Br. J. Nutr. (1977), 38, 447

Rates of rumen fermentation in relation to ammonia concentration

BY A. Z. MEHREZ*, E. R. ØRSKOV AND I. McDONALD
Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

(*Received* 30 *November* 1976 – *Accepted* 7 *April* 1977)

1. Four sheep were fed from automatic continuous feeders on whole barley fortified with graded levels of a urea solution. This approach was to a large extent successful in maintaining relatively steady states of rumen ammonia concentration.

2. Rates of barley fermentation in the rumen at various rumen NH_3 concentrations were assessed by measuring the disappearance of barley dry matter from polyester bags suspended in the rumen of these sheep.

3. The minimal NH_3 concentration for maximal rate of fermentation was estimated as 235 mg/l rumen fluid.

The optimal ammonia concentration of rumen fluid may be defined as that which results either in the maximum rate of fermentation in the rumen or that which allows the maximum production of microbial protein per unit of substrate fermented. The two definitions may not always coincide; for instance Ørskov, Fraser & McDonald (1972) showed with a barley feed that the microbial protein produced per unit of substrate fermented was not altered as a result of urea supplementation while the extent of rumen fermentation and digestibility was increased.

The rate at which rumen fermentation proceeds has a great influence on both total and digestible feed intakes (Balch & Campling, 1962) and therefore feed intake may be reduced if NH_3 concentration is limiting the rate of fermentation.

Diurnal variation in all constituents of rumen fluid, generally associated with the time of feeding, make it difficult to estimate optimal rumen NH_3 concentration. A steady state of rumen fermentation can be achieved by continuous feeding. Although urea can also be infused continuously, its absorption into grains (Ørskov, Smart & Mehrez, 1974) offers another simple method for stabilizing rumen NH_3 concentration.

The use of the 'polyester bag' technique (Mehrez & Ørskov, 1977) enabled us to determine rapidly and accurately the rate of substrate fermentation in the rumen.

This present experiment was carried out to investigate the relationship between rumen NH_3 concentration and rate of fermentation.

A brief account of this work has been published (Mehrez & Ørskov, 1976).

MATERIALS AND METHODS

Animals. The four sheep used consisted of three Suffolk × (Finnish Landrace × Dorset Horn) and one Cheviot. They were approximately 6–8 months of age and weighed 35–40 kg. Each animal was fitted with a rumen cannula 40 mm in diameter. During recovery from the operation, the animals were trained to receive their diet from an automatic continuous feeder. They had completely recovered and were well accustomed to the feeding system 3–4 weeks after the operation, and they were then allocated to the experimental treatments.

Experimental. Whole barley containing 20·9 g nitrogen/kg dry matter (DM) was used as a basal diet. It was given either unfortified or fortified with increments of 2–10 g urea/kg barley to constitute the six experimental diets. Each increment of 2 g urea/kg barley grains

* Present address: Faculty of Agriculture, University of Mansoura, El-Mansoura, Egypt.

Table 1. *The sequence of feeding for four sheep given six experimental diets which consisted of barley grains supplemented with graded levels of urea**

Sheep ... Experimental period no.	Experimental diet (g urea/kg) A	B	C	D
1	0	10	10	0
2	2	8	8	2
3	4	6	6	4
4	6	4	4	6
5	8	2	2	8
6	10	0	0	10
7	0	—	—	0

* For details of diets, see below.

resulted in increasing the N content of the grains on average by 1·1 g N/kg DM. The four sheep (A, B, C and D) were given the diets according to the sequence shown in Table 1.

Feeding and management. The sheep were kept in individual pens and each was fed from an individual continuous feeder. The absorption method described by Ørskov, Smart & Mehrez (1974) was used for fortifying the barley grains not only with urea but also with minerals and vitamins (g/kg diet; 12 $CaCl_2$, 4 Na_2O_4, 1·25 trace minerals and vitamins). The trace minerals and vitamins supplement contained the same ingredients as that used by Ørskov *et al.* (1972).

