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Rumen gases and bloat in grazing dairy cows

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SUMMARY

Results are reported from three experiments conducted at the Dairy Research Institute, Ellinbank, Australia during 1992/93 which examined the composition and kinetics of the gas in the rumen headspace of lactating dairy cows grazing white clover/perennial ryegrass pastures. Before grazing, rumen headspace gas was composed of carbon dioxide 65%, methane 31% and nitrogen 4% whereas, after one hour of active grazing, the headspace gas was composed of carbon dioxide 76%, methane 22% and nitrogen 2%. The composition of headspace gas was not affected by antibloat capsules (which release 250 mg/day of monensin). The headspace gas from bloated cows contained slightly less ($P < 0{\cdot}01$) carbon dioxide and slightly more nitrogen than that from non-bloated cows.

A novel technique which employs ethane as a tracer to measure rumen headspace volume and the kinetics of the rumen headspace gases is described. The tracer technique was used in two experiments in which the influence of grazing, antibloat capsules and bloat on the rumen headspace volume and the kinetics of the headspace gases were examined. It is concluded that our ethane tracer technique provides a simple and inexpensive way to estimate methane production by grazing ruminants.

INTRODUCTION

The rumen headspace volume, gas composition and production rate of fermentation gases in grazing dairy cows have not been extensively researched. The volume (Waghorn 1991*a*), composition (Leng 1973; Laby *et al*. 1991) and rate of production of rumen gas (Hungate 1955; Miller & Jacobson 1962; Fay *et al*. 1980; Waghorn 1991*a*) have relevance to the aetiology of ruminal bloat or tympanitis. Monensin, an ionophorous antibiotic, has long been used as an additive to ruminant feedstuffs since it improves feed conversion efficiency and reduces the incidence of feed-related bloat deaths (Raun *et al*. 1976; Lowe 1991). Lowe (1991) has attributed the reduction in bloat deaths to the fact that monensin shifts the pattern of rumen fermentation from acetic to propionic acid and this results in a reduction in methane and carbon dioxide production (Russell & Strobel 1989).

Ruminal methane (CH_4) is also of concern in nutrition because its production constitutes a waste of dietary energy (Blaxter & Clapperton 1965). Furthermore, it has been suggested that methanogenesis in ruminants constitutes a significant proportion of global CH_4 production which may contribute towards the warming of the earth's atmosphere ('the greenhouse effect') (Watson *et al*. 1992).

Ruminal CH_4 production is usually measured with animals confined in respiration chambers (IAEA 1992). Such measurements are difficult and expensive. Moreover, it has recently been concluded that it is not valid to extrapolate measurements made on animals confined in respiration chambers to grazing animals (O'Kelly & Speirs 1992). Other indirect methods which have been used to measure CH_4 production in penned ruminants have been based on tracer techniques and compartmental analysis. Bryant *et al*. (1973) employed tritium-labelled CH_4 to measure rumen CH_4 kinetics in penned sheep. Radioactive tracers are expensive and their use in lactating dairy cows is undesirable. More recently, the IAEA (1992) have advocated sulphur hexafluoride as a suitable tracer for rumen CH_4 kinetics. In this paper, we report on the composition of rumen headspace gas from bloated and non-bloated dairy cows grazing leguminous pastures. A technique is described in which ethane (C_2H_6) is used as a tracer to measure rumen gas kinetics in grazing dairy cows. The technique is used to investigate the effects of monensin antibloat capsules on the composition and kinetics of rumen headspace gas. In addition, the potential for nitrogen (N_2) to be used as a resident marker to estimate the rate of production of rumen headspace gas is examined.

MATERIALS AND METHODS

The research was conducted during the winter and spring of 1992 at the Dairy Research Institute, Ellinbank (37° 50′ S, 145° 00′ E), 110 km southeast of

Melbourne, Victoria. Two experiments (Expts 1 and 2) examined the effects of intraruminal antibloat capsules (Elanco Animal Health, 112 Wharf Road, West Ryde, New South Wales 2114) on the gas kinetics of the rumen headspace. A third experiment (Expt 3) examined the feasibility of using an intraruminal infusion (in contrast to a bolus injection) of C_2H_6 to measure gas production, and in particular methane production in the rumen. In the fourth experiment (Expt 4), the composition of rumen gas from bloating and non-bloating cows was compared. The Friesian cows used in these experiments were in early lactation producing *c*. 20 litres/day of milk, and had average bodyweights of 430 kg. Cows were milked daily at 07.00 and 16.00 h, and at other times grazed together on high quality (digestible dry matter $> 80\%$, crude protein $> 20\%$) white clover (*Trifolium repens*)/perennial ryegrass (*Lolium perenne*) pasture. The intra-ruminal capsules used in Expts 1 and 2 were designed to release 250 mg/day of monensin at a constant rate over a 100-day period. Rumen gas kinetic studies were conducted at least 2 weeks after administration of the capsules.

Experiment 1

In Expt 1, 18 cows were allocated to two groups and the cows were paired on the basis of bodyweight and milk yield. One member of each pair was administered an antibloat capsule. After the morning milking, on 9 consecutive days, all cows were allowed to graze as a herd for 2 h on white clover pasture *ad libitum*. Each morning a different matched pair of cows was herded into a temporary stockyard erected in the paddock and the cows were then individually confined in a portable cattle crush for ruminal gas measurements. While gas samples were being collected, cows were assessed for bloat by palpation of the left flank and using Johns (1954) 0–5 scale: (0, no bloat; 1, mild; 2, medium; 3, moderate; 4, severe; 5, dead). After the gas sampling procedure was completed, rumen fluid (*c*. 100 ml), was collected by means of a stomach tube. Rumen fluid was strained through two layers of cloth. The pH was measured between 30 min and 1 h after collection.

