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Estimation of microbial nitrogen in nylon-bag residues by feed ^{15}N dilution

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1. Rapeseed (*Brassica napus*), barley grain, ryegrass (*Lolium perenne*) and barley straw were labelled with ^{15}N as an internal marker. The dilution of ^{15}N was used to estimate microbial N ($RMN_{^{15}N}$) in the feed residues in nylon bags incubated in the rumen for 5, 12 and 24 h. For comparative purposes, diaminopimelic acid (DAP) content of the residues was also determined and rumen microbial N ($RMN_{DAP\text{-}N}$) in the feed residues calculated using DAP as a bacterial marker. The influence of two bag pore sizes (20 μm and 40 μm) with different sample sizes (1 g and 5 g respectively) was also studied.

2. For all feeds, the average disappearance of ^{15}N was faster than that of total N, the difference between N and ^{15}N disappearance being marked with barley, ryegrass and barley straw. The disappearance of microbially corrected dry matter (DM; correction calculated from the ^{15}N values) was, accordingly, always faster than the uncorrected DM disappearance. Except for the bag pore/sample size effect for N disappearance, significant ($P < 0{\cdot}01$–$0{\cdot}001$) feed, pore/sample size and incubation-time effects were always found for the disappearance values.

3. Errors (%) resulting from the microbial contamination (calculated from the ^{15}N values) in N-loss measurement with rapeseed, barley, ryegrass and barley straw, at 5, 12 and 24 h in 20 μm bags were respectively: $-1{\cdot}8$, $-3{\cdot}9$, $-0{\cdot}9$; $-3{\cdot}8$, $-22{\cdot}4$, $-3{\cdot}8$; $-7{\cdot}2$, $-4{\cdot}1$, $-2{\cdot}9$; $-164{\cdot}5$, $-146{\cdot}3$, $-204{\cdot}6$. In 40 μm bags the corresponding errors were respectively: $-4{\cdot}4$, $-1{\cdot}2$, $-0{\cdot}7$; $-26{\cdot}1$, $-10{\cdot}5$, $-3{\cdot}9$; $-13{\cdot}2$, $-6{\cdot}4$, $-5{\cdot}5$; $-221{\cdot}2$, $-310{\cdot}1$, $-1284{\cdot}6$.

4. The largest residual proportions of $RMN_{^{15}N}$, $RMN_{DAP\text{-}N}$ and DAP-N (% of total N) were found in barley straw, followed by barley, ryegrass and rapeseed, in that order. $RMN_{^{15}N}$ (g/kg residual DM) followed the descending order: barley, ryegrass, straw, rapeseed. $RMN_{DAP\text{-}N}$ (g/kg residual DM) and DAP (mg/kg residual DM) followed the descending order: barley, ryegrass, rapeseed and barley straw. Feed, pore/sample size and incubation-time effects were always significant ($P < 0{\cdot}001$).

5. With 40 μm bags $RMN_{^{15}N}$ values of barley, ryegrass and barley straw (expressed as % of total N or g/kg residual DM) were substantially higher than those of $RMN_{DAP\text{-}N}$. With 20 μm bags the $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$ values were generally quite close for these feeds. With rapeseed residues, $RMN_{^{15}N}$ was clearly lower than $RMN_{DAP\text{-}N}$ with 20 μm bags, but only small differences were found with 40 μm bags.

6. A more diverse microbial colonization of feed samples was indicated in the 40 μm bags compared with the 20 μm bags. It was also noticed, consequently, that in most cases larger error resulted from the microbial contamination in 40 μm bags.