The procedure used to incorporate urea and the supplement in the barley was as follows: (1) the barley was weighed and placed in a vertical mixer; (2) the required amount of $CaCl_2$ was dissolved in water (1:3, w/v) at 40–50°; (3) urea was dissolved in the $CaCl_2$ solution and then sprayed on the barley while the mixer was running. Mixing was continued for a minimum of 10 min before the addition of the next ingredient; (4) the trace minerals and vitamins mixture was mixed with water (1:3, w/v). This was added slowly to the barley in the mixer, and mixing continued for an additional 10 min; (5) finally, the Na_2SO_4 was dissolved in water (1:3, w/v) and added gradually to the barley, and mixing was continued for another 10 min.

Every animal received 1 kg of each diet/d for 1 week except when urea was not included, in which situation the sheep were offered the diet for 3 weeks. Illumination was maintained during the night to allow eating.

On each of the last 2 d of each period, four Polyester bags (Mehrez & Ørskov, 1977) containing a known amount of rolled barley DM (approximately 4·3 g) were incubated in the rumen of each sheep, being placed there at the same time of day (09.00 hours). One bag was removed after 1·5, 3, 6 and 9 h of incubation. Rumen fluid samples were obtained from each sheep initially and at the end of each incubation interval. The samples were obtained by gentle suction from different positions in the rumen. They were strained through a double layer of surgical gauze, frozen immediately and stored at −20° until they were analysed for NH_3 concentration.

After removal from the rumen, the bags were washed and dried to constant weight as described by Mehrez & Ørskov (1977) to determine DM disappearance.

Analytical methods. N content of the barley was determined by the automated Kjeldahl method described by Davidson, Mathieson & Boyne (1970). Rumen NH_3 concentration was determined by the method of Whitehead, Cooke & Chapman (1967).

Statistical methods. The relationship between DM disappearance (y; g DM disappearing/kg incubated) and rumen NH_3 concentration (x; mg/l rumen fluid) was initially described by an exponential model $y = A + Be^{-Cx}$ where A, B and C are constants. Equations of this form were fitted separately to the results for incubation intervals of 1·5, 3, 6 and 9 h, using the mean values over the two days of measurement. The fitted curves are shown in Fig. 1 together with the individual values from which they were obtained. It is evident that in the central range of NH_3 concentrations most of the points lie above the curves, and it appears that the curves approach their asymptotic values too gradually to agree with the results.

Because of the doubtful fit of the exponential model a second one was tested which assumes that as NH_3 concentration is increased the DM disappearance approaches a maximum value linearly rather than asymptotically. This may be described as the 'plateau' model, defined by two equations:

$$y = a + bx \qquad \text{for } x < x_1 \qquad (1)$$

$$y = c \quad (\text{where } c = a + bx_1) \quad \text{for } x > x_1 \qquad (2)$$

where y and x are as before, x_1 is the estimated optimal NH_3 concentration above which there was no additional DM disappearance, and a, b and c are constants. In fitting the model to the data a possible value was chosen for x_1, and the constants a, b and c were estimated by least-square methods, subject to the constraint that $c = a + bx_1$. The procedure was repeated for a series of values of x_1 within the possible range, and the best estimate was taken to be that which resulted in the best fit to the results. The final lines are shown in Fig. 1 and are clearly more consistent with the results than are the exponential curves.

RESULTS

The animals always consumed the feed available from the automatic feeders as soon as the grains fell into the troughs.

Stability of rumen NH_3 concentration

The mean NH_3 concentrations achieved at different levels of urea supplementation for five samples taken during the 2 d of sampling in each experimental period are presented in Table 2. It was apparent from the low standard deviation that the continuous feeding of barley grains supplemented with urea by the absorption method was to a large extent successful in maintaining a reasonably steady-state of NH_3 concentration, and there were no indications of variation associated with time of sampling.

Rate of barley fermentation in relation to NH_3 concentration in the rumen

Rates of rumen fermentation as determined by proportions of DM disappearing from the bags relative to the NH_3 concentration in the rumen are shown in Fig. 1 (mean values over 2 d).

Increasing the NH_3 concentration by increasing the level of urea resulted in an increase in the proportion of DM disappearance up to a maximum associated with each incubation period.

