The digestion of fresh perennial ryegrass (*Lolium perenne* L. cv. Melle) and white clover (*Trifolium repens* L. cv. Blanca) by growing cattle fed indoors

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1. Pure swards of perennial ryegrass (*Lolium perenne* L. cv. Melle) or white clover (*Trifolium repens* L.cv. Blanca) were harvested daily at three and two stages of growth respectively, and offered to housed cattle. The grass diets comprised primary growth (May) and two later regrowths of contrasting morphology (i.e. leaf:stem values of 1·54 and 2·84 respectively), and were characterized by high contents of water-soluble carbohydrate and neutral-detergent fibre and comparable in vitro dry matter (DM) digestibilities (mean 0·80). Total nitrogen content was higher on primary growth grass (34 g/kg DM) than on regrowths (23 g/kg DM) but lower than values obtained for the two clover diets (38 and 43 g/kg DM, respectively). The clover diets had lower water-soluble carbohydrate contents than the grasses, comparable cellulose, but lower neutral-detergent fibre contents and in vitro DM digestibilities of 0·70 and 0·77 respectively.

2. The experiment lasted from May until August, during which time a total of twenty-one young Friesian steers (initial average live weight 130 kg) were used to determine both nutrient supply to the small intestine (twelve animals) and apparent digestibility (nine animals). Each diet was offered at three levels of DM intake (i.e. 18, 22 and 26 g/kg live weight). A further six steers, all fed at the rate of 22 g DM/kg live weight, were used to determine the metabolizable energy contents of the five diets by means of open-circuit calorimetry.

3. The three grass diets and the later-cut clover had, as intended, quite similar in vivo organic matter digestibilities, but that of the earlier-cut clover was lower, and this was associated with a large number of flower heads in this crop at the time of feeding.

4. On the clover diets, proportionately less of the ingested organic matter appeared to be digested in the rumen (0·40) compared with the grass diets (0·58) ($P < 0·001$). On the high-N primary grass and the clover diets, substantial rumen losses of N were detected ($P < 0·01$) compared with regrowth grasses.

5. The metabolizable energy content of the primary growth of grass was 12·2 MJ/kg DM, whilst the values for the other two grass diets were lower (11·6 MJ/kg DM), despite no marked decline in overall energy digestibility. Values for the two clover diets (mean 10·5 MJ/kg DM) were considerably lower than all values noted for the grasses.

6. The amount of absorbed non-ammonia-N expressed per MJ metabolizable energy averaged 1·24 g/MJ on the grass diets, with no apparent seasonal effects, compared with 1·79 g/MJ on the two clover diets.

7. Reasons for variations in nutrient supply and the consequence of considerable rumen losses of ingested N on the high-N diets are discussed, and the contribution that white clover can make to overall protein supply in grazing ruminants is established.

Despite the fact that most of the total forage utilized by ruminants throughout the world is consumed *in situ* in the fresh form by grazing animals, remarkably few in vivo experiments have been undertaken to examine the nutritive value of such materials. Several studies have reported the digestion characteristics of frozen forages, so used to represent fresh ones, fed to sheep (Beever *et al.* 1971, 1976) and for zero grazed grasses and legumes harvested daily, also fed to sheep (MacRae & Ulyatt, 1974; Ulyatt & MacRae, 1974; Beever *et al.* 1974). Furthermore, examination of the effect of stage of growth of fresh forage on digestion and nutrient supply has been limited. Beever *et al.* (1978) examined frozen harvests of spring

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and autumn ryegrass (*Lolium perenne* L.) fed to sheep, and recently Verite *et al.* (1984) briefly reported studies in which seven harvests of fresh ryegrass were offered indoors to dairy cows to examine the sites of organic matter and protein digestion.

