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Using archaeol to investigate the location of methanogens in the ruminant digestive tract

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ABSTRACT

The quantification of archaeol, a methanogen membrane lipid, may provide an alternative method to estimate methanogen abundance. The focus of this study was to determine the location of methanogens in the ruminant digestive tract using this biomarker. Archaeol was quantified in samples obtained from four lactating cows with rumen cannulae that grazed on either white clover (WC) or perennial ryegrass (PRG) in a changeover design study with three 3-week periods. Faeces were collected over the final 5 d of each period and total rumen contents (TRC) were obtained on the final 2 days (day 1: 9 am; day 2: 3 pm). Solid-associated microbes (SAM) and liquid-associated microbes (LAM) were also isolated from the TRC. Concentrations of archaeol in the TRC showed a significant diet by time interaction, which may be related to diurnal grazing patterns and different rumen conditions associated with PRG or WC diets. There was significantly more archaeol associated with SAM than LAM, which may reflect difficulties of methanogen proliferation in the liquid phase. Faeces had higher concentrations of archaeol than SAM and LAM which was unexpected, although, losses of methanogens may have occurred during isolation (i.e., attachment to protozoa and very small particles), or the methanogens associated with SAM may have been underestimated. There was no significant relationship between faecal and TRC archaeol concentrations. Finally, there was a significant positive relationship between rumen pH and concentrations of archaeol in SAM and LAM, which may be caused by pH and/or WC diet effects. In conclusion, archaeol is potentially a useful alternative marker for determining the abundance of methanogens in the ruminant digestive tract. This work has also highlighted the difficulties associated with methanogen quantification from microbial isolates, and the need for more representative rumen sampling in future studies.

1. Introduction

This study investigated an alternative approach to study methanogen abundance involving the use of the lipid biomarker archaeol (2,3-diphytanyl-O-*sn*-glycerol). This is a core membrane lipid that is ubiquitous in methanogens, and is readily quantified by gas chromatography mass spectrometry. Previous studies have already assessed archaeol as a potential faecal biomarker for methanogens and methane production (Gill et al., 2011; McCartney et al., 2013a), where

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a significant positive relationship was found between total methanogens and methane production when looking at treatment means. Archaeol has also recently been assessed in parallel with quantitative real-time PCR (qPCR) techniques and it was found that archaeol was a useful complementary approach for estimating methanogen abundance (McCartney et al., 2013c).

The purpose of this study was to use archaeol as a total methanogen proxy to provide insights into the location and abundance of methanogens in the digestive tract of cows grazing either perennial ryegrass (PRG) or white clover (WC). Archaeol was quantified in samples from the rumen, including total rumen contents, liquid-associated microbes (LAM) and solid-associated microbes (SAM), and in faeces.

2. Materials and methods

2.1. Animal study

This study was conducted under a license issued under the UK Animal (Scientific Procedures) Act (1986). Four lactating Holstein–Friesian cows that had previously been prepared with rumen cannulae (Bar Diamond, Parma, ID) were used. At the start of the experiment the animals had an average BW of 694 kg (s.d.=46.4), a BCS of 2.7 (s.d.=0.51) and were 104 days in milk (s.d.=23.6). The BCS was determined according to Mulvany (1977). Animals were strip-grazed on pure stands of either perennial ryegrass (*Lolium perenne* cv. Fennema) or white clover (*Trifolium repens* cv. AberHerald) that had been re-growing for 21–27 days in a changeover design with three 3-week periods. In period 1, two animals grazed WC and 2 animals grazed PRG. In period 2, two animals switched diet, and in period 3 all animals switched diet. As a result two animals received the same diet in periods 1 and 2. Measurements were taken from all four animals in each period, resulting in six measurement periods per dietary treatment. All cows were supplemented with 4 kg/d of pelleted concentrates (Table 1) and received 25 g/d of poloxalene (Bloat Guard, Agrimin Ltd., Brigg, Lincolnshire, UK). Cows were milked twice-daily (approx. 08:00 and 16:00 h) with milk yields recorded electronically (Tru-Test NZ Ltd., Auckland, NZ) and samples for analysis of milk components taken on four consecutive milkings at the end of each period. Milk samples were sent to NMR laboratories (National Milk Records plc, Chippenham, UK) for infrared milk analysis of milk fat, protein and lactose.

Table 1
Chemical composition of the feeds used in this study.

Component (g/kg DM, unless stated otherwise)	Perennial ryegrass	White clover	Concentrates
DM (g/kg)	113	94.0	870
OM	899	886	914
NDF	486	231	279
ADF	269	211	142
Ether extract	30.6	19.9	54.1
Crude protein (N × 6.25)	216	309	200
Starch	–	–	283
Neutral cellulase-gammanase digestibility	–	–	823
Digestible organic matter	663	749	–
Water-soluble carbohydrates	96.1	51.0	89.2

Herbage samples (20 random snips to 6 cm) were collected daily and bulked weekly for feed composition analysis (described below). Cows were dosed via the rumen cannula with plastic pellets of different colours to identify their faeces in the field (pellets were removed prior to analysis). Faecal samples were collected daily from the field over 5 days at the end of each experimental period (bulked proportional sample of all faeces deposited over the previous 5 days) and then lyophilized and finely ground for analysis. The dried faecal samples were stored at room temperature. Rumen contents were obtained by total rumen evacuation on the final 2 days of each period (day 1: 9 am; day 2: 3 pm). A 5% sub-sample was taken throughout the evacuation procedure (every twentieth bail) and composited for subsequent analysis and the remaining rumen contents were returned immediately on completion of the evacuation procedure. The weight, DM and NDF content of the total rumen contents was determined. Rumen fluid samples were taken using automatic samplers every 2 h for the 2 days prior to rumen evacuation for ammonia and VFA analysis. The pH was recorded (Oakton PC510 pH meter; Eutech Instruments BV, Nijkerk, The Netherlands) twice a day at morning and evening milking on the final 2 days of the experimental period. All chemical analysis of feed and rumen contents was performed using methods outlined by Dewhurst et al. (2000). Briefly, the DM was determined by toluene distillation, VFA concentrations were determiend using gas chromatography, crude protein was determined using a Kjeldahl procedure with Cu catalyst on 'Kjeltec' equipment (Perstorp Analytical Ltd., Berkshire, UK). Detergent fibre analyses, NDF (with amylase) and ADF were determined using 'Fibretec' equipment (Perstorp Analytical Ltd., Berkshire, UK). Water-soluble carbohydrate were concentration was determined using an automated anthrone method (Method 9a; Technicon Industrial Systems, Tarrytown, NY), and starch by incubating with buffered amyloglucosidase prior to the automated anthrone method. Rumen ammonia concentration was determined using a test kit (No. 66-55; Sigma-Aldrich Co. Ltd., Poole, UK) on a discrete analyser. *In vitro* DOMD based on incubation with pepsin–cellulase was performed according to Jones and Hayward (1975). At the end of the experiment the animals had an average bodyweight of 665 kg (SD=29.7) and an average BCS of 2.8 (SD=0.34).

2.2. Isolating SAM and LAM

SAM and LAM fractions were obtained from the whole rumen contents collected at the 9 am rumen evacuation following the protocol of Merry and McAllan (1983). To obtain the LAM fraction, total rumen contents were hand squeezed through 4 layers of cheesecloth to obtain 1000 mL of liquid before centrifugation for 10 min at low speed (500*g*). The supernatant was then centrifuged at high speed (25,000*g*) for 25 min. After discarding the supernatant, the remaining pellet washed in saline (9 g NaCl/L) before another high-speed centrifugation step for