Rumen gas measurements

Each cow was first administered a local anaesthetic (lignocain 1·0 ml s.c.) in the left side *paralumbar fossa*. A stainless steel needle (16 gauge 150 mm long) was then inserted into the rumen and a sample of rumen headspace gas (*c*. 10 ml) was withdrawn into a gas-tight glass syringe. The glass syringe was stoppered using another needle which was already spiked into a rubber stopper. The injection needle was stoppered and left inserted in the rumen for the duration of the gas sampling period. C_2H_6 (100 ml at atmospheric pressure and ambient temperature) was then quickly injected through the needle into the rumen headspace. Additional samples (6–10) of rumen headspace gas were collected at timed intervals over the subsequent 20 min period. The needle was withdrawn and the cow was given an injection of antibiotic (20 ml i.m. containing 5·0 g procaine penicillin, 5·0 g dihydrostreptomycin as sulphate, and 0·4 g procaine hydrochloride).

The gas samples were analysed for C_2H_6 and for the principal rumen gases (CH_4, N_2, CO_2, O_2, H_2 and H_2S) by gas chromatography. A Perkin Elmer Autosystem GC was used with Porapack N and Molecular sieve 5A columns (both 2 m long, 3 mm diameter) in series. Helium (17 ml/min) was used as a carrier gas. A thermal conductivity detector was used to detect CO_2, O_2 and N_2 while a flame ionization detector was used to detect CH_4, C_2H_6, H_2 and H_2S. Standard mixtures of the above gases (CIG, 90 Bell St, Preston 3072) were used to calibrate the gas chromatograph.

Experiment 2

Expt 2 was similar to Expt 1, except that experimental measurements were made immediately after the morning milking, i.e. prior to grazing, and again on the same cows after 1 h of active grazing. Bloat scores were recorded and samples of rumen fluid collected immediately after completion of the gas sampling procedure.

Experiment 3

This experiment was conducted indoors in a feeding stall, with only one cow. The cow was held in a stanchion and offered harvested perennial ryegrass/white clover pasture. After the cow had been observed to be eating steadily for 2 h, we carried out the following gas infusion procedure. A needle with an attached three-way stopcock was inserted into the cow's rumen as described above. A sampling syringe was attached to one orifice of the stopcock and the ethane infusion line (silastic tubing, 2 mm i.d.) attached to the remaining orifice. The infusion rate of C_2H_6 was controlled by a needle valve and flow meter (Porter Instrument Co, Hatfield, Pa, USA). A sample of rumen gas was collected and then C_2H_6 was infused at 30 ml/min for 1 h. The infusion was momentarily interrupted (*c*. 10 s) during sampling of rumen gas. Rumen gas samples were collected at 5, 10, 15, 30, 46, 60, 62, 65 and 70 min from the commencement of ethane infusion. Gas samples were analysed as described above.

Experiment 4

Samples of rumen headspace gas were collected adventitiously from bloated cows which had grazed leguminous pastures. Bloated cows were herded a short distance from a paddock to stockyards, where they were confined in a cattle crush and a sample of

Table 1. *Effect of anti-bloat capsules* (*ABC*) *on rumen fluid pH, composition of rumen headspace gas and on gas kinetics of the rumen headspace* (*Expt 1*)

				Rumen gas composition				Rate of entry		
Cow no.	Treatment	pH	Headspace (litre)	CO_2 (%)	CH_4 (%)	N_2 (%)	Total gas (l/min)	CH_4 (l/min)	CO_2 (l/min)	N_2 (ml/min)
751*	Control	6·16	21·41	74·8	22·6	2·66	0·95	0·22	0·71	25·3
437	Control	6·41	8·60	76·6	22·0	1·38	2·99	0·66	2·28	57·4
707	Control	6·96	6·03	74·7	23·3	2·02	2·55	0·60	1·91	51·6
082	Control	6·44	4·41	75·2	24·2	0·59	3·26	0·79	2·45	19·2
727	Control	—	3·94	73·6	25·6	0·81	2·78	0·71	2·04	22·5
657	Control	7·02	3·05	75·6	23·0	1·36	1·40	0·32	1·06	19·0
418	Control	6·45	2·81	73·9	24·7	1·40	2·16	0·53	1·59	38·8
051	Control	6·10	1·91	77·9	21·0	1·15	1·31	0·28	1·02	15·1
691	Control	6·56	1·49	72·6	25·1	2·36	1·14	0·29	0·83	26·9
068	ABC	6·42	5·08	77·4	22·1	0·51	2·50	0·55	1·94	12·8
053	ABC	6·55	4·89	77·6	21·5	0·96	1·74	0·38	1·35	16·7
827	ABC	6·77	3·99	75·3	23·7	1·05	2·51	0·60	1·89	26·4
697	ABC	6·64	3·56	74·9	24·3	0·82	1·74	0·42	1·30	14·3
651	ABC	6·92	3·03	75·1	24·3	0·63	1·68	0·41	1·26	10·6
736	ABC	6·79	2·31	75·9	23·0	1·07	1·00	0·23	0·76	10·7
201	ABC	6·29	2·17	73·5	24·5	2·03	1·83	0·45	1·34	37·1
701	ABC	6·37	1·36	75·2	23·6	1·25	0·95	0·23	0·72	11·9
510	ABC	6·94	1·26	77·1	21·2	1·69	1·05	0·22	0·81	17·7
Mean	Control	6·51	5·96	75·0	23·5	1·63	2·06	0·49	1·54	30·6
Mean	ABC	6·63	3·07	75·8	23·1	1·11	1·67	0·39	1·26	17·6
S.E.		0·092	1·498	0·49	0·46	0·201	0·249	0·060	0·188	4·14

* Cow 751 had medium bloat (score 2) during these (D.F. = 16) measurements.

rumen headspace gas collected. When possible, samples of rumen headspace gas were also collected simultaneously from one or two non-bloated cows that had been grazing with the bloated cow. Where and when possible, the following procedure was used to collect samples of bloat foam. The procedure was similar to collecting rumen fluid. A stomach tube (2·5 m long, 30 mm i.d.) was inserted *per os* into the rumen. The tube was withdrawn, and foamy rumen contents (*c*. 250 ml) were poured to fill a glass jar of known volume. The jar was then sealed with a gas-tight lid containing a rubber septum. The jar and contents were weighed and then 0·5 ml of a silicon antifoaming solution was injected into the jar in order to collapse the foam. The foam gas was then sampled via the septum. These foam samples were collected within several minutes of taking the headspace gas samples.

