Reduced methane emissions associated with unstable rumen fermentation in sheep

GJ Faichney[1], NM Graham[2]

[1]18 Gregory Avenue, Baulkham Hills, NSW 2153
[2]14 Alleyne Close, MacGregor, ACT 2615

Summary

Two sheep which produced different amounts of methane on a pelleted, mixed diet were found to be fauna-free. In two experiments, the lower methane emission from one sheep was associated with ethanol production at low feed intake and hydrogen gas emissions at high intake but no difference in the volatile fatty acid (VFA) pattern. In the third experiment, methane emissions were greater in both sheep, the differences between the sheep were not consistent, ethanol and hydrogen production were low and VFA patterns varied. It was concluded that rumen fermentation was unstable in these sheep, perhaps because of their fauna-free status. Methane emissions were 29-78% of those predicted by a published equation in the first two experiments; they were 57-98% of those predicted in the third experiment. Reducing equivalents not accounted for may have been used to reduce CO_2 to acetate.

Introduction

Methane is one of the trace gases whose direct and indirect radiative forcing effects are responsible for the greenhouse effect. Fossil-fuel related methane, which contributes 20-30% of the methane entering the atmosphere (1), adds new carbon to the atmosphere. By contrast, methane emitted from the biosphere represents a redistribution of carbon already in the atmosphere so has a lower total radiative forcing effect than fossil methane. A major source of methane in the biosphere is fermentation in the gastro-intestinal tracts of domestic ruminants. As a by-product of the microbial degradation of carbohydrates to volatile fatty acids (VFAs) in the rumen, methane not only represents a loss of dietary energy but also, as the major sink in the rumen for reducing equivalents (2H), its synthesis allows fermentation to proceed more rapidly than it otherwise would (2). The synthesis of propionic and valeric acids requires 2H so changes in methane emissions would be expected to be associated with complementary changes in the molar proportions of these acids in the total VFAs. In this paper we examine this relationship in two sheep which had emitted different amounts of methane over several months.

Methods

Observations were made on two Border Leicester x Merino wethers which had been given the same diet for 44 weeks from weaning at two months of age yet one (sheep 2) had been found to emit 40-60% as much methane per unit of feed as the other (sheep 1) (3). Their diet consisted of pellets containing 50% ground lucerne hay, 20% wheat, 10% oat grain, 10% linseed meal and 10% cottonseed meal plus minerals at 10 g/kg of the mix.

In the first experiment, the sheep were given 400, 1000 and 1500 g/d of the diet in successive two-week periods. After at least 10 days on each intake, gas production was measured during 24 hours in closed-circuit respiration chambers (4); samples of rumen fluid were obtained by stomach tube from each sheep two hours after feeding on the day after each gas production measurement. Each sheep was then fitted surgically with rumen and abomasal cannulas and the measurements were repeated during two periods in which the sheep were given first 1000 g feed/d, then 400 g/d; rumen samples were taken through the rumen cannula at intervals throughout the feeding cycle. In a third experiment of two periods two months later, the sheep were given first 400 g feed/d, then 1000 g/d in equal meals at 12 hour intervals. Gas production measurement was followed by seven day measurement of organic matter (OM) digestibility and three days to measure the partition of OM digestion between the stomach and intestines (5).

Table 1. Methane and hydrogen emissions and rumen volatile fatty acids (VFA) and ethanol in two sheep given a pelleted, mixed diet. GEI = gross energy intake; VFA ratio = (acetate + butyrate)/(propionate + valerate)

Dry matter intake:	354 g/d		886 g/d		1329 g/d	
Sheep:	1	2	1	2	1	2
Experiment 1:						
Live weight (kg)	29	31	32	34	35	39
Methane (% GEI)	6.51	4.04	3.88	2.85	3.01	2.00
predicted (% GEI)	8.38	8.37	7.15	7.28	6.56	6.85
Hydrogen (L/d)	0.05	0.06	0.20	1.59	0.35	4.79
Ethanol (mM)	14.1	53.2	15.4	2.0	1.7	2.0
VFA ratio	4.6	5.3	3.5	3.4	3.4	3.9
Experiment 2:						
Live weight (kg)	30	30	34	34		
Methane (% GEI)	2.97	2.52	4.26	2.36		
predicted (% GEI)	8.40	8.35	7.26	7.28		
Hydrogen (L/d)	0.11	0.02	0.10	1.15		
Fermentation products (mM):						
ethanol	1.3	3.6	3.6	3.8		
acetate	36.4	42.3	88.2	49.3		
propionate	9.7	11.5	26.1	30.0		
butyrate	6.8	5.6	13.5	9.7		
valerate	0.7	0.7	3.8	3.7		
other VFA	2.6	1.7	3.7	2.3		
methane	6.8	6.5	26.0	10.3		
hydrogen	0.30	0.03	0.15	1.25		
VFA ratio	4.2	3.9	3.4	1.8		
2H recovery (%)	57.1	53.6	64.1	76.8		

Table 2. Organic matter (OM) digestion and rumen fermentation balance in two sheep given a pelleted, mixed diet. GEI = gross energy intake; VFA ratio = (acetate + butyrate)/(propionate + valerate)

