Nucleic acid metabolism in the ruminant

2.* Formation of microbial nucleic acids in the rumen in relation to the digestion of food nitrogen, and the fate of dietary nucleic acids

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1. Concentrations of nucleic acid nitrogen (NA-N) and other nitrogenous constituents were estimated in samples of rumen fluid taken from calves and cows which were either given stall diets of varying nitrogen content or were allowed to graze pasture. Concentrations of NA-N ranged from 1·5 to 27·5 mg/100 g water.

2. Ratios of RNA:DNA in rumen fluid were similar to those in rumen bacteria and were not related to those in the diets. Pure nucleic acids added to the rumen were rapidly degraded. It was therefore concluded that NA-N in rumen fluid was largely of microbial origin and provided an index of total microbial nitrogen.

3. In most experiments, with an individual animal consuming diets of various nitrogen contents, NA-N formed a fairly constant percentage (8–15 for different animals) of the total non-ammonia nitrogen in rumen fluid. This suggested that nitrogen entering the rumen fluid limited microbial growth. Consumption of a diet containing extracted decorticated groundnut meal (diet B), however, led to lower values for this percentage than did the other diets. Diet B was also exceptional in leading to marked diurnal variations in NA-N concentrations in rumen fluid, suggesting a cyclic fluctuation in the size of the microbial population.

4. Comparison of NA-N:total nitrogen ratios in rumen fluid and bacteria suggested that, for all the diets except diet B, 55–80 and 40–50% of the non-ammonia nitrogen in rumen fluid was of microbial origin for the calves and cows respectively.

The part played by nucleic acids in ruminant nutrition and the probable wastage of nitrogen resources which their presence in digesta represents has only recently received much consideration. McDonald (1954*b*) and Blaxter (1961) drew attention to this matter, but the lack of suitable techniques for reliably estimating nucleic acids in digesta delayed development in the field. Subsequent work, although sometimes based on analytical procedures which are open to criticism (see McAllan & Smith, 1969), has suggested that the production of nucleic acids in the rumen is considerable. Topps & Elliot (1965) and Radin (1965) reported estimates of total nucleic acids in the rumen fluid of sheep of up to about 50 mg/100 ml, and Ellis & Pfander (1965) showed that RNA and DNA increased in samples of rumen fluid incubated in vitro and together accounted for about 15% of the microbial nitrogen compounds (microbial-N) formed. Gaussères & Fauconneau (1965), Temler-Kucharski & Gaussères (1965) and Fauconneau & Gaussères (1966) compared the compositions of rumen contents, rumen microbes and foodstuffs and concluded that most of the DNA in rumen contents was derived from the microbes. They used DNA concentrations in digesta to estimate the contribution of the microbes to the total nitrogen present.

In our present work we have used a method for determining RNA and DNA in rumen contents which has been described previously (McAllan & Smith, 1969). The

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method has been used to investigate some of the factors influencing nucleic acid production in the rumen and the contribution of dietary nucleic acids to the total nucleic acid concentrations in the rumen. We have also made some preliminary observations on the use of nucleic acids to estimate the conversion of dietary nitrogen compounds (dietary-N) into microbial-N.

A brief report on part of this work has already been published (Smith, McAllan & Hill, 1968).

METHODS

Animals, feeding and sampling of digesta

Friesian cows aged 3·5 years and equipped with rumen cannulas (Balch & Cowie, 1962) were used in some experiments but most investigations were carried out with castrated male Friesian calves aged 15–39 weeks. The calves were weaned at 5–8 weeks on to a normal calf-rearing mixture and hay. Operations were performed at 8–15 weeks of age when the calves were fitted with a rumen cannula and sometimes with a simple cannula in the proximal duodenum. The duodenal cannulas were of Perspex as described previously (Smith & McAllan, 1966) and rumen cannulas (3·8 cm internal

Table 1. *Daily amounts (kg) of the major components of the stall diets given to the calves at the time that they weighed* 90–110 *kg*

(For animals at different live weights these amounts were increased or decreased by about 12 % for each 20 kg increment in live weight. Concentrates were given in two equal amounts at 09.00 and 17.00 h and also contained vitamin and mineral supplements. Roughages were given at 09.00 h only)

