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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24 **ABSTRACT:** Methane production from wheat or corn based dried distillers' grains with
25 solubles (CDDGS, WDDGS) was compared *in vitro*. Wheat DDGS (49 g fat/kg DM) or
26 CDDGS (115 g fat/kg DM) partially or completely replaced whole crop barley silage at
27 200, 400, 600, 800 or 1000 g/kg DM. Production of CH_4 increased linearly and
28 quadraticly ($p<0.01$) with increasing levels of CDDGS. Cumulative CH_4 production at 24
29 h was higher ($p<0.05$) for WDDGS (12.0 ± 0.5 mg/g DM) than CDDGS up to 800 g/kg
30 DM. Molar proportions of propionate in incubation fluid were higher ($p<0.05$) for CDDG
31 than for WDDGS at 200, 400 and 600 g/kg DM, respectively. *In vitro* CH_4 production
32 (mg CH_4/g DM; mg CH_4/g DMD) was lower for CDDGS than WDDGS up to 800 g/kg
33 substrate DM. The higher residual oil content in CDDGS compared to WDDGS likely
34 elicited this response.
35 **Keywords: *in vitro*, dried distillers' grains with solubles, methane**
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47 **Introduction**

48 Dried distillers' grains with solubles (DDGS) is a major by-product from the biofuel
49 industry wherein cereal grains are fermented to produce ethanol. As ethanol production
50 has increased considerably in the last decade, large amounts of DDGS are available and
51 predominantly used as feed for ruminant livestock (Klopfenstein et al., 2008). Corn based
52 DDGS (CDDGS) is the most abundant DDGS in the USA whereas in Canada wheat
53 based DDGS (WDDGS) accounts for almost one third of total DDGS production (USDA
54 Foreign Agricultural Service, 2010). As a result of the fermentation process, DDGS is
55 largely starch free, but concentrated three fold in protein, fibre and fat (Spiehs et al.,
56 2002). The fat content is higher in CDDGS (~100 g/kg dry matter [DM]; Spiehs et al.,
57 2002) than in WDDGS (~50 g/kg DM; Gibb et al., 2008) owing to the higher level of fat
58 in corn. Supplementation of ruminant diets with dietary fat reduces ruminal CH_4 through
59 a number of mechanisms including reduction in ruminal DM digestibility, direct effects
60 of fatty acids on ruminal methanogens and protozoa, and by biohydrogenation of
61 unsaturated fatty acids (Czerkawski et al., 1966; Johnson and Johnson, 1995).
62 Additionally, dietary fats often replace fermentable carbohydrates that otherwise would
63 contribute to an increase in the reducing equivalents available to reduce CO_2 to CH_4
64 (Beauchemin et al., 2008).

65 Replacing a mixture of 350 g/kg barley grain and 50 g/kg canola meal (DM basis)
66 by CDDGS (100 g fat/kg DM) in a growing high-forage diet reduced enteric CH_4
67 emissions of beef cattle from 25.3 to 21.5 g CH_4/kg DM intake, while including 400 g/kg
68 DM WDDGS (41 g fat/kg DM) had no effect on CH_4 emissions (23.9 g/kg DM intake;
69 Hünerberg et al., 2012a). In a second study by Hünerberg et al. (2012b), replacing 400

70 g/kg DM of barley grain with CDDGS (97 g fat/kg DM) in a high-grain finishing diet
71 reduced CH_4 emissions from 16.6 to 13.6 g/kg DM intake; while WDDGS (34 g fat/kg
72 DM) had no effect on enteric CH_4 production (18.4 g CH_4/kg DM intake). Results from
73 both *in vivo* trials indicate that high-fat CDDGS can effectively reduce CH_4 emissions at
74 dietary inclusion level of 400 g/kg DM. However, it is unknown how CDDGS and
75 WDDGS at inclusion level different from Hünerberg et al. (2012a; 2012b) affect CH_4
76 production. Measuring *in vivo* CH_4 production is expensive, labour intensive and time
77 consuming; while *in vitro* batch culture fermentation is an effective technique to screen
78 CH_4 production of several substrates simultaneously under standardized laboratory
79 conditions (Soliva and Hess, 2007).

