Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production

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ABSTRACT

The objective of this study was to examine the effects of including corn dried distillers grains with solubles (DDGS) in the diet at the expense of corn and soybean meal on enteric CH₄ emissions, ruminal fermentation characteristics, digestion (in sacco and apparent total-tract digestibility), N balance, and milk production of dairy cows. Twelve lactating Holstein cows were used in a triplicated 4 × 4 Latin square design (35-d periods) and fed (ad libitum intake) a total mixed ration containing (dry matter basis) 0, 10, 20, or 30% DDGS. Dry matter intake increased linearly, whereas apparent-total tract digestibility of dry matter and gross energy declined linearly as DDGS level in the diet increased. Increasing the proportion of DDGS in the diet decreased the acetate:propionate ratio, but this decrease was the result of reduced acetate concentration rather than increased propionate concentration. Milk yield increased linearly (up to +4 kg/d) with increasing levels of DDGS in the diet and a tendency was observed for a quadratic increase in energy-corrected milk as the proportion of DDGS in the diet increased. Methane production decreased linearly with increasing levels of DDGS in the diet (495, 490, 477, and 475 g/d for 0, 10, 20, and 30% DDGS diets, respectively). When adjusted for gross energy intake, CH₄ losses also decreased linearly as DDGS proportion increased in the diet by 5, 8, and 14% for 10, 20, and 30% DDGS diets, respectively. Similar decreases (up to 12% at 30% DDGS) were also observed when CH₄ production was corrected for digestible energy intake. When expressed relative to energy-corrected milk, CH₄ production declined linearly as the amount of DDGS increased in the diet. Total N excretion (urinary and fecal; g/d) increased as the amount of DDGS in the diet increased.

Efficiency of N utilization (milk N secretion as a proportion of N intake) declined linearly with increasing inclusion of DDGS in the diet. However, productive N increased linearly with increasing proportions of DDGS in the diet, suggesting better efficiency of N use by the animal. Results from this study show that feeding DDGS to dairy cows can help to mitigate enteric CH₄ emissions without negatively affecting intake and milk production.

Key words: corn distillers grains with solubles, methane, dairy cow

INTRODUCTION

Methane is a significant component of greenhouse gases, playing a key role in global warming. Enteric fermentation is a major source of on-farm CH₄ emissions, contributing up to 34% (86 million t/yr) of all agricultural emissions, and could rise up to 48% (114 million t/yr) in 2020 (EPA, 2006; FAO, 2006). Several studies have reported that increasing fat content in ruminant diets can reduce enteric CH₄ production. This has been reported with lipids from vegetable oils (McGinn et al., 2004; Beauchemin et al., 2007; Martin et al., 2008) and oilseeds (Beauchemin et al., 2009; Grainger et al., 2010; Chung et al., 2011). However, vegetable oils and oilseeds are expensive ingredients and the advantage of lowering enteric CH₄ emissions could be counterbalanced by a loss of profit when such mitigation strategy is applied at the farm level.

Recent growth of the ethanol industry in North America has resulted in increased availability of byproducts, most commonly dried distillers grains with solubles (DDGS). Corn DDGS contain 11 to 17% ether extract (Getachew et al., 2004; Penner et al., 2009; Schingoethe et al., 2009) and, therefore, DDGS can be used as a fat supplement to reduce enteric CH₄ emissions from dairy cows. Moreover, DDGS contain, on average, 30% CP of which 55% are ruminally undegradable (Janicek et al., 2008) and, thus, it can represent an interesting source of protein for dairy cow nutrition. Only a few studies

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have evaluated the potential of using DDGS to reduce enteric CH₄ production from ruminants. Birkelo et al. (2004) replaced corn and soybean meal with wet corn distillers grains (31% of dietary DM) in early lactation dairy cow diets and reported a 14% decline in CH₄ energy losses [as a proportion of gross energy (GE) intake] with no concomitant effect on milk yield. More recently, McGinn et al. (2009) reported a 24% decrease in CH₄ energy losses (percentage of GE intake) and improved feed efficiency (gain:feed ratio) in beef cattle when DDGS replaced barley grain in the diet. To our knowledge, the effect of including DDGS in the TMR of dairy cows on enteric CH₄ production has not been studied yet. Moreover, no information is available on the optimal amount of DDGS to feed to mitigate enteric CH₄ emissions without adversely affecting dairy cow performance. The purpose of this study was to examine the effects of including increasing amounts of DDGS (0, 10, 20, and 30%; DM basis) in the diet at the expense of corn and soybean meal on enteric CH₄ production, rumen fermentation, digestion, N balance, milk production, and milk composition of dairy cows.

MATERIALS AND METHODS

This study was conducted at the Dairy and Swine Research and Development Centre (Sherbrooke, Quebec, Canada). Animal procedures were conducted under the approval of the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre and were in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada; CCAC, 1993).

Cows, Experimental Design, and Diets

Twelve multiparous (2.6 ± 0.67 parity) lactating Holstein cows fitted with rumen cannulas (10 cm; Bar Diamond Inc., Parma, ID) were used in a triplicated 4×4 Latin square design (35-d periods). The cows averaged 99 ± 18 DIM at the start of the experiment, with an average BW of 645 ± 49 kg and production of 40 ± 4 kg of milk/d. During the experiment, cows were housed in individual tiestalls, and allowed free access to water. Cows were fed for ad libitum intake (5% orts, on an as-fed basis) 1 of the 4 following dietary treatments: (1) 0% DDGS, (2) 10% DDGS, (3) 20% DDGS, and (4) 30% DDGS, on a DM basis (Table 1). The DDGS replaced flaked corn (i.e., source of energy) and soybean meal (i.e., source of protein) in the diets, which were formulated to be isonitrogenous (i.e., similar dietary CP concentration) and isocaloric (i.e., similar NE_L concentration). Dried distillers grains with solubles (a single batch) were obtained from GreenField Ethanol Inc.

(Vareennes, QC). After 2 wk (i.e., 14 d) of adaptation to experimental diets, feed intake, apparent total-tract digestibility, N balance, milk production, milk composition (i.e., 7 d), in sacco ruminal degradability (i.e., 5 d), rumen fermentation characteristics (i.e., 2 d), and CH₄ production (i.e., 3 d) were measured. Only 2 air-flow controlled chambers were available for measuring CH₄ emissions in this study, which limited the number of animals that could be examined at the same time to 2 (i.e., 1 cow per chamber). Consequently, the groups of cows had to be staggered by 4 d to facilitate measurements. Cows were weighed at the beginning and the end of each experimental period on 2 consecutive days before the a.m. feeding and after the a.m. milking.

Feed Intake, Apparent Total-Tract Digestibility, and N Balance

Apparent total-tract digestibility and N outputs were measured over 7 consecutive days. Diets were offered in equal amounts twice daily (0900 and 1930 h). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows. Samples of the TMR, feed ingredients, and orts were collected daily and stored at -20°C . Samples were composited by cow within period, freeze dried, ground to pass a 1-mm screen using a Wiley mill (standard model 4; Arthur M. Thomas Co., Philadelphia, PA) and analyzed for DM, OM, total N, NDF, ADF, starch, ether extract (EE), and GE.

Total collection of feces and urine was performed by fitting cows with harnesses and tubes allowing the collection of feces and urine separately. Feces were weighed and mixed daily, and a representative sample (2%) was collected, stored at -20°C , and subsequently thawed, freeze dried, and ground to pass a 1-mm screen using a Wiley mill for later analysis of OM, total N, NDF, ADF, starch, ether extract, and GE. Total urine was collected daily into reinforced plastic containers containing an appropriate amount of H₂SO₄ (50% vol/vol) to maintain pH <2.0. A representative sample (2%) was collected and kept frozen at -20°C until analyzed for total N. Retained N was calculated by subtracting N in feces, urine, and milk from N intake. Productive N was calculated as the sum of N retained in the body and N secreted in milk.

