Production and absorption of ammonia in the sheep's stomach

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1. When rumen fluid together with small quantities of ($^{15}NH_4$)$_2SO_4$, [1-^{14}C]sodium acetate and polyethylene glycol (PEG) was introduced into the emptied rumen of a sheep, no significan change occurred in the ratio ^{15}N:^{14}C during a period of 30–60 min. The ratio ^{15}N:PEG, however, declined by 22–53%. When ($^{15}NH_4$)$_2SO_4$, [1-^{14}C]sodium acetate and PEG were introduced into the normal rumen of a sheep fed on lucerne hay the ratio ^{15}N:^{14}C in the whole rumen contents showed no appreciable change in a period of 1 h, but the ratio ^{15}N:PEG declined by 25–41%. It was therefore concluded that the rate constants for removal of ammonia nitrogen (NH$_3$-N) and volatile fatty acid (VFA) from the rumen were very nearly equal.

2. In sheep fed at 1 h intervals on lucerne hay chaff or lucerne hay pellets VFA production, VFA and NH$_3$-N concentration in the rumen fluid and the volume of fluid passed from the rumen to the omasum were determined. From these results and the information previously gained on the relative rates of removal of NH$_3$-N and VFA from the rumen, the amounts of NH$_3$-N produced in the rumen and absorbed there or at lower levels of the digestive tract were calculated to be equivalent to 23–27% of the dietary N in lucerne hay chaff and 17% of that in lucerne hay pellets; 59–66% of the NH$_3$-N was absorbed from the rumen and the remainder passed to the omasum.

Since the work of McDonald (1948) on NH$_3$ in the rumen it has been recognized that significant amounts of nitrogen in that form may be absorbed from the fore-stomachs of ruminants and that the phenomenon may have an important bearing on the value of nitrogenous compounds in the diet.

The work reported here is concerned with measurement of the quantity of ammonia nitrogen (NH$_3$-N) produced in the rumen and absorbed through its wall or passed on to the omasum. An approach to the problem was made through determination of the relative rates of removal of NH$_3$-N and VFA from the rumen.

Daily production of NH$_3$-N was determined when two forms of lucerne hay were fed to sheep.

EXPERIMENTAL

Relative rates of absorption of NH$_3$ and VFA through the rumen wall

Two series of experiments were carried out, one in sheep from which the bulk of suspended digesta had been removed from the rumen, and the other in sheep in which the rumen contents remained in their normal state. The animals used were Merino ewes aged 3–8 years and weighing 35–45 kg. The main purpose of the first series was to allow the processes of absorption to be examined at controlled pH levels. In both series the rates of removal of NH$_3$-N and VFA were examined through changes in the concentrations of isotopically labelled NH$_3$ and sodium acetate. The use of a single labelled acid as marker for total VFA has been discussed by Weller, Gray, Pilgrim & Jones (1967).

Procedure—series (*a*). The rumen contents of a fistulated sheep previously fed on finely ground and pelleted lucerne hay were removed through the rumen fistula. The fluid was separated from the bulk of the solids by filtering it through terylene voile and then returning it to the rumen. The rumen was gently massaged, its contents were again removed and the process was repeated until it was judged that only an insignificant amount of solid plant residues remained within the rumen.

Before the test period several litres of rumen fluid were collected from other sheep fed on lucerne hay, centrifuged for 20 min at 7500 g and the supernatant fluid was retained. Approximately 3 l. of this fluid were taken and to them were added (1) a mixture of acetic, propionic and butyric acids in proportions similar to those in the rumen fluid and in sufficient quantity to bring the pH to a selected starting value; (2) sufficient $(NH_4)_2SO_4$ to bring the NH_3-N concentration to a desired level, the range covered in the trials being about 20 to 40 mg NH_3-N/100 ml; (3) small quantities of [1-^{14}C]sodium acetate (activity about 5 μc) and ^{15}N-labelled $(NH_4)_2SO_4$ (about 0·01–0·02 g ^{15}N); (4) 9–10 g of PEG. The mixture was brought to a temperature of 40°, a sample removed for analysis and the remainder introduced into the previously emptied rumen. After 5–10 min a sample was withdrawn and incubated at 40° for the duration of the trial. At intervals during a test period of 30–60 min samples were taken from the rumen for determination of VFA, NH_3-N, ^{14}C in VFA, ^{15}N in NH_3-N, and PEG.

