In vitro production of methane with increasing levels of corn or wheat based dried distillers’ grains with solubles in a barley silage based diet

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ABSTRACT: Methane production from wheat or corn based dried distillers’ grains with solubles (CDDGS, WDDGS) was compared *in vitro*. Wheat DDGS (49 g fat/kg DM) or CDDGS (115 g fat/kg DM) partially or completely replaced whole crop barley silage at 200, 400, 600, 800 or 1000 g/kg DM. Production of CH$_4$ increased linearly and quadratically ($p<0.01$) with increasing levels of CDDGS. Cumulative CH$_4$ production at 24 h was higher ($p<0.05$) for WDDGS (12.0 ± 0.5 mg/g DM) than CDDGS up to 800 g/kg DM. Molar proportions of propionate in incubation fluid were higher ($p<0.05$) for CDDG than for WDDGS at 200, 400 and 600 g/kg DM, respectively. *In vitro* CH$_4$ production (mg CH$_4$/g DM; mg CH$_4$/g DMD) was lower for CDDGS than WDDGS up to 800 g/kg substrate DM. The higher residual oil content in CDDGS compared to WDDGS likely elicited this response.

Keywords: *in vitro*, dried distillers’ grains with solubles, methane
**Introduction**

Dried distillers’ grains with solubles (DDGS) is a major by-product from the biofuel industry wherein cereal grains are fermented to produce ethanol. As ethanol production has increased considerably in the last decade, large amounts of DDGS are available and predominantly used as feed for ruminant livestock (Klopfenstein et al., 2008). Corn based DDGS (CDDGS) is the most abundant DDGS in the USA whereas in Canada wheat based DDGS (WDDGS) accounts for almost one third of total DDGS production (USDA Foreign Agricultural Service, 2010). As a result of the fermentation process, DDGS is largely starch free, but concentrated three fold in protein, fibre and fat (Spiehs et al., 2002). The fat content is higher in CDDGS (~100 g/kg dry matter [DM]; Spiehs et al., 2002) than in WDDGS (~50 g/kg DM; Gibb et al., 2008) owing to the higher level of fat in corn. Supplementation of ruminant diets with dietary fat reduces ruminal CH$_4$ through a number of mechanisms including reduction in ruminal DM digestibility, direct effects of fatty acids on ruminal methanogens and protozoa, and by biohydrogenation of unsaturated fatty acids (Czerkawski et al., 1966; Johnson and Johnson, 1995).

Additionally, dietary fats often replace fermentable carbohydrates that otherwise would contribute to an increase in the reducing equivalents available to reduce CO$_2$ to CH$_4$ (Beauchemin et al., 2008).

Replacing a mixture of 350 g/kg barley grain and 50 g/kg canola meal (DM basis) by CDDGS (100 g fat/kg DM) in a growing high-forage diet reduced enteric CH$_4$ emissions of beef cattle from 25.3 to 21.5 g CH$_4$/kg DM intake, while including 400 g/kg DM WDDGS (41 g fat/kg DM) had no effect on CH$_4$ emissions (23.9 g/kg DM intake; Hünerberg et al., 2012a). In a second study by Hünerberg et al. (2012b), replacing 400
g/kg DM of barley grain with CDDGS (97 g fat/kg DM) in a high-grain finishing diet reduced CH₄ emissions from 16.6 to 13.6 g/kg DM intake; while WDDGS (34 g fat/kg DM) had no effect on enteric CH₄ production (18.4 g CH₄/kg DM intake). Results from both in vivo trials indicate that high-fat CDDGS can effectively reduce CH₄ emissions at dietary inclusion level of 400 g/kg DM. However, it is unknown how CDDGS and WDDGS at inclusion level different from Hünerberg et al. (2012a; 2012b) affect CH₄ production. Measuring in vivo CH₄ production is expensive, labour intensive and time consuming; while in vitro batch culture fermentation is an effective technique to screen CH₄ production of several substrates simultaneously under standardized laboratory conditions (Soliva and Hess, 2007).

The objective of this study was to compare in vitro CH₄ production from CDDGS and WDDGS as these by-products over a range of substitution for whole crop barley silage, and to describe responses of CH₄ and other fermentation parameters to increasing levels of both DDGS types as a substrate.

