Short Title: Methane and N excretion of finishing cattle fed DDGS

Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle

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Abstract

Hünerberg, M., McGinn, S. M., Beauchemin, K. A., Okine, E. K., Harstad, O. M., and McAllister, T. A. 2012. **Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle.** Can. J. Anim. Sci. The objective of this study was to examine the impact of corn- or wheat-based dried distillers' grains with solubles (CDDGS, WDDGS) on enteric methane (CH_4) emissions from finishing beef cattle, and to determine if any observed reductions were a result of the fat content of CDDGS. A second objective was to compare the effect of CDDGS or WDDGS on N excretion. The experiment was designed as replicated 4 × 4 Latin square with 28-d periods using 16 ruminally fistulated crossbred heifers. The control diet contained 87% barley grain, 8% barley silage and 5% supplement (dry matter; DM basis). Treatment diets were formulated by replacing 40% DM of barley grain with CDDGS, WDDGS, or corn oil supplemented WDDGS (WDDGS+oil). For the WDDGS+oil diet 6.5% corn oil was added to WDDGS (3.4% fat DM) to achieve a similar fat level as in CDDGS (9.7% DM). All diets were fed as total mixed rations once daily *ad libitum*. Total collection of urine and feces was conducted between day 18 and 21. Methane was measured between day 25 and 28 using four identical open circuit respiratory chambers. Compared with WDDGS, feeding CDDGS and WDDGS+oil reduced ($P < 0.05$) CH_4 emissions as a percentage of gross energy intake (GEI) from 5.5 to 4.0 and 4.2%, respectively. Feeding CDDGS also reduced ($P < 0.05$) CH_4 emissions compared to the control (5.0% of GEI), while WDDGS+oil tended ($P = 0.08$) to elicit a similar response. Methane (% of GEI) between WDDGS and the control did not differ ($P = 0.29$). Excretion of total N was greater ($P <0.001$) for CDDGS, WDDGS and WDDGS+oil (220, 253, and 265 g d^{-1}) compared with the control (143 g d^{-1}). Although oil appears to be responsible for reducing CH_4 emissions when DDGS is

included in the diet, increased N excretion requires that a complete life cycle assessment be conducted to assess the full impact of DDGS on greenhouse gas emissions from finishing cattle.

Key words: beef cattle, dried distillers' grains with solubles, methane, nitrogen excretion

Abbreviations: **AUC**, area under the curve; **ADF**, acid detergent fibre; **ADFD**, total tract digestibility of acid detergent fibre; **ADIN**, acid detergent insoluble nitrogen; **ARA**, acute ruminal acidosis; **BW**, body weight; **CDDGS**, corn-based dried distillers' grains with solubles; **CH_4**, methane; **control**, control diet; **CP**, crude protein; **CPD**, apparent total tract digestibility of crude protein; **DDGS**, dried distillers' grains with solubles; **DE**, digestible energy; **DM**, dry matter; **DMD**, apparent total tract digestibility of dry matter; **DMI**, dry matter intake; **GE**, gross energy; **GEI**, gross energy intake; **GHG**, greenhouse gas; **NDF**, neutral detergent fibre; **NDFD**, total tract digestibility of neutral detergent fibre; **OM**, organic matter; **OMD**, apparent total tract digestibility of organic matter; **PUN**, plasma urea nitrogen; **SARA**, sub-acute ruminal acidosis; **SD**, standard deviation; **VFA**, volatile fatty acids; **WDDGS**, wheat-based dried distillers' grains with solubles; **WDDGS+oil**, diet containing wheat-based dried distillers' grains with solubles and corn oil

INTRODUCTION

Greenhouse gas (**GHG**) emissions in the form of enteric methane (**CH_4**) as well as direct and indirect N_2O along with N losses in the form of NH_3, NO_3^- and NO_2^- are major environmental concerns arising from ruminant production (FAO 2006). Dried distillers' grains with solubles (**DDGS**) is a co-product of grain based fuel ethanol production and is used as a source of protein as well as energy in ruminant diets. As the majority of starch in the original grain is fermented to ethanol, the remaining nutrients in DDGS (fibre, crude protein [**CP**], fat and minerals) are concentrated about three fold over that in the original grain (Spiehs et al. 2002). Depending on inclusion level, the chemical composition of diets containing DDGS can differ substantially from grain diets for finishing beef cattle, supplying less starch and more CP, fibre and fat.

Incorporating corn-based DDGS (**CDDGS**) in high-forage growing diets effectively reduces CH_4 emissions (McGinn et al. 2009; Hünerberg et al. 2013). Replacing barley grain (35% dry matter [**DM**]) basis) with CDDGS **(**12.7% fat DM) in a high-forage diet (60% barley silage, DM basis) decreased enteric CH_4 in growing beef cattle from 7.1 to 5.4% of gross energy (**GEI**) (McGinn et al. 2009). Similarly, we observed a reduction in CH_4 emission from 7.8 to 6.6% of GEI when 35% barley grain and 5% canola meal DM were replaced with CDDGS (10.0% fat DM) in a high-forage diet (55% barley silage, DM basis) for growing beef cattle (Hünerberg et al. 2013). However, inclusion of 40% DM wheat-based DDGS (**WDDGS**; 4.1% fat DM) had no effect on CH_4 emissions (7.3% of GEI) in this study. In contrast to WDDGS alone, inclusion of 40% DM corn oil supplemented WDDGS (9.5% fat DM) reduced CH_4 emissions (6.3% of GEI) to the same extent as CDDGS, confirming that the oil in CDDGS was likely responsible for the reduction in CH_4 (Hünerberg et al. 2013). Although the inclusion of

CDDGS reduced CH_4 emissions, it increased total N-excretion in heifers from 170 to 206 g d^{-1} (Hünerberg et al. 2013). Methane emissions, as % of GEI or per kg of DM intake respectively, are lower for cattle fed high concentrate finishing diets as compared to high forage growing diets (Johnson and Johnson 1995). It is not known if CDDGS elicits a further reduction in CH_4 emissions in finishing cattle with comparatively low CH_4 emissions. Furthermore, N retention in finishing cattle is lower than in growing cattle, likely augmenting the negative environmental consequences associated with high levels of N excretion in cattle fed DDGS diets.

The objective of this study was to examine the effect of CDDGS and WDDGS on enteric CH_4 emissions and N excretion from finishing beef cattle. It was hypothesized that CDDGS mediated reductions in CH_4 emissions were attributable to its oil content, consequently corn oil was added to WDDGS to determine if this practice resulted in a similar reduction in CH_4 emissions.