The pH of the fluid during the trials in which it was allowed to change was recorded at each time of sampling. When the pH was to be maintained constant at a given level, a mixture of unlabelled acetic, propionic and butyric acids was introduced more or less continuously and the pH monitored, the electrodes of the pH meter remaining in the rumen throughout the experimental period.

Procedure—series (*b*). Sheep with rumen fistulas were fed a daily ration of 800 g lucerne hay pellets in equal lots at intervals of 1 h by means of an automatic feeder (Gray, Weller, Pilgrim & Jones, 1967). At the beginning of the test period 100 ml of a solution containing approximately 10 μc of [1-^{14}C]sodium acetate, 0·07 g ^{15}N in $(NH_4)_2SO_4$, and 10 g PEG, were introduced through the fistula.

Without any further feeding a period of 1–1·5 h was allowed to elapse for mixing to be completed and then samples of whole rumen contents (150–200 g) were removed at 15 min intervals over a period of 1 h. Each sample of rumen contents was immediately subsampled by a procedure previously described (Gray, Pilgrim & Weller, 1958) to give a 20–25 g sample for determination of total N and ^{15}N, and a 40–50 g sample for determination of moisture. In addition a sample of the rumen fluid was set aside for determination of the concentrations of VFA, ^{14}C and PEG.

Production of NH_3 in the rumen in sheep fed on lucerne hay

A sheep which had been fed for several months on a diet of lucerne hay chaff was given 1000 g/day in equal lots at intervals of 1 h. VFA production was measured by the isotope dilution procedure of Weller *et al.* (1967) using a constant infusion of ^{14}C-labelled sodium acetate and determining the ^{14}C concentration in the rumen acids in a composite sample of rumen fluid. Each such sample was collected automatically

over a 24 h period, and the sampling continued for 4 consecutive days. The complex of radioactive chromium with ethylenediaminetetrachloroacetic acid (^{51}CrEDTA) was included in the infusion mixture so that it could be introduced into the rumen at a constant rate for the purpose of determining the volume of fluid passed from the rumen to the omasum (Downes & McDonald, 1964). The concentrations of VFA, NH_3-N and ^{51}Cr were determined in the composite samples of rumen fluid. Calculations were then made of the amounts of NH_3-N removed from the rumen, and the amounts passed to the omasum.

The experiment was repeated with a sheep fed on pelleted lucerne hay.

Analytical methods

VFA and ^{14}C in VFA were determined as previously reported (Weller *et al.* 1967). PEG was determined by the turbidimetric method of Hydén (1955). Nitrogen in the digesta was determined by the Kjeldahl method and NH_3-N by the microdiffusion method of Conway (1950) with potassium metaborate as alkaline reagent. To measure ^{15}N in NH_3-N, NH_3 was distilled from rumen fluid over MgO, neutralized by 0·05 N-H_2SO_4 and brought to dryness for determination of ^{15}N abundance in the mass spectrometer. ^{15}N in rumen contents was determined by distilling NH_3 from the Kjeldahl digest made alkaline with NaOH, neutralizing as above and determining the ^{15}N abundance. Radioactivity in the rumen fluid samples was determined by counting in a well-type thallium-activated NaI crystal.

RESULTS

Relative rates of absorption of NH_3 and VFA through the rumen wall

Series (a)—washed rumen

Table 1 shows the concentrations of VFA and NH_3-N together with the labels ^{15}N and ^{14}C in the rumen fluid during one of the trials. Table 2 lists the values for the ratio of ^{15}N to ^{14}C for all of the trials, and also shows the extent of decline in the ratio of ^{15}N to PEG.

Throughout the series there was little or no change in the concentrations of either NH_3-N or VFA in the incubated samples, and the concentration of ^{14}C in the VFA in the rumen itself showed practically no change: it was therefore concluded that neither production nor loss of NH_3 or VFA occurred in the rumen as a result of any residual fermentation there. The ^{15}N abundance in the NH_3-N in the rumen, however, showed some lessening during each of the trials, a change to be expected if urea were entering the rumen in the saliva or through the blood supply to the rumen wall.

Rates of absorption. It is evident from Table 2 that the ^{15}N:^{14}C ratio remained substantially constant during each of these trials and therefore the rates of removal of NH_3-N and VFA (i.e. the proportions of the amounts present that were removed in unit time) were very close to one another. Removal would be partly to the omasum and partly through the rumen wall to the blood stream. Since the rates of removal through the reticulo-omasal orifice to the omasum must have been the same for both substances, it follows that the rates of absorption through the rumen wall must also have

been very nearly equal. These findings applied to rumen fluid within the pH range 6–7, whether constant or changing, and to concentrations of NH_3-N of 20–40 mg/100 ml, and VFA 40–100 m-moles/l.

