Animal Feed Science and Technology 220 (2016) 226–236

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Ruminal methanogens and bacteria populations in sheep are modified by a tropical environment

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ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form 13 July 2016
Accepted 9 August 2016

Keywords:
Rumen microbiota
Volatile fatty acids
Temperate *vs* tropical site
Sheep breed
Forage
Methane

ABSTRACT

Microbial fermentation of carbohydrates in the rumen is largely responsible for the emission of methane by ruminants. Ruminants fed tropical forages usually produce more enteric methane than ruminants fed temperate forages. The relative influence of forage type, breed and temperate *vs* tropical environment on rumen microbial populations is not known. This experiment aimed to separate these effects. We designed two parallel experiments in sheep in two sites: temperate (France) and tropical (French West Indies), using in each site two breeds, Texel (temperate origin), and Blackbelly (tropical origin) fed the same temperate forages (C3 carbon fixation, permanent grasslands of high and low quality) and tropical forages (C4 carbon fixation, permanent grasslands of high and low quality). We determined diet digestibility, ruminal end-products of fermentation and microbial groups: total protozoa, methanogens and bacteria, and selected fibrolytic bacteria. Dry matter digestibility coefficient was higher in tropical site (612 *vs* 580 g/kg on average, $P = 0.004$) but no difference was observed between C3 and C4 forages. There was no effect of site on total VFA concentration, but the acetate:propionate ratio was higher for the tropical site (4.30 *vs* 3.93 on average, $P = 0.007$). The acetate:propionate ratio was also affected by forage type with higher values for C3 than C4 forage (4.24 *vs* 3.99 on average, $P = 0.03$). Concentration of total rumen bacteria and methanogens was determined by qPCR targeting, respectively, the *rrs* (16S ribosomal RNA subunit) and *mcrA* (methyl coenzyme-M reductase) genes. For both groups, the number of gene copies per gram of DM rumen content was higher in the tropical site ($P < 0.001$). For cellulolytic bacteria, higher number of *rrs* copies per gram of DM of rumen content were detected for *Fibrobacter succinogenes* in the temperate site ($P < 0.001$), whereas no differences were observed for *Ruminococcus flavefaciens* or *Ruminococcus albus* numbers between sites, breeds and forage type. Protozoa numbers determined by counting did not vary between sites, forages or breeds, but a site × forage interaction was observed ($P = 0.01$): there were more protozoa and *R. albus* in tropical sites for tropical forages. Our results suggest that rumen microbiota was mainly influenced by environment (temperate *vs* tropical) and that forage type (C3 *vs* C4) and breed had minor effects. However, an interaction between environment and forage type was observed for some variables.

Abbreviations: CH_4, methane; VFA, volatile fatty acid; DM, dry matter; H_2, hydrogen; CO_2, carbon dioxide; H, high; L, low; NDF, neutral detergent fibre assayed without a heat-stable amylase and expressed inclusive of residual ash; ADF, acid detergent fibre expressed inclusive of residual ash; OM, organic matter; NH_3, ammonia; PBS, phosphate buffer saline; DGGE, denaturing gradient gel electrophoresis; TAE, Tris-Acetate-EDTA; MFS, methylgreen formalin saline.

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http://dx.doi.org/10.1016/j.anifeedsci.2016.08.010
0377-8401/

1. Introduction

Enteric methane (CH_4) from ruminants accounts for 39% of livestock sector greenhouse gases emissions (Gerber et al., 2013). Several studies have tested different strategies for CH_4 abatement in ruminants (Hristov et al., 2013; Martin et al., 2010). However, although ruminant diets are based on forage, there is scant information on the effect of forage type on methanogenesis. A meta-analysis (Archimède et al., 2011) showed that enteric CH_4 production per kg of dry matter (DM) intake may be higher for ruminants fed tropical forages than for those fed temperate forages. These differences in CH_4 production may be due to the constitutional variation in the chemical structure of tropical and temperate forages, which display C4 and C3 carbon fixation pathways, respectively. However, differences could also be due to the ambient environment or to animal breed. Experiments carried out with tropical forages have always been conducted in the tropics with breeds originating from and adapted to a tropical environment; likewise, experiments with temperate forages have been run in temperate areas with breeds originating from these areas.