		Diet A	Diet B	Diet C	Diet D	Diet E
Roughages	Hay	1·14	1·14	—	—	—
	Straw	—	—	1·14	1·14	—
Concentrate components	Flaked maize	1·36	0·75	—	—	1·82
	Decorticated extracted groundnut meal	—	0·95	—	—	—
	Barley	—	—	—	0·59	—
	Fish meal	—	—	—	0·17	—
	Molassine meal	—	—	—	0·21	—
	Starch	—	—	0·42	0·62	—
	Glucose	—	—	0·42	—	—
	Cellulose	—	—	0·42	—	—

diameter) were of Corosite (W. H. Uhlhorn & Co. Ltd). Results are given for samples from the rumen cannulas only. Periods of at least 3 weeks after the operation and at least 9 weeks after weaning were allowed before experiments involving the sampling of digesta were begun. During the experimental periods, the calves (live weight 85–180 kg) were given a variety of diets, all providing about the same daily net energy intake (4000 kcal at 90–110 kg live weight), as shown in Table 1. Alternatively during April or early May, the calves were sometimes allowed to graze a pasture, predominantly perennial ryegrass, which had previously been fertilized with Nitro-chalk (12 kg N/ha) and potassium chloride (8 kg K/ha). The cows (live weight about 400 kg) either grazed a pasture or were given, once a day, a diet consisting of 6 kg hay and

2 kg dairy cubes (containing 17% barley, 20% maize, 20% wheat offal, 15% decorticated extracted groundnut meal, 10% copra cake, 5% palm-kernel cake, 10% molasses +minerals and vitamins). This will be referred to as as diet F. At least 8 d were allowed between changing a diet and taking samples.

Samples from the rumen of a calf were obtained by inserting a tube (about 1 cm internal diameter) into the rumen, sucking out about 25 ml of digesta, moving the tube to a new position, taking another 25 ml and then repeating the process until about 100 ml were obtained. Rumen samples obtained from the calves by this method did not contain the coarsest material but, for ease of handling, they were also strained through surgical gauze. Preliminary experiments (four) with calves given diet A showed that the strained fluid contained 93 ± 2, 90 ± 2 and 93 ± 2% respectively of the total-N, RNA and DNA present in the unstrained material. There is good reason to suppose, therefore, that the composition of the strained rumen contents was not greatly dissimilar to that of material passing from the rumen to the omasum for total-N and nucleic acids as well as for wholly water-soluble constituents such as ammonia. Subsequent references to rumen fluid from the calves are to material which was strained through surgical gauze.

Samples from the rumen of a cow were obtained from a single site through an in-dwelling tube equipped with a polyethylene straining device which removed coarse particles to about the same extent as the surgical gauze used for the calves.

In eight experiments, pairs of samples were taken from the rumens of calves receiving diets A or B or pasture. The samples were taken at least 3 h after feeding and within a sufficiently short time of each other (less than 1 h) for little endogenous change to be expected. Analyses of these pairs of samples gave mean percentage variations, with their standard errors, about the mean values of 4 ± 2, 7 ± 2 and 11 ± 1 for total-N, RNA and DNA respectively. Results were therefore sufficiently consistent to show that the sampling technique was satisfactory and that errors due to differences between one part of the rumen and another were not serious.

All samples were collected into vessels surrounded by ice and were homogenized in an Atomix blender (MSE) before subsampling and analysis. Nucleic acid determinations were started within 2–3 h of collection.

Distribution volume of polyethylene glycol in the rumen

This was estimated by infusing 50–100 g polyethylene glycol of molecular weight 4000 (PEG) dissolved in 200–400 ml of water into about four different randomly selected sites within the rumen and then taking periodic samples of rumen fluid, starting 1·5 h after the infusion and continuing, at hourly intervals, for a further 4–5 h. The initial PEG concentration at the time of infusion was estimated by extrapolation.