In Sacco Ruminal Degradability

Ruminal degradability of timothy hay (i.e., source of fiber), soybean meal (i.e., source of protein), and flaked corn (i.e., source of starch) was determined on 1 Latin square (4 cows) using a nylon bag procedure. Feeds were ground to pass a 1-mm screen using a Wiley mill and 5-g samples (DM basis) were weighed in duplicate

Table 1. Ingredient composition (DM basis) of the experimental diets containing increasing amounts of corn dried distillers grains with solubles (DDGS)

Ingredient (%)	DDGS			
	0%	10%	20%	30%
Alfalfa silage	22.9	22.9	22.9	22.9
Corn silage	33.8	33.8	33.8	33.8
Timothy hay	3.4	3.4	3.4	3.4
Flaked corn	16.7	11.0	5.7	0.0
Soybean meal	13.2	8.8	4.4	0.0
DDGS	0.0	10.1	19.9	30.0
Beet pulp, dehydrated	7.6	7.6	7.5	7.5
Calcium carbonate	0.7	0.7	0.8	0.8
Mineral and vitamin supplement ¹	1.6	1.6	1.5	1.5

¹Contained 12.48% Ca, 6.80% P, 6.81% S, 7.72% Na, 1.97% K, 96 mg of I/kg, 2,877 mg of Fe/kg, 620 mg of Cu/kg, 2,520 mg of Mn/kg, 3,777 mg of Zn/kg, 83 mg of Co/kg, 628,000 IU of vitamin A/kg, 81,000 IU of vitamin D/kg, 3,739 IU of vitamin E/kg, and 27.8 mg of Se/kg.

in polyester bags (17 × 9 cm; 53-μm pore size) made of monofilament PeCap polyester (Sefar Nitex; Sefar AG, Heiden, Switzerland). Bags were placed in a large mesh (46 × 38 cm; 3 × 5-mm pores) retaining sac containing a 700 g anchor to keep the bags immersed in the rumen while allowing the rumen fluid to circulate freely. Bags were soaked in 37°C water for 5 min before being placed in the ventral sac of the rumen for 2, 4, 8, 16, 24, 48, and 72 h for soybean meal and flaked corn and 2, 4, 8, 16, 24, 48, 72, and 96 h for hay. Upon removal from the rumen, bags were immediately immersed in ice water to impede microbial activity and then thoroughly rinsed with cold tap water and frozen at -20°C. Bags were later thawed, washed in a domestic washing machine, and dried for 48 h at 55°C. Bags and contents were weighed and dried residues were ground through a 1-mm screen (1093 Cyclotec Sample Mill; Foss Tecator AB, Höganäs, Sweden) and stored for subsequent determination of DM and OM. Bags used for time 0 disappearance were soaked in water at 37°C for 5 min, and then treated similar to the other bags.

Kinetics of ruminal degradation of OM of hay, soybean meal and flaked corn was calculated using a non-linear model (McDonald, 1981). The PROC NLIN of SAS (SAS Institute Inc., Cary, NC) was used to fit the following model:

$$P = a + b(1 - e^{-c(t-L)}) \text{ for } t > L;$$

$$P = a \text{ for } t \leq L,$$

where P = percentage of OM disappearance from the nylon bag at time *t*, *a* = soluble and rapidly degradable fraction (%), *b* = slowly degradable fraction (%), *c* = fractional rate of disappearance of the fraction *b* (/h), *L* = lag time (h), and *t* = time of incubation (h). The model was fitted using (*a* + *b*) ≤ 100, *b* ≥ 0, *c* ≥ 0, and *L* ≥ 0 constraints.

The effective ruminal degradability (**ERD**) of OM was calculated using the following equation:

$$ERD = a + [bc/(c + kp)] \times e^{(-kpL)},$$

where *kp* is the ruminal fractional passage rate, calculated at 0.05/h for timothy hay and 0.061/h for soybean meal and flaked corn, from the equations suggested by the NRC (2001) for dry forages and concentrates.

Ruminal Fermentation Characteristics and Protozoa Enumeration

Ruminal fluid was collected from cows on 2 successive days before (0 h) and at 1, 2, 4, 6, and 8 h after the a.m. feeding. A total of 250 mL was collected from the anterior dorsal, anterior ventral, medium ventral, posterior dorsal, and posterior ventral locations within the rumen using a 50-mL syringe screwed to a stainless tube ending with a probe covered by a fine metal mesh (RT rumen fluid collection tube; Bar Diamond Inc.). Ruminal fluid pH was measured immediately after sampling (Accumet pH meter; Fisher Scientific, Montreal, QC, Canada), and samples were acidified to pH 2 with 50% H₂SO₄ and frozen at -20°C for later determination of VFA and ammonia (NH₃) concentrations.

Protozoa enumeration was carried out on rumen content collected 4 h after the a.m. feeding on 2 consecutive days. Ruminal content (1 L) was strained through 4 layers of cheesecloth and a 5-mL portion of the strained rumen fluid was preserved using 5 mL of methyl green formalin-saline solution for protozoa enumeration (Ogimoto and Imai, 1981). Protozoa samples were stored at room temperature in darkness until counting. Protozoa were microscopically enumerated using a counting chamber (Neubauer Improved Bright-Line counting cell, 0.1-mm depth; Hausser Scientific Co., Horsham, PA). Each sample was counted twice, and if the average

of the duplicates differed by more than 10%, the counts were repeated.

Milk Production and Milk Composition

Cows were milked twice daily at 0700 and 1900 h in their stalls and milk production was recorded at each milking. During digestibility measurements (i.e., 7 d), milk samples were collected from each cow at each milking, stored at +4°C with a preservative (2-bromo-2-nitropropan-1,3-diol), and then sent to a commercial laboratory (Valacta Laboratories, Sainte-Anne-de-Bellevue, QC, Canada) for analyses of fat, protein, lactose, MUN, and SCC.

Methane Production Measurement

Two air-flow controlled chambers were used for measurement of CH₄ production. The chambers measured 4.09 m length × 2.95 m width × 2.84 m height. When chambers doors were closed, air entered the chamber through a ventilation duct and exited through an exhaust. Air temperature within the chamber was maintained at 18 ± 1°C. Air flow into and out of the chamber was measured using inline mass flowmeters (FT2; Fox Thermal Instruments Inc., Marina, CA) and maintained at 180 ± 10 m³/h. Methane concentration was continuously measured at the air entrance and exhaust ducts using CH₄ analyzers (MGA3000; ADC Gas Analysis Ltd., Hoddesdon, Hertfordshire, UK). The amount of CH₄ (entering and leaving the chamber) was calculated by multiplying the concentration of CH₄ by the airflow (at entrance and at exhaust). The difference between the incoming and outgoing mass of CH₄ corresponded to the amount of enteric CH₄ emitted in each chamber by the animal. The chambers were calibrated before the experiment by releasing known amounts of CH₄ in each chamber (with no cow inside) and calculating the recovered amounts from the difference incoming and ingoing CH₄. The recovered amounts ranged from 98 to 102%. The calibration factors (i.e., to adjust each chamber to 100% recovery) were used to correct CH₄ emissions data. A small positive pressure was generated inside each chamber to prevent inflow of gases into the chambers. Cows were preconditioned to the environmental chambers before the beginning of the experiment. To reduce the effect of isolation on animal behavior, the chambers were equipped with windows and speakers so the cow in the chamber can see and hear other cows. Cows entered the chambers 18 h before starting CH₄ measurements. Methane was recorded every minute over a period of 3 consecutive days and fluxes were averaged to derive 24-h CH₄ emissions. Within each chamber, the cow was kept in a tiestall

