experienced ruminal pH below 6.0, and the average ruminal pH was 6.0, whereas Felix et al. (2012) observed that ruminal pH was below 5.3 for...
12 h. Rumen environment greatly influences the success of cellulolytic bacteria that produce cellulase (Hungate, 1966). Stewart (1977) experimented in vitro with cellulose disappearance from rumen contents at various pH and discovered that a pH reduction from 6.6 to 5.2 decreased cellulose disappearance by 31%. Although numerous studies confirm that ruminal pH and carbohydrate solubility greatly impact fiber digestion in the rumen (Huhtanen and Khalili, 1992; Mould et al., 1983; Rooke et al., 1987), few studies have examined hemicellulolytic bacteria activity in DGS-based diets.

One of the biggest differences in the 2 trials, however, is the method of alkaline agent delivery. Felix et al. (2012) treated batches of DDGS weekly instead of supplementing the alkaline agent to the diet. A plethora of previous data support the theory that pretreating fibrous feeds will increase fiber digestibility (Berger et al., 1979; Kong et al., 1992; Kim and Holtzapple, 2006); however, most of this work theorizes that the improvement occurs because lignin and hemicellulose bonding is disrupted. Although the lignin content of DGS is reportedly minimal (4.35% to 10% of the NDF; NRC 1996), there has not been any research to date on the efficacy of treating vs. supplementing alkaline agents for cattle fed DGS-based diets.

The addition of CaO reduced (P = 0.03) DMI of steers by 15% in this experiment, regardless of DGS type. This is similar to previous research by Nuñez et al. (2013), where steers fed diets containing 60% DDGS had linearly reduced DMI with increasing dietary CaO when CaO was included up to 2.4% of the diet DM. The reduction of DMI may be caused by decreased palatability with CaO addition; however, additional research would be needed to confirm this. In addition to CaO effects, moisture differences in wet vs. dry DGS can influence diet palatability and DMI as well (Ham et al., 1993). In this experiment, steers fed DDGS had a 17% increase (P < 0.01) in DMI compared to steers fed MDGS. These results confirm the observations of Ham et al. (1994) that cattle consuming wetter diets will eat less DM.

Because of the fiber content of DGS, we had hypothesized that increasing the pH of the diet would improve rumen fermentation; however, there was no CaO by time interaction (P ≥ 0.24) on ruminal VFA concentrations (Table 4). In addition, there was no effect of DGS type (P ≥ 0.25) or its interaction with time (P ≥ 0.06) on ruminal VFA concentrations. There was a tendency (P = 0.07) for decreased ruminal propionate concentration when CaO was added to the diet that increased (P = 0.04) A:P, regardless of type of DGS fed. However, acetate and total ruminal VFA at 3 h postfeeding were not different (P ≥ 0.22) when comparing steers fed 0% with those fed 1.2% CaO. Although most of the energy in DGS is in the form of fiber, cattle consuming DGS-based diets have a much different VFA profile than cattle consuming traditional fiber-based diets (Leupp et al., 2009). Cattle consuming forage-based diets typically have a VFA profile of 65% to 70% acetate, 15% to 25% propionate, and 5% to 10% butyrate (Fluharty, 2009). However, when cattle are fed grain-based diets, the VFA profile is approximately 50% to 60% acetate, 35% to 40% propionate, and 5% to 10% butyrate (Fluharty, 2009). Ruminal VFA profiles in this experiment are more similar to those of cattle fed grain-based diets with approximately 55% acetate, 33% propionate, and 14% butyrate at 3 h postfeeding.

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<thead>
<tr>
<th>Item</th>
<th>DDGS</th>
<th>MDGS</th>
<th>0% CaO</th>
<th>1.2% CaO</th>
<th>SEM</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>Cellulase activity&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
<td>0.46</td>
</tr>
<tr>
<td>0 h postfeeding</td>
<td>0.049</td>
<td>0.043</td>
<td>0.039</td>
<td>0.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h postfeeding</td>
<td>0.047</td>
<td>0.045</td>
<td>0.048</td>
<td>0.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylanase activity</td>
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<td>0.013</td>
<td>0.137</td>
<td>0.139</td>
<td>0.150</td>
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</tr>
<tr>
<td>0 h postfeeding</td>
<td>0.148</td>
<td>0.156</td>
<td>0.133</td>
<td>0.171</td>
<td></td>
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</tr>
<tr>
<td>3 h postfeeding</td>
<td>0.137</td>
<td>0.152</td>
<td>0.139</td>
<td>0.150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>CaO treatment is listed as a percentage of the dietary DM.