In vitro experiments

Experiments to investigate the extent of the degradation of nucleic acids by samples of rumen contents in vitro were carried out in the following way. A sample (about 500 g) of rumen contents was collected into a vessel surrounded by ice, a gas mixture containing 95% N_2 and 5% CO_2 was passed into the fluid for 2 min and the vessel

stoppered. Subsamples (25 g) were rapidly transferred to flasks fitted with Bunsen valves and gas inlet tubes, and 55 mg amounts of pure RNA or DNA (dissolved in 1 ml water containing a minimum amount of NaOH) added. The flasks were immediately placed in a water bath at 37°, the 95% N_2, 5% CO_2 gas mixture was passed into the fluid for 2 min and the flasks were sealed and shaken mechanically. The flasks were incubated for different times up to 4 h with further 2 min periods of passing the 95% N_2, 5% CO_2 gas mixture at intervals of 30 min. The pH of the mixtures remained within the range 6·8–7·1 during the whole procedure. At an appropriate time the whole contents of a flask were cooled to below 4°, added to an equal weight of cold 18% (w/v) trichloroacetic acid in ethanol and the mixture was homogenized. This corresponded to the first step in the nucleic acid extraction procedure (McAllan & Smith, 1969) and further treatment followed this procedure.

Analytical

Total-N. Samples (1–5 g) were digested with the sulphuric acid, potassium sulphate, mercuric oxide mixture recommended by Fleck & Munro (1965). Hydrogen peroxide (100 vol) was added dropwise to facilitate digestion of the more difficult samples. The solutions were made up to contain 1·0–10·0 mg ammonia nitrogen/100 ml. This was estimated colorimetrically in an Autoanalyzer (Technicon Instruments Co. Ltd) and using, essentially, the procedure given in the Technicon Methodology Sheet N-3b.

Nucleic acids. RNA and DNA in digesta samples were determined as described previously (McAllan & Smith, 1969). Samples of foodstuffs were ground and homogenized with about 9 parts of water, and the suspension was treated as for digesta samples. The amounts of the nucleic acids or the nitrogen which they contained were estimated by assuming equivalence to the yeast RNA or thymus DNA which were respectively used as standards.

Ammonia. This term is used here to include both un-ionized ammonia and ammonium ions. Determinations in digesta were carried out by the procedure of Conway (1957) using boric acid in the central well and titrating with HCl.

Polyethylene glycol. This was determined in rumen contents by a method previously described (Smith, 1959) except that 20 min instead of 1 h was allowed for the development of turbidity in the final stage of the procedure.

RESULTS

Concentrations of nucleic acids in rumen fluid

The composition of the rumen contents showed diurnal variations related to intermittent feeding (p. 553) but, for the purpose of comparing different diets and establishing relationships between different constituents, samples were taken during the relatively steady-state conditions obtaining 4–7 h after a meal of concentrate. Although there was no evidence of consistent diurnal variation in the animals at pasture, for comparative purposes samples were taken between 11.00 and 15.00 h from such animals. The results, which are given in Table 2, do not distinguish between experiments carried out with calves at different ages since there were no marked differences

from this cause. Nucleic acid concentrations in the samples appeared to be broadly related to the nitrogen contents of the different diets. The reason for this became clear when it was found that, for all the diets except diet B, there was, for each individual animal a close correlation between total non-ammonia-N and nucleic acid-N (RNA+DNA) in the rumen fluid. This is illustrated for calves N136 and N 122 in Fig. 1. For these calves, mean values with their standard errors (numbers

