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Changes in the ratio of tetraether to diether lipids in cattle feces in response to altered dietary ratio of grass silage and concentrates[1]

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ABSTRACT: The distinctive membrane lipids of the archaea can contain a wide range of chemical structures. The membrane lipid composition of ruminal methanogenic archaea has not yet been characterized. In this study, we analyzed proportions of the core archaeal membrane lipids dialkyl glycerol diethers (DGDG) and glycerol dialkyl glycerol tetraether (GDGT). We analyzed the feces of beef steers consuming diets that promoted differences in ruminal conditions that were either favorable (i.e., grass silage) or challenging (i.e., concentrates) for the methanogenic archaea. There was significantly less total ether lipid in the feces of cattle consuming the concentrate diet in comparison to the grass silage diet (97 vs. 218 mg/kg DM, respectively), reflecting the inhibitory effect of dietary concentrate on methanogens. Additionally, the proportion of fecal ether lipids as GDGT was much greater in feces from cattle consuming the concentrate diet than in feces from cattle fed grass silage (90% vs. 67% GDGT). A possible explanation for this adaptation is that membrane lipids composited of GDGT lipids are less permeable to protons, thereby protecting the methanogens against low ruminal pH and helping to maintain the chemiosmotic potential (which is important for ATP production, methanogenesis, and growth). The greater proportion of fecal ether lipids as GDGT may reflect adaptation of membrane lipids within the same species, a shift toward methanogens that have a greater proportion of GDGT (e.g., Thermoplasmata), or both. The effect of ruminal environment on membrane composition means that it will be important to consider the production of both DGDG and GDGT lipids when developing a proxy for methanogenesis.

Key words: Archaea, archaeol, dialkyl glycerol diether, glycerol dialkyl glycerol tetraether, ruminal methanogen

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INTRODUCTION

The core biological membrane lipids of the archaea are composed of different proportions of dialkyl glycerol diethers (**DGDG**) and glycerol dialkyl glycerol tetraethers (**GDGT**). The most common and simplest forms of the DGDG and GDGT are archaeol and caldarchaeol, respectively (Fig. 1). In vitro studies with pure cultures of archaea showed wide variation in the percentage GDGT content of archaeal lipids, with 100% GDGT in the Thermoplasmatales, approximately 60% GDGT in the Thermococcales (Schouten et al., 2013), and 8% to 40% GDGT for Methanobacteriales commonly found in the rumen (Table 1).

The GDGT lipids are less permeable to protons in comparison to DGDG lipids (Mathai et al., 2001; Slonczewski et al., 2009). Adaptation of membrane lipids is thought to be the primary basis for energy conservation of the archaea (Valentine, 2007). A greater proportion of GDGT may reduce the influx of protons, resulting in more efficient maintenance of the chemiosmotic potential, which is used to generate ATP (Mathai et al., 2001; Slonczewski et al., 2009). This adaptation may

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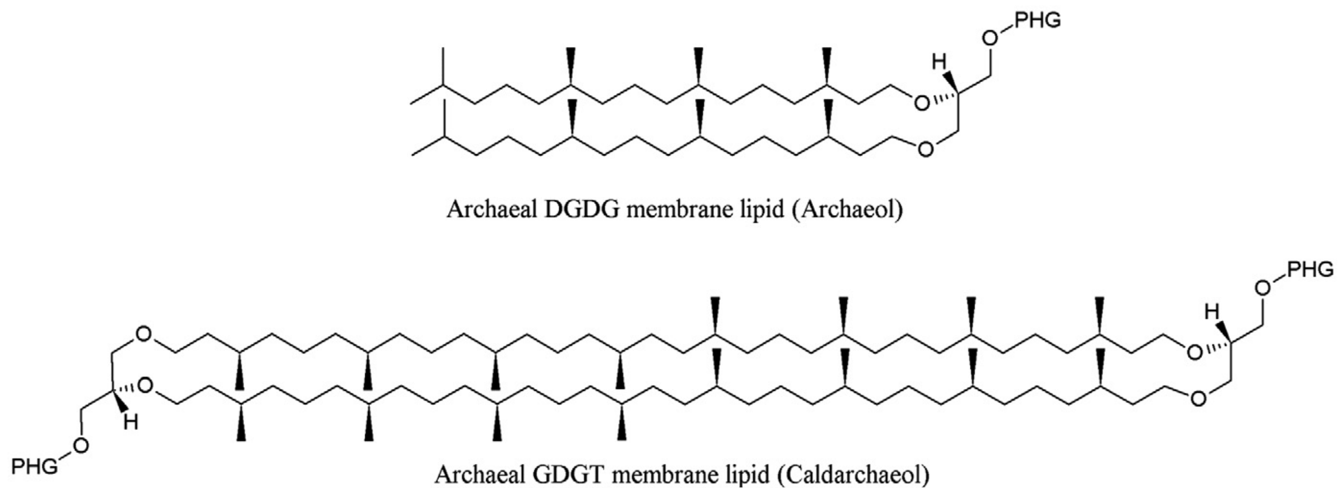

Figure 1. Structure of the core membrane lipids of the Archaea including glycerol dialkyl glycerol diether (DGDG) and glycerol dialkyl glycerol tetraether (GDGT). PHG = polar head group.

also help prevent acidification of the cytoplasm in low-pH environments (Macalady et al., 2004).