that measured 1.82 m long × 1.60 m wide, elevated from the floor by 25 cm. Manure was collected from each cow in a stainless box placed below and to the rear of each stall. Farm personnel accessed the chambers twice daily (0800 and 2000 h) to feed and milk the cows and remove the manure. The doors were kept opened for a maximum of 1 to 1.5 min to allow exchange of material in and out of the chambers. This resulted in interruptions of flux measurements for 15 to 30 min, which is the time required for gas concentrations to reach steady state. These interruptions had a little effect on daily emissions because fluxes were calculated every minute and used to derive the 24-h period emissions values. Cows in the chambers had free access to water and were fed twice daily for ad libitum intake (5% orts on an as-fed basis). Offered feed and orts were weighed daily to determine feed consumption. Samples of feed offered and orts were collected, pooled, and kept frozen for later determination of DM and GE concentrations.

Chemical Analyses

Dry matter was determined by oven drying at 55°C for 48 h. Analytical DM was determined by drying in a vacuum oven at 100°C overnight (AOAC, 1990; method 934.01). Ash content was determined by incineration at 550°C overnight in a muffle furnace (AOAC, 1990; method 942.05) and the OM content was calculated as the difference between 100 and the percentage of ash. Crude protein (N × 6.25) was determined using the macro-Kjeldahl procedure (AOAC, 1990; method 990.03). The concentration of NDF was determined as described by Van Soest et al. (1991) without the use of sodium sulfite and with the inclusion of heat-stable α-amylase. The ADF content was determined according to AOAC (1990; method 973.18). The NDF and ADF procedures were adapted for use in an Ankom200 fiber analyzer (Ankom Technology Corp., Fairport, NY). The concentration of starch was determined colorimetrically according to the procedure of Hall (2001). Ether extract was determined using the Soxtec 2047 SoxCap in combination with Soxtec extraction systems (Foss, Eden Prairie, MN) according to the AOAC (1990; method 920.39). Gross energy was determined using an oxygen bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). The concentration of N in acidified urine samples was determined by micro-Kjeldahl analysis (AOAC, 1990).

Ruminal VFA concentration was determined using HPLC Agilent Technologies 1200 Series (Agilent Technologies Canada Inc., Mississauga, ON, Canada). The HPLC was equipped with an RI detector and Aminex HPX-87H column (300 × 7.8 mm) packed with sulfonated divinyl benzene-styrene copolymer (Bio-Rad

Laboratories Ltd., Mississauga, ON, Canada). The mobile phase was 0.005 M sulfuric acid and the flow rate was 0.6 mL/min. Ammonia N concentration in ruminal fluid was determined as in Weatherburn (1967). Protein, fat, lactose, urea N, and SCC in milk samples were analyzed by infrared spectroscopy (MilkoScan FT 6000; Foss Electric A/S, Hillerød, Denmark). Milk composition was corrected for differences in milk yield between a.m. and p.m. milkings.

Statistical Analyses

Data were analyzed using PROC MIXED of SAS (SAS Institute Inc.). The statistical model included treatment and period as fixed effects and square and cows within square as random effects. Ruminal fermentation characteristics (pH, VFA, and NH₃) data were analyzed as repeated measures using the same model with the addition of the fixed effects of day, sampling time (i.e., h) and all interactions. The CH₄ and protozoa data were also analyzed as repeated measures with the inclusion of the fixed effects of day and day × treatment interaction. For CH₄ data, the fixed effect of the chamber was initially included in the model but was removed because it was not significant. In sacco ruminal degradability data were analyzed as a 4 × 4 Latin square design and the model included the fixed effects of treatment and period, and the random effect of cow. Orthogonal polynomial contrasts (linear and quadratic) were used to examine treatment effect on response variables. Significant effect of treatment on least squares means was declared when $P \leq 0.05$, whereas tendencies were declared when $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

Ingredients of the experimental diets are shown in Table 1, whereas the chemical composition of the

experimental diets, DDGS, flaked corn, and soybean meal are presented in Table 2. The DDGS used in our study contained slightly less CP (27.9 vs. 31.1 ± 1.54%; mean ± SD), more EE (16.3 vs. 11.9 ± 2.12%; mean ± SD), and similar NDF concentrations (32.1 vs. 32.7 ± 2.93%; mean ± SD) compared with previous studies (Anderson et al., 2006; Kleinschmit et al., 2007; Janicek et al., 2008; McGinn et al., 2009; Ranathunga et al., 2010). In the current study, DDGS (0, 10, 20, and 30% of dietary DM) replaced flaked corn (16.7, 11.0, 5.7, and 0% of dietary DM) and soybean meal (13.2, 8.8, 4.4, and 0% of dietary DM) and, consequently, as the DDGS proportion in the diet increased, EE and fiber (NDF and ADF) concentrations of the dietary treatments increased, whereas the starch content decreased (Table 2). Even though diets were formulated to be isocaloric and isonitrogenous, increasing DDGS in the diet resulted in a slight increase in GE and CP concentrations.

Intake and Apparent Total-Tract Digestibility

Intake of DM increased linearly ($P < 0.01$) as dietary DDGS proportion increased in the diet (Table 3). Cows offered diets with the highest DDGS proportion consumed 8% more DM than cows fed 0% DDGS diet. Intake of OM, GE, CP, NDF, and EE increased linearly ($P < 0.01$) with increasing DDGS proportions in the diet, whereas starch intake declined linearly ($P < 0.01$). The effects of feeding DDGS on feed intake have been variable among studies. Similarly to our observations, Nichols et al. (1998) replaced soybean meal and corn with DDGS (20% of dietary DM) and observed greater DMI for cows fed DDGS diet than for cows fed a control diet. More recently, Janicek et al. (2008) reported a linear increase in DMI for cows fed diets containing increasing amounts of DDGS (up to 30%; DM basis) included at the expense of forage and concentrate ingredients. Kleinschmit et al. (2006) reported no difference

Table 2. Chemical composition of the diets, corn dried distillers grains with solubles (DDGS), soybean meal, and flaked corn

Item	DDGS				Ingredient		
	0%	10%	20%	30%	DDGS	Flaked corn	Soybean meal
OM (% of DM)	93.0	92.9	92.6	92.5	95.2	98.3	92.7
CP (% of DM)	16.2	16.4	16.6	16.8	27.9	8.35	51.5
NDF (% of DM)	32.3	33.8	36.3	37.8	32.1	8.69	8.47
ADF (% of DM)	21.8	21.8	22.9	23.3	13.2	2.78	6.28
Starch (% of DM)	19.0	15.8	13.7	11.2	2.20	64.0	1.12
Ether extract (% of DM)	3.99	4.98	6.06	7.16	16.3	6.29	3.77
Gross energy (Mcal/kg of DM)	4.46	4.54	4.63	4.73	5.41	4.44	4.65
NE _L ¹ (Mcal/kg of DM)	1.62	1.63	1.65	1.66	2.29	2.09	2.13

¹Calculated according to NRC (2001).

in DMI when DDGS (CP = 30.5%, NDF = 47.8%, and EE = 10.6%) replaced corn and soybean meal in dairy cow diets (20% of the dietary DM). Contrary to our findings, Birkelo et al. (2004) reported an 11% decline in DMI when wet corn distillers grains (CP = 39.5%, NDF = 58.1%, and EE = 8.5%) were included (at 31% of dietary DM) in dairy cow diets at the expense of corn and soybean meal, whereas Anderson et al. (2006) reported a tendency ($P = 0.09$) for lower DMI for cows fed 20% of wet or dry corn distillers grains (replacing corn and soybean meal) diets compared with cows fed the control diet. Discrepancies between studies in DMI response to feeding corn distillers grains could be related to differences in experimental conditions, such as the rate of inclusion of corn distillers grains with solubles, the type of feed ingredient of the basal diet (forage or concentrate, or both) being replaced by the distillers grains, and the form (wet or dry) of this byproduct to feed. Kleinschmit et al. (2007) showed that ruminal degradability and intestinal digestibility differed widely among DDGS sources, reflecting a great variation in the quality (i.e., chemical composition) of DDGS, which may further explain the variable DMI between studies.