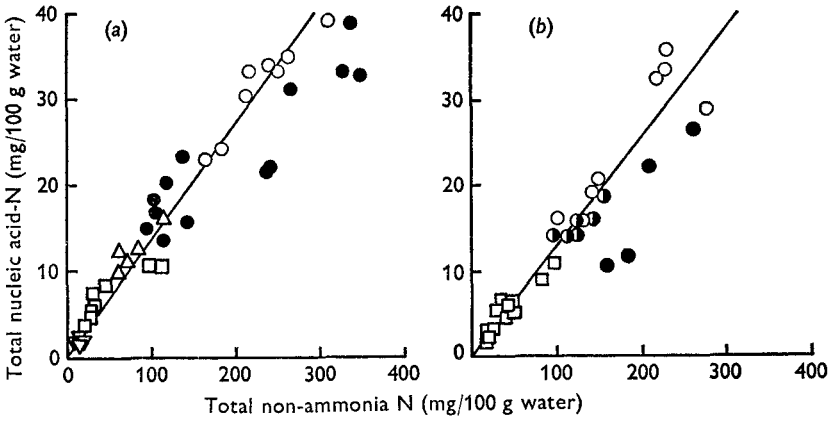

Fig. 1. Relationships between concentrations of nucleic acid-nitrogen (RNA+DNA) and total non-ammonia-N in individual samples of rumen fluid taken from (*a*) calf N 136 and (*b*) calf N 122, receiving a variety of diets as follows: diet A (□), diet B (●), diet C (▽), diet D (△), diet E (◑) and pasture (○). Details of the diets are given in Table 1. The linear curves are related only to diets other than diet B.

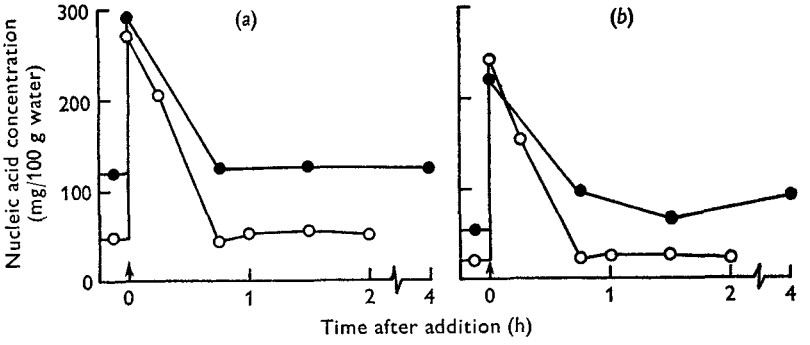

Fig. 2. Changes with time in (*a*) RNA and (*b*) DNA concentrations in rumen fluid after adding 50–75 g of the appropriate pure nucleic acid to the rumens of calves maintained on diet A (○) or pasture (●). Details of diet A are given in Table 1.

of samples in parentheses) for the percentages of total non-ammonia-N represented by nucleic acid-N were 14·4 ± 0·6 (26) and 13·0 ± 0·4 (26) respectively. Similar correlations, although with animal-to-animal differences, were shown by calves 3 K and N 137 and cows 11 K and 12 K for which corresponding mean values were 10·3 ± 0·5 (15), 14·8 ± 0·9 (21), 8·9 ± 0·7 (12) and 8·0 ± 0·5 (10) respectively. Diet B for all the calves gave mean values of about 70% of the above values.

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Table 2. *Mean concentrations with their standard errors (mg nitrogen/100 g water) for various nitrogenous constituents in rumen fluid samples taken from calves or cows 4–7 h after they received a stall diet or between* 11.00 *h and* 15.00 *h when they were at pasture*

(Details of the stall diets are given in Table 1 and on p. 547)

Animals		Diet		Rumen fluid samples				
Type	No.	Type	Approx. N content (g/100 g dry matter)	Total no. examined	Total non ammonia-N	RNA-N	DNA-N	Ammonia-N
Calves	4	A	1·6	32	49·1 ± 5·0	3·9 ± 0·3	2·0 ± 0·2	0·5 ± 0·1
	4	B	4·4	35	195 ± 17	12·2 ± 0·4	6·1 ± 0·5	13·2 ± 1·3
	2	C	0·2	8	14·6 ± 0·7	1·1 ± 0·2	0·4 ± 0·1	0·2 ± 0·0
	2	D	2·0	12	56·6 ± 5·2	5·6 ± 0·7	3·4 ± 0·4	0·4 ± 0·1
	1	E	1·7	6	128 ± 9	10·6 ± 0·6	4·3 ± 0·3	1·1 ± 0·2
	4	Pasture	2·0–4·0	33	213 ± 12	18·5 ± 1·3	8·9 ± 1·4	6·1 ± 1·0
Cows	2	F	1·9	9	44·1 ± 3·2	2·3 ± 0·1	1·2 ± 0·1	5·0 ± 0·9
	2	Pasture	2·0–4·0	20	104 ± 11	5·5 ± 0·5	3·2 ± 0·3	16·6 ± 1·5