Estimation of feed-protein rumen degradability using the nylon-bag technique (Mehrez & Ørskov, 1977) is an attractive alternative to in vivo methods, due to its simplicity and reproducibility. However, as recognized by some workers, the true feed-protein degradation values obtained are probably erroneous due to microbial contamination of the feed residues (Mathers & Aitchison, 1981; Lindberg & Varvikko, 1982; Varvikko *et al.* 1983; Rooke *et al.* 1984). Although colonization of feed particles by the rumen microbes has also been demonstrated by several microscopic studies (Akin *et al.* 1973; Akin & Amos, 1975; Cheng *et al.* 1977; Bauchop, 1979, 1981), and it has been shown to occur within 15 min in the rumen (Cheng *et al.* 1977; Bauchop, 1981), only a few attempts at estimating the microbial N in the residues in nylon bags have been reported.

Mathers & Aitchison (1981) studied the extent of microbial contamination of lucerne

(*Medicago sativa*) and sprat meal in nylon bags using ^{35}S incorporation into rumen microbes. Their results indicated that a substantial proportion of the N in the residues could be of microbial origin. Similar results with grass silage were obtained by Rooke *et al.* (1984), also using ^{35}S incorporation. Mehrez & Ørskov (1977), on the other hand, concluded that negligible amounts of microbial N were left on barley residues when diaminopimelic acid (DAP) was used as a rumen bacterial marker.

The aim of the present study was to quantify the extent of microbial contamination of feed residues in nylon bags, and the subsequent error in the feed N degradation results. For this purpose, four different feeds were labelled with ^{15}N as an internal feed N marker. The dilution of ^{15}N in the feed N was used as an indicator of the extent of microbial contamination. For comparative purposes, the DAP concentration in the feed residues was also measured and microbial N in the residues estimated using DAP as a bacterial marker. The influence of two bag pore sizes with different sample sizes was also examined.

MATERIAL AND METHODS

Animal and its diet

One non-lactating cow of Swedish Red and White breed, fitted with a 120 mm rumen cannula, was used. The animal was given 2·3 kg dry matter (DM) legume–grass silage (176 g crude protein (N × 6·25; CP) and 480 g neutral-detergent fibre (NDF)/kg DM) and 2·3 kg DM grass hay (86 g CP and 551 g NDF/kg DM)/d in two equal meals at 07.00 and 15.00 hours. An adaptation period of 3 weeks preceded the experiment.

^{15}N-labelled feed samples

In the experiment, rapeseed (*Brassica napus*), barley grain, ryegrass (*Lolium perenne*) of the second harvest and barley straw were used as the experimental feeds.

Rapeseed, barley and ryegrass were cultured in 5 litre pots and fertilized with ^{15}N-labelled ammonium nitrate (10 atom % ^{15}N) as the only N fertilizer. NH_4NO_3 was given as 100 g/kg water solution. Growing of feed plants took place in an open greenhouse and the growing periods for rapeseed, barley and ryegrass of second harvest were 100, 90 and 20 d respectively.

A DM yield of 545 g seeds was obtained for rapeseed by fertilizing a total amount of 1·0 g seeds (in sixteen pots) with 87·5 g NH_4NO_3 (32 g N). NH_4NO_3 was applied in two doses, 65·5 g (24 g N) at sowing and 22·0 g (8 g N) 45 d later. A 560 g DM yield of barley grain and somewhat less straw were gained by fertilizing 10 g seed grains (ten pots) with 41 g NH_4NO_3 (15 g N). The fertilizer was given as a single dose at sowing. The levels of NH_4NO_3 for 2·5 g ryegrass seeds (ten pots) were 27 g (10 g N) at sowing and 22 g (8 g N) on the day following the first cut. Approximately 250 g was the DM yield of the second harvest. At harvest, rapeseed and barley were separated into seeds and straw.

After harvest, the samples were all dried at 40° for 3 d. The rapeseeds were then crushed and diethyl ether-extracted before further use. All samples were milled to pass a 1·0 mm screen.

Nylon-bag procedures

Feed samples were enclosed in nylon bags with 20 μm and 40 μm apertures. The internal dimensions of the bags were 100 mm × 50 mm and 60 mm × 120 mm respectively. Details of the cloth and preparation of the bags were given previously (20 μm bags, Lindberg, 1981; 40 μm bags, Setälä, 1983). An air-dry sample (1 g) was weighed into 20 μm bags and a 5 g sample was weighed into 40 μm bags.