MATERIAL AND METHODS

This experiment was conducted using the Metabolism Barn Unit and the Controlled Environment Facility at Agriculture and Agri-Food Canada's Research Center in Lethbridge, AB, Canada. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Animals and Experimental Design

Sixteen crossbreed beef heifers (529.1 ± 41.1 kg of initial body weight [**BW**]) were used in a replicated 4 × 4 Latin square with four 28-d periods, and four dietary treatments. Heifers were

paired, such that each pair had similar BW and pairs of heifers were randomly allocated between squares. The four pairs within each square were randomly allocated to one of four treatment diets. Methane was measured using four open circuit respiratory chambers with each chamber housing two heifers. Periods were staggered by one week between square 1 and 2 as only four chambers were available at a time. The pairing of heifers was consistent throughout the experiment, such that heifers within a chamber received the same treatment. All heifers were ruminally cannulated and ovariectomized. Heifers were gradually transitioned over 4 weeks from a growing diet containing 55% DM barley silage to a finishing diet containing 8% DM barley silage.

At the beginning of each period, day 1–7 were used to transition the heifers from their previous diet to the new diet. Starting on d 8 all heifers received their intended experimental diet. Apparent total tract digestibility of nutrients and excretion of N was determined from day 18 to 21 using the eight heifers in square 1 (534.9 ± 36.5 kg of initial BW). Rumen contents of all heifers were sampled on day 21. From d 1 to 24, heifers were housed in tie stalls in the metabolism unit with individual access to feed and water. Before the morning feeding on day 25, heifers were moved to the controlled environment facility to measure CH_4 over 4 d. Except for the periods during the measurement of digestibility and CH_4 emissions, heifers were given daily exercise in an open dry lot.

Diets and Feed Sampling

High-concentrate diets were formulated to have a composition that is typical of that fed to feedlot cattle in western Canada prior to slaughter. The control diet (**control**) contained (DM basis) 8% whole crop barley silage, 87% steam rolled barley grain, 3.4% ground barley as a

carrier for a 1.6% vitamin and mineral supplement (Table 1). The three diets containing DDGS were formulated by replacing 40% of barley grain DM with CDDGS, WDDGS, or WDDGS plus corn oil (**WDDGS+oil**). For the WDDGS+oil diet, 6.5% of corn oil (Great Value; Wal-Mart, ON, Canada) was added per kg DM of WDDGS (3.4% fat DM) to achieve a fat level similar to CDDGS (9.7% fat DM). The inclusion level of 40% DDGS (DM basis) was chosen to reflect the usage of DDGS as an energy source and was within a range shown to have no negative impact on the growth performance of finishing cattle (Klopfenstein et al., 2008; Gibb et al., 2008). Heifers were fed once daily at 1100 h for *ad libitum* intake (5% refusal, as fed basis). The weight of feed offered and refused was recorded daily throughout the study.

Diets and ingredients were sampled weekly and analyzed for DM content by drying at 55°C for 48 h. Diets were adjusted if the DM content of barley silage deviated more than 3.0% from the average. Weekly subsamples of diets and ingredients were composited by period. Orts were sampled daily during the digestibility trial and CH_4 measurements and pooled by heifer at the end of each period. Samples were stored at −20°C until further analysis.

Nutrient Digestibility and Nitrogen Excretion

To ensure complete separation of urine and feces, the eight heifers in square 1 were fitted with urinary indwelling balloon catheters (Bardex® Lubricath® Foley catheter, 75 c.c. and 26 Fr.; Bard Canada Inc., Oakville, ON, Canada). Urine was acidified (pH < 2) with 4 N H_2SO_4 to prevent volatilization of NH_3. Total output of feces was collected using rubber mats positioned behind the heifers. Total output of urine and feces was measured every 24 h over 4 d. Urine samples were pooled (1% total volume) for each animal within period and a sub-sample (20 ml) was diluted with distilled water at a ratio of 1:5 at the end of each period and stored at −20°C until

analyzed. A daily sub-sample of the feces from each animal (~500 g) was oven-dried at 55°C. At the end of the digestibility experiment, a representative composite sample from each heifer for each period was obtained by pooling the dried daily fecal samples based on their original DM content.

Ruminal Fermentation Measurements, Ruminal pH and Blood Sampling

Rumen samples were collected by sampling ~500 g of rumen content from the reticulum, dorsal and ventral sac of each heifer at 0, 2, 6, 12 and 24 h after feeding. Samples from each site were mixed and squeezed through 2 layers of polyester monofilament fabric (pore size 355 μm; B. & S. H. Thompson, Ville Mont-Royal, QC, Canada) and pH of the filtrate measured (Accumet model 25; Cole-Parmer Canada Inc, Montreal, QC, Canada) immediately. Filtrate (5 mL) was mixed with 1 mL of 25% (w/v) HPO_3 for volatile fatty acids (**VFA**) and lactate analysis and with 1 mL of 1% (w/v) H_2SO_4 for NH_3 analysis. Both samples were stored at −20 ◦C until analyzed. For enumeration of ruminal protozoa, filtrate (5 mL) was mixed with 5 mL of methyl green-formalin-saline solution. The samples were stored at room temperature in the dark until examined.

Ruminal pH was recorded every min between d 25 and 28 using the LRCpH data logger system (Dascor, Escondido, CA, USA). Loggers were calibrated using buffers at pH 4 and 7 at the start and end of each measurement period. Probes were placed in the ventral sac of the rumen 2 h before the heifers entered the chambers on day 25 and removed immediately after they were returned to the metabolism unit on day 28. Ruminal pH data were summarized by day as average, minimum, maximum, and standard deviation (**SD**) of mean pH, and as duration below and area under the curve (**AUC**) for threshold values of pH 5.5 and 5.2. The AUC was calculated

as the sum of the absolute value of pH deviations below pH 5.5 or 5.2 multiplied by the duration below pH 5.5 or 5.2, and reported in pH × min. Durations and AUC for pH 5.5 and 5.2 were considered indicative of sub-acute (**SARA**) and acute ruminal acidosis (**ARA**), respectively (Penner et al. 2007). Intake corrected AUC was calculated as AUC divided by DM intake (**DMI**).

Blood samples for the determination of plasma urea nitrogen (**PUN**) were collected into 10-mL vacuum tubes containing Li-heparin solution (Vacutainer, Becton Dickinson, Mississauga, ON, Canada) by jugular vein puncture on day 28 of each period 30 min before feeding time. Blood was centrifuged (3,000 × g at 4°C for 20 min) and plasma was collected and stored at −20°C until analyzed.