Dry matter and OM and GE digestibility of the diet declined linearly ($P < 0.01$) with increasing DDGS proportions in the diet. Digestibility of ADF averaged 60.5% and was not affected ($P \geq 0.80$) by DDGS addition to the diet. Digestibility of NDF slightly increased when DDGS was supplied up to 20% of dietary DM and then declined at 30% of DDGS supplementation level, causing a quadratic ($P = 0.05$) effect of treatment. Apparent total-tract digestibility of CP ($P = 0.02$)

and EE ($P < 0.01$) increased linearly, whereas starch digestibility increased quadratically ($P < 0.01$) as the proportion of DDGS in the diet increased. Zhang et al. (2010) replaced concentrates (rolled barley and canola meal) in dairy cow diets with 20% dried distillers grains and solubles from corn and wheat. They observed a numerically higher DMI for cows fed DDGS diets, and reported a significant decrease in apparent total-tract digestibility of DM and an increase in that of NDF and EE, which agrees with our findings observed with a 20% DDGS diet. Birkelo et al. (2004) also reported a numerical decline in DM digestibility coupled with an increase in CP and EE digestibility when 31.2% wet distillers grains replaced soybean meal and corn in dairy cow diets, which also corroborates findings of the present study.

Several studies reported a decline in diet digestibility when dietary fat level increased (Beauchemin et al., 2007; Chung et al., 2011), which explains the effect of DDGS addition on DM, OM, energy, and NDF (at 30% DDGS) digestibility observed in the current study. The increase in NDF digestibility when DDGS were included up to 20% of dietary DM reflects the high degradability of NDF from DDGS. In vitro NDF digestibility of DDGS is high, ranging from 68 to 73% (Getachew et al., 2004; Robinson et al., 2008; Tedeschi et al., 2009). It has been reported that corn distillers grain-based diets reduce chewing activity (Penner et al., 2009; Zhang et al., 2010), and thus may increase ruminal passage rate (Janicek et al., 2008), which may also explain the effect of DDGS on DMI and digestibility observed in the present study. Although DMI tended to increase, eating, rumination, and total chew-

Table 3. Intake and apparent total-tract digestibility of nutrients in lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS)

Item	DDGS				SEM	P-value	
	0%	10%	20%	30%		Linear	Quadratic
Intake							
DM (kg/d)	23.4	24.4	24.8	25.2	0.63	<0.01	0.45
OM (kg/d)	21.7	22.7	22.9	23.3	0.58	<0.01	0.44
GE (Mcal/d)	104	111	115	119	2.9	<0.01	0.54
NDF (kg/d)	7.50	8.23	9.00	9.53	0.239	<0.01	0.54
ADF (kg/d)	5.06	5.30	5.65	5.87	0.153	<0.01	0.90
CP (kg/d)	3.79	4.02	4.09	4.26	0.110	<0.01	0.71
Starch (kg/d)	4.31	3.87	3.40	2.83	0.119	<0.01	0.57
EE ¹ (kg/d)	0.93	1.21	1.50	1.81	0.038	<0.01	0.62
Digestibility (%)							
DM	70.7	70.2	69.6	68.1	0.63	<0.01	0.12
OM	72.5	71.9	71.1	69.8	0.60	<0.01	0.28
GE	69.6	69.2	68.7	67.6	0.51	<0.01	0.32
NDF	56.0	56.9	57.4	54.8	1.11	0.42	0.05
ADF	60.6	60.4	60.3	60.6	1.35	0.98	0.80
CP	67.3	68.3	68.4	69.2	0.70	0.03	0.80
Starch	94.7	95.4	96.2	98.8	0.30	<0.01	<0.01
EE	53.0	53.8	57.4	59.4	1.84	<0.01	0.73

¹EE = ether extract.

Table 4. In sacco OM degradation kinetic parameters and effective degradability of flaked corn, soybean meal, and timothy hay incubated in the rumen of lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS)

Item ¹	DDGS				SEM	<i>P</i> -value	
	0%	10%	20%	30%		Linear	Quadratic
Corn, flaked							
a (%)	43.2	42.9	43.3	42.6	0.46	0.41	0.61
b (%)	56.2	56.2	54.9	56.3	0.51	0.61	0.19
c (/h)	0.055	0.059	0.060	0.061	0.0052	0.32	0.71
Lag time (h)	0.00	0.00	0.00	0.04	0.020	0.23	0.36
ERD (%)	69.4	70.4	70.5	71.4	1.21	0.13	0.94
Soybean meal							
a (%)	40.2	40.2	40.3	40.4	0.06	0.06	0.78
b (%)	59.6	59.7	59.4	59.5	0.19	0.52	0.88
c (/h)	0.126	0.104	0.085	0.086	0.0170	0.08	0.48
Lag time (h)	0.96	0.85	0.32	0.24	0.174	0.01	0.94
ERD (%)	78.8	78.3	75.6	76.0	1.49	0.03	0.65
Timothy hay							
a (%)	19.7	18.9	19.7	19.0	0.37	0.38	0.92
b (%)	51.8	60.8	61.2	62.0	4.30	0.16	0.37
c (/h)	0.033	0.022	0.019	0.017	0.0041	0.01	0.20
Lag time (h)	4.47	1.66	2.42	1.65	0.841	0.08	0.26
ERD (%)	36.1	35.1	33.4	33.3	1.24	0.03	0.56

¹a = soluble and rapidly degradable fraction; b = slowly degradable fraction; c = fractional rate of disappearance of the fraction b; ERD = effective ruminal degradability.

ing time (/kg of DMI or /kg of NDF) were reduced as DDGS was incorporated (20% of dietary DM) in dairy cow diets in replacement of barley grain (Zhang et al., 2010).

In Sacco Ruminal Degradability

In sacco ruminal OM degradation parameters of flaked corn, soybean meal, and timothy hay are presented in Table 4. The soluble and rapidly degradable fraction (a), the slowly degradable fraction (b), the fractional rate of degradation (c), and the ERD of flaked corn were not affected ($P \geq 0.13$) by increasing the proportion of DDGS in the diet and averaged 43%, 56%, 0.059/h, and 70%, respectively. The lack of a change in ruminal degradability of flaked corn (i.e., source of starch) coupled with decrease in starch intake (Table 3) as DDGS proportion increased in the diet suggests a decline in the amount of starch escaping ruminal degradation. Reynolds (2006) reported that postruminal digestion of starch declined with increasing starch outflow from the rumen, which may explain the quadratic decrease in apparent total-tract digestibility of starch.