Data analysis

Rumen headspace gas was usually found to contain $< 0{\cdot}4\%$ O_2, and this was thought to arise mostly from air contamination of samples. Accordingly, in Expts 1, 2 and 3, we used O_2 as a marker for air contamination, and using the O_2:N_2 ratio of air (21:78), a correction factor was employed to take account of this contamination in the rumen gas. The C_2H_6 data were analysed as a one-compartment model using the CONSAM [Conversational, simulation, analysis and modelling] computer program of Boston *et al*. (1988). CONSAM calculated directly the initial volume of distribution of the tracer, i.e. the rumen headspace volume, and the fractional clearance rate (FCR) of the tracer. The total rate of entry (ROE) of gas into the rumen headspace was calculated as the product of the headspace volume and the FCR. The ROE into the rumen headspace of an individual gas was calculated as the product of its proportional composition in the rumen headspace and the total ROE of rumen gas.

Analyses of variance were carried out on all data from Expts 1, 2 and 4 using the GENSTAT 5 statistical package (Genstat 5 Committee 1987). The data from Expts 1 and 2 were combined and linear regression analyses were performed between the inverse of the N_2 composition of rumen headspace gas and the ROE of total rumen gas, and also with the ROE of CH_4.

RESULTS

The results from Expts 1, 2 and 4 are shown in Tables 1, 2 and 3 respectively. Examination of the gas chromatographs of samples from two cows (Nos. 868 and 982) in Expt 2 indicated that the C_2H_6 tracer must not have been properly injected into these cows and

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Table 2. *Effect of time of sampling and anti-bloat capsules* (*ABC*) *on rumen fluid pH, composition of rumen headspace gas and on gas kinetics of the rumen headspace* (*Expt 2*). (*D.F. generally 16, but sometimes less depending on the number of missing values*)

Cow no.	Treatment	pH	Headspace (litre)	Rumen gas composition CO_2 (%)	CH_4 (%)	N_2 (%)	Total gas (l/min)	Rate of entry CH_4 (l/min)	CO_2 (l/min)	N_2 (ml/min)
				Pre-grazing						
864	Control	—	5·41	53·5	40·5	6·0	0·19	0·08	0·10	11·3
868	Control	7·71	4·53	59·9	35·4	4·7	0·39	0·14	0·23	18·4
201	Control	7·32	1·96	65·3	29·0	5·7	0·90	0·26	0·59	51·5
869	Control	7·84	2·82	61·7	34·2	4·1	0·51	0·17	0·31	20·9
736	Control	7·30	2·99	66·5	28·2	5·3	0·86	0·24	0·57	45·7
454	Control	6·96	3·78	71·0	27·1	1·9	0·82	0·22	0·58	15·5
850	Control	6·64	1·45	66·4	30·2	3·4	0·71	0·21	0·47	23·9
830	Control	7·13	3·99	68·8	28·8	3·6	0·55	0·16	0·38	12·6
501	Control	7·27	2·48	74·0	24·8	1·2	0·66	0·16	0·48	7·9
589	ABC	7·69	9·03	62·2	32·9	5·0	0·36	0·12	0·23	22·5
982	ABC	7·56	2·98	57·0	38·0	5·0	0·29	0·11	0·16	14·2
727	ABC	7·52	2·99	64·1	33·1	2·9	0·67	0·22	0·43	19·5
730	ABC	7·74	7·08	51·7	41·7	6·6	0·26	0·11	0·13	16·9
697	ABC	7·72	5·34	71·6	26·1	2·3	1·37	0·36	0·98	31·5
651	ABC	7·08	3·55	72·9	24·9	2·2	1·11	0·28	0·81	24·3
695	ABC	7·51	2·12	73·2	25·0	1·9	0·58	0·15	0·42	11·0
106	ABC	7·30	3·92	73·9	24·1	2·1	1·22	0·29	0·90	25·6
707	ABC	7·56	1·49	66·7	28·5	4·7	0·51	0·15	0·34	23·9
Mean	Control	7·27	3·27	65·2	30·9	3·8	0·62	0·18	0·41	23·1
Mean	ABC	7·52	4·28	65·9	30·5	3·6	0·71	0·20	0·49	20·6
Mean	Pre-grazing	7·40	3·77	65·6	30·7	3·7	0·66	0·19	0·45	21·8
S.E.		0·10	0·65	2·4	1·9	0·6	0·11	0·03	0·09	3·9
				Post-grazing						
864	Control	—	3·41	73·2	25·5	1·3	0·99	0·25	0·73	12·9
868	Control	7·79	—	79·1	19·0	1·8	—	—	—	—
201	Control	7·22	4·55	76·2	22·4	1·4	0·31	0·70	2·38	43·7
869	Control	—	1·12	76·4	22·0	1·6	0·84	0·18	0·64	13·4
736	Control	6·75	6·24	77·2	21·7	1·1	0·20	0·43	1·54	22·0
454	Control	6·50	4·60	76·4	22·6	1·0	0·25	0·57	1·90	14·4
850	Control	7·24	2·22	73·0	25·3	1·7	1·09	0·28	0·80	18·5
830*	Control	7·51	17·75	72·3	23·1	4·6	2·93	0·68	2·12	134·9
501	Control	7·12	3·78	80·3	18·6	1·2	1·82	0·34	1·46	21·9
589	ABC	—	4·11	79·5	19·2	1·3	1·73	0·33	1·38	22·5
982	ABC	7·10	—	77·8	21·3	0·9	—	—	—	—
727	ABC	7·00	5·76	75·8	22·6	1·6	1·69	0·38	1·28	27·1
730	ABC	7·04	6·66	79·1	19·9	1·0	2·60	0·52	2·05	26·0
697	ABC	7·16	5·20	78·5	19·3	2·1	0·81	0·16	0·64	17·0
651	ABC	6·89	3·36	75·2	23·9	0·9	1·60	0·38	1·20	14·4
695	ABC	7·71	3·43	61·3	37·8	1·0	1·27	0·48	0·78	12·7
106	ABC	7·00	2·94	77·6	20·6	1·8	1·55	0·32	1·20	27·9
707	ABC	7·16	5·30	79·9	18·6	1·6	1·05	0·19	0·83	16·7
Mean	Control	7·16	5·46	75·9	22·2	1·7	1·91	0·43	1·45	25·7
Mean	ABC	7·13	4·49	76·1	22·6	1·4	1·54	0·35	1·17	20·5
Mean	Post-grazing	7·15	5·03	76·0	22·4	1·6	1·73	0·39	1·31	23·1
S.E.		0·12	1·27	1·5	1·5	0·1	0·25	0·06	0·19	3·5