Materials and methods

Substrates, inoculum and incubation

The substrates used were mixtures of whole crop barley silage and CDDGS or WDDGS in the ratios of 800:200, 600:400, 400:600, 200:800 and 0:1000 (g/kg DM). It has to be acknowledged that DDGS concentrations above 400 g/kg DM to 600 g/kg DM are typically not fed in vivo because of adverse effects on feed intake and animal performance. The levels of DDGS used for this study were chosen to characterize in vitro
CH₄ production and fermentation parameters for a theoretically range of DDGS inclusion level of up to 1000 g/kg DM.

All substrate components were dried separately at 55°C for 24 h and ground through a 1 mm screen (Wiley mill standard model 3, Arthur H. Thomas, Philadelphia, PA, USA) before being combined. The incubation included 5 replications for each DDGS type at each inclusion level. The substrates 0.3 ± 0.005 g were weighed into ANKOM bags (model F57, ANKOM Technology, Macedon, NY, USA) and heat sealed. Bags were placed in 125 ml serum vials 1 day prior to incubation.

Rumen fluid was obtained from two ruminally cannulated non-lactating Holstein cows 2 h after feeding. Cows were fed a high forage diet (650 g/kg whole crop barley silage, 200 g/kg barley grain, 100 g/kg canola meal and 50 g/kg vitamin/mineral supplement; DM basis) ad libitum. Rumen contents were collected from three sites within the rumen (i.e., reticulum and dorsal and ventral sac), thoroughly mixed and squeezed through two layers of PeCAP® polyester 355 μm pore size screen into a preheated and insulated transport bucket. Donor cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Rumen fluid was immediately transferred to the laboratory and re-strained through 4 layers of cheesecloth. Filtrate was maintained at 39°C in a water bath and the headspace continuously flushed with CO₂. Strained rumen fluid (10 ml) was dispensed into pre-warmed 39°C culture flasks, which were preloaded with a substrate filled ANKOM bag, 40 ml of buffer solution and 0.5 ml of cysteine sulfide solution as a reducing agent (Menke et al., 1979). The incubation flasks were sealed with aluminium crimp-sealed rubber stoppers and placed on two rotary shaker platforms (Lab-Line Instruments Inc.,
Melrose Park, IL, USA) oscillating at 90 rpm in an incubator (model 1915, Sheldon Manufacturing, Cornelius, OR, USA) at 39°C. Triplicate flasks containing only rumen fluid and buffer solution were used as blank controls. All flasks were incubated for 24 h.

Gas measurement and sample collection

A pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC, Canada) attached to a 22 gauge (0.6 mm) needle was used to measure gas pressure \( P_t (kPa) \) inside the flasks by inserting the needle into the flasks after 3, 6, 12 and 24 h of incubation. Gas pressures were used to calculated gas production \( G_P (ml) \) using the equation of Mauricio et al. (1999) as:

\[
G_P = 0.18 + (3.697 \times P_t) + (0.0824 \times P_t^2)
\]

Gas production was corrected for the amount of substrate incubated and gas produced from blank controls. After each \( P_t \) measurement, a 15 ml gas sample was collected from each flask using a syringe. The gas sample was then injected into a 5.9 ml evacuated Exetainer (Labco Ltd., High Wycombe, Buckinghamshire, United Kingdom) and analyzed for CH\(_4\). The remaining gas was released from the flask after the gas sample was collected. Gas production (mL/g DM) and CH\(_4\) production per g incubated DM (mg/g DM) or digested DM (mg/g DMD) were summarized and reported for the duration of incubation.

After 24 h of incubation, flasks were opened and the pH of the incubation fluid measured using a pH meter (model Accumet 25, Denver Instrument Company, Arvada, CO, USA). Subsequently, flasks were placed on ice and a 1.6 ml subsample of fluid was removed from the bottle, acidified with 400 \( \mu \)l of metaphosphoric acid (0.25; wt/vol) and
stored at -20°C for analysis of VFA. Bags containing the residual substrate were removed
from the flasks, washed under cold tap water until the water became clear, dried at 55°C
for 48 h and weighed to estimate in vitro DM disappearance (IVDMD).

