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Grape marc reduces methane emissions when fed to dairy cows

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ABSTRACT

Grape marc (the skins, seeds, stalk, and stems remaining after grapes have been pressed to make wine) is currently a by-product used as a feed supplement by the dairy and beef industries. Grape marc contains condensed tannins and has high concentrations of crude fat; both these substances can reduce enteric methane (CH_4) production when fed to ruminants. This experiment examined the effects of dietary supplementation with either dried, pelleted grape marc or ensiled grape marc on yield and composition of milk, enteric CH_4 emissions, and ruminal microbiota in dairy cows. Thirty-two Holstein dairy cows in late lactation were offered 1 of 3 diets: a control (CON) diet; a diet containing dried, pelleted grape marc (DGM); and a diet containing ensiled grape marc (EGM). The diet offered to cows in the CON group contained 14.0 kg of alfalfa hay dry matter (DM)/d and 4.3 kg of concentrate mix DM/d. Diets offered to cows in the DGM and EGM groups contained 9.0 kg of alfalfa hay DM/d, 4.3 kg of concentrate mix DM/d, and 5.0 kg of dried or ensiled grape marc DM/d, respectively. These diets were offered individually to cows for 18 d. Individual cow feed intake and milk yield were measured daily and milk composition measured on 4 d/wk. Individual cow CH_4 emissions were measured by the SF_6 tracer technique on 2 d at the end of the experiment. Ruminal bacterial, archaeal, fungal, and protozoan communities were quantified on the last day of the experiment. Cows offered the CON, DGM, and EGM diets, ate 95, 98, and 96%, respectively, of the DM offered. The mean milk yield of cows fed the EGM diet was 12.8 kg/cow per day and was less than that of cows fed either the CON diet (14.6 kg/cow per day) or the DGM diet (15.4 kg/cow per day). Feeding DGM and EGM diets was associated with decreased milk fat yields, lower concentrations of saturated fatty acids, and enhanced concentrations of mono- and polyunsaturated fatty acids, in particular *cis*-9,*trans*-11 linoleic acid. The mean CH_4 emissions were 470, 375, and 389 g of CH_4/cow per day for cows fed the CON, DGM, and EGM diets, respectively. Methane yields were 26.1, 20.2, and 21.5 g of CH_4/kg of DMI for cows fed the CON, DGM, and EGM diets, respectively. The ruminal bacterial and archaeal communities were altered by dietary supplementation with grape marc, but ruminal fungal and protozoan communities were not. Decreases of approximately 20% in CH_4 emissions and CH_4 yield indicate that feeding DGM and EGM could play a role in CH_4 abatement.
Key words: fat, tannin, fatty acid, microbial profiling

INTRODUCTION

Methane (CH_4) is a potent greenhouse gas (IPCC, 2007). Enteric CH_4 emissions from ruminants amount to approximately 80 million tonnes annually and account for approximately 28% of global anthropomorphic CH_4 emissions (Beauchemin et al., 2008). Interest is growing in developing practical nutritional strategies for ruminants that will reduce these emissions.

One strategy that can reduce CH_4 emissions is the addition of fat to the diet of ruminants (Beauchemin et al., 2008; Moate et al., 2011). Another effective strategy for reducing enteric CH_4 emissions from ruminants involves including condensed tannins (**CT**) in their diet (Waghorn et al., 2002; Grainger et al., 2009). Grape marc is the skins, seeds, and stems that remain after grapes (*Vitis vinifera*) have been pressed to make wine. Grape marc contains high concentrations of both fat and tannin (Spanghero et al., 2009). In one experiment, when cows were fed 20% of their dietary DMI as ensiled grape marc (**EGM**), no negative effects were observed on their DMI, milk yields or milk composition (Belibasakis et al., 1996). In vitro studies have shown that tannin extracted from grape seed can reduce CH_4 production (Pellikaan et al., 2011), but little information is available concerning the nutritional value of grape

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marc as a ruminant feed and its potential to reduce CH_4 emissions from ruminants. Theoretically, the fats and tannins in grape marc may inhibit CH_4 emissions if fed to ruminants. However, how the fats and tannins in grape marc interact with the ruminal microbiota is not known.

In Australia, 2 commercially available forms of grape marc exist: dried grape marc (**DGM**) and EGM. Grape marc is usually first steam distilled for up to 15 min at approximately 94°C to extract ethanol, and the residue from this distillation (spent grape marc) is then ensiled for a period of at least 1 mo to produce EGM. Dry grape marc is made from the spent grape marc, which is stockpiled for several months before making a dry meal powder. Drying occurs as a continuous process for approximately 20 min, in a gas-fired rotary drum heated to approximately 120°C. The resulting dry meal is ground and then steam-pelleted at 85°C. (B. Mengersen, Tarac Technologies Pty. Ltd., Nuriootpa, Australia, personal communication).

In Australia, dairying is mostly pasture based, with many cows grazing all year. Cows calve in the spring when pasture is abundant and of high quality. However, during the summer, high temperatures and lack of rain generally result in low pasture growth rates and reduced milk yields. By February (late summer) and March (early autumn) all cows in the herds with seasonally concentrated calving pattern are in late lactation and poor-quality pasture and low pasture availability exacerbates low milk yields. At this time of the year, farmers may feed their cows feed supplements of concentrates (mainly wheat), but a fiber source such as hay, pasture silage, or grape marc is also required. During times of drought (1999–2009) and during most summers, purchased hay becomes very expensive. Thus, despite the low nutritional quality of grape marc (Belibasakis et al., 1996), it has potential to be incorporated into dairy cow rations because it has a high concentration of fiber, and it is currently a waste product with low cost compared with the alternative of expensive purchased hay. This experiment was designed to mimic the feeds, feeding practices, and conditions on many dairy farms in late summer in southern Australia and to examine the effect on dairy cow production and CH_4 emissions when grape marc is incorporated into such diets

The objective of this research was to compare the effects on CH_4 emissions, milk yield, milk composition, and ruminal microbiota resulting from feeding either a control diet or diets containing DGM or EGM to dairy cows in late lactation. We hypothesized that feeding DGM or EGM instead of alfalfa hay to dairy cows (1) would not influence yields of milk, milk fat, or milk protein; and (2) the 2 types of grape marc would inhibit CH_4 emissions from dairy cows to an equal extent.

MATERIALS AND METHODS

The experiment was conducted at the Department of Environment and Primary Industries, Ellinbank Centre, Victoria, Australia (**DEPI** Ellinbank; 38°14′S, 145°56′E) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2004 (NHMRC, 2004). Cow use was approved by the Animal Ethics Committee of the Department of Environment and Primary Industries (Victoria).

Cows and Diets

Thirty-two lactating, multiparous Holstein-Friesian cows, each producing 16.3 ± 1.83 L of milk/d (average ± SD) with 582 ± 54.2 kg of BW and 203 ± 72.8 DIM were allocated to 3 groups balanced for mean milk yield, BW, and yields of fat and protein according to the method of Baird (1994). Each group was then randomly allocated to 1 of 3 dietary treatments: (1) A control (**CON**) diet in which cows were individually offered 14.0 kg of alfalfa hay DM/d and 4.3 kg of concentrate mix DM/d; (2) a DGM diet in which cows were individually offered 9.0 kg of alfalfa hay DM/d, 5.0 kg of DGM DM/d, and 4.3 kg of concentrate mix DM/d; or (3) an EGM diet in which cows were individually offered 9.0 kg of alfalfa hay DM/d, 5.0 kg of EGM DM/d, and 4.3 kg of concentrate mix DM/d. The EGM used in this experiment had been ensiled for 11 mo. The concentrate mix contained 93.0% crushed wheat, 4.7% dried molasses, and 2.3% mineral mix (DM basis).

The CON treatment group had 12 cows assigned to it, whereas the DGM and EGM treatment groups each originally had 10 cows. However, 1 cow in the EGM treatment group contracted mastitis and was removed from the experiment. On d 1 to 16 of the experiment, all cows received the CON treatment so that covariate measures could be obtained. From d 17 to 19, cows allocated to the DGM and EGM treatments transitioned onto their dietary treatment, with the CON cows remaining on their CON diet. All cows then were offered their full allocated treatment diet until d 37; that is, for 18 d duration.