In the rumen microbial ecosystem, a decrease in pH inhibits methanogenic archaea, particularly when ruminants are fed high levels of rapidly fermentable carbohydrates (Van Kessel and Russell, 1996). However, the effect of these decreases of ruminal pH on the proportions of GDGT and DGDG membrane lipids in the methanogenic archaea has not yet been assessed. We hypothesize that methanogens adapt their membrane lipid composition when ruminal conditions are challenging to conserve energy and prevent acidification of the cytoplasm. Therefore, in this study, concentrations of both DGDG and GDGT lipids were determined in feces from animals consuming diets that promote different ruminal conditions that are either favorable (i.e., grass silage) or challenging (i.e., concentrates) for ruminal methanogens.

MATERIALS AND METHODS

Dried, ground fecal samples were obtained from beef steers consuming diets based on mixtures of grass silage and concentrates (McGeough et al., 2010). Six cattle consumed diets based on 72% (DM basis) grass silage, and 6 cattle consumed diets based on 89% (DM basis) concentrates. The feces (300 mg) were weighed in triplicate, and 43.3 µg of diether standard (1,2-di-*O*-hexadecyl-*rac*-glycerol; Santa Cruz Biotechnology Inc., Santa Cruz, CA) and 1.07 µg of tetraether standard (C_{46} GDGT; Huguet et al., 2006) were added to each sample. The analytical procedures to extract and fractionate the samples were described by McCartney et al. (2013b). Briefly, the total lipid extract was obtained using a modified Bligh-Dyer method, and then the polar head groups were removed from the membrane lipids by acid methanolysis. The total lipid extract was then separated into apolar and alcohol fractions by column chromatography.

For DGDG (archaeol) analyses, alcohol fractions were trimethylsilylated, dissolved in ethyl acetate, and then analyzed by gas chromatography/mass spectrometry using the method in McCartney et al. (2013b). For GDGT analyses, alcohol fractions were dissolved in hexane:isopropanol 99:1 (vol/vol) and ultrasonicated for 10 min. The extract was isolated using a 1-mL Luer lock syringe combined with 4-mm polytetrafluoroethylene syringe filters with a 0.45-µm opening, with the original sample vial further rinsed and the contents filtered 3 times. Solvent was evaporated under a gentle stream of N_2, then the residue was redissolved in 500 µL hexane:isopropanol 99:1 (vol/vol) before transfer into a HPLC vial. Analyses were performed under normal-phase chromatography conditions on a ThermoFisher Scientific TSQ Quantum Access HPLC-mass spectrometer (ThermoFisher Scientific, Hemel Hempsted, UK), fitted with a Prevail Cyano HPLC column (3-µm particle size, 150 × 2.1 mm i.d.; Alltech, Deerfield, IL). The flow rate was set at 0.2 mL/min, with the column at ambient room temperature. Initial solvent was hexane:isopropanol 99:1 (vol/vol), eluted isocratically for 7 min, followed by a linear gradient to 1.6% isopropanol over 43 min. A selected ion-monitoring acquisition was used to detect ions characteristic of specific tetraethers, the mass-to-

Table 1. Proportion and type of glycerol dialkyl glycerol tetraether (GDGT) membrane lipid in pure cultures of ruminal methanogens belonging to the Methanomicrobiales[1]

Methanogen	GDGT, %	GDGT type
Methanobrevibacter smithii	8	Caldarchaeol
Methanobrevibacter ruminantium	28	Caldarchaeol
Methanosphaera stadtmanae	40	Caldarchaeol

[1]Adapted from Schouten et al. (2013).

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charge (***m/z)*** values of which are reported in Weijers et al. (2009). Sample ionization was performed using positive ion atmospheric pressure chemical ionization. The mass spectrometry (**MS**) settings were as follows: discharge current, 3 kV; vaporization temperature, 380°C; capillary temperature, 280°C; tube lens offset, 178 V; skimmer offset, −10 V; sheath gas pressure, 15; auxiliary gas pressure, 8; ion sweep gas pressure, 1 (all gas pressures are nominal with no units).

A 1-way ANOVA with diet as a treatment factor was applied to the data using Genstat software (14th edition, VSN Int., Hemel Hempstead, UK).

