The use of monensin or formaldehyde to control the digestion of the nitrogenous constituents of perennial ryegrass (*Lolium perenne* cv. Melle) and white clover (*Trifolium repens* cv. Blanca) in the rumen of cattle

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1. Pure swards of perennial ryegrass (*Lolium perenne* cv. Melle) and white clover (*Trifolium repens* cv. Blanca) were harvested daily as primary growth (grass in May, clover in June) or regrowths (clover only in July) and offered, in the long form, to growing cattle at a feeding rate of 22 g dry matter/kg live weight. With each forage, two treatments were compared with the untreated forage (C): monensin (100 g active ingredient/kg, 250 mg/d) addition to the rumen (treatment M) or formaldehyde (30 g/kg crude protein (nitrogen × 6·25)) application to the diet (treatment F). The objective of the experiment was to examine means of manipulating N metabolism in the rumen and the duodenal non-ammonia-N (NAN) supply derived from fresh forages.

2. The apparent digestion of ingested organic matter (g/kg) in the rumen was unaffected by treatment M (C 509, M 497) but was significantly ($P < 0.01$) reduced by treatment F (443). The extent of cellulose digestion in the rumen was not affected by any of the treatments imposed and the changes in organic matter digestion were due mainly to effects on N digestion and rumen microbial synthesis. On the untreated diets, duodenal NAN supply averaged 0·74 g/g N intake and treatment M caused a small but non-significant increase (M 0·79 g/g N intake). In contrast, the effect of treatment F was much larger (F 0·91 g/g N intake; $P < 0.01$). These differences were accompanied by corresponding reductions in rumen NH_3 concentrations (mg/l; C 350, M 310, F 220; $P < 0.001$).

3. Of the increased flow of NAN to the small intestine observed on the white clover only diets with treatment F, 0·70 was accounted for by an increased net synthesis of microbial N, while treatment M had no effect on microbial N synthesis and a marginal reduction in feed N degradability only with the regrowth white clover diet. Treatment F reduced feed N degradability to a limited extent on both clover diets (C 0·82, M 0·81, F 0·77). No corresponding measurements were made for the ryegrass diets.

4. It is concluded that the extensive loss of N from the reticulo-rumen of cattle fed on fresh forages can be reduced by the use of agents to reduce protein solubility. However, the study demonstrated that treatment F may in some circumstances increase N supply to the small intestine more through enhancing microbial N synthesis within the rumen than through increasing the passage of undegraded feed N to the small intestine. The use of monensin, to manipulate proteolytic or deaminative activity in the rumen, or both, was not found to confer any beneficial effects on duodenal-NAN supply.

There have been several studies to evaluate the digestion of fresh forages and the nutrient supply arising from such diets (Beever *et al.* 1971, 1976, 1978*b*; MacRae & Ulyatt, 1974; Ulyatt & MacRae, 1974; Ulyatt & Egan, 1979; Vérité *et al.* 1984). Recent experiments with growing cattle (Ulyatt *et al.* 1981; Beever *et al.* 1985, 1986*a, b*) have demonstrated that as the amount of nitrogen in the crop increases, considerable losses of N between mouth and duodenum (up to 0·40 of N intake) can occur. Such losses have been associated with high rumen ammonia concentrations and it has been concluded that both are associated with the relatively large amounts of soluble N in fresh forages and the consequent imbalance between degraded N and degraded organic matter (OM) supply in the rumen.

The purpose of the present study was to consider ways by which this excessive rumen loss of N from fresh forages could be beneficially reduced. After an exhaustive search of the literature, two approaches were adopted. These comprised formaldehyde application to the forage before feeding, to protect partially the forage protein from rumen degradation (see Ferguson, 1975), and monensin addition to the rumen at the time of feeding. Dinius *et al.* (1976) showed monensin to cause significant reductions in rumen NH_3 concentration,

whilst Chalupa (1980) presented values from in vitro studies which suggested a reduction in the extent of amino acid degradation in response to incremental additions of monensin. Thus whilst there was no unequivocal proof of the efficacy of monensin as a modifier of rumen proteolytic or deaminative activity, there was sufficient circumstantial evidence to warrant a fuller investigation of the agent in the present study.