Feeding and Measurements

Cows were fed in individual feed stalls within a well-ventilated animal house (Williams et al., 2011). Cows were offered their dietary treatments in 2 equal portions between 0730 and 1130 h, and 1530 and 1930 h each day. Cows were offered the concentrate mix and grape marc between 0730 and 0900 h in the morning and between 1530 and 1700 h in the afternoon. The alfalfa hay was offered to the cows between 0900 and

1130 h in the morning and between 1700 and 1930 h in the afternoon. At all other times, cows did not have access to any other feed but had access to clean drinking water. For cows offered the grape marc diets, the grape marc was manually mixed with the concentrate mix before it was offered to the cows. The quantities of feeds offered and refused by individual cows were weighed at each feeding. Samples representative of the feed offered and refused were collected at each feeding and bulked by group. After almost every feeding period, cows had eaten all of the grape marc grain mixture offered to them. On the few occasions when a small number of cows refused some grape marc-concentrate mixture, it was assumed that the residue contained the same proportions (on a fresh-weight basis) of grape marc and concentrate that was offered. The concentration of DM was determined by drying feed samples in a fan-forced draft oven at 105°C for 24 h. Samples of the concentrate mix, alfalfa hay, DGM, and EGM were collected and stored at −18°C over the duration of the experiment and then bulked by feed type, freeze-dried, and ground to pass through a 0.5-mm screen.

When not in the animal house, cows were either at the milking parlor (0700–0730 and 1500–1530 h) or held in an open-air loafing pad (1130–1500 and 1930–0700 h).

Cows were weighed each morning after milking. The change in BW of individual cows was calculated as the difference in the mean BW measured during the 7 d before the start of this experiment and the mean BW during the last 3 d of the experiment.

Milk yield was measured for each cow at each milking using a DeLaval Alpro milk metering system (DeLaval International AB, Tumba, Sweden). Milk samples were collected from individual cows during morning and afternoon milkings over 4 d (Monday afternoon to Friday morning) each week of the covariate and experimental periods. Fat, protein, and lactose in these milk samples were measured by a near-infrared milk analyzer (model 2000; Bentley Instruments Inc., Chaska, MN). Somatic cells were counted by a Fossomatic SC300 cell counter (Foss, North Ryde, New South Wales, Australia). Energy-corrected milk, standardized to 4.0% fat and 3.3% protein, was calculated using the formula of Tyrrell and Reid (1965).

Milk fat was extracted from fresh samples using a method based on the Röse-Gottlieb gravimetric method (IDF, 1987) and stored at −20°C until analyzed for FA composition. Concentrations of FAME in milk fat were determined by gas chromatography following methylation with sodium methoxide (Slover and Lanza, 1979). Analyses were conducted using an Agilent 6890 gas chromatograph with autosampler, autoinjector, and flame ionization detector (Agilent Technologies Inc., Santa Clara, CA), and with a RTX-2330 column (105 m × 0.25 mm i.d. and 0.2-μm film thickness), using He as the carrier gas at a rate of 1.4 mL/min. Peak identification was by retention time comparisons with Sigma Chemical Corp. (St. Louis, MO) standards 189-19 (37 FAME 4:0–24:0) included every 15 samples.

Analysis of Feeds

Feeds were analyzed for CP, DM digestibility, NDF, ME, and crude fat by near-infrared spectroscopy at the Dairy One Forage Laboratory, Ithaca, NY, using methods detailed in AOAC International (2000; method 989.03).

The analysis for proanthocyanidin concentration (extractable and bound CT) in feed samples was performed using the HCl-butanol assay described by Hagerman (2002). The proanthocyanidin reference material was obtained from the aqueous acetone extraction of grape seed tannin, which had been further purified by selective absorption and extraction from Sephadex LH20 medium (Pharmacia, New York, NY) as described by Hagerman (2002).

Total tartaric acid (the sum of D and L enantiomers) in grape marc was measured using a HPLC system (Agilent 1100 Series High Performance Liquid Chromatograph; Agilent Technologies Inc.) by the method of Castoldi (1989). On d 35 and 36, milk samples were collected from all cows at 2 consecutive milkings (p.m. and a.m.) and then analyzed for FA by gas chromatography according to standard methods (IDF, 2001, 2002).

CH_4 Emissions

The SF_6 tracer technique of Zimmerman (1993), as modified by Grainger et al. (2009), was used to estimate CH_4 emissions from individual cows. In our experiment, the permeation tubes (NIWA, Wellington, New Zealand) were filled with about 2.3 g of SF_6 and calibrated in a dry incubator set at 39°C for 12 wk and the permeation tubes were weighed twice each week. The release rate of SF_6 was 4.1 ± 0.91 mg/d (average ± SD) and ranged from 2.6 to 5.3 mg/d. The permeation tubes containing SF_6 were placed in the rumen of the cows per os 1 wk before the first measurements of CH_4 were performed. Stainless steel canisters of 800 mL with an initial sampling rate of approximately 0.6 mL/min were used to continuously sample eructated gases. Gas samples were collected on d 34 and 36. Canisters were exchanged twice daily at 0730 and 1930 h so that each canister contained a sample of gas representing CH_4 and SF_6 emissions from an individual cow over a 12-h period. After the samples of gases had been collected into canisters, the canisters were filled with sufficient

N_2 (999.99 g of N/kg) to bring the remaining vacuum to 0 kPa before analysis by gas chromatography. The technique for collecting background gas samples in the animal house and while cows were outside the animal house was previously described in detail (Williams et al., 2011). Analysis of collected gas samples was by gas chromatography (Williams et al., 2011) and CH_4 emissions for each 12-h period were calculated using equation 2 from the study of Williams et al. (2011).

The daytime and nighttime CH_4 emissions were then added to obtain the daily CH_4 emissions (g/d). Gas samples from one of the cows fed the CON diet leaked from the canisters; therefore, results from these samples were not included in statistical analyses.

Ruminal Fermentation, Methanogens, and Protozoa

Rumen headspace gas (~400mL) was sampled from all cows on d 33 of the experiment using the rumenocentesis method of Moate et al. (1997). Analysis of the samples for H_2, CH_4, and CO_2 was by gas chromatography as described by Moate et al. (2013b).

Rumen fluid samples (~400 mL) were collected from each cow between 1100 and 1300 h (at least 4 h after the start of the morning feeding period) on d 37. Ruminal fluid samples were collected using a vacuum pump and a sampling tube placed into the rumen via the mouth. Samples were allowed to drain freely through 1 layer of cheesecloth, which separated rumen solids from the ruminal fluid. Prior to this experiment, training in this sampling technique using rumen-fistulated cows ensured that, in this experiment, the sampling tube could be placed deep into the ventral sac of the rumen such that contamination of ruminal fluid samples with saliva was negligible. The pH of the ruminal fluid was immediately measured using a Mettler-Toledo FG2 pH meter (Mettler-Toledo, Schwerzenbach, Switzerland). A 0.5-mL representative subsample of ruminal fluid was transferred to a 12-mL plastic vial and then diluted with 4.5 mL of a solution containing 4% formalin, 0.9% saline, and 0.4% methylene blue. These samples were stored at room temperature until counted for ciliate protozoa in a Mod-Fuchs-Rosenthal counting chamber using a Leica microscope (Leica Microsystems Pty Ltd., North Ryde, New South Wales, Australia). A 4-mL subsample of ruminal fluid was diluted with 1 mL of 25% H_3PO_4 and then frozen for subsequent analysis of VFA, according to the method of Packer et al. (2011). A 10-mL subsample of ruminal fluid was frozen and subsequently used for microbial profiling.