The bags were introduced into the rumen immediately before morning feeding and withdrawn after 5, 12 or 24 h incubation. After withdrawal, the bags were washed for 15 min

Table 1. *Number of bags collected for one replication of each treatment combination*

Bag pore/sample size (μm/g)...	20/1			40/5		
Incubation time (h)...	5	12	24	5	12	24
Rapeseed (*Brassica napus*)	5	6	7	1	2	3
Barley	12	11	17	2	2	5
Ryegrass (*Lolium perenne*)	5	8	16	1	1	3
Straw	5	5	5	1	1	1

with running cold tap-water in a small rotating cylinder (45 rev./min) and slightly squeezed to remove the excess water.

Four replications for each treatment combination were collected during four consecutive 2 d periods, with one replication for each treatment combination on the same 2 d period. The number of bags collected for one replication on each occasion is given in Table 1. The total number of bags was 500 (408 20 μm bags; 92 40 μm bags).

The residues from bags within single replications were pooled after drying at 45° for 12 h and thereafter stored at room temperature for chemical analyses.

Chemical analyses

DM was determined on samples (100 mg) after drying at 105° overnight.

Total N was analysed as follows: 20 ml sulphuric acid containing salicylic acid (25 mg/l) were added to a 500 mg feed sample and kept at room temperature overnight. Sodium sulphite (1·5 g) was then added and the mixture was incubated at 100° for 30 min in a water-bath. After cooling, 8·0 g potassium sulphate and 1·0 g copper sulphate were added and the temperature slowly raised to 180°. The temperature was thereafter gradually raised to 420° by 20–50° steps at 20-min intervals. The final temperature was kept constant for two more hours. After cooling, 40 ml water and 50 ml sodium hydroxide (450 g/kg) were added and the N was distilled into 0·100 M-hydrochloric acid (Tashiro indicator) and immediately titrated back to neutrality with 0·100 M-HCl.

^{15}N isotope was analysed from the ammonium chloride of the titrated distillates following the method of Ross & Martin (1970) modified by A. Jaakkola and T. Yläranta (unpublished results). The N was liberated by sodium hypobromite and then introduced to the mass spectrometer (MM 622; VG Micromass, England).

Buffer-soluble N was determined according to Lindberg *et al.* (1982) using a mineral buffer adjusted to pH 6·7–6·9 with orthophosphoric acid.

DAP was analysed according to Smith *et al.* (1978) with acid-ninhydrin. The readings were made using an AutoAnalyzer (Technicon Instruments Corp.).

Calculations

The rumen microbial N ($RMN_{^{15}N}$) in the feed residues, derived from ^{15}N values, was calculated according to the equation:

$$\left(1-\frac{^{15}\text{N \% of total residual N}}{^{15}\text{N \% of total original N}}\right)\times 100.$$

Using DAP as a microbial (bacterial) marker, rumen microbial N ($RMN_{DAP\text{-}N}$) was calculated with the average value of 0·0065 (McAllan & Smith, 1983) for the microbial DAP-N:total N value in the residues.

Table 2. *Chemical composition of the feed samples*

	Percentage of DM	Percentage of total nitrogen			
	total N	N_{Bs}	^{15}N	$^{15}N_{Bins}$	DAP-N
Rapeseed (*Brassica napus*)	5·3	24·4	9·2	9·2	0·028
Barley	1·8	23·5	8·6	8·6	0·076
Ryegrass (*Lolium perenne*)	3·2	21·1	8·9	8·9	0·044
Straw	0·6	30·4	8·0	7·7	0·098

Bs, Buffer-soluble N; Bins, ^{15}N as percentage of buffer-insoluble N; DAP-N, diaminopimelic acid-N.

The correction of residual DM for the microbial contribution was based on the assumption that rumen bacteria contain 92 g N/kg DM (Baldwin, 1970).