The soluble and rapidly degradable fraction of OM of soybean meal averaged 40.3%, whereas the slowly degradable fraction averaged 59.6% and both were not significantly affected by DDGS treatment. A tendency ($P = 0.08$) was observed for a linear decrease in the fractional degradation rate of soybean meal, whereas the lag time decreased linearly ($P = 0.01$) as the proportion of DDGS increased in the diet. These changes caused

a linear decline ($P = 0.03$) in soybean meal ERD by 0.5, 3.2, and 2.8 percentage units at DDGS 10, 20, and 30%, respectively. The decline in ruminal degradability of soybean meal (Table 4) and the concomitant increase in apparent total-tract CP digestibility (Table 3) with increasing DDGS proportions in the diet indicate an increase in postruminal digestion of dietary CP.

The response of timothy hay ruminal degradability kinetics to DDGS supplementation was similar to that of soybean meal. The soluble and rapidly degradable fraction and the slowly degradable fraction averaged 19 and 59% across treatments, respectively, and both were not affected ($P \geq 0.16$) by DDGS addition. However, a linear decline ($P = 0.01$) in the fractional rate of degradation and a tendency ($P = 0.09$) for a linear decrease in the lag time were observed with increasing DDGS proportions in the diet. As a result, timothy hay ERD declined linearly ($P = 0.03$) by 1.0, 2.7, and 2.8 percentage units at DDGS 10, 20, and 30%, respectively, suggesting that ruminal fibrolytic activity was reduced by the incorporation of DDGS in the diet. Islas and Soto-Navarro (2011) also reported a numerical decrease in NDF ruminal fractional degradation rate as DDGS proportion increased from 0 to 15% in the diet of grazing beef heifers. Leupp et al. (2009) observed a tendency for lower NDF ($P = 0.08$) and ADF ($P = 0.09$) ruminal degradabilities as the proportion of DDGS (replacing rolled corn, sunflower meal, and urea) increased (up to 60% of DM) in the diet of growing steers, associated with a quadratic increase in duodenal flows of NDF and ADF. Higher EE content of DDGS

diets compared with 0% DDGS diet (Table 2) may explain the effect of treatment on ruminal NDF degradability. In sacco ruminal degradability of barley silage was negatively affected by addition of unprotected fat to the diet (Chung et al., 2011).

Postruminal digestion of NDF when cows were fed diets containing 10 and 20% DDGS may have occurred and would be a possible explanation for the quadratic effect of DDGS on NDF apparent total-tract digestibility (Table 3). The decrease in ruminal degradability of hay is consistent with the decline observed in apparent total-tract NDF digestibility when cows were fed diets with 30% DDGS treatment (Table 3). The reduced apparent total-tract digestibility of NDF at the highest inclusion rate of DDGS could be also be explained by an increased ruminal passage rate (due to increased DMI) and a decline in digestion at postruminal sites. Data from Leupp et al. (2009) also suggest a shift in NDF digestion from the rumen to hind gut in steers fed up to 60% DDGS in the diet.

Ruminal Fermentation Characteristics and Protozoa Counts

Ruminal pH, total VFA concentration, VFA molar proportions, NH_3 concentration, and protozoa counts are presented in Table 5. Because the interactions between day and treatment were not significant for any of the variables reported, only averages of the 2-d measurements were reported. No treatment $\times$ time interaction was observed for any of the ruminal fermentation parameters measured except for acetate, propionate, and butyrate molar proportions. In the current study, despite cows being fed highly digestible diets and had high DMI levels, the mean daily ruminal pH remained relatively high (averaging 6.21) and was not affected

($P \geq 0.40$) by DDGS supplementation. The diets contained high concentrations of NDF ($>32\%$), mostly from forage, and moderate concentrations of starch ($\leq 19\%$), which ensured adequate salivation and buffering capacity of the rumen fluid. Other studies fed diets with similar characteristics (i.e., forage:concentrate ratio and NDF concentration) at the same DMI level and reported similar rumen pH values (Yang and Beauchemin, 2007; Yang and Beauchemin, 2009).

A tendency ($P = 0.06$) was observed for a quadratic increase in minimum pH as dietary DDGS level increased in the diet. This could be related to a reduction in starch and increase in NDF concentrations as the proportion of dietary DDGS increased in the diet. Ruminal degradation of NDF is much slower than starch, which causes less accumulation of VFA in the rumen and less ruminal pH fluctuation after feeding. No effect of feeding distillers grains on ruminal pH has been reported previously (Schingoethe et al., 1983; Zhang et al., 2010; Islas and Soto-Navarro, 2011).

Total VFA concentration decreased linearly ($P < 0.01$) as the proportion of DDGS increased in the diet. Other studies also reported lower ruminal total VFA concentrations when DDGS were fed to dairy or beef cattle (Nichols et al., 1998; Kleinschmit et al., 2006; Leupp et al., 2009) compared with those fed control diets. In vitro incubation (24 h) of DDGS with ruminal fluid produced 28% less total VFA than that produced from incubating corn grain (Getachew et al., 2004), reflecting lower ruminal degradability of DDGS compared with corn grain. Reduction in total VFA concentration as DDGS proportion in the diet increased are consistent with the reduced ruminal degradability of OM of hay and soybean meal (Table 4) and the decline in apparent total-tract digestibility of DM, OM, and GE observed with DDGS diets (Table 3).

Table 5. Ruminal fermentation characteristics and protozoa counts of ruminal fluid of lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS)

Item	DDGS				SEM	P-value	
	0%	10%	20%	30%		Linear	Quadratic
pH							
Mean	6.21	6.21	6.27	6.22	0.058	0.40	0.42
Minimum	5.92	5.92	5.98	5.97	0.055	0.95	0.06
Maximum	6.56	6.59	6.64	6.55	0.068	0.16	0.54
Total VFA (mM)	99.3	96.1	93.6	91.1	6.17	<0.01	0.84
VFA (mol/100 mol)							
Acetate (A)	63.4	62.7	61.8	60.1	0.83	<0.01	0.07
Propionate (P)	21.8	22.1	22.3	23.1	0.81	0.01	0.53
Butyrate	11.5	12.0	12.8	13.7	0.21	<0.01	0.15
Isobutyrate	0.83	0.76	0.74	0.70	0.029	<0.01	0.25
Valerate	1.17	1.21	1.22	1.30	0.038	<0.01	0.17
Isovalerate	1.36	1.22	1.17	1.10	0.060	<0.01	0.23
A:P	3.01	2.91	2.83	2.67	0.133	<0.01	0.56
NH_3 (mM)	8.38	7.51	6.68	6.06	0.307	<0.01	0.71
Protozoa ($\times 10^5/\text{mL}$)	5.12	5.28	5.42	4.48	0.46	0.15	0.04

Treatment $\times$ sampling time interactions for molar proportions of acetate, propionate, and butyrate were due to changes in the magnitude of differences between treatments at each sampling time. Molar proportion of acetate decreased linearly ($P < 0.01$; Figure 1), whereas molar proportions of propionate (Figure 2) and butyrate (Figure 3) increased linearly ($P < 0.01$) as the DDGS proportion in the diet increased. The reduction in acetate molar proportion could be related to the decrease in ruminal fiber digestion, as illustrated by the observed decline in ruminal degradability of timothy hay (Table 4) as the DDGS proportion in the diet increased. The increase in propionate molar proportion as dietary proportion of DDGS increases is a consequence of a decline in acetate molar proportion rather than an increase in propionate concentration. Indeed, the concentration of propionate in ruminal fluid was not affected by DDGS addition and averaged 21.5 mM ($P = 0.71$; data not shown), which was consistent with the lack of an effect of DDGS on in sacco ruminal degradability of flaked corn (Table 4). With increasing dietary DDGS inclusion, Leupp et al. (2009) reported a decline in acetate molar proportion but an increase in that of propionate, with no effect on butyrate molar proportion in beef cattle. Anderson et al. (2006) reported a numerical decrease in acetate molar proportion and an increase in propionate and butyrate proportions in dairy cows fed DDGS diets. Others reported no effect of dietary DDGS inclusion on acetate or propionate molar proportions (Kleinschmit et al., 2006; Zhang et al., 2010). The decrease in acetate proportion as DDGS inclusion rate increased caused a linear decrease ($P < 0.01$) in acetate:propionate ratio (Table 5). Similar observations have been reported in some studies (Anderson et al., 2006; Leupp et al., 2009), but not others (Nichols et al., 1998; Kleinschmit et al., 2006). It is generally assumed that supplementation of fat to ruminant diets reduces the ruminal acetate:propionate ratio (Getachew et al., 2004; McGinn et al., 2004; Chung et al., 2011). Effect of DDGS on acetate:propionate ratio is, thus, related to the increase in EE intake as DDGS dietary proportion increased (Table 3).