Laboratory analyses

Methane concentrations were analyzed using a gas chromatograph [GC (model 6890,
Agilent Technologies, Wilmington, DE, USA)] coupled to a thermal conductivity
detector. The correlation coefficients for all standard curves exceeded 99.9%. The VFA
concentrations were determined by GC as described by Holtshausen et al. (2009).
Analytical DM was determined by drying at 135 ºC for 2 h (AOAC, 2005; method
930.15), followed by hot weighing. Organic matter (OM) was calculated as the weight
lost upon ignition at 550°C for 5 h (AOAC, 2005; method 942.05). Crude fat was
determined by ether extraction (method 920.39; AOAC, 1995) using a hot extraction unit
(model E-816 HE, Buchi Labortechnik AG, Flawil, Switzerland). Total N was
determined by combustion analysis (model NA 1500, Carlo Erba Instruments, Milan,
Italy). Neutral detergent fibre (NDF) and acid detergent fiber (ADF) were quantified as
described by Van Soest et al. (1991), using conventional filtration through fritted glass
crucibles, and expressed inclusive of residual ash. Neutral detergent fibre was determined
with inclusion of a heat stable amylase and sodium sulphite. Starch was determined as
described by Rode et al. (1999). Chemical analyses were completed on each sample in
duplicate (Table I).

Statistical analysis
Data were analyzed using the mixed model procedure of SAS (2001). The incubation flask was the experimental unit for all variables. The statistical model was:

\[ y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \]

where: \( y_{ij} \) was the dependant fermentation variable; \( \mu \) the overall mean; \( \alpha_i \) the fixed effect of type of DDGS \( i \) (CDDGS or WDDGS); \( \beta_j \) the fixed effect of DDGS inclusion level \( j \) (200, 400, 600, 800 or 1000 g/kg DM); \( (\alpha\beta)_{ij} \) the interaction of DDGS type \( i \) by inclusion level \( j \); and \( \varepsilon_{ij} \) the residual error term. Denominator degrees of freedom were estimated using the Kenward-Roger option in the model statement. Pre-planned comparisons between CDDGS and WDDGS at the same inclusion level were completed using the contrast statement. Polynomial contrasts were used to determine linear and quadratic responses of dependent variables to increasing level of CDDGS or WDDGS. Data are presented as least squares means ± standard error of means. Differences were declared significant if \( p < 0.05 \).

**Results and Discussion**

The IVDMD (Table II) decreased linearly \( (p < 0.01) \) with increasing levels of CDDGS or WDDGS in the diet, likely attributable to the higher concentrations of EE in CDDGS (115 g EE/kg DM) and WDDGS (49 g EE/kg DM) compared to barley silage (25 g EE/kg DM). Elevated dietary fat levels can depress *in vitro* fibre and OM digestion by exerting toxic effects on protozoa and cellulolytic bacteria (Henderson, 1973), and by limiting microbial attachment to feed particles (McAllister et al., 1994). The depression in IVDMD was higher \( (p < 0.05) \) for CDDGS than for WDDGS at inclusion levels above
400 g/kg DM, which corresponds with the lower \((p<0.05)\) gas production (as ml/kg DM) for CDDGS compared to WDDGS at all inclusion levels.

Production of \(\text{CH}_4\) (mg/g DM) increased \((p<0.05)\) from 5.7 to 10.0 mg \(\text{CH}_4/g\) DM as the concentration of CDDGS increased from 200 to 800 g/kg DM. However, this response is not typical of that observed \textit{in vivo} as increased levels of concentrate in the diet are usually associated with lower \(\text{CH}_4\) emissions per unit feed intake (Johnson and Johnson, 1995). However, it is important to consider that substitution of DDGS for barley silage also results in a substantial change in both the protein content and the nature of the fibre within the mixed substrate. Our results suggest that substitution of DDGS for barley silage results in an increase in the amount \(\text{CH}_4\) produced/g DM fermented.

