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Preliminary study of non-methanogenic hydrogenotrophic microflora in the rumen of newborn lambs

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Hydrogen is an important intermediate in the rumen ecosystem. Produced by numerous bacterial, fungal, and protozoal species, it serves as electron donor in the reduction of CO_2 to methane. The replacement of methanogenesis, which constitutes a loss of energy for the ruminant, by alternative hydrogenotrophic pathways has been proposed as a strategy to improve utilization of feed energy by the animal. In this context, and based on previous results (Fonty *et al*, 1987), we have initiated an investigation of the hydrogen-utilizing microflora potentially present in the rumen prior to the establishment of methanogens.

Rumen contents were sampled from lambs kept in sterile isolators 1 day after birth and associated with the rumen microflora of 2, 3 or 5 day-old flock-reared lambs. Hydrogen utilization experiments were performed using washed rumen bacteria in anaerobic dilution solution under H_2/CO_2 or N_2/CO_2 (4:1) gas at initial overpressure of 0.5 bars. Incubations were at 39 °C on a rocking agitator. The incorporation of ^{13}C- or ^{14}C-labeled CO_2 was followed. Pure cultures of H_2-utilizing bacteria were obtained by repeated streaking, transfers and final isolation from roll-tubes using a medium (Balch *et al*, 1979) containing 30 % rumen fluid.

A production of methane from H_2/CO_2 was observed for the microflora of 2-day-old lambs or older. No methane was produced by 24 h microflora, while an H_2-dependent incorporation of CO_2 took place (table I). The $^{14}CO_2$ label was primarily incorporated in the VFA fraction (86 %). This was absent after a treatment killing all bacteria (80 °C, 30 min). ^{13}C NMR showed that double labeled acetate was the major product. Strains of hydrogen-utilizing bacteria were isolated from the 24 h microflora. These were pleiotrophic and produced acetate as the main fermentation product.

We propose data supporting the hypothesis that hydrogenotrophic bacteria pre-exist in the rumen prior to the establishment of methanogens. These are able to use H_2 to reduce CO_2 to soluble products.

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Table I. Fermentation products of rumen bacterial suspensions incubated *in vitro* for 24 h.

Lamb	*Microflora/ lambs aged*	*Incubation under*	*Fermentation products (mM)*				
			CH_4	*Acetate*	*Propionate*	*Butyrate*	*Valerate*
A	24 h	N_2:CO_2	0.0	28.7	8.7	2.6	1.1
		H_2:CO_2	0.0	49.2	10.7	3.0	1.1
B	48 h	N_2:CO_2	1.4	27.5	9.4	2.0	1.0
		H_2:CO_2	10.0	29.4	10.1	2.3	1.1