Table 3. *Percentages of nucleic acid-N and total-N in the dry matter of various foodstuffs (mean values with their standard errors) and estimated mean values for the diets detailed in Table 1*

	No. of samples	RNA-N	DNA-N	Total-N
Hay	12	0·107 ± 0·006	0·041 ± 0·002	1·56 ± 0·06
Straw	2	0·086 ± 0·002	0·009 ± 0·002	0·495 ± 0·115
Flaked maize	6	0·028 ± 0·003	0·006 ± 0·001	1·65 ± 0·08
Decorticated extracted groundnut meal	8	0·102 ± 0·011	0·006 ± 0·001	9·35 ± 0·12
Fish meal	1	0·067	0·042	11·6
Pasture*	6	0·106 ± 0·023	0·056 ± 0·005	2·54 ± 0·24
Diet A		0·064	0·022	1·62
B		0·086	0·020	4·40
C		0·039	0·004	0·24
D		0·064	0·011	1·96
E		0·028	0·006	1·65

* Samples of pasture were cut by hand from areas near which the calves were grazing.

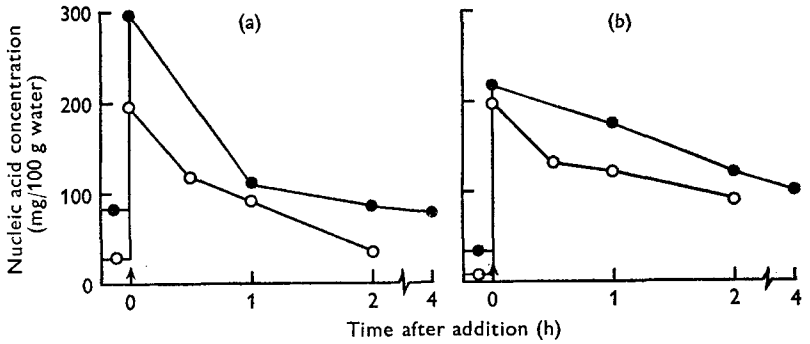

Fig. 3. Change with time in (*a*) RNA and (*b*) DNA concentrations in samples of rumen fluid, incubated in vitro at 37° in an atmosphere of 95 % N_2, 5 % CO_2 after adding a solution of the appropriate pure nucleic acid. Values were corrected for the small dilution produced by adding the nucleic acid solutions. The rumen samples were obtained from calves receiving either diet A (○) or pasture (●). Details of diet A are given in Table 1.

Variations in relative amounts of RNA and DNA

Individual samples showed considerable variations in RNA:DNA ratio, which ranged between about 1·2 and 3·2. There were no marked differences between either different diets (Table 2) or different animals in this ratio. Mean results with their standard errors for RNA:DNA ratios in samples (number in parentheses) from individual animals were 2·07 ± 0·09 (39), 2·24 ± 0·03 (33), 2·19 ± 0·11 (30), 2·01 ± 0·13 (21), 1·92 ± 0·19 (12) and 1·73 ± 0·08 (10) for calves N 136, N 137, N 122 and 3 K and cows 11 K and 12 K respectively.

Origin of the nucleic acids in the rumen

The diets contained enough DNA and RNA (Table 3) to account for up to about 20 and 50% respectively of these nucleic acids in rumen fluid. However, if dietary nucleic acids survived in the rumen it would be expected that RNA:DNA ratios in rumen fluid would be influenced by differing dietary ratios. A comparison of the values in Tables 2 and 3 shows that this was not so and suggests that the dietary nucleic acids were, at least partially, degraded in the rumen.