80 The objective of this study was to compare *in vitro* CH_4 production from CDDGS
81 and WDDGS as these by-products over a range of substitution for whole crop barley
82 silage, and to describe responses of CH_4 and other fermentation parameters to increasing
83 levels of both DDGS types as a substrate.

84

85 **Materials and methods**

86 *Substrates, inoculum and incubation*

87 The substrates used were mixtures of whole crop barley silage and CDDGS or WDDGS
88 in the ratios of 800:200, 600:400, 400:600, 200:800 and 0:1000 (g/kg DM). It has to be
89 acknowledged that DDGS concentrations above 400 g/kg DM to 600 g/kg DM are
90 typically not fed *in vivo* because of adverse effects on feed intake and animal
91 performance. The levels of DDGS used for this study were chosen to characterize *in vitro*

92 CH_4 production and fermentation parameters for a theoretically range of DDGS inclusion
93 level of up to 1000 g/kg DM.
94 All substrate components were dried separately at 55°C for 24 h and ground through
95 a 1 mm screen (Wiley mill standard model 3, Arthur H. Thomas, Philadelphia, PA, USA)
96 before being combined. The incubation included 5 replications for each DDGS type at
97 each inclusion level. The substrates 0.3 ± 0.005 g were weighed into ANKOM bags
98 (model F57, ANKOM Technology, Macedon, NY, USA) and heat sealed. Bags were
99 placed in 125 ml serum vials 1 day prior to incubation.
100 Rumen fluid was obtained from two ruminally cannulated non-lactating Holstein
101 cows 2 h after feeding. Cows were fed a high forage diet (650 g/kg whole crop barley
102 silage, 200 g/kg barley grain, 100 g/kg canola meal and 50 g/kg vitamin/mineral
103 supplement; DM basis) *ad libitum*. Rumen contents were collected from three sites within
104 the rumen (*i.e.,* reticulum and dorsal and ventral sac), thoroughly mixed and squeezed
105 through two layers of PeCAP® polyester 355 μm pore size screen into a preheated and
106 insulated transport bucket. Donor cows were cared for in accordance with the guidelines
107 of the Canadian Council on Animal Care (1993).
108 Rumen fluid was immediately transferred to the laboratory and re-strained through 4
109 layers of cheesecloth. Filtrate was maintained at 39°C in a water bath and the headspace
110 continuously flushed with CO_2. Strained rumen fluid (10 ml) was dispensed into pre-
111 warmed 39ºC culture flasks, which were preloaded with a substrate filled ANKOM bag,
112 40 ml of buffer solution and 0.5 ml of cysteine sulfide solution as a reducing agent
113 (Menke et al., 1979). The incubation flasks were sealed with aluminium crimp-sealed
114 rubber stoppers and placed on two rotary shaker platforms (Lab-Line Instruments Inc.,

115 Melrose Park, IL, USA) oscillating at 90 rpm in an incubator (model 1915, Sheldon
116 Manufacturing, Cornelius, OR, USA) at 39°C. Triplicate flasks containing only rumen
117 fluid and buffer solution were used as blank controls. All flasks were incubated for 24 h.
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119 *Gas measurement and sample collection*
120 A pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC,
121 Canada) attached to a 22 gauge (0.6 mm) needle was used to measure gas pressure [P_t
122 (kPa)] inside the flasks by inserting the needle into the flasks after 3, 6, 12 and 24 h of
123 incubation. Gas pressures were used to calculated gas production [G_P (ml)] using the
124 equation of Mauricio et al. (1999) as:
125 $$G_P = 0.18 + (3.697 \times P_t) + (0.0824 \times P_t^2)$$
126 Gas production was corrected for the amount of substrate incubated and gas produced
127 from blank controls. After each P_t measurement, a 15 ml gas sample was collected from
128 each flask using a syringe. The gas sample was then injected into a 5.9 ml evacuated
129 Exetainer (Labco Ltd., High Wycombe, Buckinghamshire, United Kingdom) and
130 analyzed for CH_4. The remaining gas was released from the flask after the gas sample
131 was collected. Gas production (mL/g DM) and CH_4 production per g incubated DM
132 (mg/g DM) or digested DM (mg/g DMD) were summarized and reported for the duration
133 of incubation.
134 After 24 h of incubation, flasks were opened and the pH of the incubation fluid
135 measured using a pH meter (model Accumet 25, Denver Instrument Company, Arvada,
136 CO, USA). Subsequently, flasks were placed on ice and a 1.6 ml subsample of fluid was
137 removed from the bottle, acidified with 400 µl of metaphosphoric acid (0.25; wt/vol) and

138 stored at -20°C for analysis of VFA. Bags containing the residual substrate were removed

139 from the flasks, washed under cold tap water until the water became clear, dried at 55°C

140 for 48 h and weighed to estimate *in vitro* DM disappearance (IVDMD).