Methane Emission Measurements

Four identical open circuit respiratory chambers (4.4 m wide × 3.7 m deep × 3.9 m tall, 63.5 m^3 volume, C1330, Conviron Inc., Winnipeg, MB, Canada) were used to measure CH_4 emissions from each pair of heifers over four consecutive days. Two heifers in each chamber were placed in individual tie stalls equipped with rubber mats. Both heifers within each chamber had free access to feed and water. The chambers were vented using fresh-air intakes and chamber exhaust ducts with dedicated fans for each individual duct. The air volume of each chamber was exchanged every 5 min. Air temperature within the chambers was maintained at 10°C throughout the experiment. Air from the fresh-air intake and exhaust air duct of each chamber was sampled sequentially for 3 min each, every 27 min, by pumping 1 L min^{-1} (TD3LS7; Brailsford and Company, Rye, NY, USA) through a common infrared gas analyzer (Ultramat 6; Siemens, Karlsruhe, Germany) via a set of solenoids controlled by a data logger (CR23X;

Campbell Scientific, Logan, UT, USA). Before entering the gas analyzer the air stream was dried using magnesium perchlorate. After each 24 min cycle (8 ducts × 3 min each), pure N_2 gas was introduced into the gas analyzer for 3 min. Data generated during this time period was used to account for any drift in the analyzer between measurement cycles. The difference in the concentration of CH_4 between the incoming and outgoing flow in the fresh-air intake and exhaust duct respectively, was used to calculate the amount of CH_4 produced by each pair of heifers within each chamber as described by Beauchemin and McGinn (2006). Air velocity in each intake and exhaust duct was continuously monitored (model 8455 Air Velocity Transducer, TSI Inc., Shoreview, MN, USA). Air flow rates in the ducts were adjusted to generate a slight positive pressure (approximately 2 Pa) inside each chamber (model 265 Pressure Sensor; Setra, Boxborough, MA, USA). The chambers were opened once daily at 1100 h for cleaning and to feed the heifers. Methane emission data corresponding to the door opening times (~30 min d^{-1} to clean and feed all four chambers) as well as the time needed for each chamber to reequilibrate (15 min after the door was closed) were omitted from the analysis. The gas analyzer was calibrated daily, immediately after feeding using N_2 as zero and 405 mg kg^{-1} of CH_4 as span standard gases. The system was calibrated before the beginning of the experiment by sequentially releasing 0, 0.1, 0.2 and 0.3 L min^{-1} of CH_4 separately into each empty chamber using a mass-flow meter (Omega Engineering, Stamford, CT, USA). The slopes of the best fit four point regressions (actual against calculated CH_4 emission) were used to correct for variability between chambers. The r^2 value of this four point regression exceeded 0.99 in all four chambers.

Laboratory Analyses

Samples of composited ingredients, diets, orts and feces were oven dried at 55°C and ground through a 1 mm screen (Cutting Mill SM100; Retsch, Haan, Germany). Analytical DM was determined by drying at 135°C for 2 h (AOAC, 2005; method 930.15), followed by hot weighing. Organic matter (**OM**) was calculated as the difference between 100 and the percentage of ash (AOAC, 2005; method 942.05). Neutral detergent fibre (**NDF**) and acid detergent fibre (**ADF**), both expressed inclusive of residual ash were quantified as described by Van Soest et al. (1991) using amylase and sodium sulfite for the NDF analysis. Fat was determined according to AOAC (2006; method 2003.05) using ether extraction (Extraction Unit E-816 HE; Büchi Labortechnik AG, Flawil, Switzerland). Gross energy (**GE**) in diets, orts and feces was determined using a bomb calorimeter (model E2k; CAL2k, Johannesburg, South Africa). For the measurement of CP (N × 6.25) and starch, ground samples were reground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). Total urinary N was analyzed in the same fashion using freeze dried urine. Ball ground ADF residues of CDDGS and WDDGS were analysed for N to determine acid detergent insoluble N (**ADIN, Table 2**). Starch content of the diets was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Rode et al. (1999).

Concentrations of VFA and lactate in ruminal fluid were quantified using gas chromatography (model 5890, Hewlett Packard, Wilmington, DE, USA) with a capillary column (30 m × 0.32 mm × 1 μm; ZB-FFAP, Phenomenex Inc., Torrance, CA, USA) and flame ionization detection. Internal standards were crotonic acid for VFA and malonic acid for lactate analysis. Lactate samples were methylated with BF_3-MeOH (10% w/w) prior to GC analysis. Concentration of NH_3 in urine and rumen fluid was determined by the salicylate-nitroprusside-

253 hypochlorite method (Sims et al. 1995) using a flow injection analyzer (Technicon Autoanalyzer
254 II, Technicon Instruments, Tarrytown, NY, USA). Concentration of urea in urine and blood
255 plasma was analyzed using micro-Segmented Flow Analysis (model Astoria2; Astoria Pacific
256 Inc., Clackamas, OR, USA). Ruminal protozoa were enumerated under a light microscope as
257 described by Ogimoto and Imai (1981) using a counting chamber (Neubauer Improved Bright-
258 Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA, USA). Duplicate
259 preparations of each sample were counted. If values differed from the average by more than
260 10%, a third sample was enumerated.
261
262 **Calculations and Statistical Analyses**
263 The data were analyzed using a Mixed procedure (SAS 2001). Heifer was the
264 experimental unit for intake, digestibility, N excretion, and ruminal fermentation variables as
265 these data were obtained from individual heifers. For ruminal fermentation variables, the model
266 included the fixed effect of diet and the random effects of square, heifer nested within square,
267 and period nested within square. For ruminal fermentation variables sampling time (0, 2, 6, 12
268 and 24 h after feeding) was treated as a repeated measure. Protozoa data were $\log_{10}$-transformed
269 prior to statistical analysis. Data for N excretion and total tract digestibility were analyzed using
270 the same model, but without the random effect of square because only square1 heifers were used
271 and sampling day (1-4) was treated as a repeated measure. The chamber, representing data from
272 two heifers, was the experimental unit for CH_4 measurements. Cumulative daily CH_4 emission
273 from each chamber was calculated for each of the 4 d within each period. Methane emission was
274 expressed per unit of DMI and as a proportion of gross energy (**GE**) intake and digestible energy
275 (**DE**) intake of the two heifers within the chamber on that same day. The GE content of CH_4 was

assumed to be 55.6 MJ kg^{-1}. Methane as % of DE was calculated based on the diet specific GE digestibility values determined between day 18 and 21 shown in (Table 5). The model used for CH_4 production variables included the fixed effect of diet and the random effects of square, period nested within square, and chamber nested within square. Day of sampling (day 1-4) within each period was treated as a repeated measure. For all analyses, the best time series covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Denominator degrees of freedom were estimated using the Kenward-Roger option in the model statement. The PDIFF option adjusted by the Tukey method was included in the lsmeans statement to enable multiple comparisons. Treatment effects were declared significant at $P < 0.05$, and trends were discussed at $P < 0.10$.

RESULTS

Ruminal Fermentation and pH

Feeding CDDGS, WDDGS or WDDGS+oil as compared to the control diet decreased ($P < 0.05$) the concentration of total VFA in rumen fluid (Table 3). In addition, feeding CDDGS decreased the concentration of acetate compared to the control; while feeding WDDGS resulted in lower concentration of propionate compared to the control. Concentrations of butyrate, isovalerate, valerate, isobutyrate as well as the acetate:propionate ratio were similar among treatments ($P > 0.10$). Feeding WDDGS or WDDGS+oil compared to the control or CDDGS resulted in a higher ($P < 0.001$) ruminal concentration of NH_3 and an increase ($P < 0.05$) in total numbers of rumen protozoa.