This possibility was examined by investigating the fate of pure nucleic acids added to the calf rumen via a cannula and to rumen fluid in vitro. Results, given in Figs. 2 and 3, showed that the added amounts of both RNA and DNA were almost completely destroyed within 1 h of adding to the rumen and that they were also destroyed fairly rapidly on in vitro incubation. We have shown (Smith & McAllan, 1970) that the destruction led to accumulations of polynucleotides of low molecular weight (mainly from DNA), mono- and/or oligonucleotides, and further degradation products. These substances were, however, found in the rumen for only a short time after addition of the pure nucleic acids. They were not present to an appreciable extent in the rumen fluid of calves receiving normal diets.

Table 4. *Effect of diet on the distribution volume of polyethylene glycol within the rumen of different calves expressed per unit of live weight (l/100 kg)*

(Mean values with their standard errors are given for experiments carried out between 9 and 33 weeks after weaning. The numbers of experiments are shown in parentheses. Details of the diets are shown in Table 1)

Diet	Calf N 136	Calf N 137	Calf N 122
A	14·1 ± 0·6(2)	15·0 ± 1·3(4)	14·9 ± 1·4(4)
B	10·3 ± 0·6(4)	11·2 ± 0·8(4)	—
C	19·9 ± 1·1(2)	25·9 ± 0·3(2)	—
D	12·1 ± 0·6(3)	15·4 ± 0·5(3)	—
E	—	—	6·2 ± 0·3(4)
Pasture	7·8 ± 0·4(4)	8·7 ± 0·5(3)	8·3 ± 0·8(4)

Fluid volumes and absolute amounts of nucleic acids in the rumen

Values for the distribution volume of PEG within the rumen of calves receiving different diets are shown in Table 4. These, although probably underestimates (Warner & Stacy, 1968; Czerkawski & Breckenridge, 1969), give an approximate measure of the total fluid volumes in the rumen which can be regarded, at least for comparative purposes, as approximating to the distribution volumes of the nucleic acids of the rumen fluid. It is probable that locally high concentrations of microbes, and therefore of nucleic acids, occur near the surface of coarse solid material but we are mainly concerned with the fluid likely to pass out of the rumen into the lower alimentary tract. The results in Table 2 taken in conjunction with those in Table 4 show for the various diets examined that, although higher nucleic acid concentrations in the rumen were often accompanied by lower total rumen fluid volumes, there were considerable differences between diets in the absolute amounts of nucleic acids (and therefore presumably in the total microbial population) present in the rumen fluid at any one

time. Mean approximate values for total nucleic acid-N (RNA + DNA) varied from 0·34 g/100 kg live weight for calves receiving diet C to 2·27 g/100 kg live weight for calves at pasture.

Diurnal variations in the concentrations of nitrogenous components in rumen fluid

Results given previously have been for samples taken from animals at pasture or during a period 4–7 h after a concentrate feed. Diurnal variations in rumen composition have not been investigated in detail but sufficient observations have been made to indicate that such samples represented a reasonably steady state with respect to the percentages of total-N present as nucleic acid-N and usually with respect to absolute concentrations of nucleic acids also. Of the diets examined, only diet B led to any

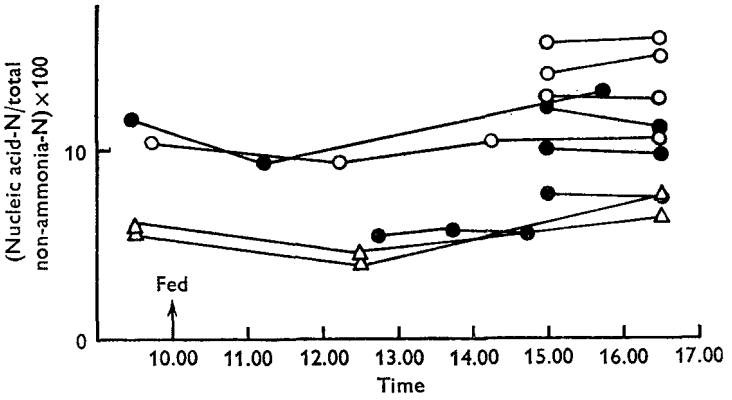

Fig. 4. Diurnal changes in the amounts of total nucleic acid-nitrogen expressed as a percentage of total non-ammonia-N in the rumen fluid of calves given diet A (○) or diet B (●) or of cows given diet F (△). Each experiment (indicated by linked values) was carried out three to six times with an individual animal and the results shown are the means of these replications. The diets are described in Table 1 or on p. 547.