The molar proportion of valerate increased linearly ($P < 0.01$), whereas the proportion of branched-chain VFA (isobutyrate and isovalerate) declined linearly ($P < 0.01$) with increasing DDGS proportions in the diet (Table 5). Ruminal NH₃ concentration also decreased linearly ($P < 0.01$) as the proportion of DDGS in the diet increased (Table 5). Similarly to our study, other studies (Nichols et al., 1998; Anderson et al., 2006; Kleinschmit et al., 2006) also reported reductions in ruminal NH₃ concentration and branched-chain VFA molar proportions when DDGS replaced partially the concentrate portion of dairy cow diets. Decreases in

Figure 1. Ruminal acetate molar proportion in lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS; ■ 0%, □ 10%, ● 20%, ○ 30%).

ruminal NH₃ and branched-chain VFA are an indication of a reduction in ruminal degradation of protein, as illustrated by the decline in ERD of soybean meal observed in the current study for cows fed DDGS diets as compared with those fed the 0% DDGS diet (Table 4).

Supplementation with DDGS caused a quadratic ($P = 0.04$) response in protozoa count, being lowest at 30% DDGS (Table 5), which may be explained by the higher EE concentration (Table 2) and intake (Table 3) at this supplementation level. Incorporating fat in the diet of dairy cows has been reported to reduce protozoa numbers in ruminal fluid both in vitro and in vivo (Machmüller et al., 1998; Beauchemin et al., 2009; Chung et al., 2011). The reduction in total protozoa

Figure 2. Ruminal propionate molar proportion in lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS; ■ 0%, □ 10%, ● 20%, ○ 30%).

Figure 3. Ruminal butyrate molar proportion in lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS; ■ 0%, □ 10%, ● 20%, ○ 30%).

count may also explain at least partially the decrease in NH_3 concentration (Table 5) and the resulting reduction in in sacco ruminal degradation of soybean meal (Table 4). Reducing ruminal protozoa contributes to improving N efficiency by decreasing ruminal protein degradation and intraruminal N recycling, and increasing microbial N uptake and microbial protein synthesis (Ushida and Jouany, 1985; Williams and Coleman, 1988; Oldick and Firkins, 2000).

Milk Production and Milk Composition

Milk production increased linearly ($P < 0.01$) with increasing DDGS proportions in the diet. Cows offered the 30% DDGS diet produced 4 kg/d more milk than when offered the diet with 0% DDGS supplementation. This increase in milk yield is consistent with the higher DM and energy intakes of cows fed DDGS diets compared with cows fed the 0% DDGS diet (Table 3).

Fat and protein percentages decreased linearly ($P < 0.01$) with increasing DDGS inclusion in the diet, whereas a quadratic effect ($P < 0.01$) of DDGS addition on MUN concentration was observed. Milk urea nitrogen is an indicator of BUN, which is formed from NH_3 absorbed from the rumen, or AA catabolism at the tissue level. Ruminal NH_3 concentration declined linearly with the addition of DDGS in the diet, which explains the decline in MUN when DDGS were included up to 20% of the diet. The increase in MUN concentration at 30% DDGS may be due to the increased supply of AA at the intestinal level and the eventual use of AA for gluconeogenesis, which might have increased AA deamination and the concentration of BUN. The decline in milk fat content can be related to the decrease in ruminal molar proportion of acetate

(Table 5). According to Bauman and Griinari (2001), diets causing a decrease in milk fat content and yield also alter ruminal lipid metabolism, resulting in the formation of specific biohydrogenation intermediates of the *trans*-10 pathway that directly inhibit milk fat synthesis. Such changes may explain the linear decrease in milk fat content observed in the current study. A tendency for a quadratic effect of DDGS on FCM ($P = 0.09$) and ECM ($P = 0.10$) yields was observed, as cows fed DDGS diets produced, on average, 2 kg/d more FCM or ECM than cows fed the 0% DDGS diet. Fat yield was not significantly ($P > 0.05$) changed by experimental treatment, although it tended ($P = 0.08$) to respond quadratically to increasing inclusion of DDGS in the diet. Protein ($P = 0.03$) and lactose ($P < 0.01$) yields increased linearly when cows were fed increasing proportions of DDGS, indicating no negative effect of increasing EE intake in the diet (via DDGS addition) on production parameters.

A similar positive effect of DDGS on dairy cow performance has been reported previously (Anderson et al., 2006; Kleinschmit et al., 2006; Janicek et al., 2008). According to a review by Schingoethe et al. (2009), adding corn distillers grains with solubles to dairy cattle diets either improved or had no effect on milk production parameters. Milk yield of high-producing dairy cows in early to mid lactation responded positively to incorporating corn distillers grains in their diets, but not in low-producing, late-lactation cows (Janicek et al., 2008; Hollmann et al., 2011), which supports our findings. The observed increase in milk yield reported in this study was also noted by Janicek et al. (2008) but not by Kleinschmit et al. (2006) and Anderson et al. (2006). In the last 2 studies, the higher milk production was, in fact, due to the greater energy density of the diets containing corn distillers grains.

Reported effects of DDGS on milk fat concentration have been variable among studies. For instance, and similarly to our findings, Leonardi et al. (2005) observed no change in milk fat yield when the proportion of DDGS increased (from 0 to 15%, DM basis) in the diet; however, milk fat concentration decreased linearly due to increased milk production in that study. In the same study, the authors observed a reduction in the proportions of short-chain FA coupled with an increase in the proportions of long-chain FA in milk, suggesting that de novo FA synthesis in the mammary gland was inhibited, but this was offset by uptake and increased secretion of long-chain FA, more likely absorbed from the diet (Leonardi et al., 2005). Other studies (Kleinschmit et al., 2006; Janicek et al., 2008) reported no changes in milk fat content when cows were fed DDGS diets. In a meta-analysis study, Hollmann et al. (2011) reported that the negative effect of corn

distillers grains on milk fat concentration occurs more likely when milk fat concentration of cows fed control treatments is greater than 3.6%, which is in agreement with findings of the present study. The same study also reported that FCM yield increased by up to 2.2 kg/cow per day when 20% DDGS were incorporated in the diet, compared with the 0% DDGS diet, which also agrees with our findings.

In the current study, the higher milk protein yield observed as dietary DDGS proportion increased in the diet may be explained by a greater supply of AA due to (1) an increased feed N intake coupled with an increase in CP digestibility and (or) (2) a reduction in protein degradation in the rumen as reflected by declines in ruminal NH₃ concentration, branched-chain VFA molar proportions, protozoa numbers (Table 5), and the ERD of soybean meal (Table 4). Gehman and Kononoff (2010) observed enhanced microbial protein synthesis when cows were fed 25% corn wet distillers grains (replacing corn silage or alfalfa silage, corn, soybean meal, and heat-treated soybean), which may further explain the greater protein secretion in milk observed in the current study. Higher intestinal availability and higher arterial concentrations of essential and nonessential AA were reported when cows were fed diet containing corn distillers grains compared with a control diet containing soybean meal (Nichols et al., 1998; Mjoun et al., 2010).