* Cow 830 had mild bloat (score 1) during these measurements.

consequently their tracer data are not reported. In Expts 1, 2 and 3, the disappearance of C_2H_6 either injected or infused into the rumen headspace could in all but the two cases mentioned above, be well described using single exponentials and be fitted to a simple one-compartment model (Figs 1 and 2).

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Table 3. *The bloat score and gas composition of the rumen headspace and of bloat foam of cows grazing white clover pasture (Expt 4)*

Cow no.	Bloat score	Rumen gas composition CO_2 (%)	CH_4 (%)	N_2 (%)	O_2 (%)
589	0	73·1	17·7	7·47	1·75
982	0	77·3	21·2	1·42	0·15
727	0	75·0	22·4	2·39	0·22
730	0	73·8	18·6	6·17	1·48
697	0	78·1	19·2	2·57	0·12
651	0	74·4	23·7	1·67	0·22
695	0	78·5	18·9	2·24	0·41
106	0	77·2	20·6	2·15	0·10
864	0	72·7	25·4	1·85	0·16
868	0	76·5	18·4	4·41	0·74
201	0	75·9	22·3	1·71	0·08
869	0	75·5	21·7	2·50	0·25
736	0	72·8	20·5	5·47	1·24
454	0	75·2	22·5	2·22	0·09
850	0	72·2	25·0	2·51	0·24
501	0	76·1	17·6	5·12	1·13
830	1	69·3	22·1	7·68	0·93
117	1	65·2	25·5	8·52	0·83
707	1	74·2	23·1	2·48	0·13
751	2	73·8	22·3	3·59	0·27
856	2	65·9	24·5	8·58	1·02
856 (foam)*	2	64·9	26·7	8·10	0·30
603	3	55·1	26·5	16·50	1·9
603 (foam)*	3	51·6	26·1	20·80	1·4
643	3	65·3	24·0	9·38	1·33
575	3	59·7	21·7	15·34	3·25
773†	4	70·2	19·2	9·48	1·23
Mean bloated	2·3	66·5	23·2	9·06	1·21
Mean non-bloated	0	75·3	21·0	3·24	0·52
S.E. (23 D.F.)	0·23	1·19	0·703	0·916	0·210

* (Foam) data not included in means.
† Cow 773 died from bloat several minutes after collection of this sample.

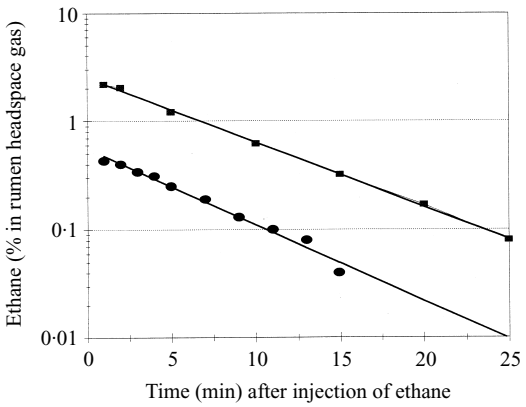

Fig. 1. Single exponential changes in the concentration of rumen gas tracer after two separate injections of 100 ml of ethane into the rumen of cow 830 (Expt 2), immediately before (■) and after (●) 1 h of active grazing. This cow had mild bloat (score 1) after grazing.

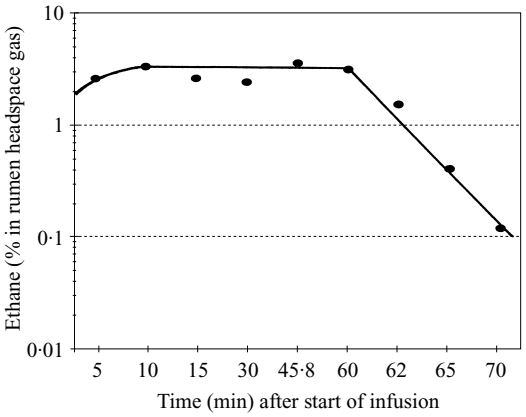

Fig. 2. The changes in the concentration of ethane in rumen gas during and after a 1 h infusion of ethane (30 ml/min) into the rumen headspace. The solid line is predicted by a simple one-compartment model and (●) depicts the measured concentrations of ethane.

In Expts 1 and 2, the rumen fluid pH, rumen headspace, and ROE of rumen gas, CO_2, and CH_4 and their concentrations were not significantly ($P > 0·05$) affected by antibloat capsules. In Expt 1, the ROE of N_2 was significantly ($P < 0·05$) reduced in cows administered the capsules, however, this effect was not apparent in Expt 2. In Expt 2, where measurements were made pre- and post-grazing, rumen fluid pH and concentrations of CH_4 and N_2 were significantly ($P < 0·01$) decreased after grazing, but the concentration of CO_2 and the ROE of fermentation gases were significantly increased ($P < 0·01$).

