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Evidence for methane oxidation in rumen fluid in vitro

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Although one of the major activities of the rumen fermentation is methanogenesis, some oxygen enters the rumen and it is possible that methane oxidation also occurs. Methane-utilising bacteria were isolated from the rumen (Stock and McCleskey, 1964, J Bacteriol, 88, 1071), but their role was never established. The present experiments were undertaken to evaluate the extent of methane oxidation in rumen fluid in vitro.

Rumen fluid was obtained from three canulated sheep fed a diet of hay, barley, fishmeal and molasses (500, 229.5, 100, 91 and 9.5 g/kg dry matter, respectively). Strained rumen fluid (10 ml) plus buffer (40 ml) was incubated with the above diet (200 mg, ground to pass though a 1 mm sieve) at 39°C under an atmosphere of CO_2 (headspace 70 ml) for 24 h and methane production was compared in the presence and absence of methylfluoride, a potent inhibitor of methane oxidation. Low additions (4.5 µmol) of methylfluoride increased methane production from 373 ± 25.2 µmol to 403 ± 22.0 ($P < 0.05$). A tenfold higher addition of methylfluoride inhibited methane production. The addition of 446 µmol of ^{13}C methane, in the absence of diet or methylfluoride, resulted in the formation of 1.3 ± 0.1 µmol $^{13}CO_2$ (see table). The possibility that this $^{13}CO_2$ was formed by reversal of methanogenesis was eliminated by the finding that 20 mM bromoethanosulfonic acid, an inhibitor of methanogenesis, caused only a 16% fall in the production of $^{13}CO_2$.

Thus it appears that some methane production is possible in rumen fluid, but that is unlikely to exceed 8% of net methane production.

	Enrichment of headspace gas after 24 h incubation	
Addition	Atom % $^{13}CO_2$	µmol of $^{13}CO_2$
446 µmol of $^{12}CH_4$	1.0759	20.64
446 µmol of $^{13}CH_4$	1.0861	21.96

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