Feed efficiency (expressed as kilogram of ECM produced per kilogram of DMI) averaged 1.51 and was not affected ($P > 0.19$) by increasing the level of DDGS in the diet. In contrast, Anderson et al. (2006) and Kleinschmit et al. (2006) reported higher feed efficiency when cows were fed diets containing 20% of wet or dry corn distillers grains with solubles. In those 2 studies, DMI was not changed (Kleinschmit et al., 2006) or tended to decrease (Anderson et al., 2006), whereas milk yield increased in cows fed corn distillers grains with solubles, explaining the improved feed efficiency compared with our findings. On the other hand, weight gain (kg/d) increased linearly ($P < 0.01$) when cows were fed 10, 20, and 30% DDGS diets by 21, 162, and 227% compared with 0% DDGS (Table 6). Thus, cows also displayed higher productivity when DDGS diets were fed.

Methane Production

No interactions were observed between day and treatment for CH₄ measurements and, therefore, only averages over the 3-d measurements were reported (Table 7). Methane production was 495 g/d for cows consuming 0% DDGS diet and declined linearly ($P = 0.03$) as DDGS proportion in the diet increased. When expressed on DMI basis, CH₄ production declined lin-

early ($P < 0.01$) by 2, 4, and 8% at DDGS proportion 10, 20, and 30%, respectively. This indicates that, on average, a 1% increase in fat decreased CH₄ production by 0.5 g/kg of DMI (0.5, 0.4, and 0.7 for 10, 20, and 30% DDGS, respectively). The model [CH₄ (g/kg of DMI) = 24.55 (± 1.029) - 0.102 (± 0.0147) dietary fat (g/kg); diets containing <80 g of fat/kg of DM] of Grainger and Beauchemin (2011) predicted a 1 g of CH₄/kg of DMI decrease for each 1% increase in dietary fat. This may suggest that the proposed model does not allow accurate predictions of enteric CH₄ emission changes under a wide range of dietary lipid-supplementation conditions (e.g., DDGS supplementation).

Methane energy losses as a percentage of GE intake decreased linearly ($P < 0.01$) by 5, 8, and 14% in cows fed 10, 20, and 30% DDGS diets, respectively, compared with cows fed the 0% DDGS diet. Similarly, when corrected for digestible energy intake, CH₄ decreased linearly ($P < 0.01$) as the amount of DDGS in the diet increased. When expressed on a milk-yield basis (g of CH₄/kg of milk), CH₄ production declined linearly by 9, 13, and 15% for 10, 20, and 30% DDGS diets, respectively. Similarly, the amount of CH₄ produced per kilogram of FCM ($P = 0.04$) or ECM ($P = 0.02$) decreased linearly by an average of 9% in cows fed increasing proportions of DDGS in the diet. A quadratic effect ($P = 0.05$) of DDGS on CH₄ production was observed, related to kilograms of milk fat produced. When expressed on the basis of kilograms of milk protein produced, CH₄ production declined linearly ($P < 0.01$) as the DDGS proportion increased in the diet.

Studies on the effect of DDGS on CH₄ production in cattle are scarce. Using the head box technique, Birkelo et al. (2004) reported a 14% decline in CH₄ energy losses (% of GE intake) when cows were fed 31% wet corn distillers grains, which is similar to the extent of decrease observed in the present study. In that study, the lower CH₄ production was associated with a decrease in DMI. More recently, McGinn et al. (2009) used the sulfur hexafluoride (SF₆) tracer technique and observed a 24% decrease in CH₄ production (% of GE intake) with no concomitant effect on DMI when DDGS (35% of dietary DM) replaced barley grain in growing beef cattle diets.

The observed decline in CH₄ production could be attributed mainly to the high EE content in DDGS diets compared with the 0% DDGS diet (Table 2). Feeding 10, 20, and 30% DDGS diets increased total EE intake by 30, 61, and 95% compared with 0% DDGS diet (Table 3). Several studies have reported decreases in enteric CH₄ emissions when cattle diets were supplemented with unprotected fat (Beauchemin et al., 2009; Grainger et al., 2010; Chung et al., 2011). The negative effect of increasing dietary EE intake on

Table 6. Milk production, milk composition, BW, and BW gain of lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS)

Item	DDGS				SEM	P-value	
	0%	10%	20%	30%		Linear	Quadratic
Production (kg/d)							
Milk	32.6	35.1	35.8	36.6	1.97	<0.01	0.22
4% FCM ¹	32.1	34.5	34.1	33.7	2.16	0.22	0.09
ECM ²	35.3	37.8	37.3	37.1	2.20	0.16	0.10
Composition (%)							
Fat	3.93	3.91	3.69	3.47	0.184	<0.01	0.17
Protein	3.49	3.41	3.31	3.31	0.047	<0.01	0.31
Lactose	4.60	4.63	4.59	4.58	0.027	0.42	0.26
SCC ($\times 10^3$ /mL)	75	82	133	89	12.6	0.23	0.19
MUN (mg/dL)	11.1	10.0	9.9	10.6	0.52	0.23	≤ 0.01
Yield (kg/d)							
Fat	1.27	1.36	1.32	1.27	0.099	0.80	0.08
Protein	1.13	1.19	1.18	1.20	0.057	0.03	0.36
Lactose	1.50	1.62	1.65	1.68	0.096	<0.01	0.17
Feed efficiency							
Milk/DMI	1.40	1.44	1.44	1.45	0.056	0.23	0.51
FCM/DMI	1.37	1.42	1.37	1.33	0.068	0.20	0.17
ECM/DMI	1.51	1.55	1.50	1.46	0.067	0.19	0.23
BW							
Initial (kg)	700	701	697	698	15.8	0.41	0.87
Final (kg)	710	714	724	730	16.2	<0.01	0.74
Gain (kg/d)	0.29	0.35	0.76	0.95	0.150	<0.01	0.43

¹4% fat-corrected milk = $0.4 \times \text{milk yield (kg/d)} + 15 \times \text{fat yield (kg/d)}$.

²Energy-corrected milk = $0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{fat yield (kg/d)} + 7.2 \times \text{protein yield (kg/d)}$.

protozoa population (Table 5) may partially explain the observed reduction in CH₄ production. Methanogens are present on and have a symbiotic relationship with protozoa. Hydrogen produced by protozoa during feed fermentation in the rumen is utilized by methanogens to produce CH₄. Substantial methanogenic activity in the rumen has been linked to protozoa related methanogens (Vogels et al., 1980; Finlay et al., 1994; Newbold et al., 1995). Furthermore, EE from DDGS could have a toxic effect on other methanogens in the rumen, as

observed with EE from linseed and soybean (Martin et al., 2008; Yang et al., 2009). The observed decline in the acetate:propionate ratio (Table 5) supports the speculation of a change in microbial population in the rumen toward less acetate-producing bacteria and less methanogenesis.