It seemed from the values shown in Fig. 1 that the DM disappearance was either 'flattening off' exponentially or was increasing linearly up to a maximum after which it remained nearly constant with increasing NH_3 concentration.

Estimation of the NH_3 concentration necessary for maximum rate of fermentation in the rumen

It is clear from Fig. 1 that the minimal NH_3 concentration (x_1) necessary for maximal DM disappearance was within the range of 200–270 mg/l rumen fluid. A series of values of x_1

Table 2. *The effect of continuous feeding of barley grains supplemented with graded levels of urea* to sheep on diurnal rumen ammonia concentrations*

(Mean values and standard deviations for sets of five observations)

		NH_3 concentration (mg/l rumen fluid)			
	Level of urea supplementation	Day 1		Day 2	
Sheep	(g/kg diet)	Mean	SD	Mean	SD
A	0	77	4·5	52	4·3
	2	135	10·0	158	4·4
	4	214	20·5	181	16·4
	6	253	1·9	205	9·5
	8	309	3·6	304	12·8
	10	375	26·5	564	28·5
	0	129	16·6	150	7·9
B	0	81	0·7	74	3·1
	2	152	2·8	179	4·3
	4	200	28·1	215	24·4
	6	269	20·3	246	15·7
	8	382	24·4	299	33·2
	10	360	42·2	356	11·2
C	0	76	4·4	71	6·9
	2	129	11·2	149	—
	4	235	8·9	204	13·8
	6	250	25·6	328	25·1
	8	341	12·2	302	7·9
	10	330	59·8	445	38·8
D	0	104	15·6	128	9·9
	2	151	16·1	179	9·5
	4	192	6·0	145	1·5
	6	207	6·4	216	7·3
	8	219	25·4	252	4·7
	10	283	43·2	268	43·3
	0	74	6·2	72	5·7

* For details of diets, see p. 437.

within this range were therefore used in fitting the 'plateau' model to the results for each incubation interval. The residual standard deviations (RSD) of the fitted equations are presented in Table 3. The estimate of x_1 which gave the lowest values of RSD was 235 mg/l rumen fluid, and this is therefore our estimate of the minimal NH_3 concentration for maximum DM disappearance. The equations fitted to the values for each incubation interval were as follows:

Incubation interval (h)	for $x < 235$	for $x > 235$
1·5	$y = 0{\cdot}52x+29$	$y = 151$
3	$y = 1{\cdot}26x+52$	$y = 348$
6	$y = 1{\cdot}89x+44$	$y = 488$
9	$y = 1{\cdot}86x+168$	$y = 605$

From Table 3 it can be seen that x_1 could be varied between 230 and 240 mg NH_3/l rumen fluid with very little change in the quality of fit of the 'plateau' model to the results, but that values as low as 210 or as high as 270 mg/l fitted substantially less well, the RSD's being increased by over 10% and the residual variances, therefore, by over 20%.

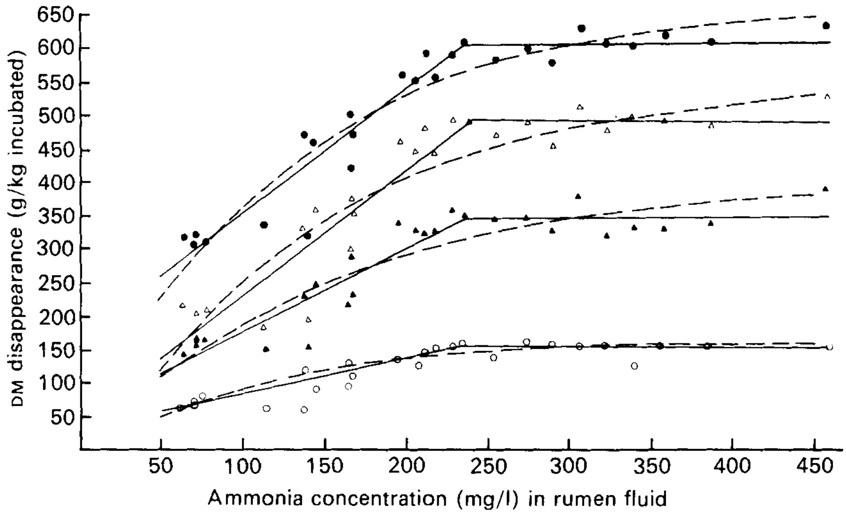

Fig. 1. The relationship between rumen ammonia concentration and the disappearance of dry matter of barley from Dacron bags incubated in the rumen of sheep for 1·5 h (○), 3 h (▲), 6 h (△), 9 h (●) (for experimental details see p. 438). (– – –), exponential model; (——), 'plateau' model. (For details of equations see p. 439.) The results plotted are the means for two incubation days.