A preliminary report of this work has been published (Beever *et al.* 1984).

MATERIALS AND METHODS
Management of swards
Two pasture species were used; perennial ryegrass (*Lolium perenne* cv. Melle) and white clover (*Trifolium repens* cv. Blanca). Management of the swards and harvesting procedures were as described by Beever *et al.* (1985) with the exception that the ryegrass received 120 kg N/ha instead of 80 kg N/ha in early May in order to enhance total N content in the crop. Three contrasting forages were examined between mid-May and late-July, comprising primary growth perennial ryegrass (R1 in May), primary growth white clover (W1 in June) and regrowth white clover (W2 in July). To ensure that all regrowth forage was 4 weeks old at the time of harvest, a cutting sequence similar to that used by Beever *et al.* (1985) was adopted.

Animals
A total of nine Friesian steers (initially 10 months old and 270 kg live weight (LWT)) were used. Before the experiment, each animal was prepared with a PVC cannula (i.d. 38 mm) into the dorsal sac of the reticulo-rumen and a PVC 'T' piece cannula (i.d. 20 mm) into the proximal duodenum anterior to the bile duct, according to the techniques described by Beever *et al.* (1978*a*).

During the experiment, all animals were held unrestrained in individual pens and fresh water and mineral blocks were available at all times. Temperature was controlled not to exceed 14° and there was artificial lighting at all times. All animals were weighed weekly.

Experimental design
The experiment comprised three periods corresponding to the three forages. Within each period (forage) a group of three animals was allocated to each of three treatments. These comprised the untreated forage (control C), the untreated forage with a twice daily intrarumen addition of monensin (treatment M) and the same forage treated with formaldehyde (treatment F) before feeding. Before the start of the experiment, the nine animals were allocated according to LWT to give three groups of approximately equal mean weight. Once allocated, the animals remained in their groups throughout the experiment, with each group being reallocated to treatments between the three periods according to a 3 × 3 Latin square design.

Each period lasted 5 weeks and comprised 2 weeks' adaptation to the forage, during which time no treatments were applied, followed by a 3-week period when the treatments were applied. All experimental measurements were confined to the last 2 weeks after a minimum period of 7 d adaptation to the treatments.

Throughout the experiment, feeding level was fixed at 22 g dry matter (DM)/kg LWT and feed allowances were adjusted weekly in response to changes in LWT.

Preparation and feeding of diets

All forages were cut daily at 07.30 hours by means of a rotary-drum mower and harvested without chopping by means of a forage wagon. The harvested forage was delivered to the animal house and a rapid DM determination was performed (Beever *et al.* 1985). Appropriate amounts of fresh forage were then weighed and, during the 2-week adaptation period, these were offered to the animals without further treatment in two equal amounts at 11.30 hours and 16.30 hours, with the afternoon feed being held in a cold room at 2° until required. During the experimental measurement period, sufficient fresh forage was weighed out for the three animals receiving the formaldehyde-treated forage and spread thinly onto a polyethylene sheet before spraying with a solution of formaldehyde (380 ml/l)–water (1:3, v/v) at a rate equal to 30 g F/kg estimated crude protein (nitrogen × 6·25) content. To optimize contact of the spray with the forage, the total spray volume was applied in three equal amounts, with the forage being mixed thoroughly between each spraying. The treated forage was then weighed into daily allowances for each animal, along with untreated forage for the remaining six animals. The daily allowance was divided for the morning and afternoon feeds in the ratio 3:7, and placed immediately in a cold room at 2° (at approximately 08.30 hours) until it was offered to the animals at 11.30 and 16.30 hours respectively. At these times, the three animals on treatment M received, via the rumen cannula, 125 mg monensin (100 g active ingredient/kg) wrapped in filter paper.

Feed refusals, if any, were removed daily at 10.00 hours and oven-dried immediately to determine actual DM intakes. During the two periods when white clover was offered, all animals received an oral dose (5 ml) of poloxalene (980 ml/l; Smith, Kline & French, Welwyn, Herts) at each feeding time to prevent legume bloat.