In Expt 4, samples of bloat gas were collected from nine cows. In two of these cows, considerable difficulty was experienced in obtaining bloat gas since the sampling needle repeatedly became blocked with bloat foam. The sampling needle had to be manipulated to the top of the rumen in order to obtain a sample of free headspace gas. Although the concentrations of the major fermentation gases in the headspace of bloated cows were similar to those in non-bloated cows, in bloated cows there was significantly ($P < 0·01$) less CO_2 and significantly ($P < 0·05$) more CH_4, N_2 and O_2 than in non-bloated cows (Table 3). Bloat foam collected from two cows (Nos. 603 and 856) was found to be similar in composition to free headspace gas taken simultaneously from the same cows. The density of the foam from cow 603 was measured to be 0·57 g/ml. In Expt 4, despite drenching

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with bloat oil and performing a rumenotomy, cow 773 died as a result of bloat. At autopsy, her rumen contents had no foam.

DISCUSSION

Rumen gas composition

The gas composition of the rumen headspace of the grazing cows in these experiments (Tables 1, 2 and 3) was similar to that reported previously for penned cattle (Kleiber *et al.* 1943; McArthur & Miltimore 1961). In Expt 2, the concentrations of CO_2, CH_4 and N_2 were significantly different ($P < 0{\cdot}01$) between pre- and post-grazing. These findings are consistent with published reports on the effects of feeding on the composition of rumen headspace gas (Washburn & Brody 1937; Czerkawski & Clapperton 1968). Approximately 40% of the daily pasture intake of grazing dairy cows occurs in each of the 3-h periods following the morning and afternoon milkings (D. Dalley, personal communication). Consequently, we consider that the degree of rumen-fill could explain the different gas compositions pre- and post-grazing. Furthermore, in contrast to stall feeding, the variability in gas composition between cows in the grazing situation could also be explained by the variability in rumen-fill, the possibility of greater diet selection and also intermittent intake of cold pasture and water which may quench rumen fermentation and hence gas production. Small quantities of H_2 (0·01–4%) and H_2S (0·1–0·16%) in rumen gas have previously been reported (Washburn & Brody 1937; Kleiber *et al.* 1943; Czerkawski & Clapperton 1968). The gas analysis technique used in the work presented here could have detected these gases at concentrations of 0·1%, yet no trace of these gases was detected. It is speculated that in these experiments, the pasture diet was not conducive to the production of these gases. In Expts 1 and 2, the antibloat capsules (which release 250 mg/day of monensin into the rumen), had no significant effect on the ratio of CH_4:CO_2 in fermentation gas. In Expts 1 and 2, the steady state concentration of monensin in rumen fluid would have been *c.* 1 mg/l. These findings are consistent with those of Chalupa *et al.* (1980), who found that, *in vitro*, monensin at 1·0 mg/l in rumen fluid had no significant effect on the ratio of CH_4:CO_2 in fermentation gas.

Tracer method

The work described in this paper appears to be the first report of tracer techniques being used to measure the gas kinetics of the rumen headspace in grazing dairy cows. Initially, it was intended to use C_2H_6 as a tracer for CH_4 only. However, since the proportions of all the rumen headspace-gases did not vary significantly over the short 20 min period of each tracer study, ethane was used as a tracer of rumen headspace gas *in toto*, and also for CO_2, CH_4 and N_2 individually. Since C_2H_6 was injected into the rumen headspace, this technique mainly measures the kinetics of the rumen headspace as distinct from gas kinetics of the entire rumen. Eructation is the major pathway by which gases leave the rumen headspace, but since CO_2 is soluble in water, absorption of CO_2 back into the rumen fluid and across epithelial tissue into the blood may also be important (Waghorn 1991*b*). C_2H_6, like CH_4 and N_2, is relatively insoluble in water and ethane should therefore be an ideal tracer of CH_4, even if not for CO_2. Furthermore, since eructation of CH_4 from the rumen headspace accounts for > 93% of the CH_4 produced in the rumen (Murray *et al.* 1976), the C_2H_6 tracer technique can provide a good estimate of the rate of production of CH_4 in the rumen.

In a previous tracer experiment on rumen CH_4 (Bryant *et al.* 1973), the tracer (tritium-labelled CH_4 contained in 25 ml of water) was injected as a bolus into the rumen contents, and considerable time must have necessarily elapsed before the tracer was uniformly mixed throughout the rumen. Shipley & Clarke (1972) have pointed out that an important assumption underlying the compartmental analysis/tracer technique is that the tracer must almost instantaneously mix uniformly throughout the primary compartment (i.e. the compartment into which the tracer is injected). Since C_2H_6 is a gas, when injected into the rumen headspace, it must mix almost instantly and uniformly throughout the headspace, particularly when there is little foam. Much recent research has focused on the measurement of methane production from various sources because of its greenhouse global warming potential. The IAEA (1992) have recommended sulphur hexafluoride (SF_6) as a suitable tracer of rumen CH_4. However, SF_6 has a global warming potential > 1000 times that of low molecular weight hydrocarbons such as C_2H_6 and CH_4 (IPCC 1992; Pearce 1996). It would be ironic if 'greenhouse researchers' used such a potentially damaging tracer when a benign one can suffice. C_2H_6 was chosen as a tracer for this research since it is closest to CH_4 in the alkane homologous series and therefore has similar physical and chemical properties to CH_4. We could find no evidence in the literature that C_2H_6 can be metabolized within the rumen and we have not been able to detect any trace of C_2H_6 occurring naturally in the rumen. All the rumen gases and C_2H_6 are easily measured by gas chromatography and because C_2H_6 is a non-radioactive tracer, there is no need for expensive counting instruments and no problem in using C_2H_6 in lactating cows. Furthermore, C_2H_6 is relatively cheap (< $A 0·20 per cow for a 100 ml injection).