Table 3. *The residual standard deviations of dry matter* (DM) *disappearance* (y; *g* DM *disappearing/kg incubated*) *according to the exponential model and also according to the 'plateau' model for different assumed optimal values* (x_1) *of ammonia concentration* (x; *mg/l rumen fluid*) *in the rumen of sheep fitted with Dacron bags containing a known amount of barley* DM

	Incubation interval (h)			
	1·5	3	6	9
Exponential model ($y = A+Be^{-Cx}$)*				
	16·3	37·9	48·3	39·4
'Plateau' model ($y = a+bx$ for $x < x_1$; $y = a+bx_1$ for $x > x_1$)†				
Assumed value of x_1				
210	15·4	35·2	44·9	39·4
220	14·5	33·5	42·4	36·7
230	14·0	32·3	40·7	34·7
235	13·9	32·3	40·5	34·1
240	13·9	32·4	40·7	34·0
250	14·2	33·2	41·7	34·6
260	14·6	34·4	33·4	36·1
270	14·9	35·7	45·3	37·7

* A, B and C are fitted constants.
† *a* and *b* are fitted constants.

The RSD's of the exponential model are also given in Table 3 and are about 17 % higher than the corresponding minimum values for the 'plateau' model, indicating a substantially inferior fit to the results.

DISCUSSION

It might have been possible to maintain steady NH_3 levels in the rumen by continuous feeding of a basal diet and continuous infusion of urea solution into the rumen. The continuous feeding of barley grains supplemented with urea solution by the 'absorption'

Table 4. *Absolute (A g/kg incubated) and relative (R) proportions of maximal disappearance as predicted from the plateau model* for dry matter* (DM) *disappearance at various levels of ammonia concentration in the rumen of sheep fitted with Dacron bags containing a known amount of barley* DM

Incubation interval (h)	Values	Rumen ammonia (mg/l of rumen fluid) 50	100	150	200	⩾235
1·5	A	55	81	107	133	151
	R	0·36	0·54	0·71	0·88	1·00
3	A	115	178	241	304	348
	R	0·33	0·51	0·69	0·87	1·00
6	A	139	232	328	422	488
	R	0·28	0·48	0·67	0·86	1·00
9	A	261	354	447	540	605
	R	0·43	0·58	0·74	0·89	1·00
Mean	R	0·35	0·53	0·70	0·88	1·00

* See page 439 for details.

method resulted in fairly constant levels of rumen NH_3 concentration. This approach avoided technical problems associated with continuous infusion of urea into the rumen.

Once microbial needs for N are met no further increase in rate of fermentation would be expected (Ørskov, Fraser & McDonald, 1974). The point at which the response in rate of fermentation 'levels off' should indicate the minimal NH_3 concentration for maximal rate of fermentation. The minimal NH_3 concentration predicted by the 'plateau' model was 235 mg/l rumen fluid. The 'plateau' model fitted the values better than the exponential model for any incubation interval as shown in Table 3. The 'plateau' model had the further advantage for giving a direct estimate of the optimal NH_3 concentration. Any estimate of the optimum from the exponential model necessarily involves an arbitrary decision on when the DM disappearance may be considered sufficiently near to its asymptotic value.