297 The mean ruminal pH of heifers fed WDDGS+oil was higher ($P < 0.05$) than for those
298 offered the control diet, but was below 6.0 in all diets (Table 4). Feeding CDDGS, WDDGS and
299 WDDGS+oil resulted in higher ($P < 0.05$) minimum pH as compared to the control diet, whereas
300 heifers fed WDDGS or WDDGS+oil had lower ($P < 0.05$) SD from the daily mean pH as
301 compared to those fed the control diet. Additionally, feeding heifers CDDGS, WDDGS and
302 WDDGS+oil reduced the time below a pH threshold of 5.5 (SARA; $P < 0.05$) and 5.2 (ARA; $P <$
303 0.05) and a decreased AUC expressed as pH × min d^{-1} at pH 5.5 ($P < 0.05$) and 5.2 ($P < 0.001$)
304 as compared to those fed the control diet. In contrast, the AUC adjusted for DMI (kg DMI^{-1}) was
305 lower ($P < 0.05$) for heifers offered CDDGS and WDDGS+oil as compared to heifers fed the
306 control diet.

307

308 **Apparent Total Tract Digestibility**

309 The DMI of heifers fed WDDGS was 13.7% lower ($P < 0.01$) than those fed the control diet
310 (Table 5), resulting in lower ($P < 0.05$) OM and GE intake for heifers fed WDDGS as compared
311 to the control. Intake of DM, OM and GE among heifers fed the three diets containing DDGS
312 was similar. Feeding CDDGS, WDDGS and WDDGS+oil as compared to the control diet
313 resulted in higher ($P < 0.001$) intakes of CP and N (Table 6), with these levels being higher ($P <$
314 0.05) in diets containing WDDGS than CDDGS. Feeding WDDGS+oil resulted in highest ($P <$
315 0.05) intake of NDF, while ADF intake of heifers offered CDDGS, WDDGS and WDDGS+oil
316 was higher ($P < 0.05$) compared to the control diet. Feeding CDDGS, WDDGS and WDDGS+oil
317 reduced the apparent total tract digestibility of DM (**DMD**; $P < 0.001$), OM (**OMD**; $P < 0.05$)
318 and GE ($P < 0.05$) as compared to the control diet. The addition of corn oil in the WDDGS+oil

diet resulted in a further reduction ($P < 0.05$) of DMD, OMD, DE and CP (**CPD**) as compared to CDDGS and WDDGS. Total tract digestibility of NDF (**NDFD**) was similar among all diets. In contrast, total tract digestibility of ADF (**ADFD**) in heifers fed CDDGS and WDDGS was greater compared to those fed WDDGS+oil or the control diet ($P < 0.001$).

Nitrogen Excretion

Feeding WDDGS and WDDGS+oil resulted in greater ($P < 0.05$) total N intake and excretion (both g d^{-1}) compared to CDDGS, while feeding diets containing DDGS increased N intake and excretion ($P < 0.001$) compared to the control (Table 6). Consequently, feeding diets containing DDGS increased fecal ($P < 0.05$) as well as urinary N excretion (both g d^{-1}; $P < 0.001$) compared to the control diet. In addition, heifers fed WDDGS+oil excreted more fecal N ($P < 0.05$) than those fed CDDGS or WDDGS while both diets containing WDDGS exhibited higher ($P < 0.05$) urinary N excretion as compared to CDDGS. When the loss of N in feces or urine was expressed as percentage of total N excretion, heifers offered CDDGS and WDDGS excreted less ($P < 0.05$) N through feces, but more ($P < 0.05$) through urine compared to those fed the control and WDDGS+oil diet. Excretion of urea N (g d^{-1}), NH_3 N output (g d^{-1}) as well as PUN (mg dL^{-1}) of heifers fed diets containing DDGS were substantially higher ($P < 0.001$) compared to heifers fed the control diet. Additionally, feeding WDDGS alone or WDDGS+oil resulted in higher daily excretion of urea N ($P < 0.05$) over CDDGS and NH_3-N losses from heifers offered WDDGS were higher ($P < 0.05$) compared to those fed WDDGS+oil.

Methane Emissions

In contrast to the digestibility trial, where differences in DMI between WDDGS and control diet were measured, DMI during the period of CH_4 measurement was similar among diets (Table 7). Feeding CDDGS or WDDGS+oil reduced ($P < 0.05$) CH_4 emission (g d^{-1}) compared to WDDGS alone by 17.5 and 14.3%, respectively. Methane emissions (g d^{-1}) of heifers fed diets containing WDDGS did not differ from those offered the control diet. The reduction in CH_4 emission of heifers fed CDDGS ($P = 0.001$) or WDDGS+oil ($P = 0.006$) compared to heifers fed WDDGS was still evident when expressed as g kg^{-1} of DMI. Adjusting for numerical differences in DMI resulted in lower ($P < 0.05$) CH_4 emissions g kg^{-1} DMI for heifers fed CDDGS compared to those offered WDDGS alone or the control diet. Feeding CDDGS also reduced ($P < 0.05$) CH_4 emissions expressed as % of GE intake compared to WDDGS alone and the control diet, while there was also a trend ($P = 0.08$) for a similar response with WDDGS+oil. When corrected for differences in DE intake, heifers fed CDDGS produced less ($P < 0.001$) CH_4 than those fed WDDGS. Furthermore, feeding WDDGS tended to increase ($P = 0.06$) CH_4 emissions (% of DE intake) as compared to the control, while feeding CDDGS tended ($P = 0.08$) to decrease it. Heifers fed CDDGS or WDDGS+oil had similar CH_4 emissions, regardless of how emissions were expressed. Addition of corn oil to the WDDGS+oil diet reduced CH_4 emissions expressed as g kg^{-1} of DMI ($P = 0.006$), % of GE intake ($P = 0.002$) and % of DE intake ($P = 0.009$) as compared to WDDGS alone.

DISCUSSION

In the last 10- 15 years, mandatory inclusion of renewable fuel in conventional gasoline has led to exponential growth in grain-based ethanol production in North America. Whereas ethanol

363 production in the United States is almost exclusively from corn, wheat is used to produce 31% of
364 the ethanol in Canada, resulting in yearly production of ~ 1.2 million tonnes of DDGS in Canada
365 from both grains (USDA Foreign Agricultural Service 2010). Due to its high fibre content,
366 DDGS is predominantly utilized as a feed for ruminants, with inclusion being the highest in beef
367 cattle diets (Klopfenstein et al. 2008). Even though DDGS is mainly used as protein source for
368 ruminants, depending on price, DDGS can also serve as an energy source, replacing either grain,
369 silage or both in the diet (Klopfenstein et al. 2008; Li et al. 2011).