Statistical analyses

The design of the experiment was $4 \times 2 \times 3$ factorial (feed, bag pore/sample size, incubation time) and the values were subjected to a standard analysis of variance. Standard errors for the variates were based on the respective interactions between replications and treatments.

RESULTS

The abundance of ^{15}N (% of total N) in the original unincubated samples is given in Table 2. The results are summarized in Table 3.

N and DM

The pore/sample size effect for N disappearance was not significant ($P > 0{\cdot}05$). However, the feed, pore/sample size and incubation-time effects were statistically significant ($P < 0{\cdot}01$–$0{\cdot}001$) for N and ^{15}N disappearance as well as for uncorrected and microbially corrected DM disappearance. The disappearance of N, ^{15}N and DM (both uncorrected and microbially corrected) from rapeseed and barley was, on average, clearly faster from the 40 μm bags than from the 20 μm bags.

N disappearance was slower than ^{15}N disappearance with all the labelled feed samples, the difference being, generally, quite marked with barley, ryegrass and barley straw. Although the corrected values for DM disappearance were always higher compared with the respective uncorrected values, the values did not differ markedly from each other on any occasion.

RMN and DAP

Rumen microbial N (both $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$), DAP-N and DAP showed statistically significant ($P < 0{\cdot}001$) feed, pore/sample size and incubation-time effects at all times.

On average, the largest proportions of $RMN_{^{15}N}$, $RMN_{DAP\text{-}N}$ and DAP-N (expressed as percentage of residual N) were found with straw, followed by barley, ryegrass and rapeseed. The $RMN_{^{15}N}$ (expressed as g/kg residual DM) in the feeds followed the descending order: barley, ryegrass, straw, rapeseed. $RMN_{DAP\text{-}N}$ (expressed as g/kg residual DM) and DAP (mg/kg residual DM) were also highest in barley and ryegrass, in that order, but the lowest values were obtained with straw.

$RMN_{^{15}N}$ (both as percentage of total residual N and g/kg residual DM) in rapeseed, barley and ryegrass accumulated clearly more in 40 μm bags than in 20 μm bags, while with straw, differences between the two bag pore sizes were negligible. Substantially more DAP

Table 3. *Total nitrogen, ^{15}N, uncorrected dry matter (DM) and corrected DM disappearance (%), rumen microbial N ($RMN_{^{15}N}$, $RMN_{DAP\text{-}N}$; % of total N, g/kg residual DM), diaminopimelic acid (DAP)-N (% of total N) and DAP (mg/kg residual DM) contents of rapeseed* (Brassica napus), *barley, ryegrass* (Lolium perenne) *and barley straw incubated in the rumen for 5, 12 and 24 h in nylon bags with a pore/sample size (μm/g) of 20/1 or 40/5*

(Mean values of four samples)

Feed...	Rapeseed						Barley					
Pore/sample size (μm/g)...	20/1			40/5			20/1			40/5		
Incubation time (h)...	5	12	24	5	12	24	5	12	24	5	12	24
Disappearance (%)												
N	51·1	82·7	92·4	63·9	91·8	93·6	79·5	67·9	89·8	71·3	86·1	93·7
^{15}N	52·0	85·9	93·2	66·7	92·9	94·3	82·5	83·1	93·2	89·9	95·1	97·4
DM, uncorrected	43·5	72·2	81·7	56·8	81·4	83·4	82·4	75·3	90·0	79·1	87·2	91·6
DM, corrected	44·2	72·7	82·2	58·4	82·0	83·9	84·1	78·4	90·6	82·8	89·1	92·3
Contents												
% of residual N												
$RMN_{^{15}N}$	1·9	6·0	10·8	8·1	13·3	12·1	14·4	48·0	33·6	65·2	64·8	58·4
$RMN_{DAP\text{-}N}$	6·9	16·7	19·3	8·3	16·8	19·2	18·2	52·4	34·2	8·0	12·8	19·0
DAP-N	0·045	0·108	1·126	0·054	0·109	0·125	0·118	0·341	0·223	0·052	0·083	0·123
g/kg residual DM												
$RMN_{^{15}N}$	0·9	1·6	2·4	3·5	3·1	2·5	3·3	11·4	6·3	16·9	13·1	8·1
$RMN_{DAP\text{-}N}$	3·1	4·7	4·2	3·6	3·9	3·9	4·1	12·5	6·4	2·0	2·5	2·7
mg/kg residual DM												
DAP	133	196	174	152	159	162	173	518	268	84	109	111