marked diurnal variation in the absolute concentrations. For example, in four experiments with one calf given diet B, mean values in rumen fluid (expressed as mg total nucleic acid-N/100 g water with standard errors) increased from 10·9 + 1·5 to 25·9 ± 1·0 between 1 and 6 h after feeding. This increase was accompanied by a nearly proportionate increase in the concentration of total-N and was presumably due to dispersion of coarse particles of dietary decorticated extracted groundnut meal (DCGM) into the rumen fluid leading to a temporary increase in microbial growth. The other diets examined in this way (pasture for the cows and calves, diet F for the cows and diet A for the calves) did not lead to appreciable diurnal variations in the concentrations of nucleic acids in rumen fluid (which were measured at the times shown in Fig. 4). After feeding the stall-fed animals, there was a small decrease in the ratio of nucleic acid-N to total-N, presumably because of the sudden entry of a fresh supply of food nitrogen into the rumen, but after about 4 h it appeared that this ratio reached a fairly steady state once more (Fig 4). No such consistent variation was seen in the animals at pasture. For such animals, overall mean values (three animals, two experiments on each) for the percentage of total-N present as nucleic acid-N were 8·2, 8·9 and 9·7 for samples taken at 10.00, 12.30 and 16.30 h respectively.

The short-term effects on nucleic acid concentration of adding large amounts of available protein to the rumen were investigated in experiments in which sodium caseinate (350 g/feed), starch (450 g/feed), or both were added to the rumen of a calf (live weight 90–110 kg) receiving diet A. The supplements, made into a thick paste with water, were added through a cannula during the periods that the calf was consuming its concentrate feeds. It was found that starch alone had no marked effect but that the nucleic acid concentration increased by $65 \pm 15\%$ (two experiments) 5·5 h after a single casein-supplemented feed and by $165 \pm 10\%$ (two experiments) 5·5 h after the last of three successive casein-supplemented feeds (given on 2 successive days). Casein with starch added gave an increase (95%, 5·5 h after a single feed) similar to that of casein alone.

DISCUSSION

Our results with calves and cows show that, with many diets, about 8–15% of the non-ammonia-N in strained rumen fluid (the composition of which probably approximates to that of material passing from the rumen to the omasum) is in the form of nucleic acid-N. It seems likely that much of this nitrogen is of no nutritional value to the animal. The relatively high excretion of allantoin in the urine of ruminants compared with non-ruminants was pointed out by Blaxter (1961), and Topps & Elliott (1965) showed a significant correlation between the concentration of nucleic acids in the rumen and excretion of allantoin in the urine of sheep. Our own preliminary experiments (Smith, McAllan & Hill, 1969) have suggested that of the nucleic acid-N which is digested in the small intestine (about 75–85% of that leaving the abomasum) most is excreted in the urine as allantoin or urea. Production of nucleic acids in the rumen, therefore, appears to lead to a considerable wastage of nitrogen resources, the extent of which depends mainly upon the overall size of the microbial population. Rumen bacteria contain about 75–85% of their total-N in the form of proteins, peptides or free amino acids (Weller, 1957; Purser & Buechler, 1966) and 15–25% in the form of nucleic acids (Smith, 1969) so that these substances together account for most of the bacterial nitrogen. On average it appears that for every four parts of dietary nitrogen converted into bacterial protein or amino acids approximately one part is converted into nucleic acids.

The results (Figs. 2, 3) showing rapid degradation of pure nucleic acids added to the rumen strongly suggest that dietary nucleic acids, unless protected by association with other resistant cell structures, do not contribute appreciably to the nucleic acids in rumen fluid. Thus as the proportion of total nitrogen occurring as nucleic acid-nitrogen does not vary greatly between rumen bacteria of different species or between mixed populations from different sources (Smith, 1969) it appears that the nucleic acid concentration in rumen fluid can be regarded as an approximate measure of the density of the microbial population and could be used to estimate approximately the microbial nitrogen in the rumen. This leaves out of account the protozoa, but their nucleic acid-N : total-N ratios do not appear to differ greatly from those of the bacteria. Fauconneau & Gaussères (1966) showed that ratios in samples of rumen protozoa were about 80% of those in samples of rumen bacteria and we have found (unpublished

results) that two samples of Entodinium (one grown in pure culture, the other separated from the rumen) had nucleic acid-N:total-N ratios of 0·11 and 0·12 respectively compared with a mean value of 0·19 for rumen bacteria (Smith, 1969).