In the rumen, CH_4 production results from microbial fermentation of carbohydrates. The main end products of this fermentation are volatile fatty acids (VFA), hydrogen (H_2) and carbon dioxide (CO_2). A large diverse population of microorganisms drives this process, especially bacteria and protozoa. The H_2 produced is mainly used by methanogenic archaea to reduce CO_2 to CH_4. The activity, diversity and concentration of microbes harboured in the rumen are influenced by diet, breed, and the environment (King et al., 2011). However, to our knowledge, the relationships linking these three factors and their relative importance have not been studied.

The aim of this experiment was to compare rumen fermentation variables and microbial community structures of two breeds of sheep (Texel *vs* Blackbelly) fed C3 and C4 forages (permanent grasslands of high and low quality) in a temperate and a tropical site. Special attention was paid to methanogens and hydrogen-producing protozoa, which are associated with methanogenesis increase. Total bacteria were also studied, with a focus on cellulolytic bacteria, which play an important role in cell wall degradation and so can favour CH_4 production.

2. Materials and methods

2.1. Animals, diet, management and experimental design

The study was conducted in parallel in two research sites located in temperate and tropical areas. The temperate site was in Auvergne, France, at 45.70° North latitude and 3.03° West longitude. Where the animals were housed, the average daily temperature ranged between 11.2 °C and 15.9 °C, and the average relative humidity ranged between 32% and 46% during the experiment. The tropical area was in the French West Indies at 16.16° North latitude and 61.30° West longitude. The average daily temperature ranged between 21.0 °C and 25.0 °C and the average relative humidity ranged between 83% and 88% during the experiment.

In each site, 4 Texel wethers (temperate origin) and 4 Blackbelly rams (tropical origin) were used in two 4×4 Latin square designs. Sheep were born in the site where the experiment took place. Sheep were 2 years old and were fitted with a rumen cannula. Their body weight was 60.2 ± 1.5 kg for the Texel sheep and 51.3 ± 4.3 kg for the Blackbelly sheep in the temperate site; and 44.7 ± 0.7 kg for the Texel sheep and 44.4 ± 2.1 kg for the Blackbelly sheep in the tropical site. Management of experimental animals followed the guidelines for animal research of the French Ministry of Agriculture and other applicable guidelines and regulations for animal experimentation in the European Union (European Commission, 2010).

In both sites, sheep were fed the same forage from permanent grasslands, one grown in the temperate area and one grown in the tropical area. For each forage, there were two maturity stages that determined forage quality, high (H) and low (L), so that a total of 4 forages were studied in each site. Temperate forage was a semi-mountain permanent grassland, first cycle, flowering stage harvested in late June (L), and second cycle harvested in late August (H), both from the same parcel. The tropical forage was permanent grassland rich in *Dichanthium spp*; regrowths of 36 days (H) and 91 days (L), harvested in October. Transport of forage from one site to the other was by ship. Chemical composition of forages is presented in Table 1.

Each experimental period lasted 4 weeks: 2 weeks for adaptation to the forage, 1 week for CH_4 measurements, and 1 week for digestibility and rumen sampling. Detailed results on digestibility and CH_4 enteric productions arising from this study have been reported in a preliminary communication (Archimède et al., 2013) and will be fully published in a second paper (Archimède et al., unpublished).

Table 1
Average dry matter content and chemical composition of experimental forages[a].

	Temp H	Temp L	Trop H	Trop L
Dry matter, g/kg fresh matter[b]	875	879	866	878
Organic matter, g/kg dry matter	878	926	912	929
NDF, g/kg dry matter	586	623	742	742
ADF, g/kg dry matter	410	370	470	540
Crude protein, g/kg dry matter	134	83	120	69

[a]Temp H = temperate forage high quality, Temp L = temperate forage low quality, Trop H = tropical forage high quality, Trop L = tropical forage low quality.
[b]Dry matter content of forages was on average 1.3 percentage unit higher in temperate site than in tropical site.