Experimental procedures

During each 3-week experimental period, the following sequence was adopted. On day 7, rumen contents were sampled manually at hourly intervals between 11.00 and 24.00 hours and, on each occasion, 10 ml well-mixed rumen fluid from each animal were acidified with concentrated sulphuric acid and held frozen until required for analysis of total and individual volatile fatty acids (VFA) and NH_3 concentrations. This was immediately followed on day 8 by the commencement of an intrarumen infusion of ruthenium phenathroline (RuP) and chromium EDTA which was maintained until day 18. Infusion solutions were adjusted to provide 16 mg Ru and 120 mg Cr/kg DM intake, contained in a total volume of 500 ml/d which was infused continuously into the reticulo-rumen (20 ml/h) using the portable infusion pumps described by Evans *et al.* (1981*a*). For periods 2 and 3 when the white clover diets were offered, intrarumen infusions of ^{15}N-labelled ammonium sulphate (95% enriched) were made over a 5 min period approximately 1 h after each feeding time (total dose 1 g $(^{15}NH_4)_2SO_4/d$) to permit estimation of microbial N synthesis.

Duodenal digesta collections were undertaken over the last 2 d of the intrarumen infusions of RuP and CrEDTA using the portable samplers described by Evans *et al.* (1981*b*). Collections commenced at 09.00 hours on each day and collected samples were processed according to the procedures described by Beever *et al.* (1985). Digesta collected on the second day was also used to isolate a duodenal microbial fraction for each animal using the centrifugation procedures outlined by Siddons *et al.* (1982).

Representative samples of the feeds offered were taken daily for estimation of DM content by oven drying, whilst during days 15–18 of each measurement period fresh samples were frozen and retained for subsequent chemical analysis.

Sample preparation and analysis

All feed, total and centrifuged duodenal digesta and isolated duodenal microbial samples were freeze-dried and finely ground through a small laboratory mill before analysis. Subsequently these samples plus intraruminal infusates were analysed, as appropriate, for OM, water-soluble carbohydrate, cellulose, lignin, total N, NH_3, gross energy, Ru, Cr and ^{15}N enrichment using previously described techniques (Beever *et al.* 1978*a*; Siddons *et al.* 1982; Beever *et al.* 1985).

VFA concentrations in the rumen fluid samples were determined by gas–liquid chromatography according to the procedure described by Kellaway *et al.* (1978) and rumen NH_3 contents by the alkaline phenate method of Gehrke *et al.* (1968).

Calculation of results

Nutrient flow to the small intestine was estimated according to the dual-phase-marker method developed by Faichney (1975), and the proportion of duodenal non-NH_3-N (NAN) present as microbial N by the procedures outlined by Siddons *et al.* (1982). Estimates of the amount of undegraded feed protein entering the small intestine were derived from the flows of non-microbial NAN, less an arbitrary allowance of 2 g endogenous N/kg DM consumed per day (Harrop, 1974).

Statistical analysis

Results were analysed by analysis of variance with three animals per treatment, for comparisons between forages (which were confounded with periods), treatments and their interaction. There were ten df associated with residual error variance for significance testing. Values in Table 4 (p. 64) were obtained only from periods 2 and 3 thus giving 4 residual error df.

RESULTS

Chemical composition of the diets

Values relating to chemical composition of the diets offered are presented in Table 1.

The grass diet was characterized by higher contents of OM, water-soluble carbohydrate and cellulose but lower contents of lignin and total N than the two clover diets, which were broadly similar despite the different stages of harvest. Gross energy contents were similar on all diets. Despite the high, late application of N fertilizer to the grass, the total N content was not markedly increased in relation to similar diets used in previous studies at this laboratory.

Rumen fermentation measurements

Values relating to the concentrations of total VFA, individual molar proportions of VFA and concentrations of NH_3 on the three treatments for each forage are given in Table 2.