Using a value of 0·19 for microbial nucleic acid-N:total-N ratio it can be calculated for any one animal (and excluding samples taken within 4 h of giving a stall feed) that the mean percentages of total non-ammonia-N which were present in the rumen fluid as microbial-N were about the same for all the diets studied except diet B. These percentages were 50–80 for the different calves and 40–50 for the cows. For calves receiving diet B corresponding values were 30–60. These calculations assume all the microbes to be bacteria and the percentages are therefore probably underestimates. It appears, however, that the extent of underestimation would be only about 15% even if 50% of the microbial-N were protozoal.

When diet B was given the increases in the concentrations of both total-N and nucleic acid-N in rumen fluid between 1 and 6 h after feeding suggested that there was a cyclic fluctuation in the total microbial population associated with the pattern of feeding. When the other diets were given there was little diurnal variation in the concentrations in rumen fluid of nucleic acid-N, and presumably therefore the concentrations of total microbial-N showed little diurnal change. This does not imply that the composition of the microbial population did not change. Our results provide little information on this subject and, although different samples of rumen fluid showed marked differences in RNA:DNA ratio, which might suggest changes in microbial composition, we did not observe any consistent differences associated with any particular dietary or other factor.

In view of the variations observed in RNA:DNA ratio and the fact that RNA shows less variation relative to total-N in different rumen microbes than either DNA or total nucleic acid (Smith, 1969), it seems that RNA alone may be a better index of microbial-N in digesta than either of the other criteria. This will be considered in more detail in a subsequent publication but for the mean results obtained from the present experiments there was very little difference between values calculated using RNA as a basis and those reported above using total nucleic acid.

Our results suggest that DCGM protein was utilized less readily by the microbes than were the nitrogen sources in the other diets. The reason for this is not clear. It is possible that the protein may have been broken down to particles small enough to enter the strained rumen fluid (and to leave the rumen) but was still in such a form as to be relatively slowly attacked by the microbes. It appears that zein resists microbial attack in this way since it has been shown that the majority of zein added to ruminant diets survives unchanged to the duodenum (McDonald, 1954*a*; Ely, Little, Woolfolk & Mitchell, 1967). Such a low availability in the rumen can be nutritionally advantageous if the protein is digestible in the lower alimentary tract and several methods of treating proteins to protect them from degradation in the rumen have been investigated (Chalmers, Jayasinghe & Marshall, 1964; Leroy, Zelter & Francois, 1964; Ferguson, Hemsley & Reis, 1967). Estimation of the amounts of nucleic acids produced in the rumen seems to offer a useful way of assessing the degree of protection. The extent of conversion into microbial protein is, however, not determined solely by the

properties of the nitrogen source. The nitrogen intake was higher with the DCGM diet than with the other stall diets and, although it may sometimes have been similar to the nitrogen intake with pasture, it is possible that the amount of protein provided was excessive in relation to the intake of other nutrients. Most of the diets used in our experiments supplied considerable amounts of readily available carbohydrates and our results (Fig. 1 and the rapid increase in nucleic acid concentrations following the addition of casein to the rumen) strongly suggest that, for these diets, the amounts of nitrogen entering the rumen fluid limited microbial growth. It is probable that with excessive nitrogen but a relative deficiency of other nutrients (which could, for example, be energy supply or trace elements) then these other nutrients would limit microbial growth. Such a situation may have obtained in the experiments of Topps & Elliott (1965), who reported higher nucleic acid concentrations in the rumens of sheep receiving high-concentrate diets than in those receiving high-roughage diets. Total-N values were not, however, measured in these experiments and differences in these may have been, at least partly, responsible.

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