Feed...	Ryegrass						Barley straw						SEM
Pore/sample size (μm/g)...	20/1			40/5			20/1			40/5			
Incubation time (h)...	5	12	24	5	12	24	5	12	24	5	12	24	(69 df)
Disappearance (%)													
N	54·4	80·9	91·6	54·7	75·2	90·4	21·4	27·0	24·0	18·2	14·9	5·2	2·66
^{15}N	58·3	84·2	94·3	61·9	80·0	95·4	56·6	66·5	73·1	58·6	61·1	72·0	1·41
DM, uncorrected	54·6	76·0	85·8	56·5	70·5	87·4	23·8	34·3	46·5	22·1	29·3	45·8	1·54
DM, corrected	55·9	77·1	86·8	59·1	72·2	89·1	26·1	37·1	49·8	24·7	32·4	50·3	1·46
Contents													
% of residual N													
$RMN_{^{15}N}$	8·6	17·2	32·4	16·0	20·6	52·0	44·2	54·1	64·5	48·5	53·8	70·9	1·89
$RMN_{DAP\ N}$	17·0	19·2	25·1	11·8	12·9	20·3	31·1	41·5	55·5	41·0	38·4	36·1	3·05
DAP-N	0·111	0·125	0·163	0·077	0·084	0·132	0·202	0·270	0·361	0·266	0·251	0·234	0·0199
g/kg residual DM													
$RMN_{^{15}N}$	2·8	4·4	6·1	5·4	5·5	12·6	2·8	3·7	6·0	2·9	4·0	7·4	0·49
$RMN_{DAP\text{-}N}$	5·4	4·9	4·7	3·9	3·4	4·8	2·0	2·7	4·8	2·5	2·8	3·8	0·52
mg/kg residual DM													
DAP	229	210	199	168	148	229	82	117	205	108	115	156	21·1

$RMN_{^{15}N}$, calculated from ^{15}N values; $RMN_{DAP\text{-}N}$, calculated from DAP-N.

(mg/kg residual DM), DAP-N (% of total N) and, accordingly, $RMN_{DAP\text{-}N}$ (% of total residual N and g/kg residual DM) were found in barley, ryegrass and straw enclosed in 20 μm bags compared with the respective feeds enclosed in 40 μm bags. With rapeseed, differences in these variates between bags were very small.

$RMN_{^{15}N}$ of barley, ryegrass and barley straw (both as percentage of total residual N and g/kg residual DM) in 40 μm bags always clearly exceeded $RMN_{DAP\text{-}N}$, while with 20 μm

bags the respective $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$ values were generally quite close. $RMN_{^{15}N}$ in rapeseed residues was always lower than $RMN_{DAP\text{-}N}$, the difference being larger with residues in 20 μm bags.

DISCUSSION

The colonization by rumen microbes of the forage-plant fragments has been repeatedly demonstrated by using different electron microscopic as well as light microscopic techniques. Akin & Amos (1975) and Cheng *et al.* (1977) showed that bacterial attachment to the thick-walled bundle sheath and epidermal cells by an electron-dense extracellular substrate or rod-like appendages preceded the rumen microbial degradation of these plant tissues. A rapid colonization and subsequent direct ingestion of thin-walled forage tissues by a protozoa, *Epidinium*, was demonstrated by Bauchop (1979). Bauchop (1981) also demonstrated the invasion by anaerobic fungi of vascular tissues of various fibrous-plant samples suspended in nylon bags. The fungal colonization was mediated by flagellated zoospores which later were attached to the plant fragments by rhichoids.