370 The nutrient composition of DDGS is largely dependent on grain source. Wheat is higher
371 in CP, but lower in fat (14.2% CP and 2.3% fat DM basis; NRC 2000) than corn (9.8% CP and
372 4.1% fat DM basis; NRC 2000), WDDGS is naturally higher in CP (~40 vs. ~30% DM) and
373 lower in fat (~5 vs. ~10% DM) than CDDGS (Table 2; Gibb et al. 2008; Klopfenstein et al.
374 2008). An inclusion level of 40% DDGS (DM based) was chosen for this study because it is at
375 the high end of the range that has been shown to have no negative impact on growth
376 performance or carcass traits of finishing cattle (Gibb et al. 2008; Klopfenstein et al. 2008). The
377 control diet was typical of the barley-based finishing diets routinely fed to cattle in western
378 Canadian feedlots.

379 The lower DMI of heifers fed WDDGS compared to those fed the control diet, has been
380 previously reported for finishing diets containing > 30% (DM basis) WDDGS (Li et al. 2011;
381 Walter et al. 2012). Addition of oil to the WDDGS alleviated this difference in DMI, suggesting
382 that this response may have been related to the energy density of the diet. Moving the cattle from
383 the metabolism unit into the respiratory chambers reduced DMI of heifers fed the control,
384 CDDGS, WDDGS and WDDGS+oil diets by 16.2, 3.4, 7,5 and 8,1% respectively. As reductions
385 in DMI typically result in increased CH_4 emissions (Blaxter and Clapperton, 1965; Johnson and

386 Johnson, 1995), emissions need to be interpreted with respect to the reduction in DMI caused by
387 the change in housing conditions. However, such reductions in DMI are common in chambered
388 cattle, a response that we attempted to minimize by housing the heifers as pairs within chambers.
389 However, this precaution did not completely alleviate the impact of the change in housing
390 environment on DMI.

391 As expected, higher CP and NDF intake of heifers fed diets containing DDGS compared
392 to the control reflect differences in ingredient composition. The DDGS diets had similar NDF
393 content; therefore higher NDF intake of heifers fed WDDGS+oil as compared to CDDGS and
394 WDDGS must be due to differences in sorting behaviour rather than diet composition. The
395 control diet resulted in the highest starch intake as it contained 55.0% starch (DM basis) as
396 compared to 34.7, 31.9 and 33.2% of starch (DM basis) in the CDDGS, WDDGS and
397 WDDGS+oil diet, respectively. Starch from dry-rolled barley is highly digestible, with 80.7 ±
398 3.9% (mean ± SD) of it being fermented in the rumen and total tract digestibility frequently
399 exceeding 95% (Huntington 1997). Therefore, the lower DMD and OMD of heifers fed DDGS
400 diets may be attributable to their lower starch content as compared to the control diet. Lower
401 DMD and OMD, together with a depression in CPD and ADFD with WDDGS+oil as compared
402 to WDDGS alone may reflect the negative impact of corn oil on rumen fermentation. Despite the
403 fact that total and individual VFA concentrations in the rumen fluid did not differ between
404 WDDGS+oil and WDDGS, we propose that the depression in DM, OM, CP and ADF digestion
405 occurred ruminally rather than post-ruminally. The greater impact on nutrient digestibility of
406 added oil in WDDGS+oil as compared to CDDGS may reflect the impact of extensive
407 processing (e.g. heating and drying) at the ethanol plant on the ruminal activity of oil in CDDGS,
408 although both of these by-products caused a similar reduction in CH_4 emissions. The similar

409 NDFD of the three diets containing DDGS compared to the control is somewhat surprising. The
410 NDFD of DDGS is generally higher than the NDFD of barley grain due to extensive processing
411 prior to ethanol production and possibly as a result of alteration in the digestibility of fiber
412 during the fermentation process (Ham et al. 1994). Consequently, others have reported higher
413 NDFD of finishing diets with up to 40% CDDGS or WDDGS than those containing primarily
414 barley (Li et al. 2011; Walter et al. 2012). The fact that ADFD of heifers fed CDDGS and
415 WDDGS was higher than the control diet in our study, suggests that the processing of the grain
416 feedstock for ethanol production may have a more positive impact on the digestibility of
417 cellulose than hemicellulose.

418 Although cattle fed high-concentrate diets produce less methane (g kg^{-1} DMI) than those
419 fed high-forage diets (Johnson and Johnson 1995), the amount of GHG emitted during the
420 growing and finishing stages within the western Canadian beef production cycle is similar
421 (Beauchemin et al. 2010). This mainly reflects the longer duration of the finishing phase of the
422 production cycle and emphasizes the need to explore CH_4 mitigation strategies throughout the
423 beef production cycle. Methane emissions of heifers offered the control and WDDGS diets were
424 25.0 and 37.5%, higher respectively, than the IPCC tier 2 estimates of 4.0% of GE for diets
425 containing ≥ 90% concentrate (IPCC 2006). Similarly, we found that our emission estimates
426 (7.8% GEI) in heifers fed a barley silage diet were slightly higher than the IPCC estimate of
427 6.5% GEI for cattle fed high-forage diets (IPCC 2006). The IPCC tier 2 default emission factors
428 for high-concentrate diets are mainly derived from corn grain diets (Johnson and Johnson 1995),
429 and these values may underestimate CH_4 emissions from cattle fed barley grain, owing to its
430 higher fibre content than corn (Beauchemin and McGinn 2005). Furthermore, corn is generally
431 less extensively digested in the rumen than barley and a shift in the site of digestion from the

rumen to the lower intestinal tract would be expected to lower CH_4 emissions (Johnson and Johnson 1995). Consequently, Beauchemin and McGinn (2005) reported 29.8% higher CH_4 (g kg^{-1} DM intake) emissions from barley- compared to corn-based finishing diets consisting of 9.0% barley silage and 81.4 % grain (DM basis).

Lower CH_4 emissions from heifers fed CDDGS and WDDGS+oil as compared to those fed WDDGS alone appear to be related to the level of fat in the diet (5.4 and 5.1 vs. 3.1% fat; DM based). Fat that is unprotected from ruminal fermentation reduces CH_4 production primarily by lowering the quantity of organic matter fermented in the rumen (Johnson and Johnson 1995; Beauchemin et al. 2008). In the current study, a depression in OMD may account for lower CH_4 emissions from heifers fed WDDGS+oil as compared to WDDGS alone. However, total tract digestibility of nutrients between CDDGS and WDDGS did not differ suggesting that factors other than depression of ruminal digestion, such as a direct toxic effect of fatty acids on methanogens may have contributed to this response.

Fat exerts toxic effects on methanogens as well as protozoa (Johnson and Johnson 1995). Methanogens and protozoa exist in a synergistic relationship involving inter-species hydrogen transfer (Finlay et al. 1994). Consequently, a reduction in protozoa numbers or activity is frequently associated with reduced CH_4 production (Martin et al. 2010). Addition of corn oil to the WDDGS+oil diet did not reduce protozoa numbers, possibly because of the low level of oil added (2.6% corn oil; DM basis). Protozoa in heifers fed the control diet were almost exclusively *Entodinium*, an observation typical of high barley grain diets (Hristov et al. 2001). Adding fat to the diet also enhanced the production of propionic acid and as formation of this VFA requires reducing equivalents, it decreases the amount of H available to reduce CO_2 to CH_4 (Janssen 2010). The inverse relationship between propionate formation and CH_4 production was apparent

455 in this study as feeding WDDGS resulted in the lowest concentration of propionate and the
456 highest level of CH_4 production.