For the three forage species, irrespective of treatments, total VFA concentrations were significantly ($P < 0.001$) higher with diet W1 (mean 104·4 mmol/l) than with diets W2 (88·3 mmol/l) and R1 (77·7 mmol/l) which were not significantly different. With respect to treatments, both treatment M (mean 90·9 mmol/l) and treatment F (78·3 mmol/l) reduced VFA concentration compared with the control (101·2 mmol/l) (F < C, $P < 0.01$). In general, molar proportions of propionate were lower ($P < 0.01$) on the clover (W1, W2) diets compared with the rye grass (R1) diet, whilst butyrate and isobutyrate proportions were higher ($P < 0.001$). Acetate proportions were unaffected by forage species. For all three forages, treatment F had no effect on acetate and propionate proportions compared

Table 1. *Chemical composition of the diets as offered (g/kg dry matter (DM))*

Diet...	Perennial ryegrass (*Lolium perenne* cv. Melle) (R1)	White clover (*Trifolium repens* cv. Blanca)	
		W1	W2
Organic matter	916	886	881
Water-soluble carbohydrate	118	65	49
Cellulose	268	208	220
Lignin	21·1	30·0	33·9
Total nitrogen	24·2	41·2	40·1
Gross energy (MJ/kg DM)	18·3	18·4	18·6

R1 and W1, primary growth and W2, regrowth, of the two forage species

with the control forages, whilst treatment M caused a significant ($P < 0.001$) increase in molar proportion of propionate (C 0·19, M 0·24) and a significant ($P < 0.001$) reduction in acetate (C 0·68, M 0·64). Both treatments M and F caused a significant ($P < 0.01$) reduction in butyrate proportions.

Rumen NH_3 levels on diet R1 averaged 158 mg/l compared with significantly higher ($P < 0.001$) values on diets W1 (358 mg/l) and W2 (365 mg/l). Effects due to treatment F were seen on all diets (mg/l; C 350, M 311, F220; $P < 0.001$) with the reduction being most marked on diet W2, equivalent to almost 50% of the concentration observed on the untreated clover. As can be seen, effects due to treatment M were much less pronounced and restricted mainly to diets R1 and W1.

Nutrient supply

Values in Table 3 refer to the rumen digestion and duodenal flow of DM, cellulose and N (or NAN) in relation to amounts consumed for the three diets and fed alone, with treatment M or after treatment F.

During the experiment, LWT of the animal ranged between 270 and 320 kg and levels of DM intake achieved averaged 17·7, 19·4 and 20·4 g DM/kg LWT on forages R1, W1, W2 irrespective of treatment; only on diet R1 was an effect due to F application observed (g/kg LWT; C 18·4, F 16·3).

For the nine separate experimental diets, mean OM intakes ranged from 4·37 to 5·68 kg/d and, irrespective of treatment, the extent of OM digestion in the rumen in relation to OM intake averaged 531 g/kg with diet R1 compared with 481 and 437 g/kg with diets W1 and W2 respectively (all significantly different, $P < 0.05$). M addition had only a small negative effect on the extent of OM digestion in the rumen (g/kg OM intake; C 509, M 497) whilst an effect due to treatment F (g/kg intake; C 509, F 443; $P < 0.01$) was detected on all diets and especially diet W2. The extent of digestion of ingested cellulose in the rumen exceeded 740 g/kg on all diets. It tended to be higher with diet W1 (792 g/kg) than with diets W2 (765 g/kg) and R1 (749 g/kg) but was unaffected by either of the treatments imposed (g/kg; C 770, M 768, F 769).

Reflecting the differences in forage N contents, N intakes ranged from 116 to 260 g/d, whilst duodenal NAN flows showed a slightly narrower range from 102 to 229 g/d. As expected, NAN flows (g/d) were higher with diets W1 (181) and W2 (194) than with diet R1 (109, $P < 0.001$), and whilst treatment M had no effect on NAN supply (C 150, M 154),