The predicted optimal level of NH_3 concentration for maximal rate of fermentation was very high in comparison to most published values for optimal NH_3 concentration for maximal microbial protein synthesis per unit of substrate fermented. For example, several studies made in vitro have shown that the NH_3 concentration required for maximal microbial protein synthesis per unit of substrate fermented is approximately 50–60 mg/l rumen fluid (Bryant & Robinson, 1961; Henderson, Hobson & Summers, 1969; Allison, 1970; Satter & Slyter, 1974). Studies made in vivo are usually preferable and have shown that the corresponding NH_3 concentration is 88–133 (Hume, Moir & Somers, 1970) or 289 mg/l rumen fluid (Miller, 1973). The high value reported by Miller (1973) is difficult to comment on since details of the experimental procedure used to derive it have not been described.

When N is not limiting to microbial growth there is good evidence to suggest that the amount of microbial protein synthesized is directly related to the amount of substrate fermented (Hungate, 1966). As far as the host animal is concerned, the rate at which substrate is fermented in the rumen has an important bearing on its voluntary intake.

The results of this experiment (Fig. 1) indicated that when NH_3 concentration in the rumen was less than 235 mg/l rumen fluid the fermentation proceeded at less than maximal rate. The critical rumen NH_3 concentration is that which is associated with a certain rate of fermentation which does not result in a high gut fill and consequently in a reduced voluntary food intake.

In an attempt to illustrate the effect of NH_3 concentration on rate of fermentation, the DM disappearance at various levels of rumen NH_3 concentration was predicted from the 'plateau' model equations. Beyond the optimum NH_3 concentration (235 mg/l rumen fluid), the DM disappearance would be maximal. The predicted values are presented in Table 4.

It appears that under practical feeding conditions the NH_3 concentration in the rumen need not be 235 or more mg/l rumen fluid at all times since this concentration is only necessary for maximal rate of fermentation. With high-quality feeds of a low particle size, however, NH_3 concentrations giving less than maximal rate of fermentation may result in reductions in digestibility.

Finally, the optimal NH_3 concentration for maximal rate of fermentation of roughage diets might be different from that recorded in this study for barley, since it might be dependent on the prevalent pH conditions and on the availability of energy-yielding substrate. While the pH was not measured here previous experience would indicate that with feeding of whole grain to sheep the pH would normally be in the range from 6·0–6·2. This aspect is at present being investigated.

REFERENCES

Allison, M. J. (1970). In *Physiology of Digestion and Metabolism in the Ruminant*, p. 456 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
Balch, C. C. & Campling, R. C. (1962). *Nutr. Abstr. Rev.* **32**, 669.
Bryant, M. P. & Robinson, I. M. (1961). *Appl. Microbiol.* **9**, 96.
Davidson, J., Mathieson, J. & Boyne, A. W. (1970). *Analyst, Lond.* **95**, 181.
Henderson, C., Hobson, P. N. & Summers, R. (1969). In *Proceedings of Fourth International Symposium on the Continuous Cultivation of Micro-organisms*, pp. 189–204. [I. Malik, editor]. Prague: Czeckoslovakia. London & New York: Academic Press.
Hume, I. D., Moir, R. J. & Somers, M. (1970). *Aust. J. agric. Res.* **21**, 283.
Hungate, R. E. (1966). *The Rumen and its Microbes.* New York & London: Academic Press.
Mehrez, A. Z. & Ørskov, E. R. (1976). *Proc. Nutr. Soc.* **35**, 40A.
Mehrez, A. Z. & Ørskov, E. R. (1977). *J. agric. Sci., Camb.* **88**, 645.
Miller, E. L. (1973). *Proc. Nutr. Soc.* **32**, 79.
Ørskov, E. R., Fraser, C. & McDonald, I. (1972). *Br. J. Nutr.* **27**, 491.
Ørskov, E. R., Fraser, C. & McDonald, I. (1974). *Br. J. Nutr.* **31**, 89.
Ørskov, E. R., Smart, R. & Mehrez, A. Z. (1974). *J. agric. Sci., Camb.* **83**, 299.
Satter, L. D. & Slyter, L. L. (1974). *Br. J. Nutr.* **32**, 199.
Whitehead, R., Cooke, G. H. & Chapman, B. T. (1967). *Automation in Analytical Chemistry, Technicon Symposium*, vol. 2, p. 377. Basingstoke, Hants: Technicon Instruments Co. Ltd.

Printed in Great Britain