Dry matter intake:	359 g/d		876 g/d	
Sheep:	1	2	1	2
Experiment 3:				
Live weight (kg)	31.5	31.8	36.5	37.2
Digestible OM intake (g/d)	245	242	542	533
OM digestibility	0.738	0.726	0.666	0.655
OM digested in stomach (g/d)	143	150	336	336
Methane (% GEI)	4.83	8.21	6.42	5.45
predicted (% GEI)	8.44	8.40	7.39	7.42
Hydrogen (L/d)	0.02	0.02	0.15	0.16
Fermentation products (mM):				
ethanol	2.7	1.7	1.9	1.9
acetate	32.5	60.8	53.1	52.8
propionate	17.9	18.8	20.0	19.3
butyrate	7.9	10.8	16.3	13.5
valerate	1.8	1.9	1.5	2.3
other VFA	2.2	4.7	2.1	3.9
methane	13.2	31.6	28.0	22.8
hydrogen	0.04	0.05	0.18	0.18
VFA ratio	2.1	3.5	3.2	3.1
2H recovery (%)	93.3	102.2	97.4	89.5

Rumen fluid samples were taken at intervals throughout the 12 hour feeding cycle on the 10th day. At the end of each sampling period in each experiment, samples of rumen fluid were prepared and rumen microbes were examined using methods similar to those of Warner (6).

At the end of each gas production period, the gas in the respiration chambers was analysed, for methane by infra-red spectrometry before and after oxidation (7) and for hydrogen by gas chromatography. Rumen fluid was analysed for VFAs (8) and ethanol (9). The VFA ratio was calculated as (acetate + butyrate)/(propionate + valerate). Weighted mean concentrations of rumen metabolites during the feeding cycle were calculated in Experiments 2 and 3. The recovery of 2H was calculated from the stoichiometry of rumen fermentation (10). Methane and hydrogen emissions were converted to rumen 'concentrations' by assuming that fermented OM was equivalent to OM digested in the stomach and that the rumen contributed 90% of the methane emitted (11). In order to predict methane emissions (7), it was assumed that the OM digestibilities measured in Experiment 3 applied to Experiments 1 and 2. To convert methane emissions (L/d) to % gross energy (GE) intake, it was assumed that diet dry matter contained 18.4 MJ GE/kg and that the energy value of methane was 36.59 kJ/L.

Results

In Experiment 1, sheep 2 emitted only 62-74% as much methane as sheep 1 (Table 1) but the expected decrease in the VFA ratio did not occur. However, sheep 2 showed much higher rates of hydrogen gas emissions and, at the lowest intake, higher rumen ethanol concentrations than sheep 1; hydrogen emissions increased with feed intake. In the first period (higher intake) of Experiment 2, sheep 2 emitted only 55% as much methane as sheep 1 (Table 1) but propionate and valerate levels had increased and that of acetate decreased so that the VFA ratio was only 1.8. By contrast, the ratio in sheep 1 was 3.4 and the VFA concentration in the rumen was 42% higher than in sheep 2. Hydrogen emissions had decreased in both sheep but were still high in sheep 2. There was no consistent change in ethanol concentrations. In the second period (lower intake), methane emissions from sheep 2 were only 15% less than from sheep 1; hydrogen emissions fell in sheep 2 and ethanol concentrations fell in sheep 1, but the VFA concentrations were similar in the two sheep. The calculated 2H recovery was 54-77%.

In Experiment 3, there were no differences between the sheep in OM digestion (Table 2). By contrast with Experiments 1 and 2, it was sheep 1 which emitted less methane at the lower intake and this was associated with lower total VFA and acetate concentrations and higher propionate concentrations than in sheep 2. However, at the higher intake, sheep 2 emitted less methane but VFA concentrations and patterns were similar. Hydrogen emissions and ethanol concentrations did not differ between sheep in experiment 3. The calculated 2H recovery was 90-102%.

The prediction equation overestimated methane emissions. For Experiments 1 and 2, emissions were 29-78% of the predicted values (Table 1); they were 57-98% of predictions in Experiment 3. Omitting values for sheep 1 on the low intakes, the ranges were 29-59% and 74-98%.

Microscopic examination of the preserved rumen fluid samples showed no ciliate protozoa and no large morphologically distinctive bacteria in any sample. The bacterial populations differed appreciably between animals and between successive samples from the same animal but there were no obvious consistent differences either between animals or periods.

Discussion

The variations observed here in the disposition of 2H between the VFAs, methane, hydrogen gas and ethanol and in the rumen bacterial population are consistent with rumen fermentations being unstable in both sheep. The sheep were found to be fauna-free, perhaps as a result of their being fasted several times during the preceding 12 months for measurement of fasting heat production.

Cattle have been shown to emit more methane when faunated than when ciliate-free (12). However, in the present study methane emissions were quite different between the two sheep and were low in Experiments 1 and 2 but increased to near normal levels in Experiment 3. The calculated 2H recoveries in Experiment 3 averaged 96%, only marginally higher than the expected value of about 90% (10), but were much lower in Experiment 2, particularly on the lower intake, indicating the existence of a significant, unidentified 2H sink. If reductive acetogenesis were this sink and the CO_2-reducing bacteria responsible produced 10-15% of the acetate, 2H recovery would have averaged 90-95%.

Ethanol is not found normally as an end product of rumen fermentation (13) although it has been reported in rumen fluid from cattle given purified diets (14). The decrease in ethanol concentrations from Experiment 1 to Experiment 3 was associated with an increase in valeric acid and the highest levels of valeric acid were associated with the highest levels of propionic acid. These associations are consistent with the presence in the rumen of reactions that produce valeric acid with ethanol as an intermediate (14).

The observed methane emissions in Experiments 1 and 2 were substantially less than those predicted but were closer to the predictions for Experiment 3. Empirical prediction equations cannot allow for reduced emissions during periods of spontaneous instability in the rumen. Such events do not appear to have been reported so their frequency and thus their impact on the accuracy of prediction of methane emissions at the farm level remains uncertain.

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