Rumen Microbial Profiling

Total nucleic acid was extracted from 10 mL of freeze-dried ruminal fluid using a modification (Torok et al., 2008) of a South Australian Research and Development Institute (SARDI, West Beach, Australia) proprietary method (Stirling et al., 2004), as previously described by Torok et al. (2014). Terminal restriction fragment (**T-RF**) length polymorphism was conducted to investigate the bacterial, archaeal, fungal, and protozoan communities. The PCR primers used for bacterial profiling were 27F/907R (Torok et al., 2008), whereas Ar109f/Ar912r were used for Archaea, ITS1-F/ITS4 for fungi, and CS322F/EU929R for protozoa (Torok et al., 2014). For each primer pair, one primer was 5′ labeled with 6-carboxyfluorescein (**FAM**; 27F, Ar109f, ITS1-F, and EU929R) to enable subsequent detection of T-RF. The PCR targeting bacteria were done according to Torok et al. (2008), whereas PCR targeting Archaea, fungi, and protozoa were done according to Torok et al. (2014). Following PCR, amplification products were digested with the following restriction endonucleases (Genesearch Pty Ltd., Arundel, Queensland, Australia) according to the manufacturer's recommendations: 2 U of *Msp*I and 1 μg/μL of BSA (27F-FAM/907R amplicon), 1 U of *Mbo*I (Ar109f-FAM/Ar912r amplicon), 5 U of *Hin*fI (ITS1-F-FAM/ITS4 amplicon), and 1 of U *Hpy*188III and 1 μg/μL BSA (CS322F/EU929R-FAM amplicon). Lengths of the resulting fluorescently labeled T-RF were determined by comparison with an internal size standard (GeneScan 1200 LIZ; Applied Biosystems, Mulgrave, Victoria, Australia) following separation by capillary electrophoresis on an ABI 3730 automated DNA sequencer (Applied Biosystems). Data were analyzed using GeneMapper software (version 3.7; Applied Biosystems) and a custom-built database containing queries to validate data and generate data sets for statistical analysis (Torok et al., 2008).

Statistical Analyses

Feed intake, production traits, and CH_4 data were analyzed by ANOVA or, where corresponding covariate data were available, by ANCOVA, using GenStat 14 software (VSN International Ltd., Hemel Hempstead, UK). The statistical model was

$$y_{ij} = \mu + \tau_j + \beta x_{ij} + \varepsilon_{ij},$$

where y_{ij} is the response for animal i on treatment j, μ is a constant, τ_j is an effect of treatment j, βx_{ij} is a linear adjustment for covariate x_{ij} (if available), and ε_{ij} is an independent random error. Contrasts (t-tests) were used to test differences between treatment means and differences between grape marc (i.e., the average of DGM and EGM) and CON. Distributional assumptions of normality and constant variance were checked visually using graphs of residuals against fitted values,

and histograms and normal quantile plots of residuals. Analysis of variance *F*-statistic *P*-values were derived by Monte Carlo permutation. Somatic cell count data were logarithmically transformed before analysis.

The data obtained from microbial profiling were analyzed using multivariate statistical techniques (PRIMER 6 and PERMANOVA+β1; Primer-E Ltd., Lutton, Ivybridge, UK). Bray-Curtis measures of similarity were calculated to examine similarities between ruminal microbial communities of cows from the T-RF length polymorphism-generated data matrices, following standardization and fourth root transformation. Analysis of similarity (ANOSIM; Clarke, 1993) was used to test if ruminal microbial communities were significantly different between dietary treatments. Unconstrained ordinations were done to graphically illustrate relationships between treatments using nonmetric multidimensional scaling (Shepard, 1962; Kruskal, 1964). Constrained canonical analyses of principal coordinate (**CAP**) biplots (Anderson and Willis, 2003) were constructed to investigate the relationship between T-RF associated with diet and CH_4 yield (g of CH_4/kg of DMI). The a priori hypothesis that ruminal microbial communities were different between diets was tested in CAP by obtaining a *P*-value using permutation procedures (999 permutations) on the canonical test statistic (squared canonical correlation). The number of principal coordinates axes was chosen to achieve the maximum proportion of correct allocations (% of trace [groups]) of samples to diet. Pearson correlation was calculated between the first canonical axis (CAP1) and CH_4 yield.

Extraction of Published Data

To obtain the Cartesian data in Figure 1 of Chilliard et al. (2009), the relevant subfigures were electronically scanned and the data digitally extracted by means of UN-SCAN-IT software (version 5.0; Silk Scientific Inc., Orem, UT).

RESULTS

Diets and Feed Intake

Nutritive characteristics of the main ingredients in the diets are shown in Table 1. Cows offered each diet had similar total DMI (Table 2). One cow in the EGM group consistently ate less than 60% of the feed offered to her, so her data was excluded from all analyses.

Based on the recorded intake of individual dietary ingredients and the concentration of CP in each, the average concentrations of CP in the diets consumed by cows in each treatment group were 159 g/kg of DM for the CON diet, 150 g/kg of DM for the DGM diet, and 150 g/kg of DM for the EGM diet. The average concentrations of crude fat in diets consumed were similarly calculated as 26 g/kg of DM for the CON diet, 57 g/kg of DM for the DGM diet, and 52 g/kg of DM for the EGM diet.

Milk Production and Milk FA

The average milk yields and ECM from cows offered the CON and DGM diets were not different ($P > 0.05$) and were higher ($P < 0.01$) than their counterpart measurements in cows offered the EGM diet (Table 2). Milk fat concentration was lower ($P < 0.001$) for cows offered DGM diet than for cows offered either the CON or EGM diets. Despite this, fat yields were similar ($P > 0.05$) for cows fed the DGM and EGM diets, which were lower ($P < 0.001$) than fat yields from cows fed the CON diet. Protein and lactose concentrations were similar for cows offered all diets, but both protein and lactose yields were lower ($P < 0.01$) for cows offered the EGM diet in comparison to the yields of these components from cows offered the CON and DGM diets. Somatic cell counts ($\log_{10}$) were not affected ($P > 0.05$) by dietary treatment. Cows fed the CON diet gained significantly ($P < 0.001$) more BW than cows fed either the DGM or EGM diets.

Mean concentrations of milk FA from cows offered the CON, DGM, and EGM diets are presented in Table 3. Compared with the CON diet, the 2 grape marc diets resulted in reduced ($P < 0.01$) concentrations of C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C15:0, C16:0, C16:1, and C17:0 but increased ($P < 0.01$) concentrations of C4:0, C18:0, C20:0, and all of the C18:1 and C18:2 isomers, with the exception of *cis*-9,*trans*-11 C18:2. Overall, these changes resulted in reduced ($P < 0.01$) concentrations of total SFA and increased concentrations of both MUFA and PUFA as well as total C18 FA in the milk fat from cows offered the DGM and EGM diets compared with the CON diet. The grape marc diets also resulted in reduced ($P < 0.001$) concentrations of de novo synthesized FA compared with the CON diet. The individual FA concentrations in milk from cows fed the EGM diet were almost always intermediate to the corresponding concentrations in the milks of cows fed the CON and DGM diets.

CH_4 Emissions

Cows fed DGM and EGM diets had lower ($P = 0.001$) CH_4 emissions (g/cow per day) and CH_4 yield (g/kg of DMI) than cows offered the CON diet (Table 4). Cows offered the DGM diet also had a lower ($P = 0.03$) CH_4 yield than cows offered the EGM diet (Table 4). Although no difference ($P > 0.05$) existed in CH_4