Reduction in ruminal fiber degradation may have also contributed to reduced CH₄ production. Decreases in timothy hay in sacco ruminal degradability (Table 4) and the acetate molar proportion (Table 5) as DDGS

Table 7. Methane production of lactating cows fed diets supplemented with increasing amounts of corn dried distillers grains with solubles (DDGS)

Item	DDGS				SEM	P-value	
	0%	10%	20%	30%		Linear	Quadratic
DMI, ¹ kg/d	24.2	24.6	24.4	25.3	0.80	0.10	0.55
CH ₄ g/d ¹	495	490	477	475	21.0	0.03	0.84
g/kg of DMI ¹	20.6	20.1	19.7	18.9	0.62	<0.01	0.43
% of GE intake ¹	6.09	5.80	5.61	5.23	0.173	<0.01	0.56
% of DE intake ²	8.75	8.39	8.17	7.74	0.241	<0.01	0.79
g/kg of milk ³	15.6	14.2	13.6	13.2	0.60	<0.01	0.18
g/kg of FCM ³	15.7	14.3	14.3	14.4	0.53	0.04	0.07
g/kg of ECM ³	14.3	13.1	13.0	13.0	0.47	0.02	0.09
g/kg of milk fat ³	396	363	372	390	19.1	0.87	0.05
g/kg of milk protein ³	446	415	411	400	15.5	<0.01	0.29

¹Determined for 3 consecutive days during which cows were in the chambers.

²DE = digestible energy (estimated from energy digestibility measured over the 7-d collection period).

³Yields of milk, FCM, ECM, milk fat, and milk protein measured for 7 consecutive days (i.e., performance measurements).

proportion in the diet increased support this assumption. Ruminal degradation of fiber is known to promote acetate production in the rumen, which liberates hydrogen that is used by methanogens to form CH₄ (Van Soest, 1994). This can be also related to the higher EE concentrations in DDGS diets.

Several studies reported a decline in total-tract NDF digestibility coupled with a decrease in CH₄ emissions when unprotected fat was added to ruminant diets (Martin et al., 2008; Beauchemin et al., 2009; Chung et al., 2011), which agrees with what is observed for 30% DDGS treatment in the current study. In agreement with our observations, Leupp et al. (2009) reported tendencies for quadratic effects on ruminal digestion ($P = 0.08$) and apparent total-tract digestibility ($P = 0.13$) of NDF in steers fed up to 60% DDGS in the diet. Contrarily, Birkelo et al. (2004) reported an increase in apparent total-tract digestibility when cows were fed diets containing 31% wet corn distillers grains. Discrepancies could be related to the amount of distillers grains included in the diet and, hence, the amount of EE added to the dietary DM. In the present study, the 0% DDGS diet contained 4% EE, which increased up to 7.2% in the 30% DDGS diet (Table 2). In the Birkelo et al. (2004) study, the total EE concentration of the control and the distillers grain diet were 3.1 and 5.1%, respectively. Thus, the amount of EE ingested by cows in the experiment of Birkelo et al. (2004) may not have been sufficient to negatively affect NDF digestion. In a meta-analysis, Eugène et al. (2008) showed that increasing the lipid content of the diet decreased enteric CH₄ production and linked this decrease to a decline in DMI. However, the reduction in CH₄ production observed in the current study was not associated with a decrease in DMI (Table 3), suggesting that changes in ruminal methanogenesis was a result of disturbances of rumen fermentation caused by supplemental EE (i.e., DDGS).

N Balance

Daily N intake increased linearly ($P < 0.01$) with increasing DDGS proportion in the diet, which led to linear increases (g/d) of N output in feces ($P = 0.05$), urine ($P < 0.01$), N secretion in milk ($P = 0.03$), and body N retention (Table 8). The higher DMI and the slightly higher dietary N content when cows were fed DDGS diets (Table 3) led to the increase in N intake. When expressed as a proportion of N intake, fecal N excretion declined linearly ($P = 0.03$), whereas urinary N output remained unaffected ($P \geq 0.35$) by the dietary addition of increasing levels of DDGS. Total N excretion expressed as a percentage of N intake tended ($P = 0.07$) to decrease with increasing DDGS propor-

tions in the diet. Efficiency of N use (g of N used for milk protein synthesis and building body reserve/g of N intake) tended ($P = 0.07$) to increase with increasing DDGS proportions in the diet. Retained N as a proportion of N intake increased (up to +3.6 percentage units at 30% DDGS), whereas the proportion of N secreted in milk slightly declined (up to -1.7 percentage units at 30% DDGS) as dietary DDGS proportion increased. Assuming that the body tissue contains about 20% protein (NRC, 2001), the retention of 60 g of N/d (i.e., 375 g of protein/d) should have resulted in a BW gain of 1.85 kg/d. Considering the inherent errors associated (e.g., volatile N losses from urine and feces during collection) with N balance studies (MacRae et al., 1993; Spanghero and Kowalski, 1997), true N retention was likely overestimated, as illustrated by the modest BW change recorded (Table 2). Several studies also reported overestimation of N retention during N balance determination trials (Ruppert et al., 2003; Benchaar et al., 2007; Colombini et al., 2012).

Very few studies have investigated in detail the effects of including corn distillers grains with solubles in dairy cow diets on N outputs and whole-body N partitioning. In one study (Gehman and Kononoff, 2010), feeding corn wet distillers grains to dairy cows increased N secretion in milk, N retention, and efficiency of N utilization for productive uses, which agrees with our findings.

CONCLUSIONS

Enteric CH₄ production (g/d) was reduced when increasing proportions of DDGS were incorporated in the diet of dairy cows at the expense of corn and soybean meal. Intake of DM and milk yield responded positively to increasing DDGS proportions in the diet. Thus, when expressed as a percentage of GE intake, CH₄ production was decreased by up to 14% at the highest inclusion rate (i.e., 30% of dietary DM) of DDGS. Similar extents of decrease were also noted when CH₄ production was corrected for digestible energy intake (up to 12% at 30% DDGS supplementation). When related to FCM or ECM yield, CH₄ production (g/kg of FCM or ECM) declined linearly with increasing DDGS proportions in the diet. Increasing the EE supply as DDGS proportion in the diet increased and its effects on ruminal fiber degradation, acetate:propionate ratio, and protozoa numbers is considered the main reason for the reduction in CH₄ production. Thus, inclusion of DDGS in the diet of dairy cows has the potential to reduce enteric CH₄ production. Although the efficiency of N utilization (i.e., higher retained N and productive N) was improved by the addition of DDGS in the diet, the resulting greater N excretion in the manure needs

Table 8. Nitrogen balance in lactating cows fed diets supplemented with increasing amounts of corn distillers grains with solubles (DDGS)

Item	DDGS				SEM	P-value	
	0%	10%	20%	30%		Linear	Quadratic
Intake N, g/d	606	642	655	682	17.5	<0.01	0.71
Fecal N							
g/d	198	204	207	211	7.2	0.05	0.89
% of N intake	32.7	31.7	31.6	30.8	0.70	0.03	0.80
Urinary N							
g/d	204	209	213	223	8.6	<0.01	0.59
% of N intake	33.7	32.7	32.7	32.6	1.28	0.35	0.51
Total N excretion							
g/d	402	413	419	434	13.0	<0.01	0.81
% of N intake	66.4	64.4	64.3	63.5	1.47	0.07	0.56
Milk N							
g/d	177	187	185	189	8.9	0.03	0.36
% of N intake	29.4	29.1	28.2	27.7	1.02	<0.01	0.83
Retained N							
g/d	33	42	51	60	9.9	0.05	0.99
% of N intake	5.29	6.53	7.58	8.85	1.51	0.08	0.99
Productive N ¹							
g/d	204	229	236	248	12.3	<0.01	0.53
% of N intake	34.6	35.6	35.7	36.5	1.47	0.07	0.56

¹Retained N + milk N.

to be taken into account for better assessment of the overall environmental impact of feeding DDGS to dairy cows.

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