457 Previous work in our laboratory found that substitution of 40% CDDGS (5.4% fat in the
458 diet) or 40% WDDGS+oil (5.6% fat in the diet; all DM basis) for barley grain in a 55% barley
459 silage growing diet reduced CH_4 (g kg^{-1} DMI) by 6.3 and 6.4% respectively, for each percentage
460 of added fat (Hünerberg et al. 2013). This magnitude of CH_4 reduction was greater than that
461 observed in the current finishing study, as CH_4 (g kg^{-1} DMI) was reduced by 5.3% and 4.1% for
462 each percentage increase in fat in these respective diets. Ruminal degradation of forages results
463 in higher CH_4 production per kg DM as compared to concentrates (Johnson and Johnson 1995);
464 therefore it is not unexpected that the reduction CH_4 as a result of added fat to the diet is greater
465 for high-forage than high-concentrate diets.

466 The fact that CDDGS was slightly more effective than WDDGS+oil in reducing CH_4
467 emissions relative to the control diet might be due to the slightly lower fat level in the
468 WDDGS+oil diet. Based on a meta-analysis using data from 27 studies over a practical range of
469 fat feeding (<8% fat; DM basis), the relationship between concentration of fat in the diet and
470 CH_4 yield was not affected by form of supplemented fat, fatty acid profile, or fat source,
471 suggesting that the level of fat in the diet may be the most important factor influencing
472 methanogenesis (Grainger and Beauchemin 2011). The authors calculated that an increase from
473 5.0 to 6.0% dietary fat (DM based) decreased CH_4 (g kg^{-1} DMI) in cattle by 5.1% (Grainger and
474 Beauchemin 2011). In the present study, the calculated reduction in CH_4 from feeding CDDGS
475 (5.4% fat in the diet) as compared to WDDGS+oil (5.1% fat in the diet; both DM basis) was
476 substantially higher, as a 0.3% difference was associated with a 6.6% reduction in CH_4 (g kg^{-1}
477 DMI). The finding that CH_4 production in response to DDGS inclusion is heavily dependent on

478 the fat content of DDGS is important as a number of ethanol plants are installing enhanced
479 extraction technologies that lower the oil content by as much as 6%, resulting in a slight increase
480 in the CP, NDF and ADF content of low-oil CDDGS (U.S. Grains Council 2012). Replacing
481 conventional CDDGS with low-oil CDDGS could reduce the lipid mediated reduction in CH_4
482 emissions while at the same time increasing N excretion and possibly contributing to increased
483 N_2O emissions.

484 Similar CH_4 emissions in heifers fed WDDGS vs the control diet are somewhat
485 surprising as the WDDGS diet contained more NDF, ADF and considerably less starch. Not
486 unlike starch, the fibre in wheat DDGS is highly fermentable in the rumen (Walter et al. 2012),
487 but in this study it still resulted in a fermentation profile that was lower in propionate than the
488 control diet. The small particle size of WDDGS may also have increased the flow of fibre from
489 the rumen to the lower intestinal tract, reducing CH_4 yield. However, if this response occurred it
490 did not result in a decrease in the digestibility of fiber in heifers fed WDDGS.

491 Higher NH_3 concentration in the rumen fluid of heifers fed WDDGS and WDDGS+oil
492 as compared to CDDGS and the control diet likely arise from differences in CP content and
493 ruminal CP degradability. Corn protein is mainly composed of zein, which is more resistant to
494 ruminal degradation than gluten in wheat (Little et al. 1968), a relationship confirmed by Boila
495 and Ingalls (1994). Levels of ADIN in CDDGS and WDDGS were relatively low (Table 2), and
496 thus unlikely to contribute to differences in ruminal N degradability. However, higher protozoa
497 numbers in heifers fed WDDGS and WDDGS+oil as compared to the other two diets may have
498 increased ruminal NH_3, through deamination of amino acids that arose from the predation of
499 bacteria (Wallace et al. 1987; Koenig et al. 2000).

Reducing the starch content of the diet by replacing rapidly fermentable, cereal grains with less rapidly fermentable DDGS has been proposed as an approach to modulate ruminal pH and reduce the incidence of SARA in feedlot cattle (Klopfenstein et al. 2008). This is supported by our results as feeding CDDGS, WDDGS and WDDGS+oil reduced the time below a pH of 5.5, an indicator of SARA and pH 5.2, an indicator of ARA. Higher total VFA concentrations in rumen fluid from heifers fed the control diet as compared to those fed DDGS, suggests that DDGS were not as rapidly fermented in the rumen as barley grain. An increase in the SD of ruminal pH in heifers fed the control diet as compared to those fed WDDGS and WDDGS+oil may also be indicative of a greater risk of acidosis as previously documented by Bevans et al. (2005). Nevertheless, differences in mean pH among diets were limited, with WDDGS+oil being the only diet that exhibited a higher daily mean pH than the control. Ruminal concentrations of lactate were low for all diets indicating that even though heifers fed the control had longer durations of ruminal pH below 5.5 and 5.2, ARA did not occur as lactate concentration typically exceed > 50 m*M* with this condition (Nagaraja and Titgemeyer 2007).

Walter et al. (2012) fed a barley-based finishing diet supplemented with 20 or 40% DM CDDGS or WDDGS and found no decrease in daily mean pH or a reduction in SARA when CDDGS or WDDGS replaced barley grain. Similarly, replacement of barley grain with increasing levels of WDDGS (7, 14, 21% DM) in a finishing diet for feedlot steers did not lead to higher mean rumen pH or reduced SARA (Beliveau and McKinnon 2009). Both studies attributed the lack of an increase in pH with DDGS to its high ruminal fermentability and a reduction in rumination and saliva secretion owing to its small particle size. Van Kessel and Russell (1996) reported that methanogens are sensitive to low ruminal pH and that CH_4 production ceases *in vitro* at a pH < 6.0. This is clearly not applicable *in vivo* as, even when

ruminal pH of both animals within a chamber dropped well below 6, CH_4 was still produced. Methanogens within cattle adapted to high-concentrate diets appear to be less sensitive to low pH than those from cattle fed diets with higher forage content (Hook et al. 2011). Although the origin of this response is unclear, it may arise from these pH tolerant methanogens having a higher affinity for hydrogen or a greater endosymbiotic relationship with protozoa, where they would be less affected by the low pH within surrounding rumen fluid (Hook et al. 2011).