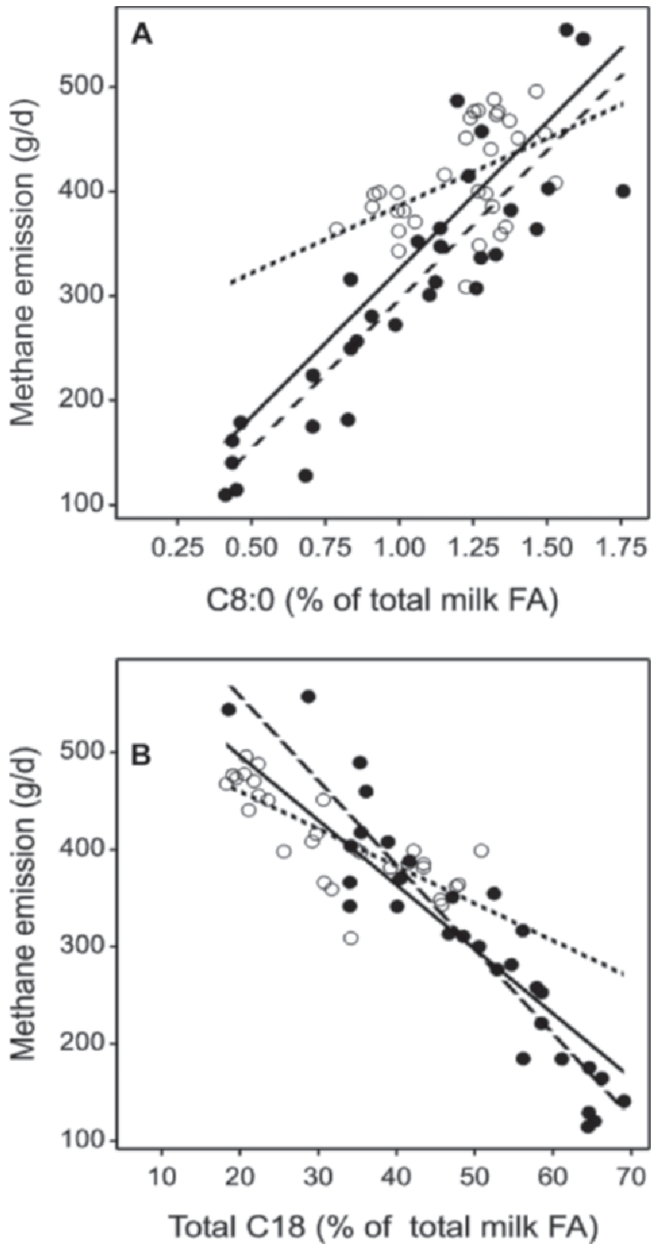

Figure 1. The relationships between the concentrations of specific FA in milk fat and CH_4 emissions from individual cows: (A) C8:0 as a percentage of total milk fat; (B) total C18 FA as a percentage of total milk fat. The circles represent individual cow data from the current experiment and the solid dots represent data published by Chilliard et al. (2009). The dotted lines represents the regression lines for data from the current experiment, the dashed lines are the regression lines for data from Chilliard et al. (2009), and the solid lines represent the regression for data combined from the present experiment and from Chilliard et al. (2009). The equations relating CH_4 production to the concentrations of C8:0 and total C18 in milk fat are as follows: Chilliard et al. (2009): CH_4 (g/d) = 11 ± 29.2 + 282 ± 26.6 × C8:0 (R^2 = 0.81; n = 31); current experiment: CH_4 (g/d) = 257 ± 51.7 + 129 ± 42.3 × C8:0 (R^2 = 0.22; n = 30); combined data: CH_4 (g/d) = 43 ± 29.2 + 282 ± 25.2 × C8:0 (R^2 = 0.67; n = 61); Chilliard et al. (2009): CH_4 (g/d) = 729 ± 30.8 − 8.7 ± 0.61 × total C18 (R^2 = 0.88; n = 31); current experiment: CH_4 (g/d) = 536 ± 18.2 − 3.8 ± 0.54 × total C18 (R^2 = 0.63; n = 30); combined data: CH_4 (g/d) = 629 ± 17.8 − 6.6 ± 0.41 × total C18 (R^2 = 0.81; n = 61).

intensity (g/kg of milk) between cows offered the CON and EGM diets, cows offered the DGM diet had lower ($P < 0.01$) CH_4 intensity than those offered the CON diet (Table 4). Daily CH_4 emissions from individual cows were positively related to the concentration of caprylic acid (C8:0) in milk fat, and negatively related to the concentration of total C18 FA in milk fat (Figure 1).

Ruminal Fermentation

The ruminal fluid pH from cows offered the DGM diet was lower ($P < 0.01$) than the pH in ruminal fluid from cows offered the CON diet (Table 5). Dietary treatment did not influence ($P > 0.05$) the concentrations of NH_3 or total VFA in ruminal fluid (Table 5). However, the ruminal fluid from cows offered the DGM diet contained a lower ($P < 0.01$) molar percentage of acetic acid and a greater molar percentage of caproic acid than the ruminal fluid from cows offered the CON diet. No dietary effect was detected on the total number of ciliated protozoa or the numbers of *Entodinia* spp., and *Dasytricha* spp. in ruminal fluid. However, the numbers of *Isotricha* spp. were less ($P < 0.05$) in the ruminal fluid of cows offered the EGM diet compared with the CON diet. Dietary treatment had no effect ($P > 0.05$) on the concentrations of H_2, CH_4, and CO_2 in the rumen headspace.

Ruminal bacterial (Global R = 0.12; $P = 0.001$) and archaeal (Global R = 0.18; $P = 0.003$) communities were altered by diet (where Global R is a global sample statistic that investigates whether there is a global difference among all treatments). Dietary supplementation with either EGM (R = 0.28; $P = 0.001$) or DGM (R = 0.22; $P = 0.002$) altered bacterial communities in ruminal fluid compared with those in the ruminal fluid of cows fed the CON diet (where R is a statistic that investigates the individual pairwise differences between treatments). Ruminal bacterial communities were not different in the ruminal fluid of cows fed the 2 different forms of grape marc (R = 0.031; $P = 0.22$). The ruminal archaeal communities were altered by the DGM diet (R = 0.34; $P = 0.004$) but not the EGM diet (R = 0.034; $P = 0.27$) compared with the communities in the ruminal fluid of cows fed the CON diet. Furthermore, ruminal archaeal communities were different between the EGM and DGM treatments (R = 0.18; $P = 0.025$). Based on DNA profiling, the ruminal fungal (Global R = 0.038; $P = 0.14$) and protozoan (Global R = 0.024; $P = 0.14$) communities were not altered by dietary supplementation with grape marc. Differences observed in ruminal bacterial and archaeal communities associated with dietary treatment are graphically demonstrated in Figure 2.

Table 1. Composition (g/kg of DM, unless otherwise noted) of main dietary ingredients

Parameter	Crushed wheat	Alfalfa hay	Dried grape marc	Ensiled grape marc
CP	132	168	131	133
Soluble protein (% of CP)	34	44	25	23
ADF	33	444	477	531
NDF	114	514	507	535
Lignin	16	89	369	422
NFC	731	216	183	177
Starch	557	8	6	3
Ash	14	101	85	77
TDN	85	53	53	48
Extractable CT[1]	ND[2]	0.4	0.3	0.3
Bound CT	ND	1.4	20.1	7.8
Ca	0.5	8.9	6.3	6.1
Mg	1.4	2.0	1.0	1.2
Na	0.14	1.65	0.23	0.20
K	3.5	28.9	21.3	19.4
Cl	1.5	5.6	0.8	2.5
Cu (mg/kg of DM)	5	5	33	34
S	1.6	2.4	1.7	1.8
Tartaric acid	ND	ND	63.5	31.8
Crude fat	24	27	142	126
Triglyceride	22	19	97	94
FA (% of total FA)				
C14:0	0	1.2	0.1	0.3
C16:0	19.6	32.7	9.4	9.2
C16:1	0	2.0	0.3	0.2
C18:0	1.3	5.3	4.6	4.6
C18:1	12.9	3.0	15.8	15.2
cis-11 C18:1	0.9	0	0.8	0.8
C18:2n-6	60.4	20.0	66.7	66.3
C18:3	4.3	26.5	1.5	1.1
C20:0	0	1.6	0.5	0.4
C20:1	0.6	0	0.2	0.2
Other	0	7.7	0	1.6

[1]CT = condensed tannin.

[2]ND = not detected.