Table 2. *Mean molar concentrations of total volatile fatty acids (VFA), molar proportions of individual VFA and concentrations of ammonia in the rumen of cattle receiving the three fresh forages alone (treatment C), supplemented with monensin (treatment M) or treated with formaldehyde before feeding (treatment F)*

| Diet... | Perennial ryegrass (Lolium perenne cv. Melle) R1 | | | White clover (Trifolium repens cv. Blanca) | | | | | | SEM |
| | | | | W1 | | | W2 | | | |
Treatment...	C	M	F	C	M	F	C	M	F	
Mean VFA concentrations (mmol/l)	83·3	78·8	71·2	115·8	103·7	93·8	104·6	90·4	69·9	6·16
Mean molar proportions of individual VFA										
Acetate	0·69	0·64	0·69	0·67	0·64	0·71	0·69	0·65	0·69	0·0105
Propionate	0·20	0·26	0·22	0·18	0·24	0·17	0·18	0·23	0·18	0·0084
Butyrate	0·10	0·08	0·08	0·13	0·10	0·10	0·11	0·10	0·10	0·0051
Iso-butyrate	0·01	0·02	0·02	0·02	0·02	0·02	0·02	0·02	0·02	0·0016
Mean ammonia concentration (mg/l)	186·4	147·5	141·1	415·7	366·0	290·2	448·7	418·0	229·4	28·49

R1 and W1, primary growth and W2, regrowth, of the two forage species.

Table 3. *The mean quantities of organic matter, cellulose and nitrogen consumed and entering the small intestine of cattle receiving the three fresh forages alone (treatment C), supplemented with monensin (treatment M) or treated with formaldehyde before feeding (treatment F); also values for the extent of rumen digestion and animal live weights*

Diet...	Perennial ryegrass (*Lolium perenne* cv. Melle) R1			White clover (*Trifolium repens* cv. Blanca) W1			W2			SEM
Treatment...	C	M	F	C	M	F	C	M	F	SEM
Mean live weight (kg)	273	275	284	295	296	301	320	317	312	2·49
Organic matter (kg/d)										
Consumed	4·57	4·56	4·37	5·25	5·04	5·02	5·68	5·63	5·65	0·114
Entering small intestine	2·13	2·16	2·13	2·51	2·61	2·84	3·03	2·95	3·58	0·119
OM digested in rumen (g/kg OM intake)	556	536	500	498	504	440	472	450	388	21·3
Cellulose (kg/d)										
Consumed	1·34	1·34	1·28	1·24	1·18	1·17	1·42	1·41	1·41	0·033
Entering small intestine	0·35	0·34	0·31	0·24	0·26	0·25	0·34	0·31	0·34	0·021
Cellulose digested in rumen (g/kg cellulose intake)	744	750	753	801	780	795	764	774	758	13·0
N (g/d)										
Consumed	121	119	116	243	235	235	260	255	258	3·3
Entering small intestine*	102	113	116	170	173	200	178	176	229	8·7
NAN flow (g/g N intake)	0·843	0·950	1·000	0·700	0·736	0·851	0·685	0·690	0·888	0·0507

R1 and W1, primary growth and W2, regrowth, of the two forage species; NAN, non-ammonia-N.
* N in the form of NAN.

Table 4. *The mean quantities (g/d) of microbial nitrogen and estimated undegraded dietary nitrogen entering the small intestine of cattle receiving the two fresh forages alone (treatment C), supplemented with monensin (treatment M) or treated with formaldehyde before feeding (treatment F); also the calculated efficiencies of microbial N synthesis and estimates of the degradability of feed N*

	White clover (*Trifolium repens* cv. Blanca)						
	W1			W2			
Diet...							
Treatment...	C	M	F	C	M	F	SEM
Duodenal flow of							
NAN	170	173	200	178	176	229	9·5
Microbial N	119	125	143	116	107	148	13·4
Undegraded dietary N*	39	37	45	48	56	68	15·5
Efficiency of microbial N synthesis							
g/kg OMADR	45·4	51·7	62·5	42·4	42·1	71·1	7·10
g/kg OMTDR	31·2	33·7	38·0	29·5	29·6	41·5	2·81
Degradability of feed N (g/g)*	0·834	0·846	0·811	0·817	0·776	0·737	0·0633

W1, W2, primary growth and regrowth crops of white clover; NAN, non-ammonia-N; OMADR, organic matter apparently digested in the rumen; OMTDR, organic matter truly digested in the rumen.
  * Assuming an endogenous N secretion of 2 g/kg dry matter intake .

treatment F gave an average increase of 20% (F 180; $P < 0.01$). If the results are expressed as NAN flows per unit N intake to account for the small variations in N intake which occurred, the size of the treatment effect of treatment F is well illustrated (C 0·743, M 0·792, F 0·913; $P < 0.01$).