Methane production (mg) per g/DM and g/DMD from CDDGS was lower \((p<0.05)\) than from WDDGS when DDGS was included at levels of 200 to 800 g/kg, with the difference being more pronounced at lower DDGS inclusion levels. In contrast, \(\text{CH}_4\) production (mg/g DM; mg/g DMD) was similar when WDDGS or CDDGS were the sole substrate incubated. Decreased \(\text{CH}_4\) emissions (mg/g DM; mg/g DMD) from samples containing 200 to 800 g/kg CDDGS as compared to WDDGS likely reflect the higher fat content in CDDGS, which could have lowered OM fermentation and exerted toxic effects on methanogens and protozoa (Czerkawski et al., 1966). Additionally, biohydrogenation of fatty acids in CDDGS may have directed reducing equivalents away from reduction of \(\text{CO}_2\) to \(\text{CH}_4\) formation, as previously described \textit{in vitro} (Jenkins 1987; Getachew et al., 2001).

Total VFA production and proportions of acetate were consistently higher \((p<0.05)\) in samples containing WDGGS compared to CDDGS. Addition of CDDGS increased
propionate proportions at levels of 200, 400 and 600 g/kg DM compared to WDDGS. This resulted in higher (p<0.05) acetate to propionate ratios for WDDGS compared to CDDGS at levels up to 600 g DDGS/kg DM and likely reflects reduced fibrolytic activity (Getachew et al., 2004) with CDDGS. Higher concentrations of propionate and lower acetate to propionate ratios, in batch culture in vitro incubation of 200 g/kg DM CDDGS compared to WDDGS have been reported by others (Au et al., 2010; McKeown et al., 2010). Production of CH₄ and propionate are closely linked since both pathways utilize reducing equivalents. Therefore, increased propionate production in diets containing CDDGS compared to WDDGS may have been responsible for the lower CH₄ concentration at DDGS inclusion rates up to 600 g/kg DM. Culture pH remained above 6.4 in all incubations and was only lower (p<0.05) in WDDGS versus CDDGS at an inclusion level of 200 g/kg DM.

Results of this in vitro study suggest that compared with WDDGS, adding CDDGS to whole crop barley silage at dietary inclusion levels of up to 800 g/kg DM could reduce CH₄ production in vivo. The lower CH₄ production was due to greater reduction in IVDMD/unit CDDGS compared to WDDGS, as well as higher concentrations of propionate when up to 600 g/kg DM CDDGS was included in the diet. These predictions were subsequently confirmed in vivo when WDDGS and CDDGS were included in barley silage-based diets at 400 g/kg DM (Hünerberg et al., 2012a; 2012b).

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References


dried grains with solubles produced from new ethanol plants in Minnesota and South

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neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.
Table I. Chemical composition (g/kg DM) of barley silage, corn and wheat dried distillers’ grains [CDDGS, WDDGS (means ± SD; n=2)].

<table>
<thead>
<tr>
<th></th>
<th>Barley silage</th>
<th>CDDGS</th>
<th>WDDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, g/kg</td>
<td>433 ± 4.3</td>
<td>917 ± 3.2</td>
<td>917 ± 2.8</td>
</tr>
<tr>
<td>Organic matter</td>
<td>921 ± 0.1</td>
<td>965 ± 0.1</td>
<td>937 ± 0.5</td>
</tr>
<tr>
<td>Crude protein</td>
<td>121 ± 1.4</td>
<td>315 ± 2.4</td>
<td>457 ± 1.8</td>
</tr>
<tr>
<td>ADF¹</td>
<td>345 ± 4.2</td>
<td>143 ± 5.0</td>
<td>144 ± 2.7</td>
</tr>
<tr>
<td>NDF²</td>
<td>522 ± 10.5</td>
<td>474 ± 14.1</td>
<td>352 ± 8.5</td>
</tr>
<tr>
<td>Crude fat</td>
<td>25 ± 1.2</td>
<td>115 ± 4.2</td>
<td>49 ± 0.6</td>
</tr>
<tr>
<td>Starch</td>
<td>247 ± 7.9</td>
<td>43 ± 0.4</td>
<td>10 ± 0.2</td>
</tr>
</tbody>
</table>

¹ADF, acid detergent fibre inclusive residual ash.