Another assumption of the tracer technique is that only a trivial amount of tracer is used so as not to perturb compartment size and 'steady-state kinetics'.

It could be argued that in the present work, since 100 ml injections of C_2H_6, (equivalent to *c*. 3% of rumen headspace volume) were used, that this was not a trace amount. However, when considered in relation to the magnitude of individual eructations, 3% of rumen headspace is not large. Furthermore, in all 53 of the individual cow tracer studies described here, the disappearance of C_2H_6 from the rumen headspace could, in each case, be described by a single exponential (see Figs 1 and 2 for typical examples). Single exponential disappearance of tracer has also been observed in tracer studies on penned sheep (Bryant *et al*. 1973). By convention, this is interpreted as indicating that the tracee exists in a single compartment, and that the conventional compartmental analysis methodology applies (Shipley & Clarke 1972). We interpret this to mean that the C_2H_6 tracer technique mainly applies to the rumen headspace gas and that it does not detect rumen gas dissolved in rumen fluid or trapped within bubbles in the rumen contents. We hypothesise that the presence of a considerable amount of foam within the rumen would result in a multi-exponential disappearance of gas tracer from the rumen. However, we were unable to test this hypothesis since, in Expts 1 and 2, no foam was detected, even in the rumen fluid and rumen gas from the two bloated cows. In bloated cows, the normal pattern of steady frequent eructations is interrupted. Under such non 'steady-state' conditions, the use of conventional compartmental modelling to estimate the ROE of gas into the rumen headspace may not be entirely appropriate.

Headspace volume

The volume of rumen headspace gas of grazing dairy cows does not appear to have been previously reported in the scientific literature. In the experiments reported here, the volume of rumen headspace averaged 3·6 litres in Expt 1, and in Expt 2, 3·8 litres pre-grazing and 5·0 litres post-grazing. One cow which bloated to score 1 (Expt 2) had a headspace volume of 17·7 litres and another cow which bloated to score 2 (Expt 1) had a headspace volume of 21·4 litres. In comparison, Waghorn (1991*b*) inflated latex weather balloons in cows' rumens and found that gas inputs of between 15 and 50 litres were needed to elicit score 1 bloat.

Rate of entry of fermentation gas into the rumen headspace

The average ROE of gas into the rumen headspace in Expt 1 was 1·87 litres/min after 2 h of grazing, and in Expt 2, 1·73 litres/min after 1 h of grazing. In contrast, in Expt 2, before grazing, the average ROE of gas was only 0·66 litres/min. In individual cows, the ROE of gas varied from as little as 0·19 litres/min before grazing, up to 3·26 litres/min after 2 h of active grazing. These estimates of ROE of gas into the rumen headspace are consistent with measured rates of gas production of $< 0{\cdot}20$ litres/min in fasted animals (Washburn & Brody 1937; Hoernicke *et al*. 1964) and up to 2·00 litres/min following feeding (Washburn & Brody 1937; Hungate *et al*. 1955; Colvin *et al*. 1957; Hoernicke *et al*. 1964).

In Expt 1, and in Expt 2 after grazing, the average ROE of total gas and of CH_4 were *c*. 20% less in cows administered the antibloat capsule compared with control cows, yet this difference was not significant ($P > 0{\cdot}05$) because of considerable between-cow variation. Nevertheless, the results from these experiments are consistent with those of Thornton & Owens (1981), who showed *in vivo* in steers, that monensin significantly reduced the rate of CH_4 production by *c*. 20% at 2 h post-feeding, and that this inhibition by monensin declined with time postprandially.

Our failure to demonstrate that antibloat capsules cause a significant ($P < 0{\cdot}05$) depression in ROE of CH_4 is primarily due to the large (300%) between-cow variation in this parameter. This large between-cow variation probably occurred because gas kinetics were measured over a short 20 min period. In contrast, when CH_4 production has been measured in animals held in calorimeter chambers, the measurement has usually been made over a 24 h period, and between-cow variation of *c*. 20% has been recorded (Thornton & Owens 1981; Rumpler *et al*. 1986). In addition, animals in calorimeter chambers are usually fed a uniform diet, whereas it is likely that individual grazing animals may select quite different diets and have varying degrees of rumen-fill at the time of measurement. This latter issue may be of considerable importance when animals are grazing clover/grass pastures. In comparison to grasses, clovers usually contain higher concentrations of calcium and lower sodium, and Rumpler *et al*. (1986) have shown that dietary cation concentration has considerable affect on rumen methanogenesis in steers fed diets with and without ionophores. Furthermore, the experiments reported here were conducted in winter and spring, and intermittent intake of large quantities of cold water or cold pasture by the grazing cows could have temporarily stifled rumen fermentation and this may explain some of the low values for the ROE of rumen gas.

Infusion of ethane

In Expt 3, the cow continued eating and ruminating during the 1 h infusion of C_2H_6. No problems were encountered during the infusion; however, occasionally, due to rumen movements, the infusion needle had to be repositioned in order to prevent its rejection from the rumen. By using the CONSAM computer program and a one-compartment model, the rumen headspace was estimated to be 2·5 litres. The ROE of rumen gas and CH_4 were also estimated to be 1·0 and

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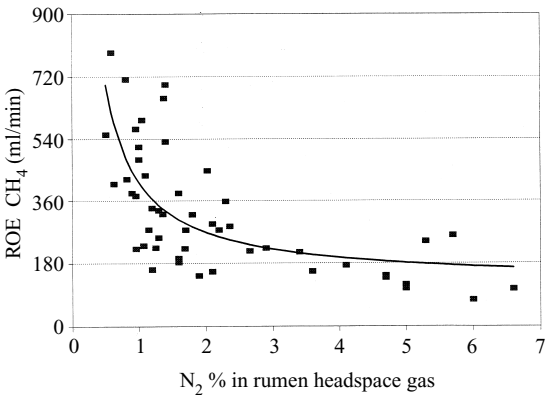