Extent of microbial N synthesis and feed N degradation
Microbial N synthesis and the extent of feed N degradation were only estimated with the two white clover diets, and these values are presented in Table 4.

On both untreated diets, microbial N represented between 0·65 and 0·7 of duodenal NAN, and the degradability of feed N was estimated to exceed 0·8; the efficiencies of microbial N synthesis averaged 43·9 g/kg OM apparently digested in the rumen (OMADR) or 30·4 g/kg OM truly digested in the rumen (OMTDR). Treatment M, which was shown to have no effect on NAN supply, equally appeared to have no effect on microbial N synthesis with respect to total amount (g/d; C 118, M 116) or efficiency (46·9 g/kg OMADR and 31·7 g/kg OMTDR), whilst there was indication of a small reduction in feed N degradability on diet W2. In contrast, treatment F enhanced microbial N synthesis by 24% as compared with the control diets (146 *v.* 118 g N/d) and this accounted for almost 70% of the increased NAN flow noted with the diet after treatment F. Consequently, efficiencies of microbial N synthesis were increased on this treatment (66·8 g/kg OMADR or 39·8 g/kg OMTDR) compared with treatments C and M, whilst degradability of feed N tended to be reduced with both diets (g/g; overall C 0·823, M 0·812, F 0·774). However, due to limited replication, none of the differences due to F application was statistically significant.

DISCUSSION

In a recent review Beever & Siddons (1986) discussed the extensive degradation of dietary N in the rumen of animals grazing high-N-containing pastures and drew attention to three possible undesirable effects on the nutrition of the host animal. First the direct loss of N from the rumen represents a net reduction in the potential supply of amino-N to the host animal and may explain the production responses which have been observed in ruminants consuming fresh forages and supplemented with protected or abomasally-infused protein (Black *et al.* 1979; Barry *et al.* 1982). Second over-supply of NH_3 to the liver may be potentially toxic and the studies of Symonds *et al.* (1981) have clearly demonstrated the consequence of hepatic NH_3 leakage when NH_3 load to the animal's tissues was elevated experimentally. Third the incidence of bloat, as seen with legume diets, may in part be related to the rapid rates of digestion and protein solubilization of fresh forage which occur in the rumen following ingestion (Beever *et al.* 1986*b*). In these regards the results of the present experiment with respect to the untreated forages confirm previous experimental findings (Beever *et al.* 1985, 1986*a*, *b*; Ulyatt *et al.* 1981).

Monesin has been used widely in the USA as a feed additive to enhance the performance of feed-lot cattle. Part of this improvement has been related to the reductions in the rumen acetate : propionate value which occur as a result of monensin inclusion in the diet (Chalupa, 1980), but evidence on the effect of monensin on protein digestion in the rumen remains equivocal. Dinius *et al.* (1976) and Poos *et al* (1979) have reported significant reductions in rumen NH_3 levels when diets are supplemented with monensin, but caution must be applied to the interpretation of such observations. Allen & Harrison (1979) examined the effect of monensin on the digestion of dried-grass pellets by sheep and reported that a reduction in acetate : propionate values from 2·99 (control) to 2·37 (treated) was associated with a 42% reduction in rumen dilution rate, an 8% increase in total VFA production and OM digestion in the rumen, and an 18% reduction in the efficiency of microbial N synthesis. Unfortunately, it is not possible from the work of Allen & Harrison (1979) to estimate the effect of monensin on NAN supply to the small intestine, but suggestions of an increased supply of undegraded dietary protein to the small intestine have been made by others (Chalupa, 1980).

In contrast, the present study demonstrated that the effect of treatment M on the digestion of fresh forage was limited to changes in the acetate : propionate value (C 3·67, M 2·65) and small depressions in rumen NH_3 concentrations. No effects on NAN supply to the small intestine, microbial N synthesis or the efficiency of microbial synthesis were detected, but in the absence of information on the effect of monensin on bacterial and protozoal numbers and their metabolic activities, and on the rates of proteolysis and deamination, it is not possible to elaborate further on this finding.