Table 1. *Absorption of ammonia nitrogen* (NH_3-*N*) *and volatile fatty acids* (*VFA*) *from rumen fluid introduced into the emptied rumen of the sheep: concentrations of* NH_3-*N, VFA and markers*

Time (min)	pH	NH_3-N (mg/100 ml)	VFA (m-moles/100 ml)	PEG (g/100 ml)	^{15}N (mg/100 ml)	^{14}C (µc/100 ml)	Ratio ^{15}N:^{14}C	Ratio ^{15}N:PEG
0	6·2	18·9	7·84	0·283	0·375	0·132	2·84	1·33
10	6·5	17·6	7·18	—	0·345	0·120	2·88	—
20	6·7	16·5	6·98	—	0·317	0·112	2·83	—
30	6·9	15·9	6·38	0·277	0·288	0·104	2·77	1·04
Incubated sample at 30 min		18·9	7·87	—	—	0·132	—	—

PEG, polyethylene glycol.

Table 2. *Ratio of* ^{15}*N to* ^{14}*C and decline in the ratio of* ^{15}*N to polyethylene glycol* (*PEG*) *during absorption of ammonia nitrogen* (NH_3-*N*) *and volatile fatty acids* (*VFA*) *from rumen fluid introduced into the empty rumen of the sheep*

Sheep no.	pH	Ratio ^{15}N:^{14}C in rumen fluid at times (min): 0	10	20	30	40	50	60	Mean	SD	% decline in ratio ^{15}N:PEG
1	6·3–6·9	4·10	3·92	3·77	3·85	—	—	—	3·91	(0·141)	22
2	6·4–7·0	3·88	3·81	3·69	3·45	—	—	—	3·71	(0·189)	30
1	6·2–6·9	2·84	2·88	2·83	2·77	—	—	—	2·83	(0·050)	22
1	6·1–6·9	4·86	4·77	5·20	5·10	4·99	—	—	4·98	(0·174)	22
4	6·1	4·64	4·81	5·09	5·10	5·45	5·85	5·98	5·27	(0·507)	21
5	6·2	7·63	7·34	7·54	7·63	7·53	7·98	8·16	7·69	(0·284)	22
	6·2	4·23	4·12	4·28	4·26	4·41	—	—	4·26	(0·104)	20
3	6·4	4·82	5·03	5·71	5·12	5·84	5·80	6·49	5·54	(0·584)	31
3	6·4	3·92	3·77	3·84	3·89	4·27	4·10	3·94	3·96	(0·170)	33
1	6·4	3·68	3·56	3·66	3·91	3·94	3·56	3·82	3·75	(0·144)	27
4	6·6	4·04	3·84	3·79	3·84	3·91	3·63	4·00	3·86	(0·137)	33
3	6·8	3·91	4·05	3·93	4·18	4·00	3·82	3·44	3·90	(0·234)	53
1	6·8	4·19	4·33	4·40	3·86	3·64	3·74	3·79	3·99	(0·307)	39

Extent of absorption through rumen wall. The ratios of ^{15}N and ^{14}C to the unabsorbed marker PEG showed a decline with time due to absorption of NH_3-N and VFA (Table 2). The extent of this decline varied from 20 to 53 % of the initial values during the series.

Series (*b*)—*normal rumen*

The ^{15}N:^{14}C ratios for all the trials in this series are given in Table 3. It is again evident from the constancy of the ratios that the rates of removal of NH_3-N and VFA from the rumen were very nearly equal. There was substantial absorption during the experimental period as indicated by the decline that occurred in the ^{15}N:PEG ratio in 1 h. The sheep in this series were fed throughout the day and the pH of the rumen fluid remained fairly steady between 6·1 and 6·3.

Production of NH₃ in the rumen of sheep fed on lucerne hay

Results for the 4 consecutive days of each trial are given in Table 4, and the following example illustrates the calculations made in arriving at the findings given in Table 5, which summarizes the results of the two trials.

Sheep given 1000 g lucerne hay chaff: $N = 27 \cdot 2$ g/day.
Production of VFA in rumen $= 3 \cdot 98$ moles/day.
Mean concentration NH_3-N in rumen fluid $= 0 \cdot 173$ g/l.
Mean concentration VFA in rumen fluid $= 0 \cdot 102$ moles/l.