Table 2. Effect of feeding dairy cows diets containing either dried grape marc (DGM) or ensiled grape marc (EGM) on their DMI, milk yield, milk composition, yields of milk components, and change in BW[1]

	Treatment				Contrast (*P*-value)	
Parameter	CON	DGM	EGM	SEM	CON vs. GM	DGM vs. EGM
Number of cows	12	10	9	—	—	—
Feed intake (kg of DM/cow per day)						
Alfalfa hay	13.2	8.9	8.8	—		
Concentrate mix	4.1	4.1	4.0	—		
DGM	0	4.9	0	—		
EGM	0	0	4.7	—		
Total	17.3	17.9	17.5	—		
Milk (kg/cow per day)	14.6[a]	15.4[a]	12.8[b]	0.39	0.34	<0.001
ECM (kg/cow per day)	16.5[a]	15.6[a]	14.1[b]	0.35	0.002	0.005
Fat (%)	4.94[a]	3.99[b]	4.90[a]	0.143	0.005	<0.001
Protein (%)	3.56	3.52	3.47	0.053	0.34	0.53
Lactose (%)	4.70	4.66	4.68	0.034	0.40	0.75
$\log_{10}$ SCC	2.49	2.49	2.51	0.066	0.91	0.77
Fat (g/cow per day)	720[a]	613[b]	608[b]	16.3	<0.001	0.84
Protein (g/cow per day)	517[a]	540[a]	437[b]	10.5	0.035	<0.001
Lactose (g/cow per day)	687[a]	717[a]	598[b]	19.5	0.26	<0.001
Change in BW[2] (kg)	29.4[a]	−2.1[b]	0.1[b]	2.50	<0.001	0.98

[a,b]Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

[1]CON = control; GM = grape marc.

[2]Change in BW measured over 18 d.

Table 3. Effect of feeding dairy cows diets containing either dried grape marc (DGM) or ensiled grape marc (EGM) on the FA composition of milk (g/100 g of FA)

	Treatment				Contrast (*P*-value)	
Parameter	CON	DGM	EGM	SEM	Treatment	GM vs. CON
Number of cows	12	10	9	—	—	—
C4:0	4.09[a]	4.61[b]	4.37[ab]	0.130	0.022	0.013
C6:0	2.45[b]	2.11[a]	2.42[b]	0.054	<0.001	0.001
C8:0	1.34[b]	1.03[a]	1.26[b]	0.043	<0.001	0.001
C10:0	3.21[c]	2.05[a]	2.65[b]	0.117	<0.001	<0.001
C12:0	3.94[c]	2.41[a]	3.07[b]	0.133	<0.001	<0.001
C13:0	0.24[c]	0.00[a]	0.11[b]	0.017	<0.001	<0.001
C14:0	12.81[c]	9.39[a]	11.59[b]	0.234	<0.001	<0.001
C14:1	1.57[c]	0.98[a]	1.27[b]	0.092	<0.001	0.001
C15:0	1.39[c]	0.90[a]	1.08[b]	0.034	<0.001	<0.001
C16:0	43.39[c]	24.89[a]	35.66[b]	0.918	<0.001	<0.001
C16:1n-7	2.61[c]	1.49[a]	1.98[b]	0.117	<0.001	<0.001
C17:0	0.87[c]	0.61[a]	0.73[b]	0.022	<0.001	<0.001
C18:0	5.16[a]	11.13[c]	8.48[b]	0.275	<0.001	<0.001
C18:1 (unknown)	0.00[a]	0.62[c]	0.20[b]	0.022	<0.001	<0.001
trans-9 C18:1	0.00[a]	0.51[c]	0.11[b]	0.016	<0.001	<0.001
trans-10 C18:1	0.00[a]	1.34[c]	0.26[b]	0.034	<0.001	<0.001
trans-11 C18:1	0.53[a]	3.23[c]	0.88[b]	0.084	<0.001	<0.001
C18:1 (unknown)	0.00[a]	0.86[c]	0.23[b]	0.021	<0.001	<0.001
cis C18:1n-9	13.86[a]	24.11[c]	19.87[b]	0.655	<0.001	<0.001
cis C18:2n-6	1.52[a]	4.85[c]	2.52[b]	0.125	<0.001	<0.001
cis-9,*trans*-11 C18:2	0.33[a]	2.07[c]	0.57[b]	0.042	<0.001	<0.001
C18:3n-3	0.68[ab]	0.72[b]	0.59[a]	0.029	0.013	0.51
C20:0	0.01[a]	0.11[b]	0.12[b]	0.015	<0.001	<0.001
Total C18	15.64[a]	35.07[c]	24.77[b]	0.759	<0.001	<0.001
Total SFA	78.90[c]	59.23[a]	71.53[b]	0.885	<0.001	<0.001
Total MUFA	18.57[a]	33.13[c]	24.78[b]	0.763	<0.001	<0.001
Total PUFA	2.53[a]	7.64[c]	3.69[b]	0.168	<0.001	<0.001
Total de novo FA	31.04[c]	23.47[a]	27.80[b]	0.502	<0.001	<0.001

[a–c]Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

[1]CON = control; GM = grape marc.

Canonical analysis of principal coordinates was used to investigate the correlation between significant diet related shifts in ruminal microbiota and CH_4 production. Constrained CAP analysis, done on ruminal bacterial (Figure 3) and archaeal (Figure 4) communities, produced biplots from the first CAP axis (CAP1) against CH_4 yield (g of CH_4/kg of DMI) of individual cows. A correlation existed between ruminal bacterial community composition and CH_4 yield when the CON diet was compared with the EGM diet (r = 0.69; Figure 3A) or DGM diet (r = 0.60; Figure 3B). Furthermore, a correlation existed between ruminal archaeal community composition and CH_4 yield when the CON diet was compared with DGM diet (r = 0.68; Figure 4A). Differences in archaeal community composition between the EGM and DGM diets were not correlated with CH_4 yield (r = 0.31; Figure 4B).

DISCUSSION

Milk yield of cows was affected by feeding grape marc but the effect was dependent on its form. Cows fed

Table 4. Effect of feeding dairy cows diets containing either dried grape marc (DGM) or ensiled grape marc (EGM) on their CH_4 emissions[1]

	Treatment				Contrast (*P*-value)	
Parameter[2]	CON	DGM	EGM	SEM	CON vs. GM	DGM vs. EGM
Number of cows	11	10	9	—	—	—
CH_4 (g/cow per day)	470[a]	375[b]	389[b]	8.1	0.001	0.26
CH_4 (g/kg of DMI)	26.1[a]	20.2[c]	21.5[b]	0.39	0.001	0.030
CH_4 (g/kg of milk)	35.3[a]	26.1[b]	35.2[a]	1.9	0.055	0.003
Milk yield (kg/d)	13.4[ab]	15.0[a]	11.5[b]	0.65	0.89	0.001

[a–c]Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

[1]CON = control; GM = grape marc.

[2]The DMI and milk yields referred to in this table relate to the 2 d during which CH_4 emissions were measured.

Table 5. Effect of feeding dairy cows diets containing either dried grape marc (DGM) or ensiled grape marc (EGM) on rumen fluid pH, concentrations of rumen fluid constituents, rumen protozoa, and composition of rumen headspace gas[1]

	Treatment				Contrast (*P*-value)	
Parameter	CON	DGM	EGM	SEM	CON vs. GM	DGM vs. EGM
Number of cows	12	10	9	—	—	—
Rumen fluid						
pH	6.87[b]	6.54[a]	6.60[ab]	0.107	0.021	0.71
NH_3 (mg/L)	76.6	66.7	75.8	5.56	0.45	0.29
Total VFA (m*M*)	63.4	68.2	71.1	3.74	0.17	0.62
Individual VFA (m*M* %)						
Acetic	69.0[b]	68.1[a]	68.6[ab]	0.27	0.045	0.20
Propionic	15.1[ab]	15.4[b]	14.7[a]	0.21	0.72	0.031
Isobutyric	0.92	0.87	0.92	0.022	0.46	0.11
n-Butyric	11.3[a]	11.8[ab]	12.2[b]	0.21	0.013	0.17
Isovaleric	1.73	1.68	1.58	0.077	0.31	0.40
n-Valeric	1.65	1.75	1.63	0.059	0.59	0.21
Caproic	0.33[a]	0.50[b]	0.42[b]	0.028	0.001	0.054
Protozoa (no./μL of rumen fluid)						
Entodinia spp.	160	174	165	16.7	0.62	0.71
Epidinia spp.	18	20	22	5.0	0.62	0.90
Isotricha spp.	3.7[b]	1.1[ab]	0.6[a]	1.08	0.041	0.80
Dasytricha spp.	9.3	7.2	1.9	2.93	0.22	0.24
Other	0.9	1.1	0.0	0.87	0.85	0.66
Total	192	203	190	19.1	0.82	0.61
Rumen headspace gas (mL/100 mL)						
H_2	0.11	0.13	0.11	0.009	0.33	0.23
CH_4	37.1	39.8	38.6	1.11	0.15	0.51
CO_2	62.8	60.1	61.3	1.11	0.14	0.48

[a,b]Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

[1]CON = control; DGM = dried grape marc; EGM = ensiled grape marc; GM = grape marc.