As expected, the increased CP content of DDGS resulted in heifers fed these diets having a higher N intake than those fed the control diet. Likewise, differences in N intake between CDDGS and WDDGS reflect the higher CP content of WDDGS. All diets containing DDGS exceeded the protein requirements of finishing beef cattle by two fold (NRC 2000), resulting in a dramatic increase in N excretion (g d^{-1}). In DDGS diets, PUN levels also exceeded 8 mg dL^{-1}, indicating that digestible N intake exceeded requirements (Cole et al. 2003). Walter et al. (2012) found a similar response in feedlot steers fed diets containing 40% DM of CDDGS or WDDGS, reporting excretions of 207 and 266 g N d^{-1}, respectively. Protein fed in excess of requirements is an environmental concern as N is predominantly excreted as urea in urine. Upon urination, urea is rapidly hydrolyzed to NH_3 by bacterial urease and disperses into the atmosphere (Mobley et al. 1995). Once in the atmosphere, NH_3 is a precursor for particulate matter and has a negative impact on air quality and respiratory health (U.S. Environmental Protection Agency 2009). Additionally, NH_3 can be re-deposited to the soil surface and contribute to eutrophication, acidification and the formation of N_2O (IPCC 2006). Excess N can also be lost in the form of NO_3^- through leaching and run-off and contaminate water bodies and be transformed to N_2O via aquatic denitrification (U.S. Environmental Protection Agency 2010). As a result, N and NH_3

volatilization from manure both directly and indirectly contribute to GHG emissions (Hristov et al. 2011).

As there were no differences in CPD among CDDGS, WDDGS and the control diet it can be assumed that greater percentage of urinary N excretion in heifers fed CDDGS or WDDGS was associated with the higher total N intake of these diets. In contrast, CPD in heifers offered WDDGS+oil was reduced as compared to the other diets. Heifers fed this diet also excreted less urinary N as a % of N intake, even though they had the highest N intake. It is likely that these responses arise due to a decrease in ruminal CP and OM digestibility as result of the addition of corn oil, leading to a reduction in N loss in urine and an increase in N loss in feces. This could have environmental implications as urinary N is more susceptible to leaching and volatile losses than fecal N (Bussink and Oenema 1998).

CONCLUSION

This study completes the first full assessment of the impact of dietary CDDG and WDDG inclusion on both CH_4 emissions and N excretion from feedlot cattle. Results show that CH_4 production in response to DDGS inclusion is heavily dependent on the fat content of DDGS. Therefore, enhanced fat extraction from CDDGS could reduce its ability to mitigate enteric CH_4 emissions in ruminants. Using DDGS as an energy source exceeds CP requirements, dramatically increasing N excretion in both urine and feces. In order to reduce the environmental impact of DDGS in feedlot cattle production, it is critical that manure be applied on the basis of N requirements of the crop.

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Table 1. Ingredient composition and chemical composition of the experimental diets

Item	Treatment[z]			
	Control	CDDGS	WDDGS	WDDGS+oil
Ingredient (% of the dietary DM)				
Barley silage	8	8	8	8
Barley grain, dry-rolled	87	47	47	47
CDDGS[y]		40		
WDDGS[x]			40	37.4
Corn oil				2.6
Barley grain, ground[w]	3.4	3.4	3.4	3.4
Calcium carbonate	1.25	1.25	1.25	1.25
Salt	0.15	0.15	0.15	0.15
Molasses, dried	0.13	0.13	0.13	0.13
Mineral and vitamin premix[v]	0.06	0.06	0.06	0.06
Vitamin E (500,000 IU kg^{-1})	0.003	0.003	0.003	0.003
Flavouring agent[u]	0.003	0.003	0.003	0.003
Chemical composition[t]				
OM (% of DM)	95.4 ± 0.5	95.6 ± 0.1	94.0 ± 0.1	94.1 ± 0.4
CP (% of DM)	12.2 ± 0.7	19.6 ± 0.7	23.1 ± 0.8	22.1 ± 1.1
NDF[s] (% of DM)	19.4 ± 1.0	27.9 ± 0.8	24.5 ± 1.4	24.4 ± 1.5
ADF[r] (% of DM)	7.3 ± 0.4	13.7 ± 0.7	14.2 ± 0.4	12.9 ± 0.5
Fat[q] (% of DM)	2.0 ± 0.1	5.4 ± 0.2	3.1 ± 0.4	5.1 ± 0.1
Starch (% of DM)	55.0 ± 1.3	34.7 ± 2.7	31.9 ± 3.3	33.2 ± 1.8
GE (MJ kg^{-1} of DM)	18.4 ± 0.1	19.0 ± 0.2	18.5 ± 0.2	18.9 ± 0.3

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.4% wheat dried distiller' grains plus solubles + 2.6% corn oil (DM basis).
[y]Corn-based dried distillers' grains with solubles.
[x]Wheat-based dried distillers' grains with solubles.
[w]Carrier for the pelleted vitamin and mineral supplement.
[v]Supplied kg DM^{-1}: 65 mg of Zn, 28 mg of Mn, 15 mg of Cu, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6,000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.
[u]Anise 422 powder containing ground cumin, fennel, fenugreek, silicon dioxide and wheat bran (Canadian Bio-Systems Inc., Calgary, Alberta, Canada).
[t]Determined using samples pooled by diet within each period (n = 4; mean ± SD).
[s]Neutral detergent fibre, assayed with a heat stable amylase and expressed inclusive residual ash.
[r]Acid detergent fibre, expressed inclusive of residual ash.
[q]Determined using ether extraction.

Table 2. Chemical analysis of major diet ingredients

	Ingredient		
Item[x]	Barley grain	CDDGS[z]	WDDGS[y]
OM (% of DM)	97.7 ± 0.1	96.9 ± 0.1	94.1 ± 0.1
CP (% of DM)	12.3 ± 1.0	31.4 ± 0.5	38.7 ± 1.1
NDF[w] (% of DM)	20.4 ± 2.3	38.0 ± 1.2	28.0 ± 0.7
ADF[v] (% of DM)	5.2 ± 0.6	23.0 ± 0.5	21.5 ± 0.4
ADIN[u] (% of total N)	ND[s]	14.3 ± 1.9	9.3 ± 0.5
Fat[t] (% of DM)	1.8 ± 0.1	9.7 ± 0.3	3.4 ± 0.2
Starch (% of DM)	56.7 ± 1.2	4.4 ± 0.3	2.5 ± 0.3

[z]Corn-based dried distillers' grains with solubles.
[y]Wheat-based dried distillers' grains with solubles.
[x]Determined using samples pooled by period (n = 4; mean ± SD).
[w]Neutral detergent fibre, assayed with a heat stable amylase and expressed inclusive residual ash.
[v]Acid detergent fibre, expressed inclusive of residual ash.
[u]Acid detergent insoluble nitrogen.
[t]Determined using ether extraction.
[s]Not determined.