141

142 *Laboratory analyses*

143 Methane concentrations were analyzed using a gas chromatograph [GC (model 6890,

144 Agilent Technologies, Wilmington, DE, USA)] coupled to a thermal conductivity

145 detector. The correlation coefficients for all standard curves exceeded 99.9%. The VFA

146 concentrations were determined by GC as described by Holtshausen et al. (2009).

147 Analytical DM was determined by drying at 135 °C for 2 h (AOAC, 2005; method

148 930.15), followed by hot weighing. Organic matter (OM) was calculated as the weight

149 lost upon ignition at 550°C for 5 h (AOAC, 2005; method 942.05). Crude fat was

150 determined by ether extraction (method 920.39; AOAC, 1995) using a hot extraction unit

151 (model E-816 HE, Buchi Labortechnik AG, Flawil, Switzerland). Total N was

152 determined by combustion analysis (model NA 1500, Carlo Erba Instruments, Milan,

153 Italy). Neutral detergent fibre (NDF) and acid detergent fiber (ADF) were quantified as

154 described by Van Soest et al. (1991), using conventional filtration through fritted glass

155 crucibles, and expressed inclusive of residual ash. Neutral detergent fibre was determined

156 with inclusion of a heat stable amylase and sodium sulphite. Starch was determined as

157 described by Rode et al. (1999). Chemical analyses were completed on each sample in

158 duplicate (Table I).

159

160 *Statistical analysis*

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

163
$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

164 where: y_{ij} was the dependant fermentation variable; μ the overall mean; α_i the fixed effect
165 of type of DDGS i (CDDGS or WDDGS); β_j the fixed effect of DDGS inclusion level j
166 (200, 400, 600, 800 or 1000 g/kg DM); $(\alpha\beta)_{ij}$ the interaction of DDGS type i by inclusion
167 level j; and ε_{ij} the residual error term. Denominator degrees of freedom were estimated
168 using the Kenward-Roger option in the model statement. Pre-planned comparisons
169 between CDDGS and WDDGS at the same inclusion level were completed using the
170 contrast statement. Polynomial contrasts were used to determine linear and quadratic
171 responses of dependent variables to increasing level of CDDGS or WDDGS. Data are
172 presented as least squares means ± standard error of means. Differences were declared
173 significant if $p<0.05$.
174

175 **Results and Discussion**

176 The IVDMD (Table II) decreased linearly ($p<0.01$) with increasing levels of CDDGS or
177 WDDGS in the diet, likely attributable to the higher concentrations of EE in CDDGS
178 (115 g EE/kg DM) and WDDGS (49 g EE/kg DM) compared to barley silage (25 g
179 EE/kg DM). Elevated dietary fat levels can depress *in vitro* fibre and OM digestion by
180 exerting toxic effects on protozoa and cellulolytic bacteria (Henderson, 1973), and by
181 limiting microbial attachment to feed particles (McAllister et al., 1994). The depression
182 in IVDMD was higher ($p<0.05$) for CDDGS than for WDDGS at inclusion levels above

183 400 g/kg DM, which corresponds with the lower ($p<0.05$) gas production (as ml/kg DM)

184 for CDDGS compared to WDDGS at all inclusion levels.

185 Production of CH_4 (mg/g DM) increased ($p<0.05$) from 5.7 to 10.0 mg CH_4/g DM as

186 the concentration of CDDGS increased from 200 to 800 g/kg DM. However, this

187 response is not typical of that observed *in vivo* as increased levels of concentrate in the

188 diet are usually associated with lower CH_4 emissions per unit feed intake (Johnson and

189 Johnson, 1995). However, it is important to consider that substitution of DDGS for barley

190 silage also results in a substantial change in both the protein content and the nature of the

191 fibre within the mixed substrate. Our results suggest that substitution of DDGS for barley

192 silage results in an increase in the amount CH_4 produced/g DM fermented.