DGM produced more milk than cows fed the CON diet, whereas cows fed EGM produced less milk than those fed the CON diet. Cows fed the DGM and EGM diets produced less milk fat than cows fed the CON diet and cows fed the EGM diet produced less milk protein than cows fed ether the CON or DGM diets. Thus, for cows in late lactation, we reject our hypothesis that the feeding of either DGM or EGM would not influence their yields of milk, milk fat, or milk protein. This finding contrasts with the findings of the only other relevant experiment in this area in which the replacement of 3.5 kg of maize silage DM with EGM in the diet of dairy cows in early lactation had no effect on milk production, yields of milk fat, and milk protein and concentrations of milk fat and milk protein (Belibasakis et al., 1996). Over the 18 d of this experiment, cows fed the CON diet gained more BW than cows fed either the DGM or EGM diets, but changes in rumen fill cannot be excluded as a possible explanation for this finding.

In the current experiment, we measured a 20% decrease in CH_4 emissions and a 23% decrease in CH_4 yield when either DGM or EGM was fed to dairy cows. These findings support our second hypothesis. This is the first report in the scientific literature to document that the feeding of grape marc to ruminants results in reductions in CH_4 emissions and CH_4 yield. Further, reductions of this magnitude in CH_4 emissions and CH_4 yields as a result of any dietary intervention have rarely been reported (Beauchemin et al., 2008; Moate et al., 2011).

These findings raise the question as to which components of grape marc are responsible for these significant reductions in CH_4 emissions and CH_4 yield. Grape marc contains at least 4 substances that theoretically could be partly responsible for the observed effects: fat, lignin, tannins, and DL-tartaric acid.

High concentrations of dietary fat have been reported to result in reduced production of CH_4 (Beauchemin et al., 2008; Moate et al., 2011). Using the prediction equation of Moate et al. (2011) that each 10 g/kg of DM increase in dietary fat concentration results in a 3.5% reduction in CH_4 yield, the higher fat concentrations in the DGM diet compared with the CON diet would reduce CH_4 yield by 11.2% (i.e., 53 g of CH_4/cow per day), whereas for EGM, the reduction would be 9.5% (i.e., 45 g of CH_4/cow per day). However, these predicted reductions in CH_4 yields only account for about 55% of the measured reductions in CH_4 emissions. Therefore, this calculation suggests that fat is not the only CH_4-suppressing constituent in grape marc.

Unlike alfalfa hay, the DGM and the EGM both contained substantial concentrations of lignin (Table 1). Lignin is poorly fermented in the rumen (Akin and Benner, 1988) and in vivo CH_4 emissions are not

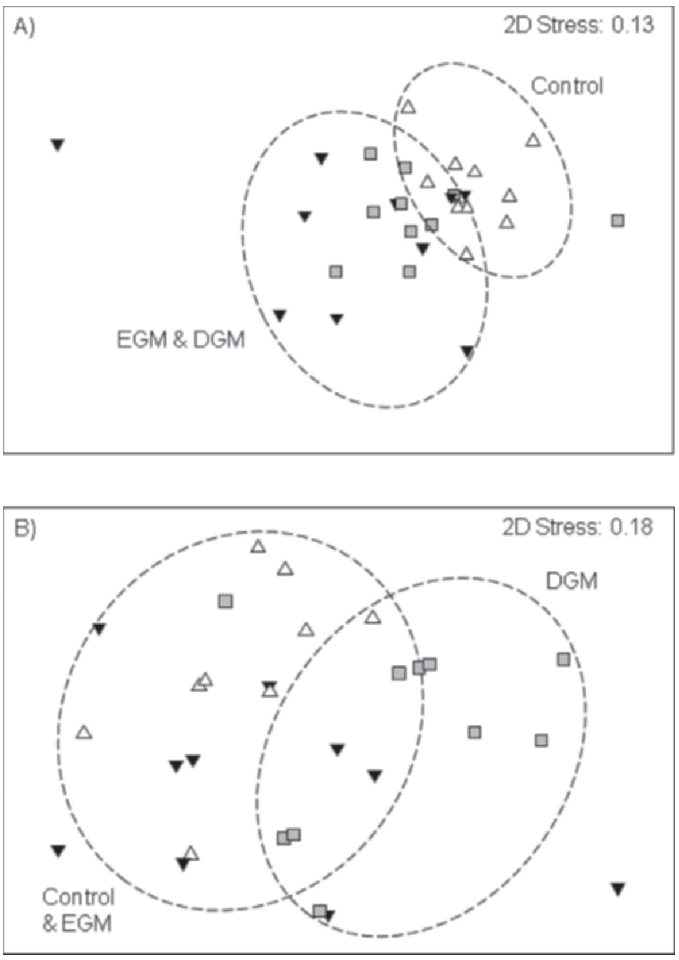

Figure 2. Nonmetric multidimensional scaling ordination of microbial communities from 32 dairy cows. (A) Bacterial communities within the rumen; (B) archaeal communities within the rumen. Δ = control diet; ▼ = ensiled grape marc (EGM); □ = dried grape marc (DGM).

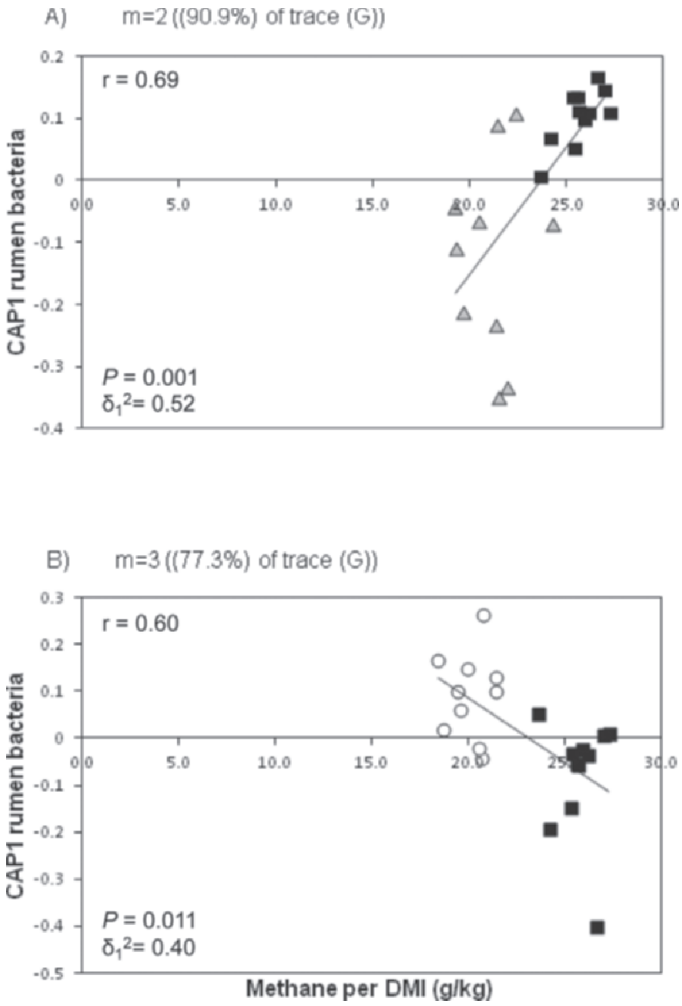

Figure 3. Canonical analyses of principal coordinates (CAP) of diet-associated rumen bacterial communities related to CH_4 production per kilogram of DMI: (A) CAP-versus-CH_4 biplots for cows on the control and ensiled grape marc (EGM) diets; (B) CAP-versus-CH_4 biplots for cows on the control and dried grape marc (DGM) diets. The CAP analysis was based on Bray-Curtis similarities calculated from fourth-root transformed species abundances. The number of principal coordinates axes (m) achieves the maximum proportion of correct allocations (% of trace [groups, G]) of samples to diet. ■ = control diet; Δ = EGM; ○ = DGM; δ_1^2 = squared canonical correlation (canonical test statistic).

correlated with lignin intake (Ellis et al., 2007). The mean intakes of lignin for cows fed the CON, DGM, and EGM diets were approximately 1.2, 2.7, and 2.8 kg/cow per day, respectively. For most diets containing relatively low amounts of lignin, CH_4 yield is generally approximately 23.0 g of CH_4/kg of DMI (Dijkstra et al., 2011). Assuming that grape marc lignin is not fermented in the rumen, then lignin, by taking the place of other potentially fermentable DM, could account for approximately 30 g/d of the measured inhibition in CH_4 emissions.