Table 2. Effects of dietary treatment on the concentrations and ratio of diether (archaeol) and tetraether (caldarchaeol) in feces and methane production

	Dietary treatment			
Item	Concentrates	Grass silage	SED	*P*-value
Fecal ether lipids, mg/kg DM				
Archaeol	9.4	71.1	6.57	<0.001
Caldarchaeol	87	147	36.9	0.138
Total ether lipids	97	218	42.1	0.016
Caldarchaeol:archaeol ratio	10.4	2.09	1.95	0.002
Methane production, g/kg DMI	17.4	28.1	3.45	0.012

RESULTS AND DISCUSSION

Major Ether Lipids in Cattle Feces

Previous studies on ruminal samples have only focused on DGDG membrane lipids to assess the location and abundance of methanogens in the ruminant digestive tract (McCartney et al., 2013a, 2014) and to assess its suitability as a proxy for methanogenesis (McCartney et al., 2013b). The measurement of ether lipids in the feces is assumed to be an indirect measurement of ruminal methanogens on the basis of the study by Gill et al. (2010), which found that these lipids are only detectable in the feces of foregut fermenters. Furthermore, our recent observation that the concentration of DGDG in feces was 2 to 2.5 times that in ruminal digesta (McCartney et al., 2014) suggests that there is little digestion and absorption of these membrane lipids in the digestive tract.

There were significantly lower concentrations of both archaeol and caldarchaeol in feces from animals consuming the concentrate diet (Table 2), which is probably due to ruminal conditions that were unfavorable for methanogens and methanogenesis. McGeough et al. (2010) showed that methane production was significantly reduced for the concentrate diet (15.3 g/kg DMI) in comparison with the grass silage diet (35.6 g/kg DMI), and this was most likely related to low ruminal pH (Beauchemin et al., 2008). Caldarchaeol was the only GDGT detected in feces from these steers. This is in agreement with previous studies where caldarchaeol was also the only GDGT detected in pure cultures of important ruminal methanogen species such as *Methanobrevibacter smithii*, *Methanosphaera stadtmanae*, and *Methanobrevibacter ruminantium* (Table 1).

Relationship between Rumen Methane Production and Fecal Ether Lipids

The high and variable GDGT content in the methanogen membrane may help explain the nonlinearity of the relationship between fecal archaeol and methane production noted by McCartney et al. (2013b). Concentrations of caldarchaeol and total ether lipids were more proportional to measured methane production for these samples than archaeol concentrations (Table 2). For example, when animals consumed the grass silage diet, methane emissions, caldarchaeol, and total ether lipids concentrations doubled, but fecal archaeol concentrations increased by nearly a factor of 8.

Proportion of Fecal Ether Lipids as GDGT in Cattle Feces

The percentage of ether lipids present as GDGT was 90% for the concentrate diet and 67% for the grass silage diet. These are much greater proportions of GDGT than were obtained previously for predominant ruminal species, particularly those in the Methanobacteriales order (Table 1). The highest proportion of ether lipids as GDGT was in the feces of cattle consuming the concentrate diet. This diet would have created a more challenging environment for methanogens, particularly by lowering ruminal pH (Van Kessel and Russell, 1996) but also by increasing production of propionic acid (an alternative hydrogen sink; Moss et al., 2000) and reducing production of hydrogen (O’Hara et al., 2003).

It is unclear whether the greater proportion of GDGT results from a change in ruminal microbiota composition or changes in membrane composition of individual methanogen species already established in the rumen. Sprott et al. (1991) showed changes in the membrane composition of *Methanococcus jannaschii* in response to growth temperature, whereas Shimada et al. (2008) showed changes in *Thermoplasma acidophilum* when exposed to low pH and high temperature. An alternative explanation for the changes in the proportion of fecal ether lipids as GDGT is a change in species composition of the microbiota. Macalady et al. (2004) observed that extreme acidophiles, such as members of the Thermoplasmatales family, are composed exclusively of GDGT, whereas extreme alkaliphiles are composed exclusively of DGDG. Therefore, the drop

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in ruminal pH after consumption of a high-concentrate diet may cause a shift in the ruminal microbiota toward acidophilic bacteria composed mostly of GDGT membrane lipids. Furthermore, a high-concentrate diet has been linked to increased levels of methylamine in the rumen (Ametaj et al., 2010), which is an important substrate for Thermoplasmatales (Poulsen et al., 2013) and could further promote its proliferation. Other archaeal species may also be responsible for the increase of GDGT, but these are difficult to identify because of the difficulties associated with isolating and culturing ruminal archaea. Greater proportions of GDGT may protect methanogens against low ruminal pH (Macalady et al., 2004), but the reduced lateral mobility of the GDGT membrane lipids may limit the rate of electron transport (Valentine, 2007), and this could inhibit the methanogen growth because electron transport is central to ATP production by methanogenesis (Schäfer et al., 1999).

Further work should continue detailed analysis of membrane lipids to identify the basis for variation in the proportion of fecal ether lipids as GDGT and its relationship with methane yield.

Conclusions

A change in the proportion of GDGT in methanogen membranes was associated with altered ruminal conditions when feeding a high level of concentrates. This was mostly likely because increased GDGT will help methanogens under conditions of low pH, reducing the permeability of cell membranes and thus conserving energy for growth. The effect of ruminal environment on membrane composition means that it will be important to consider the production of both DGDG and GDGT lipids in developing a proxy for methanogenesis.

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