193 Methane production (mg) per g/DM and g/DMD from CDDGS was lower ($p<0.05$)

194 than from WDDGS when DDGS was included at levels of 200 to 800 g/kg, with the

195 difference being more pronounced at lower DDGS inclusion levels. In contrast, CH_4

196 production (mg/g DM; mg/g DMD) was similar when WDDGS or CDDGS were the sole

197 substrate incubated. Decreased CH_4 emissions (mg/g DM; mg/g DMD) from samples

198 containing 200 to 800 g/kg CDDGS as compared to WDDGS likely reflect the higher fat

199 content in CDDGS, which could have lowered OM fermentation and exerted toxic effects

200 on methanogens and protozoa (Czerkawski et al., 1966). Additionally, biohydrogenation

201 of fatty acids in CDDGS may have directed reducing equivalents away from reduction of

202 CO_2 to CH_4 formation, as previously described *in vitro* (Jenkins 1987; Getachew et al.,

203 2001).

204 Total VFA production and proportions of acetate were consistently higher ($p<0.05$) in

205 samples containing WDGGS compared to CDDGS. Addition of CDDGS increased

206 (p<0.05) propionate proportions at levels of 200, 400 and 600 g/kg DM compared to
207 WDDGS. This resulted in higher (p<0.05) acetate to propionate ratios for WDDGS
208 compared to CDDGS at levels up to 600 g DDGS/kg DM and likely reflects reduced
209 fibrolytic activity (Getachew et al., 2004) with CDDGS. Higher concentrations of
210 propionate and lower acetate to propionate ratios, in batch culture *in vitro* incubation of
211 200 g/kg DM CDDGS compared to WDDGS have been reported by others (Au et al.,
212 2010; McKeown et al., 2010). Production of CH_4 and propionate are closely linked since
213 both pathways utilize reducing equivalents. Therefore, increased propionate production in
214 diets containing CDDGS compared to WDDGS may have been responsible for the lower
215 CH_4 concentration at DDGS inclusion rates up to 600 g/kg DM. Culture pH remained
216 above 6.4 in all incubations and was only lower (p<0.05) in WDDGS *versus* CDDGS at
217 an inclusion level of 200 g/kg DM.

218 Results of this *in vitro* study suggest that compared with WDDGS, adding CDDGS
219 to whole crop barley silage at dietary inclusion levels of up to 800 g/kg DM could reduce
220 CH_4 production *in vivo*. The lower CH_4 production was due to greater reduction in
221 IVDMD/unit CDDGS compared to WDDGS, as well as higher concentrations of
222 propionate when up to 600 g/kg DM CDDGS was included in the diet. These predictions
223 were subsequently confirmed *in vivo* when WDDGS and CDDGS were included in
224 barley silage-based diets at 400 g/kg DM (Hünerberg et al., 2012a; 2012b).

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231 **References**

232 Association of Official Analytical Chemists (AOAC) (2005). Official Methods of
233 Analysis, 18th ed. AOAC International, Gaithersburg, MD, USA.
234 Au, F., McKeown, L. E., McAllister, T. A. & Chaves, A. V. (2010). Fermentation
235 characteristics of corn-, triticale-, and wheat-based dried distillers' grains with
236 solubles in barley-based diets determined using continuous and batch culture systems.
237 *Journal of the Science of Food and Agriculture, 90,* 2074-2082.
238 Beauchemin, K. A., Kreuzer, M., O'Mara, F. & McAllister, T. A. (2008). Nutritional
239 management of enteric methane abatement: a review. *Australian Journal of*
240 *Experimental Agriculture, 48,* 21-27.
241 Canadian Council on Animal Care (CCAC). (1993). Guide to the care and use of
242 experimental animals. Vol. 1. 2nd ed. E. D. Olfert, B. M. Cross, and A. A.
243 McWilliam, ed. CCAC, Ottawa, ON, Canada.
244 Czerkawski, J. W., Blaxter, K. L. & Wainmann, F. W. (1966). The metabolism of oleic,
245 linoleic and linoleic acids by sheep with reference to their effects on methane
246 production. *British Journal of Nutrition, 20,* 349-362.
247 Getachew, G., DePeters, E. J., Robinson, P. H. & Taylor, S. J. (2001). *In vitro* rumen
248 fermentation and gas production: influence of yellow grease, tallow, corn oil and their
249 potasium soaps. *Animal Feed Science and Technology, 93,* 1-15.