Table 3. Ruminal fermentation variables of ruminally cannulated beef heifers (*n* = 16) fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil

	Treatment[z]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	*P*-value
Total VFA (m*M)*	174.7a	155.2b	156.9b	157.7b	6.96	0.008
Individual VFA (m*M*)						
Acetate (A)	80.6a	71.7b	78.2ab	75.3ab	2.92	0.016
Propionate (P)	65.2a	57.2ab	48.8b	54.2ab	4.68	0.016
Butyrate	18.5	16.7	21.3	19.2	1.86	0.216
Isovalerate	2.53	2.32	1.94	1.93	0.351	0.481
Valerate	4.97	4.78	4.29	4.44	0.655	0.775
Isobutyrate	1.89	1.75	1.63	1.81	0.342	0.301
A: P ratio	1.50	1.44	1.86	1.56	0.163	0.177
Lactate	0.083	0.108	0.116	0.122	0.0239	0.636
NH_3-N	4.24b	5.12b	10.77a	8.90a	0.859	<0.001
Protozoa ($\times 10^5$ cell mL^{-1})	3.8b	4.1b	8.8a	7.1a	1.00	<0.001

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[a-b]Within a row, means without a common letter differ, $P < 0.05$.

Table 4. Ruminal pH of ruminally cannulated beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (*n* = 16 per treatment)

	Treatment[z]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	*P*-value
Ruminal pH[y]						
Mean	5.79b	5.94ab	5.89ab	5.96a	0.062	0.039
Minimum	5.01b	5.18a	5.20a	5.24a	0.067	0.001
Maximum	6.80	6.76	6.71	6.77	0.051	0.388
SD of mean pH	0.47a	0.42ab	0.39b	0.38b	0.026	0.003
Duration of pH (h d^{-1})						
<5.5[x]	8.6a	5.5b	5.5b	4.5b	1.19	0.009
<5.2[w]	4.6a	2.1b	2.3b	1.4b	0.73	0.004
AUC[v], pH x min d^{-1}						
<5.5	171.5a	84.7b	93.2b	61.2b	26.98	0.003
<5.2	52.2a	15.7b	25.8b	9.9b	9.19	<0.001
AUC kg DMI^{-1} (pH x min)						
<5.5	19.0a	9.2b	11.0ab	6.9b	2.90	0.005
<5.2	5.7a	1.7b	2.8ab	1.1b	1.07	0.005

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[y]Ruminal pH determined for 4 d during which the heifers were in the chambers.
[x]Threshold level indicative of sub-acute ruminal acidosis.
[w]Threshold level indicative of acute ruminal acidosis.
[v]AUC = area under the curve.
[a-b]Within a row, means without a common letter differ, $P < 0.05$.

Table 5. Nutrient intakes and total tract digestibility measured in beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (*n* = 8 per treatment)

	Treatment[z]					Treatment
Item[y]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	*P*-value
Intake						
DM (kg d^{-1})	10.15a	9.13ab	8.76b	9.51ab	0.511	0.008
OM (kg d^{-1})	9.68a	8.71ab	8.22b	8.95ab	0.488	0.004
CP (kg d^{-1})	1.23c	1.69b	1.95a	2.01a	0.115	<0.001
NDF (kg d^{-1})	1.98b	2.06b	2.09b	2.40a	0.157	<0.001
ADF (kg d^{-1})	0.75b	1.19a	1.21a	1.21a	0.065	<0.001
GE (MJ d^{-1})	185.7a	172.8ab	161.5b	179.3ab	10.11	0.017
Digestibility (%)						
DM	82.0a	77.3b	76.8b	73.2c	1.10	<0.001
OM	83.7a	78.9b	78.5b	74.9c	1.08	<0.001
CP	77.0ab	78.9a	78.1a	74.5b	1.08	0.003
NDF	54.6	50.5	52.6	49.0	2.87	0.179
ADF	36.0b	56.6a	52.1a	41.6b	2.64	<0.001
GE	82.2a	78.1b	77.4b	73.9c	1.06	<0.001

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).

[y]Nutrient intakes and total tract digestibility determined over 4 d.

[a-c]Within a row, means without a common superscript letter differ, $P < 0.05$.

Table 6. Nitrogen intake, excretion, and plasma urea N concentration of ruminally cannulated beef heifers (*n* = 8) fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil

	Treatment[z]					
Item[y]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	*P*-value
N intake (g d^{-1})	197c	271b	312a	322a	18.5	<0.001
N excretion (g d^{-1})	143c	220 b	253a	265a	15.9	<0.001
Fecal excretion						
Output (kg d^{-1})	1.8b	2.1b	2.0b	2.5a	0.19	<0.001
Fecal N (g d^{-1})	44.9c	58.1b	67.6b	80.9a	5.99	<0.001
Total N (% N intake)	23.0ab	21.1b	21.9b	25.5a	1.08	0.003
Total N (% N excretion)	31.4a	25.9b	26.7b	30.6a	1.35	<0.001
Urinary excretion						
Output (L d^{-1})	7.1b	10.1a	11.2a	10.5a	0.99	<0.001
Urinary N (g d^{-1})	98.3c	162.0b	185.3a	183.7a	11.30	<0.001
Total N (% N intake)	50.8b	61.4a	60.7a	57.6a	1.99	<.0001
Total N (% N excretion)	68.6b	74.1a	73.3a	69.4b	1.35	<0.001
Urea N (g d^{-1})	52.0c	100.2b	116.4a	120.1a	8.16	<0.001
NH_3 N (g d^{-1})	2.7c	7.1ab	7.6a	5.9b	0.82	<0.001
Plasma urea N[x] (mg dL^{-1})	6.0b	10.0a	10.0a	10.5a	0.50	<0.001

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).

[y]Nitrogen intakes and excretion were measured over 4 d (*n* = 8 per treatment).

[x]Samples taken on d 21 (*n* =16 per treatment).

[a-c]Within a row, means without a common superscript letter differ, $P < 0.05$.

Table 7. Daily methane emissions from ruminally cannulated beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (*n* = 8 per treatment)

	Treatment[z]					
Item[y]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	*P*-value
DMI (kg d^{-1})	8.51	8.82	8.10	8.74	0.672	0.202
Methane						
g d^{-1}	136.2ab	119.0b	144.3a	123.6b	10.06	0.008
g kg^{-1} of DMI	16.6ab	13.6c	18.4a	14.5bc	1.60	<0.001
% of GE[x] intake	5.0ab	4.0c	5.5a	4.2bc	0.47	<0.001
% of DE[w] intake	6.1ab	5.1b	7.1a	5.7b	0.60	<0.001

[z]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).

[y]Methane emissions and corresponding dry matter intake (DMI) determined over 4 d during which the heifers were in the chambers. Chamber (data for 2 heifers) was the experimental unit.

[x]Gross energy.

[w]Methane as % of digestible energy (DE) was calculated based on DE values determined between d 18 and 21 (Table 4).

[a-c]Within a row, means without a common letter differ, $P < 0.05$.