In the absence of information on microbial N synthesis, it would be reasonable to conclude that the response to treatment F was associated with an increased flow of undegraded dietary N from the rumen, as reported by Ferguson (1975), Beever *et al.* (1977), Thomson *et al.* (1981), Siddons *et al.* (1984) for concentrate diets or grass-silage diets. However, the findings for the two clover diets indicate clearly that the major part of the increased flow of NAN was due to an enhanced synthesis of microbial N. Preliminary investigations before this experiment showed that the level of formaldehyde used, and the exposure time before feeding (average 6 h) reduced buffer solubility of the dietary N by over 50%, and in the absence of information relating to proteolytic and deaminative activity in the rumen, it must be concluded that this led to a more controlled release of the dietary N. In turn this would modify the pattern of degraded N : degraded OM release in the rumen, as discussed by Beever *et al.* (1986*b*) and provide a more suitable environment for the

Table 5. *The effect of forage species, stage of harvest, monensin addition to the rumen or formaldehyde application to the diet, on the absorption of non-ammonia-nitrogen (NAN) from the small intestine in relation to metabolizable energy supply (g NAN/MJ ME per d)*

Diet...	Perennial ryegrass (*Lolium perenne* cv. Melle)	White clover (*Trifolium repens* cv. Blanca)	
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Treatment	R1	W1	W2
Control	1·09	1·68	1·53
Monensin	1·19	1·73	1·59
Formaldehyde	1·30	1·97	2·01

R1 and W1, primary growth and W2, regrowth, of the two forage species.

effective capture of degraded N by the rumen micro-organisms, thus leading to an enhanced synthesis of microbial N. For the white clover diets examined by Beever *et al.* (1986*b*), microbial synthesis averaged 0·51 g/g N intake whilst in the present study values averaged 0·47 g/g N on treatments C and M and 0·59 g/g on treatment F. Further evidence that treatment F had its major effect on microbial synthesis is available from the findings relating to the rumen digestion of cellulose, which gave no indication of an impaired digestion on any of the three treated forages, whilst at least two-thirds of the extra OM flowing into the small intestine on the two white clover diets could be accounted for by an increased synthesis of microbial OM, assuming a N:OM value in microbial biomass of 0·1 (R. C. Siddons, personal communication).

In the present study no estimates of digestible energy (DE) or metabolizable energy (ME) contents of the diets were obtained, but using the values for digestibility and ME:DE provided by Beever *et al.* (1985) and Cammell *et al.* (1986) for similar diets, along with an average availability of duodenal NAN in the small intestine of 0·63 (MacRae & Ulyatt, 1974), the effect of forage species and the treatments imposed on absorbed NAN/ME supply can be estimated. These values are presented in Table 5. On the untreated diets values ranged from 1·09 (R1) to 1·53 (W2) and 1·68 (W1) g NAN/MJ ME, which were marginally lower than the values of 1·26 and 1·82 g N/MJ for grasses and clovers respectively reported by Beever *et al.* (1985). Treatment M gave a 9% increase in the supply of NAN/MJ ME on diet R1 whilst the values on diets W1 and W2 were not significantly different from those obtained on the control diet. Treatment F, on the other hand, was estimated to increase NAN supply per MJ ME by 19, 17 and 31% respectively on the three diets, such that across all nine treatments, a twofold range in values (1·09–2·01 g/MJ) was detected in response to change in forage species and treatment F.

Clearly such results indicate that microbial degradation and assimilation of N in the rumen of cattle offered fresh forages can be beneficially manipulated by formaldehyde treatment. However, the approach does not offer a practical means by which the effect can be achieved, particularly since the effects would be of greatest economic importance to animal production systems which place heavy reliance on the use of grazed forages. Appropriate technology to reduce N loss on fresh forages does not appear to exist at present, but the sizes of the responses observed in the present study suggest that development of such technology would be a worthwhile objective for those interested in improving the nutritive value of grazed forage.

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