250 Getachew, G., Robinson, P. H., DePeters, E. J. & Taylor, S. J. (2004). Relationships
251 between chemical composition, dry matter degradation and in vitro gas production of
252 several ruminant feeds. *Animal Feed Science and Technology, 111,* 57-71.
253 Gibb, D. J., Hao, X. & McAllister, T. A. (2008). Effect of dried distillers grains from
254 wheat on diet digestibility and performance of feedlot cattle. *Canadian Journal of*
255 *Animal Science, 88,* 659-665.
256 Henderson, C. (1973). The effect of fatty acids on pure cultures on rumen bacteria. *The*
257 *Journal of Agricultural Science (Cambridge), 81,* 107-112.
258 Holtshausen, L., Chaves, A.V., Beauchemin, K. A., McGinn, S. M., McAllister, T. A.,
259 Odongo, N. E., Cheeke, P. R. & Benchaar, C. (2009). Feeding saponin-containing
260 Yucca schidigera and Quillaja saponaria to decrease enteric methane production in
261 dairy cows. *Journal of Dairy Science, 92,* 2809-2821.
262 Hünerberg, M., McGinn, S. M., Beauchemin, K. A., Okine, E. K., Harstad, O. M. &
263 McAllister, T. A. (2012a). Use of dried distillers' grains with solubles to reduce
264 enteric methane emissions from growing beef cattle. GHG seminar NJF 453.
265 November 5-6, 2012, Oslo, Norway.
266 Hünerberg, M., McGinn, S. M., Beauchemin, K. A., Okine, E. K., Harstad, O. M. &
267 McAllister, T. A. (2012b). Effect of dried distillers' grains plus solubles on enteric
268 methane emissions and nitrogen excretion from finishing beef cattle. GHG seminar
269 NJF 453. November 5-6, 2012, Oslo, Norway.
270 Jenkins, T.C. (1987). Effect of fats and fatty acid combinations on ruminal fermentation
271 in semi continuous *in vitro* cultures. *Journal of Animal Science, 64,* 1526-1532.

272 Johnson, K. A. & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of*
273 *Animal Science, 73,* 2483-2492.
274 Klopfenstein, T. J., Erickson, G. E. & Bremer, V. R. (2008). Use of distillers by-products
275 in the beef cattle feeding industry. *Journal of Animal Science, 86,* 1123-1231.
276 Mauricio, R. M., Mould, F. L., Dhanoa, M. S., Owen, E., Channa, K. S. & Theodorou, M.
277 K. (1999). A semi-automated in vitro gas production technique for ruminant feedstuff
278 evaluation. *Animal Feed Science and Technology, 79,* 321-330.
279 McAllister, T. A., Bae, H. D., Jones, G. A. & Cheng, K. J. (1994). Microbial attachment
280 and feed digestion in the rumen. *Journal of Animal Science, 72,* 3004-3018.
281 McKeown, L. E., Chaves, A.V., Oba, M., Dugan, M. E. R., Okine, E. & McAllister, T. A.
282 (2010). Effects of corn-, wheat- or triticale dry distillers' grains with solubles on in
283 vitro fermentation, growth performance and carcass traits of lambs. *Canadian Journal*
284 *of Animal Science, 90,* 99-108.
285 Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W. (1979).
286 The estimation of the digestibility and metabolizable energy content of ruminant
287 feedingstuffs from the gas production when they are incubated with rumen liquor *in*
288 *vitro*. *Journal of Agricultural Science (Cambridge), 93,* 217-222.
289 Rode, L. M., Yang, W. Z. & Beauchemin, K. A. (1999). Fibrolytic enzyme supplements
290 for dairy cows in early lactation. *Journal of Diary Science, 82,* 2121-2126.
291 SAS Institute. (2001). System for Windows Release 9.1. SAS Inst. Inc., Cary, NC, USA.
292 Soliva, C. R. & Hess H. D. (2007) Measuring methane emissions of ruminants by in vitro
293 and in vivo techniques. In Measuring Methane Production from Ruminants; Makkar,
294 H. P. S., Vercoe, P. E., (Eds.), Springer Netherlands, 15-32.