Fig. 3. Relationship between the rate of entry of CH_4 into the rumen (ROE CH_4) as measured by the ethane technique (■) and the N_2 % in rumen headspace gas (data pooled from Expts 1 and 2).

$$\text{ROE } CH_4 = 176 \text{ (S.E.} \pm 155) + 246 \text{ (S.E.} \pm 54)/N_2\% \qquad R^2 = 0{\cdot}46$$

0·20 litres/min respectively. In addition, it was found that the following simple equation could be used to estimate the rate of production of rumen CH_4 during the period when the concentration of C_2H_6 in rumen gas had plateaued:

$$\text{ROE } CH_4 = (\text{Avg } CH_4\%/\text{Avg } C_2H_6) \times \text{IR} \quad (1)$$

where Avg CH_4 % and Avg C_2H_6 are the average concentrations of CH_4 and C_2H_6 during the period in which the concentration of C_2H_6 in rumen gas had plateaued, and IR is the C_2H_6 infusion rate (30 ml/min). In Expt 3, since Avg CH_4 %, Avg C_2H_6 and IR were 20·0, 2·86 and 30 respectively, ROE of CH_4 by this calculation was also 0·20 litres/min. It is concluded that this modification of the C_2H_6 technique is suitable for measuring the rumen gas kinetics of penned animals.

Nitrogen

Based on theoretical considerations, Kleiber (1956) estimated the ROE of N_2 into the rumen of a cow from the blood at *c.* 55 litres/day. From Expt 1, the estimate of the mean ROE of N_2 into the rumen of control cows was 33·6 ml/min (48·4 litres/day), a value consistent with Kleiber's theoretical estimate. It is hypothesised that since the concentration of N_2 in blood is relatively constant throughout the day, the rate of entry of N_2 into the rumen is also likely to be relatively constant and that N_2 gas may therefore prove useful as an endogenous marker for estimating the rate of production of rumen gas, and in particular the rate of production of CH_4. In attempting to verify this hypothesis, all the data from Expts 1 and 2 were combined and it was found that the ROE of gas into the rumen headspace (ROE gas, ml/min) was inversely correlated to the N_2 % in rumen gas as described by Eqn (2) below:

$$\text{ROE gas} = 418 \text{ (S.E.} \pm 580) + 1356 \text{ (S.E.} \pm 203)/N_2\% \qquad R^2 = 0{\cdot}48 \quad (2)$$

In addition, the ROE of CH_4 into the rumen headspace (ROE CH_4, ml/min) was also inversely correlated to the N_2 % in the rumen headspace (Fig. 3). For N_2 to have general applicability as a resident marker of rumen gas production, it would have to be shown that the ROE of N_2 into the rumen is relatively constant, and is not affected by diurnal variation, feeding or other external factors such as monensin supplementation. In this respect, in Expt 2, even though the ROE of rumen gas increased > 300 % from pre- to post-grazing, the ROE N_2 was not affected by grazing (21·8 and 23·1 ml/min pre- and post-grazing respectively). However, the findings with respect to monensin are equivocal. In Expt 1, the ROE of N_2 was significantly decreased in cows administered antibloat capsules (17·6 ml/min) compared with control cows (30·7 ml/min), whereas, in Expt 2, the ROE of N_2 was not influenced by antibloat capsules.

Although N_2 does act as an endogenous marker for measuring rumen gas production, the authors consider it to be substantially inferior to C_2H_6. With the exception of the C_2H_6 tracer, the N_2 method requires the same apparatus, takes only marginally less work, yet is likely to be much less accurate, since it could be greatly affected if samples were contaminated with air or affected by other factors.

Bloat

Bloat is a serious disorder of dairy and beef cattle, especially when they are grazing temperate pastures containing legumes. Legume or foamy bloat is caused by the formation of a persistent foam in the reticulorumen that inhibits the eructation of fermentation gases (Reid 1960). In contrast to foamy bloat, in free-gas bloat, the rumen fluid is not foamy, there is little or no foam in the rumen headspace and consequently the bloat must be caused by factor(s) as yet unknown, which inhibit eructation. Mild or subclinical bloat can reduce the productivity of cattle, while in severe cases, the accumulation of gas in the reticulorumen causes increased pressure on internal organs and death by cardiorespiratory failure (Clarke & Reid 1974). Common means of bloat prophylaxis involve spraying pastures with antibloat oil, drenching cattle with detergents and the administration of the Elanco antibloat capsules. The antibloat capsules are usually administered at the end of winter, and since they release medicament for *c.* 100 days, this covers the major risk period for bloat. Research in Australia

and New Zealand has shown that the antibloat capsules can reduce the incidence of visible bloat and bloat deaths by *c*. 80% (Moate *et al*. 1990; Cameron & Malmo 1991; Lowe *et al*. 1991). The extent of the economic significance of bloat is unknown, but each year, in Australia and New Zealand, many thousands of cattle die as a result of bloat, in spite of the recent widespread use of antibloat capsules (Bryant 1991; Cameron & Malmo 1991; Dickens 1991; Laby 1991).

Reid's (1960) seminal paper 'Bloat: the foam hypothesis' is the cornerstone on which, for the last 35 years, bloat research and antibloat prophylactic/therapeutic treatments have been based. With the success of these agents in controlling bloat, it is often assumed that most bloat in Southern Australia is legume or foamy bloat. In the past this may have been true, but in the current research and in many other cases investigated by the authors, the majority of bloated cows have not had foamy rumen fluid nor could foam be detected within the rumen headspace. It is concluded that in these experiments, the major cause of bloat was failure to eructate or impaired eructation rather than a high rate of gas production or the presence of a persistent foam. Notwithstanding this, the authors acknowledge the efficacy of current antibloat treatments with putative modes of action that target foam and gas production. However, from the above observations, it is concluded that these antibloat agents which are effective against free-gas bloat must also have other mechanisms of action such as stimulating eructation. This highlights the need for an improved understanding of the aetiology of bloat, especially free-gas bloat.