²NDF, neutral detergent fibre assayed with heat stable amylase and expressed inclusive residual ash.
Table II. Effect of inclusion level of corn or wheat dried distillers’ grains with solubles on *in vitro* dry matter disappearance (IVDMD), gas and CH\textsubscript{4} production, pH and volatile fatty acids (VFA) after 24 h *in vitro* incubation.

<table>
<thead>
<tr>
<th></th>
<th>CDDGS</th>
<th>WDDGS</th>
<th>CDDGS</th>
<th>WDDGS</th>
<th>CDDGS</th>
<th>WDDGS</th>
<th>CDDGS</th>
<th>WDDGS</th>
<th>SEM</th>
<th>Type(^1)</th>
<th>Level(^2)</th>
<th>Type × Level(^3)</th>
<th>L(^4)</th>
<th>Q(^5)</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVDMD, g/kg DM</td>
<td>492.6</td>
<td>508.5</td>
<td>482.9</td>
<td>496.2</td>
<td>446.9</td>
<td>494.5*</td>
<td>429.9</td>
<td>457.6*</td>
<td>9.74</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.16</td>
<td>&lt;0.01</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.42</td>
</tr>
<tr>
<td>Gas, mL/g DM</td>
<td>128.2</td>
<td>177.9*</td>
<td>130.2</td>
<td>183.2*</td>
<td>143.1</td>
<td>180.9*</td>
<td>147.1</td>
<td>174.1*</td>
<td>4.89</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0.02</td>
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<tr>
<td>CH\textsubscript{4}, mg/g DM</td>
<td>5.7</td>
<td>12.5*</td>
<td>7.4</td>
<td>12.4*</td>
<td>8.8</td>
<td>12.2*</td>
<td>10.0</td>
<td>11.5*</td>
<td>0.30</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td>&lt;0.01</td>
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<tr>
<td>pH</td>
<td>6.45*</td>
<td>6.41</td>
<td>6.42</td>
<td>6.41</td>
<td>6.43</td>
<td>6.43</td>
<td>6.44</td>
<td>6.45</td>
<td>0.005</td>
<td>0.03</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.11</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.55</td>
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<tr>
<td>Total VFA, mM</td>
<td>68.3</td>
<td>81.5*</td>
<td>72.1</td>
<td>79.6*</td>
<td>73.4</td>
<td>80.0*</td>
<td>73.5</td>
<td>77.0*</td>
<td>0.85</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.56</td>
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<tr>
<td>VFA, mol/100</td>
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<tr>
<td>Acetate (A)</td>
<td>49.3</td>
<td>51.4*</td>
<td>50.3</td>
<td>51.6*</td>
<td>50.6</td>
<td>52.0*</td>
<td>51.2</td>
<td>51.9*</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.55</td>
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<tr>
<td>Propionate (P)</td>
<td>22.3*</td>
<td>19.4</td>
<td>21.3*</td>
<td>19.4</td>
<td>20.3*</td>
<td>19.5</td>
<td>19.7</td>
<td>19.5</td>
<td>0.14</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.63</td>
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<tr>
<td>Butyrate</td>
<td>18.1</td>
<td>17.9</td>
<td>17.8</td>
<td>17.7</td>
<td>17.8*</td>
<td>17.1</td>
<td>17.6*</td>
<td>17.0</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.41</td>
<td>&lt;0.01</td>
<td>0.52</td>
</tr>
<tr>
<td>A:P ratio</td>
<td>2.21</td>
<td>2.65*</td>
<td>2.36</td>
<td>2.66*</td>
<td>2.50</td>
<td>2.66*</td>
<td>2.60</td>
<td>2.66*</td>
<td>0.022</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.95</td>
</tr>
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</table>

\(^1\)Type = CDDGS or WDDGS.  
\(^2\)Level = 200, 400, 600, 800 and 1000 g/kg DM of DDGS.  
\(^3\)Type × Level = interaction of DDGS type × inclusion level.  
\(^4\)L = linear and  
\(^5\)Q = quadratic effects of different types of DDGS.  
\(^*\)Means within an inclusion level differ at (*; \(p<0.05\)).