Concerning the estimation of microbial N in the feed residues in the present study, there are some points of importance that need to be mentioned. First, the major sites for the bacterial and protozoal action in forages are cut or damaged cell-wall surfaces (Latham *et al.* 1978; Bauchop, 1979). In connection with the nylon-bag technique this is noteworthy, as the samples incubated in bags are milled mostly to pass a fine-mesh screen (in this experiment 1·0 mm) thus increasing vastly the effective surface for the microbial activities.

Second, estimation of RMN from the residual: original value of feed ^{15}N might be biased by an uneven distribution of ^{15}N isotope in the original plant material. However, very small changes in ^{15}N concentration in the buffer-incubated samples compared with the original unincubated samples (Table 2) indicated the ^{15}N isotope to be quite uniformly distributed in the plant material and, furthermore, that ^{15}N: total N value in the feed material could be expected to remain unchanged during the rumen incubation. Also, calculation of microbial colonization of feed residues in nylon bags using ^{15}N dilution might lead to an underestimate due to the re-use of the feed ^{15}N degraded inside the bags. Because of continuous free-flow of rumen fluid through the bag pores (the exception being 20 μm bags with barley) this underestimation, however, seems unlikely. Similar values for $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$ in feeds enclosed in 20 μm bags also support this conclusion. Further, the bacteria known to predominate in degrading (and adhering to) the plant cell wall in the rumen (e.g. *Bacteroides succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*; Bryant, 1973) utilize ammonia as a main source of N but do not produce NH_3. Therefore, contribution of ^{15}N from feed samples to adhering microbes is probably insignificant.

A third point is the washing of the bags after rumen incubation. In the nylon-bag technique, the general assumption is that the soluble and small particulates added to the bags from the rumen are removed by washing the bags in running tap-water. This procedure is often poorly specified and rarely standardized. In the present experiment a standardized washing method was used, in which the bags were placed in a small rotating cylinder after withdrawal from the rumen. Washing for 15 min in cold running tap-water was considered (judged by the variation between replicates) to be adequate to remove all the added material from the bags.

Finally, measurements made in the present study provide estimates of variation within one animal only. It seems likely that the variation between replications obtained from different animals would have been larger than those obtained from the single animal. Also, the diet given to the animal during the sample collection most likely has an influence on the extent of microbial-N enrichment of the feed particles enclosed in the nylon bag.

According to the present results it seems obvious that, in the nylon-bag technique,

Table 4. *Errors (%)* in the feed-nitrogen-loss estimates resulting from the microbial contamination of the feed sample residues*

Bag pore/sample size (μm/g)....	20/1			40/5		
Incubation time (h)....	5	12	24	5	12	24
Rapeseed (*Brassica napus*)	−1·8	−3·9	−0·9	−4·4	−1·2	−0·7
Barley	−3·8	−22·4	−3·8	−26·1	−10·5	−3·9
Rye-grass (*Lolium perenne*)	−7·2	−4·1	−2·9	−13·2	−6·4	−5·5
Straw	−164·5	−146·3	−204·6	−221·2	−310·1	−1284·6

* Calculated from the values in Table 3 according to the equation:

$$\left(1-\frac{^{15}\text{N disappearance}}{\text{total N disappearance}}\right)\times 100.$$

microbial attachment to the feed residues may be an important source of error in the study of quantitative feed-protein degradation and, to a lesser extent, feed-DM degradation. The degradation results also show that the microbial attachment occurs not only on forage material, but also on starchy and protein-rich feeds. However, the magnitude of error (%) in the N-loss measurements depends on the N content of the feeds and on the rumen degradation rate. The calculations made in Table 4 suggest that with straw, a feed with a low N content and slow degradation rate, the errors in N loss measurements can be enormous, owing to the large proportion of microbial N, which also increased absolutely the residual total N content. The errors in barley N loss estimates were also quite substantial during the first 12 h of rumen incubation.