Considerable evidence exists that tannins can inhibit CH_4 production in vitro (Pellikaan et al., 2011). However, the in vivo effects of dietary tannins on CH_4 production from ruminants have been equivocal (Beauchemin et al., 2007; Grainger et al., 2009). In the current experiment, the concentrations of extractable and bound CT were 0.3 and 20.1 g/kg of DM in DGM and 0.3 and 7.8 g/kg of DM in EGM. It is likely that the grape marc fed in this experiment contained tannins from skins and stems as well as seeds, so the appropriateness of the grape seed tannin standards could be questioned. Furthermore, the heating of grape marc during its manufacture and oxidation during ensilation are likely to have resulted in chemical changes to the CT in the grape marc, which would reduce its ability to inhibit enteric methanogenesis. Nevertheless, if the CT in the DGM and EGM diets caused all of the measured

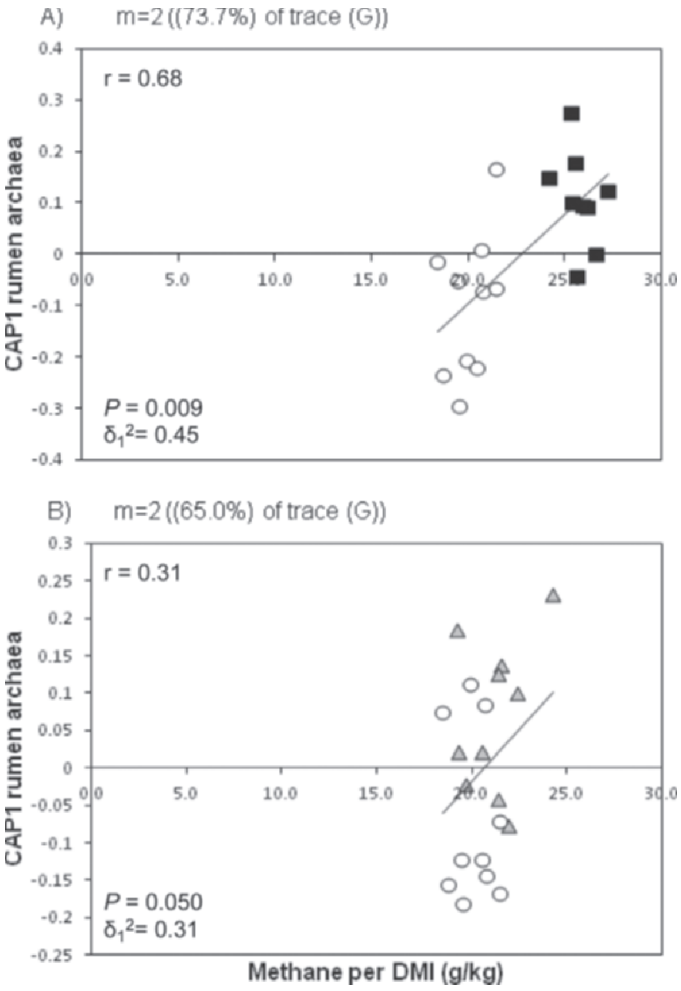

Figure 4. Canonical analyses of principal coordinates (CAP) of diet-associated rumen archaeal communities related to CH_4 production per kilogram of DMI: (A) CAP-versus-CH_4 biplots for cows on the control and dried grape marc (DGM) diets; (B) CAP-versus-CH_4 biplots for cows on the ensiled grape marc (EGM) and DGM diets. The CAP analysis was based on Bray-Curtis similarities calculated from fourth-root transformed species abundances. The number of principal coordinates axes (m) achieves the maximum proportion of correct allocations (% of trace [groups, G]) of samples to diet. ■ = control diet; Δ = EGM; ○ = DGM; δ_1^2 = squared canonical correlation (canonical test statistic).

reduction in CH_4 yield that was not attributable to fat and lignin (i.e., approximately 15 g of CH_4/d), then the CT in grape marc may have had only a minor inhibitory effect on CH_4 emissions.

Another constituent of grape marc that we speculate could be involved in CH_4 suppression is tartaric acid. L-Tartaric acid occurs in grape marc. Tartaric acid is similar in size and structure to both malic acid and fumaric acid, both of which have potent antimethanogenic properties in vivo (Foley et al., 2009; Wood et al., 2009). Grape marc typically contains a substantial concentration of tartaric acid and the DGM and EGM used in the current experiment contained 63.5 and 31.8 g of total tartaric acid/kg of DM, respectively. Thus, the DGM and EGM treatments provided 311 and 223 g of total tartaric acid/cow per day, respectively. Other constituents of grape marc such as Cu, *p*-coumaric acid, and resveratrol might also have contributed to the measured suppression in CH_4 production and CH_4 yield; however, additional in vivo research will be required to identify the relative importance of various constituents of grape marc with respect to CH_4 mitigation.

Regardless of the components of grape marc responsible for inhibition of enteric methanogenesis, the causative mechanisms must involve an alteration or an effect on ruminal fermentation, or at least an effect on ruminal microbiota. Ruminal bacterial and archaeal communities were shown to be altered by dietary supplementation with grape marc, although changes observed in the archaeal communities were related more to the form of grape marc used. The DGM diet caused a decrease in the pH of ruminal fluid, whereas the EGM diet was associated with a tendency for ruminal fluid pH to be decreased (Table 5). Low pH in ruminal fluid is reported to inhibit ruminal methanogenesis (Lana et al., 1998) but, in the current experiment, the measured mean pH values in ruminal fluid were above 6.5 for all treatments and, therefore, low ruminal pH is unlikely to be the cause of the decreased CH_4 emissions from cows fed the DGM and EGM diets. When diets rich in rapidly fermentable starch are fed to ruminants, ruminal fermentation generally alters to increase the production of propionic acid and reduce the production of both acetic acid and CH_4 (Lana et al., 1998). In the current experiment, the DGM and EGM had no effects on the total concentrations of VFA and only very small effects, if any, on the molar percentages of acetic and propionic acid in ruminal fluid (Table 5). We conclude that an alteration in the production of propionic acid was not the mechanism responsible for the grape marc-mediated reduction in CH_4 emissions.

The mechanism(s) by which CT in diets reduce CH_4 emissions are not yet known. It has been speculated that CH_4 emissions may be reduced either indirectly by reduced fiber digestion (Carulla et al., 2005) or by a direct effect on methanogens per se (Field et al., 1989). Condensed tannins are also known to reduce the numbers of ciliated protozoa in ruminal fluid, and these protozoa are known to harbor methanogenic microbes (Ushida and Jouany, 1996). In the current experiment, the DGM and EGM diets had no effect on the counts of the 2 most common ciliated protozoa: *Entodinia* and *Epidinia*, or on total protozoa in ruminal fluid (Table 5). Furthermore, the structure of the ruminal protozoan community, as measured by DNA microbial profiling, was not altered by these dietary treatments. Thus, this

mechanism can be considered as not causing the grape marc-mediated suppression in methanogenesis.