Table 3. *Ratio of ^{15}N to ^{14}C and decline in the ratio of ^{15}N to polyethylene glycol (PEG) during absorption of ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) from normal rumen contents of the sheep*

Ratio ^{15}N:PEG in rumen contents at 15 min intervals

Sheep no.	Time (min)					Mean	SD	% decline in ratio ^{15}N:PEG
	0	15	30	45	60			
1	11·4	11·3	12·5	12·2	10·2	11·5	(0·899)	41
2	10·3	10·7	10·0	9·7	9·4	10·0	(0·507)	28
1	10·4	11·2	11·0	12·0	12·7	11·5	(0·899)	39
2	12·4	11·0	9·4	10·3	10·3	10·7	(1·12)	29
3	11·0	11·2	11·4	12·4	12·6	11·7	(0·729)	25

Table 4. *Production of volatile fatty acids (VFA) and concentrations of VFA and ammonia nitrogen (NH_3-N) in the rumen of sheep fed lucerne hay*

Day no.	VFA produced (moles/day)	VFA conc. in rumen fluid (m-moles/l.)	NH_3-N (mg/l.)	^{51}CrEDTA		
				Volume infused (ml)	Activity in infused solution (counts/sec ml)	Activity in rumen fluid (counts/sec ml)
			Sheep fed lucerne hay chaff—27·2 g N/day			
1	3·98	102	173	1030	26·6	1·95
2	3·46	124	250	1000	26·6	2·80
3	3·59	108	192	995	26·6	1·94
4	3·70	96·6	190	980	26·6	1·83
Mean	3·68	108	201			
			Sheep fed lucerne hay pellets—28·4 g N/day			
1	3·26	106	154	1050	26·6	2·27
2	3·29	82·2	124	1060	26·6	1·79
3	3·05	105	170	1039	26·6	2·53
4	2·98	103	170	980	26·6	2·62
Mean	3·15	99·0	155			

EDTA, ethylenediaminetetrachloroacetic acid

Since the rate constants of removal of NH_3-N and VFA from the rumen were found to be equal

$$\frac{NH_3\text{-N removed (g)}}{NH_3\text{-N (g/l.)} \times V(l.)} = \frac{VFA\text{ removed (moles)}}{VFA\text{ (moles/l.)} \times V(l.)},$$

where V = volume of rumen fluid. And since the amounts produced in the day can be considered equal to the amounts removed,

$$\text{NH}_3\text{-N produced} = \text{NH}_3\text{-N removed} = \frac{3 \cdot 98 \times 0 \cdot 173}{0 \cdot 102} = 6 \cdot 75 \text{ g N/day},$$

^{51}CrEDTA: infusion 1030 ml/day: radioactivity $26 \cdot 6 \equiv$ counts/sec per ml, rumen fluid radioactivity $\equiv 1 \cdot 95$ counts/sec per ml.

Therefore   volume of fluid passed from rumen to omasum = $14 \cdot 1$ l./day.

Therefore NH_3-N passed to omasum = $14 \cdot 1 \times 0 \cdot 173 = 2 \cdot 43$ g N/day.

Thus of the $6 \cdot 75$ g NH_3-N produced in and removed from the rumen, $4 \cdot 32$ g were absorbed through the rumen wall and $2 \cdot 43$ g were passed to the omasum. It can be seen from Table 5 that production of NH_3-N varied very little between the 4 days; it was equivalent to 23–27% of the dietary N when lucerne hay chaff was fed, and to 17% when the same hay was given in pellet form; 59–66% of this NH_3-N was absorbed from the rumen itself.

Table 5. *Production of ammonia nitrogen (NH_3-N) in the sheep fed lucerne hay*

| Day no. | Fluid passed to omasum (l./day) | Production of NH₃-N | | | |
		NH_3-N absorbed from rumen (g/day)	NH_3-N passed to omasum (g/day)	Total production (g/day)	Production as % of dietary N
		Sheep fed lucerne hay chaff—total N 27·2 g/day			
1	14·1	4·32	2·43	6·75	25
2	9·5	4·60	2·38	6·98	26
3	13·7	3·76	2·62	6·38	23
4	14·2	4·57	2·71	7·28	27
Mean	12·9	4·31	2·53	6·84	25
		Sheep fed lucerne hay pellets—total N 28·4 g/day			
1	12·3	2·84	1·89	4·73	17
2	15·7	3·01	1·95	4·96	17
3	10·9	3·08	1·86	4·94	17
4	9·9	3·23	1·69	4·92	17
Mean	12·2	3·04	1·85	4·89	17

DISCUSSION

The pH of the rumen contents of sheep can be said commonly to lie between 6 and 7 although on some diets lower values are found, and during starvation or when large amounts of urea are given it often rises above 7.