There is little information in the scientific literature regarding the composition of rumen headspace gas from bloated cows and no information available regarding the gas composition of bloat foam. Kleiber *et al*. (1943) found that the rumen headspace gas from one bloated cow was similar in composition to the gas from two non-bloated cows. Furthermore, Hungate *et al*. (1955), who investigated *in vitro* the fermentation of rumen fluid from normal and bloated cows, found that the proportions of acid, CO_2 and CH_4 were similar in the two groups. In the current research, although the composition of the rumen headspace gas from nine bloated cows was similar to that from 16 non-bloated cows, it nevertheless contained less CO_2, more CH_4 and more than double the concentrations of O_2 and N_2 than that from non-bloated cows (Table 3). Furthermore, this paper reports for the first time that the composition of the gas of bloat foam is similar to the composition of free headspace gas taken simultaneously from the same animal. These findings dispel at least one theory for the development of bloat. Leng (1973) has hypothesised that ruminal bloat may be triggered by a sudden burst in fermentation, producing a rapid increase in the concentration of volatile fatty acids, a sudden fall in rumen fluid pH and a consequential massive release of CO_2 from solution into the rumen headspace. The measurements of pH, gas composition of bloat foam, and the finding of a significantly lowered concentration of CO_2 in the rumen headspace gas from bloated cows does not support Leng's hypothesis. Since in Expts 1 and 2 the concentrations of these gases were not affected by antibloat capsules, it is concluded that the mode of action of the antibloat capsule does not involve modification of the composition of rumen fermentation gas.

In Expt 4, in the two cases of foamy bloat, the composition of the gas in the headspace and corresponding foam were similar and contained in one case *c*. 65% and in the other *c*. 52% CO_2 (Table 3). Laby *et al*. (1991) and Moate *et al*. (1991) using an *in vitro* foam generating apparatus, and purified leaf protein, examined the effect of various gases and CH_4:CO_2 ratio on the strength and persistence of foam. They found that persistent foams could be generated with CH_4 and N_2 but not with CO_2 and not when there was $> 30\%$ CO_2 in a mixture of CO_2 and CH_4. We therefore speculate that for foamy bloat to occur, there must sometimes be present in the rumen powerful foam-stabilizing agents which can cause a persistent foam even at relatively high concentrations of CO_2. In Expt 4, the rumen headspace of bloated cows contained a significantly ($P < 0{\cdot}01$) higher concentration of N_2 (8·1%) than the headspace from non-bloated cows (3·2%) (Table 3). It is possible that some N_2 in rumen gas may be derived from air ingested with food and that some bloated cows may swallow N_2 while attempting to eructate. Another possibility is that in contrast to CO_2, N_2 is retained by foam in the rumen. This explanation is also supported by *in vitro* foam studies (Laby *et al*. 1991). We can only speculate as to whether the higher N_2% in bloat gas is a cause or a result of bloat. Also, in Expt 1, the reduced ROE of N_2 in cows administered the antibloat capsule might result from reduced subclinical bloat in these cows, or it may indicate a possible mechanism by which the antibloat capsule controls bloat.

Hungate *et al*. (1955) measured the fermentation rate *in vitro* of rumen fluid from six bloated and four non-bloated steers and found significantly higher rates of production of both CO_2 and CH_4 in bloated compared with non-bloated animals grazing the same pasture. Waghorn (1991*a*) asserted that 'the rate of gas production may be important in the aetiology of bloat' and concluded that a rate of gas production of between 1·6 and 3·3 litres/min is sufficient to produce moderate bloat, depending on losses to eructation and absorption. Using the C_2H_6 method, in Expt 1 the ROE of gas into the rumen headspace was 0·95 litres/min in the cow with score 2 bloat, and in Expt 2, 2·93 litres/min in the cow with score 1 bloat. However, these rates of gas production do not appear exceptional, especially in comparison with two non-

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bloated cows with ROE > 3 litres/min. It is concluded that gas production rate *per se* is not a critical trigger for either form of bloat.

Using the gas composition data from Expt 4, correcting for O_2 content and using Eqn (2), the estimated mean ROE of gas into the rumen headspace of bloated cows is only *c*. 0·57 litres/min compared with *c*. 1·46 litres/min in non-bloated cows. This reduced ROE of gas into the rumen headspace in bloating cows may appear anomalous. However, in bloating cows, the accumulation of headspace gas leads to increased intraruminal pressure. Under such conditions, the egress of gas from the rumen headspace by absorption pathways may increase. For example, the water-soluble gases, and in particular CO_2, may be expected to diffuse more readily back into the rumen fluid or into surrounding tissues, resulting in lowered proportions of CO_2 and higher proportions of water-insoluble gases in the rumen headspace. Consistent with this, the concentration of CO_2 was significantly lower in the headspace of bloating cows than in non-bloating cows (66·5 *v*. 75·3 % respectively).

The use of C_2H_6 as a tracer to measure the kinetics of CH_4 in the rumen headspace enables the estimation of the production of CH_4 in ruminants. In comparison with other methods, this method is simple, inexpensive in that it does not require costly respiration chambers, radioisotopes or fistulated animals, and it allows measurements to be made on grazing animals. In the experiments reported here, rates of production of CH_4 and CO_2 and the ratio of CH_4:CO_2 in rumen headspace gas were not substantially affected by bloat or by the monensin antibloat capsule. Therefore, it is concluded that the mechanism by which the antibloat capsules reduce bloat remains unknown. The absence of foam in all but two of the bloat cases observed in this work suggests that factors other than foam may lead to bloat. Furthermore, with respect to the development of bloat in these experiments, it is concluded that failure to eructate, or impaired eructation was the cause of bloat rather than being a result of a high rate of production of gas or the presence of a persistent foam. Improved methods for the control of bloat, especially free-gas bloat, could result from future research which should focus on feed factors and chemotherapeutic agents with eructogenic properties.

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