No effect of the DGM and EGM diets was detected on the concentrations of H_2, CH_4, and CO_2 in rumen headspace gas (Table 5) and the concentrations of H_2, CH_4, and CO_2 in rumen headspace gas are similar to those previously reported in the scientific literature (Moate et al., 1997). This observation, although not definitive, suggests that no major changes existed in the relative magnitudes of the fermentation pathways involved in the production of CO_2 and CH_4. This finding is consistent with a lignin effect whereby reduced CH_4 production simply reflects a reduction in the amount of fermentable carbohydrate in the diet.

Feeds rich in polyphenolics or tannins have previously been shown to alter the composition of gut bacterial, and archaeal communities but these changes have not been investigated in relation to actual enteric CH_4 emissions (Vasta et al., 2010; Mohammed et al., 2011; Tan et al., 2011). In the current study, changes in bacterial community composition in response to dietary supplementation with grape marc were shown to be linked to significant reduction in enteric CH_4 yields in vivo. Interestingly, the EGM and DGM did not affect the bacterial communities differently. Yet, when the archaeal communities were investigated, it was only the DGM that resulted in significant changes in the ruminal archaeal communities compared with the CON treatment, and was shown to be linked to CH_4 yield reductions. This suggests that whereas DGM acted on both the H_2 producing and utilizing bacterial communities and the methanogenic archaeal communities to reduce enteric CH_4 yields, the EGM predominantly acted on the ruminal bacterial communities as the mechanism to reduce CH_4 yields. It is noted that reduction in CH_4 production due to EGM treatment may have been due to changes at the functional level in the ruminal Archaea. These would not be detected by our profiling analysis, which investigates phylogenetic or community structural changes only. Although methanogenic Archaea are the only known organisms capable of CH_4 production, they rely on bacteria, protozoa, and fungi to provide digestive products for methanogenesis. Hence, these organisms also have an indirect influence on CH_4 production, as they are either involved in H_2 metabolism or because they affect the methanogens or other members of the microbiota (Bauchop, 1989; Kamra, 2005; Hook et al., 2010).

In this experiment, we used nonfistulated cows and rumen fluid was obtained by a sampling tube placed down the cow's throat to the rumen. This is an important feature of this experiment because Moate et al. (2013a) recently showed that CH_4 production in rumen-fistulated cows was reduced by approximately 7% compared with nonfistulated cows. Furthermore, Moate et al. (2013a) showed that substantial quantities of air enter the rumen of fistulated cows. Thus, we consider that in the current experiment, no possibility existed for our results to be influenced by an aerobically compromised rumen.

For milk from cows fed the CON, DGM, and EGM diets, the mean concentrations of all of the major FA reported in Table 3 are within the ranges previously reported in the scientific literature (Moate et al., 2007). The scientific literature is equivocal in terms of the effect of dietary tannins on milk fat concentration and the FA profile of milk fat. Benchaar et al. (2008) investigated the effects of feeding CT from Quebracho and reported no effect on milk fat concentration. In contrast, Molle et al. (2009) reported that feeding ewes CT extracted from *Hedysarum coronarium* resulted in reduced milk fat concentration. In the current experiment, the feeding of DGM but not EGM was associated with a significant decrease in milk fat concentration. Milk fat depression is generally mediated by the amount of *trans*-10,*cis*-12 C18:2 reaching the mammary gland (Bauman and Griinari, 2003), but in the current experiment, *trans*-10,*cis*-12 C18:2 could not be detected in any of the milk samples.

In the current experiment, the concentrations of most individual FA in milk fat were significantly altered by feeding both forms of the grape marc. Concentrations of total C18 FA were substantially increased in the milk fat of cows fed the DGM and EGM diets, reflecting the high concentration of crude fat and the high percentage of C18 FA, especially linoleic acid in the fat of DGM and EGM diets (Moate et al., 2008). The feeding of grape marc also lowered the proportions of those FA produced de novo in the mammary gland, namely the SFA from C8:0 to C16:0. The amount of de novo synthesized FA in milk has been shown to be related to the dietary intake of fermentable carbohydrate (Moate et al., 2008). The reduced proportion of de novo FA and, in particular, the reduced proportion of C8 FA in milk fat from cows fed the DGM and EGM diets likely occurred because the grape marc contained high amounts of lignin, which is not fermented in the rumen. It can also be explained by dilution of the de novo synthesized FA with the diet-derived total C18 FA (Moate et al., 2008).

Turner et al. (2005) fed dairy cows birdsfoot trefoil (*Lotus corniculatus*) and interpreted their findings as indicating that the CT in the birdsfoot trefoil inhibited ruminal biohydrogenation of long-chain FA and resulted in a net reduction in the concentration of SFA in milk fat. In our experiment, dietary supplementation with grape marc resulted in reduced SFA and increased MUFA and PUFA concentrations in the milk of cows,

suggesting that ruminal biohydrogenation was indeed inhibited.

In this experiment, *trans*-9 C18:1, *trans*-10 C18:1, and *trans*-11 C18:1 concentrations were all substantially increased in the milk fat of cows fed the DGM diet. High temperatures and steam are involved in the drying and pelleting used to produce the DGM pellets. The enhancement of *trans* FA in the milk fat of cows fed the DGM diet is consistent with reports that when heat-treated or extruded oil seeds are fed to dairy cows, the resulting milk fat is enhanced in *trans* FA (Chouinard et al., 1997). This is of interest because intake of high amounts of *trans* FA is reputed to be associated with cardiovascular disease in humans (Mozaffarian et al., 2006). Of note, the feeding of DGM to dairy cows was associated with a 6-fold increase in the concentration in milk of *cis*-9,*trans*-11 CLA. This effect is consistent with the intake of high quantities of linoleic acid from the DGM diet (Kay et al., 2004). This finding may be important because CLA is considered to be anticarcinogenic, antiatherosclerotic, antidiabetic, and have numerous other health benefits for humans (Belury, 2002).

Recently, several researchers have attempted to find relationships between the concentrations of specific FA in milk fat and the CH_4 emissions of dairy cows (Chilliard et al., 2009; Dijkstra et al., 2011). In particular, Chilliard et al. (2009) reported that for diets containing linseed meal, a strong positive relationship exists between the concentration of C8:0 (caprylic acid) in milk fat and daily CH_4 emissions, and also a strong negative relationship between the concentration of total C18 FA and CH_4 emissions. In the present research, the slope (129 ± 42.3) relating CH_4 production to the concentration of C8:0 in milk fat was significantly different ($P < 0.01$) from the slope (285 ± 26.6) reported by Chilliard et al. (2009). Similarly, the slope (-3.8 ± 0.54) we found relating CH_4 production to the concentration of total C18 FA in milk fat was different ($P < 0.01$) from the slope (-8.7 ± 0.61) reported by Chilliard et al. (2009). Nevertheless, similar to Chilliard et al. (2009), we found a strong positive relationship between the CH_4 production rate and the concentration of C8:0, and a strong negative relationship between CH_4 production rate and the concentration of total C18 in milk fat (Figure 1). Thus, the findings of the current research (Figure 1) support those of Chilliard et al. (2009) and extend their findings to diets that contain grape marc, a feedstuff rich in linoleic acid instead of linolenic acid, as occurs in linseed. Further research is required to determine if these or other similar equations have application to a wider range of diets.

CONCLUSIONS

The feeding of DGM instead of alfalfa hay to dairy cows in late lactation had no effect on milk yield or concentrations of protein and lactose in milk, but milk fat concentration and, consequently, yield of milk fat was reduced. In contrast, the feeding of EGM instead of alfalfa hay to dairy cows in late lactation had no effect on concentrations of milk fat, milk protein, and milk lactose, but milk yield and, consequently, yields of milk fat, milk protein, and milk lactose were all reduced. The feeding of both DGM and EGM resulted in milk fat with enhanced concentrations of MUFA, PUFA, and *cis*-9,*trans*-11 linoleic acid. The important and novel finding of this research was that the feeding of both DGM and EGM to dairy cows in late lactation reduced CH_4 emissions by approximately 20%, without a concomitant reduction in DMI. Furthermore, CH_4 yield was reduced by 23% in response to feeding DGM, and by 18% in response to feeding EGM, and these reductions in CH_4 emissions were associated with changes in the ruminal bacterial and archaeal communities.

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