The uncorrected disappearance values for barley obtained with 20 μm bags were quite exceptional among the feeds. Not only the total N but also the DM content increased in the residues during the incubation period from 5 to 12 h. At the same time, the disappearance of ^{15}N was almost zero. These observations suggest a biased growth of a microbial population inside the nylon bag, and also that the microbes inside the bag were not of protein degraders, unless the ^{15}N degraded from the barley was re-used by the microbes. The microbial growth was probably promoted by the change in the micro-environment inside the bag as a result of the blockage of the bag pores. The blockage of the pores in 20 μm bags with barley noticed in this experiment was similar to that found with 10 μm bags in an earlier study (Lindberg & Varvikko, 1982).

Nearly one-third (32 g/kg DM) of the assumed N content (91·2 g/kg DM) for the microbial matter was calculated to be adequate to cause an increasing corrected DM-degradation curve for barley from a 5–12 h incubation period. If this lower microbial N content had been generally applied in estimating the microbial contribution to the residual DM of feeds, the difference between uncorrected and corrected DM degradation values would become considerably wider.

Mathers & Aitchison (1981) concluded that N degradation values for protein-rich feeds of low degradability might be less affected by the microbial contamination. In this experiment the rapeseed sample, although not a feed of low degradability, had the lowest proportion of microbial N (calculated from ^{15}N) in residues. The corrected feed N (^{15}N) disappearance values for rapeseed were only slightly different from those of the uncorrected (total N) disappearance. Also, the absolute microbial colonization ($RMN_{^{15}N}$ g/kg residual DM) was smallest in the rapeseed residues. This could be due to the relatively high content of lignin and polyphenolic fractions (up to 360 g/kg DM; Åman, 1979) in the dark rapeseed hulls. In forages, the lignified plant fragments have been shown to have only a few adherent

bacteria when the feeds were exposed to rumen microbial degradation in vitro (Cheng *et al.* 1977).

Recently, the reliability of DAP as a rumen bacterial marker has been discussed because of varying DAP concentrations found in a number of feeds (Czerkawski, 1974; Rahnema *et al.* 1979; Dufva *et al.* 1982; Theurer, 1982). In the present experiment, the relative DAP-N values (% of total N; Table 2) in the original barley and forage samples were of the same magnitude as those reported by Czerkawski (1974), and they were 4 (rapeseed meal) to 16% (barley straw) of the DAP-N values previously reported for the rumen microbes (Czerkawski, 1974; Dufva *et al.* 1982). Although DAP-N:total N values for different microbial fractions (Czerkawski, 1974) or single bacterial species (Dufva *et al.* 1982) vary widely, comparatively similar values (range 0·0056–0·0069) with different diets have been published (McAllan & Smith, 1983) for mixed rumen bacteria. Therefore, an average value of 0·0065 for DAP-N:total-N of rumen microbes (bacteria) seemed justified in the present experiment.

Generally, rather close $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$ values in the feed residues enclosed in 20 μm bags suggest that the microbial contamination of the residues was mainly due to bacteria. In 40 μm bags, non-bacterial N may have also been involved, as indicated by the large difference between $RMN_{^{15}N}$ and $RMN_{DAP\text{-}N}$. This suggests that a more diverse rumen microbial population containing more protozoa (Lindberg *et al.* 1984) and also anerobic fungi (Bauchop, 1979, 1981) are entering the 40 μm bags. Subsequently, a more dynamic breakdown of feed N and DM can take place in the 40 μm bags.

It can be concluded that when using the N and DM degradation values obtained with the nylon-bag technique, it must be borne in mind that the uncorrected values may be erroneous. Markedly false rumen N degradation results are probable with a starchy or fibrous vegetable feed supplement of an ordinary type. With protein-rich feeds, e.g. rapeseed used in the present study, error due to microbial contamination seems moderate. The errors reflecting the DM degradation are probably too small to be biologically significant.

The influence of rumen microbial colonization on the actual feed amino acid degradation studies using the nylon-bag technique will be presented in another paper.

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