<sup>2</sup>The P-values in the row with the enzyme name are from the repeated-measures model, where D = the main effect of distillers grains (DGS) type, C = the main effect of CaO treatment, D × T = the interaction of DGS × time, and C × T = the interaction of CaO × time. There was no interaction (P ≥ 0.70) for D × C. When an interaction of C × T occurred (P < 0.05), the SLICE option (SAS Inst. Inc., Cary, NC) was used to compare treatments at each time period. The SEM shown is associated with the main effect × T interaction.

<sup>3</sup>Enzyme activity is reported as the release of glucose equivalents over time.
pH decreases the activity of methanogenic archaea and subsequently reduces ruminal methane emissions (Moe and Tyrrell, 1979). We had hypothesized, therefore, that the acidic ruminal pH associated with DGS-based diets (Felix and Loerch, 2011) would decrease methane production and that CaO addition would raise ruminal pH, altering ruminal fermentation as discussed above, subsequently increasing methane concentrations. However, no differences ($P \geq 0.48$) were found in methane concentration when comparing steers fed 0% CaO to steers fed 1.2% CaO or when comparing steers fed MDGS to steers fed DDGS-based diets (Fig. 2). Behlke et al. (2007) conducted in vitro experiments replacing forage with DDGS as an energy source and found that increasing DDGS in rumen fluid in vitro resulted in decreased methane production. However, when corn was replaced with DDGS in vitro, methane production increased as DDGS inclusion increased (Behlke et al., 2008). Although dietary DGS inclusions were not varied in the present study, the ruminal pH was increased with 1.2% CaO inclusion in the diet with no effect on methane concentrations. The variability of ruminal methane concentrations (SE = 1.09%) in this experiment may have prevented us from finding significance.

The sulfuric acid content of DGS and acidic rumen pH associated with feeding DGS can make including DGS in beef cattle diets challenging. Acidic ruminal pH, even for a short time, can shift rumen microbial populations and decrease fiber digestibility. As much of the energy in DGS-based diets comes from the fiber in DGS, it is important to control rumen pH to ensure successful fiber digestion in the rumen. In this experiment, adding CaO in DGS-based diets neutralized the acidity in DGS and increased rumen pH at some time points. However, increased ruminal pH did not successfully increase fiber disappearance in situ. Further research investigating greater dietary inclusions of CaO may be necessary to improve utilization of DGS in beef cattle diets.

### Table 4. Effects of supplementing 0% CaO or 1.2% CaO (DM basis) to steers fed dry (DDGS) or modified wet (MDGS) distillers grains with solubles based diets on ruminal VFA concentrations over time

<table>
<thead>
<tr>
<th>Item</th>
<th>DDGS</th>
<th>MDGS</th>
<th>0% CaO</th>
<th>1.2% CaO</th>
<th>SEM</th>
<th>$P$-value $^{1,2,3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h postfeeding</td>
<td>42.7</td>
<td>44.9</td>
<td>41.8</td>
<td>45.7</td>
<td>2.06</td>
<td>0.25 0.22 0.73 0.61</td>
</tr>
<tr>
<td>3 h postfeeding</td>
<td>53.5</td>
<td>56.9</td>
<td>54.1</td>
<td>56.3</td>
<td>2.36</td>
<td>0.61 0.07 0.06 0.24</td>
</tr>
<tr>
<td>Propionate, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h postfeeding</td>
<td>17.5</td>
<td>18.4</td>
<td>20.1</td>
<td>15.8</td>
<td>3.64</td>
<td>0.29 0.04 0.25 0.57</td>
</tr>
<tr>
<td>3 h postfeeding</td>
<td>34.6</td>
<td>30.5</td>
<td>36.2</td>
<td>28.9</td>
<td>0.17</td>
<td>1.70 2.0 1.7 2.1</td>
</tr>
<tr>
<td>A:P $^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h postfeeding</td>
<td>2.7</td>
<td>2.9</td>
<td>2.5</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h postfeeding</td>
<td>1.7</td>
<td>2.0</td>
<td>1.7</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h postfeeding</td>
<td>74.2</td>
<td>77.0</td>
<td>75.0</td>
<td>76.2</td>
<td>4.64</td>
<td>0.87 1.00 0.58 0.72</td>
</tr>
<tr>
<td>3 h postfeeding</td>
<td>109.3</td>
<td>108.2</td>
<td>109.4</td>
<td>108.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 $D =$ the main effect of distillers grains (DGS) type, $C =$ the main effect of CaO treatment, $D \times T =$ the interaction of DGS of time, and $C \times T =$ the interaction of CaO of time.

2 No other VFA were significant and therefore were not reported.

3 Effect of time was significant for all VFA at $P < 0.01$. There was no interaction ($P \geq 0.12$) of $D \times C$ or $D \times C \times T$ for ruminal VFA or enzyme activity. When an interaction of $C \times T$ occurred ($P < 0.05$), the SLICE option (SAS Inst. Inc., Cary, NC) was used to compare treatments at each time period.

4 Ratio of acetate to propionate.
LITERATURE CITED