It is known that VFA are absorbed faster at low than at high pH values in the rumen fluid and it has been suggested that this is due to the fact that un-ionized molecules of the acids may penetrate the lipoid layers of cell membranes whereas VFA ions may be limited to diffusion through water-filled pores (Danielli, Hitchcock, Marshall & Phillipson, 1945). Examination of the dissociation curves of the acids

Table 6. *Loss of dietary nitrogen as ammonia calculated to be absorbed from the digestive tract of the sheep*

Authors	Method	Diet	N consumed (g/day)	NH_3-N in rumen fluid (mg/100 ml)	Dietary N lost as NH_3 (%)
Gray et al. (1958)	Single marker for N	Lucerne hay chaff	23·0	30 (approx.)	52
Hogan & Weston (1967)	Double marker for N	Lucerne hay chopped	30·9	24	23[*]
		Lucerne hay ground	30·7	23	21[*]
Present work	NH_3-N production	Lucerne hay chaff	27·2	17–25	< 23–27[†]
		Lucerne hay pellets	28·4	12–17	< 17[†]

[*] Calculated from non-ammonia N leaving abomasum.
[†] NH_3-N production exceeds dietary N lost, because of recycling of urea.

indicates that on this basis there could be marked changes in the rate of absorption between pH 6 and pH 4, but relatively little within the range pH 6–7 (Aafjes, 1967). While it is unlikely that the absorption can be explained fully in this way it appears that pH does affect the rates of absorption; but there is no evidence that the effect would be at all marked in the pH range used in the work reported here.

There is also little doubt that one of the main factors that could influence the rate of absorption of NH_3, apart from its concentration, is the pH of the rumen fluid and this has been attributed to the fact that the ammonium ion does not penetrate cell membranes rapidly in comparison with the undissociated molecule (Hogan, 1961; Coombe, Tribe & Morrison, 1960). The effect of pH on NH_3 absorption would therefore be expected to be opposite to that on VFA absorption. But again it cannot be concluded that absorption would be much affected in the pH range 6–7. It has been pointed out that other factors may play a part in absorption and that the effective pH, i.e. the pH at the site of absorption, may be different from that of the rumen fluid in general (Sperber, 1968).

All of the trials reported here were carried out within the pH range 6–7 and the results indicate that any effects that pH may have had on the rates of absorption of ^{15}N or ^{14}C were insufficient to change the ratio of the one to the other in the rumen.

The experiments have provided information on the amounts of NH_3-N produced in the rumen and absorbed there or at lower levels of the digestive tract. There is, however, always some recycling of urea to the rumen and consequently the quantities of NH_3-N reported in Table 5 represent only the upper limits to the loss of useful N to the animal through NH_3 formation.

A number of attempts have been made to approach the problem of such losses by measuring the amounts of N passing through the abomasum or small intestine. Recent reports (Balch, 1957; Harris & Phillipson, 1962; Clarke, Ellinger & Phillipson, 1966; Hogan & Weston, 1967) are in agreement with the earlier finding of Gray & Pilgrim (1956) that when the N content of the diet is low there can be a net gain of N in the rumen, presumably through microbial use of recycled urea, while at higher levels of N in the diet there can be a net loss due to absorption of NH_3. It has been shown, however, that the use of N:lignin ratios on which the original finding was based must lead to overestimation of the loss of N by NH_3 formation (Bruce, Goodall, Kay, Phillipson & Vowles, 1966; Hogan & Weston, 1967). Although the results reported here are not intended to indicate net losses of useful N they may be compared with the findings of Hogan & Weston (1967) on the passage of N from the abomasum to the small intestine during the feeding of a similar diet. It can be seen from Table 6 that the values for NH_3-N production and absorption are consistent with the later report rather than with the original work based on N:lignin ratios (single marker method). But it should be noted that the amount of N given was smaller and the concentration of NH_3-N greater in the early experiment and this would be expected to lead to a greater proportion of dietary N being lost through NH_3 absorption.

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