295 Spiehs, M. J., Whitney, M. H. & Shurson, G. C. (2002). Nutrient database for distiller's
296 dried grains with solubles produced from new ethanol plants in Minnesota and South
297 Dakota. *Journal of Animal Science, 80,* 2639-2645.
298 USDA Foreign Agricultural Service. (2010). Canada: Annual Biofuels. Global
299 Agricultural Information Network GAIN Report Number: CA0023. [Online]
300 Available: http://gain.fas.usda.gov/Pages/Default.aspx [2012 Aug. 27].
301 Van Soest, P.J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber,
302 neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.
303 *Journal of Dairy Science, 74,* 3583-3597.

304 **Table I.** Chemical composition (g/kg DM) of barley silage, corn and wheat dried distillers' grains [CDDGS, WDDGS (means ± SD; n=2)].

	Barley silage	CDDGS	WDDGS
Dry matter, g/kg	433 ± 4.3	917 ± 3.2	917 ± 2.8
Organic matter	921 ± 0.1	965 ± 0.1	937 ± 0.5
Crude protein	121 ± 1.4	315 ± 2.4	457 ± 1.8
ADF[1]	345 ± 4.2	143 ± 5.0	144 ± 2.7
NDF[2]	522 ± 10.5	474 ± 14.1	352± 8.5
Crude fat	25 ± 1.2	115 ± 4.2	49 ± 0.6
Starch	247 ± 7.9	43 ± 0.4	10 ± 0.2

305 [1]ADF, acid detergent fibre inclusive residual ash.

306 [2]NDF, neutral detergent fibre assayed with heat stable amylase and expressed inclusive residual ash.

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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_4 production, pH and volatile fatty acids (VFA) after 24 h *in vitro* incubation.

	Dried distillers' grains with solubles, g/kg DM											p-values[6]						
	200		400		600		800		1000		Pooled				CDDGS		WDDGS	
	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	SEM	Type[1]	Level[2]	Type × Level[3]	L[4]	Q[5]	L	Q
IVDMD, g/kg DM	492.6	508.5	482.9	496.2	446.9	494.5*	429.9	457.6*	392.2	445.7*	9.74	<0.01	<0.01	0.16	<0.01	0.23	<0.01	0.42
Gas, mL/g DM	122.8	177.9*	130.2	183.2*	143.1	180.9*	147.1	174.1*	146.0	162.0*	4.89	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.02
CH_4, mg/g DM	5.7	12.5*	7.4	12.4*	8.8	12.2*	10.0	11.5*	9.9	9.5	0.30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CH_4, mg/g DMD	10.9	23.3*	14.5	23.5*	18.7	23.4*	22.0	23.8*	21.6	20.2	0.74	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	0.02
pH	6.45*	6.41	6.42	6.41	6.43	6.43	6.44	6.45	6.45	6.45	0.005	0.03	<0.01	<0.01	0.11	<0.01	<0.01	0.55
Total VFA, mM	68.3	81.5*	72.1	79.6*	73.4	80.0*	73.5	77.0*	73.7	75.8*	0.85	<0.01	0.16	<0.01	<0.01	<0.01	<0.01	0.56
VFA, mol/100																		
Acetate (A)	49.3	51.4*	50.3	51.6*	50.6	52.0*	51.2	51.9*	51.4	52.2*	0.13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.55
Propionate (P)	22.3*	19.4	21.3*	19.4	20.3*	19.5	19.7	19.5	19.4	19.7	0.14	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	0.63
Butyrate	18.1	17.9	17.8	17.7	17.8*	17.1	17.6*	17.0	17.5*	16.4	0.09	<0.01	<0.01	<0.01	<0.01	0.41	<0.01	0.52
A:P ratio	2.21	2.65*	2.36	2.66*	2.50	2.66*	2.60	2.66†	2.64	2.65	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	0.95	0.62

[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.

[6]Means within an inclusion level differ at (*; $p<0.05$).