Whilst limited in number, these experiments have established that both the species of forage and their stage of growth can have dramatic effects on nutrient digestion and absorption, particularly with respect to the fate of the nitrogenous fractions (Beever & Siddons, 1985). The present study comprised part of a larger study to investigate the effect of forage species and stage of growth on nutritive value. In this experiment, three growth stages of perennial ryegrass, followed by two of white clover (*Trifolium repens* L.), all of intended similar digestibility, were harvested daily and fed in the fresh, unchopped form to growing cattle in order to obtain quantitative information on the digestive fate of the organic matter, carbohydrate and protein components. Part of this work has been briefly reported (Beever *et al.* 1981).

EXPERIMENTAL

Management of swards and diet preparation

The two pasture species used were perennial ryegrass (cv. Melle) and white clover (cv. Blanca). The perennial ryegrass pasture (5·5 ha) was sown in July 1976. On 23 March 1979, before the experiment, the crop received a top dressing of 120 kg nitrogen/ha as a N–phosphorus–potassium (20:10:10, w/w) compound fertilizer and the field was subsequently divided into ten plots: five of 0·4 ha each for cutting for the indoor feeding experiment reported in the present paper and five of 0·65 ha to provide feed for a concurrent grazing experiment (M. J. Ulyatt, D. J. Thomson and D. E. Beever unpublished results). Subsequently, a further application of 80 kg N/ha as Nitrochalk was given on 2 May 1979. The white-clover pasture (5·7 ha) was sown in July 1978 and purity was achieved by spraying a selective herbicide (Kerb, Pan Brittanica Industries, Waltham Cross, Herts; 1·4 kg/ha) in February 1979. The clover received a top dressing of 250 kg/ha of N–P–K (0:24:24, w/w) on 5 April 1979 and was also divided into ten plots: five of 0·44 ha for the indoor feeding experiment and five of 0·65 ha for the concurrent grazing experiment. The ryegrass plots, which were harvested for indoor feeding, received a further application of 60 kg N/ha following each mechanical defoliation.

Perennial ryegrass was harvested at three distinct stages of growth to provide three grass feeds of contrasting chemical and morphological characteristics. Primary growth was offered during period 1 (2–23 May) (R1) and this was followed by a regrowth crop (period 2, 24 May–13 June) (R2) after the sward had been lightly topped by mechanical means in late April. Finally, a second regrowth was examined in period 3 (14 June–4 July) (R3). In order to establish regrowth materials of an average maturity, a weekly grass cutting sequence was initiated 3 weeks in advance of the animal feeding, both in periods 2 and 3. However, grass (and also clover; see below) required during the final 2 weeks in each of these periods was pre-trimmed on the same date so that for the penultimate and the final week of each period the animals were offered material of 3–4 weeks and 4–5 weeks growth respectively. This procedure was adopted to ensure that abrupt changes in composition of the feeds did not occur during measurement periods.

White clover was fed at two stages of growth in period 4 (5 July–1 August) (W1) and period 5 (2–30 August) (W2). All clover plots were lightly trimmed (to 20 mm) on 19 June and those plots used for period 5 were mechanically harvested on 12 July before establishment of the requisite crops. The clover plots were irrigated (to 25 mm (1 in) soil moisture deficit) as necessary during a period when rainfall was negligible.

Each plot of forage was planned to provide feed for 1 week. Surplus material at the end of each week was removed with a forage harvester.

For the feeding experiment, forage was cut once daily at 07.30 hours with a drum mower at a height approximately 50 mm above ground level. The cut herbage was then picked up, without chopping, by a self-loading forage wagon and delivered to the animal house. Each forage was then mixed and the following samples taken: (*a*) duplicate samples (100 g), which were dried for 24 min in a modified microwave oven (Toshiba) to provide a rapid measure of dry matter (DM) content for calculating daily DM allowances; (*b*) triplicate samples (200 g), dried at 100° for 24 h in a forced-draught oven for accurate estimate of DM content; (*c*) a sample (1 kg), stored frozen for subsequent botanical and chemical analysis.

When the rapid DM content was known the daily allowances were calculated and weighed out as two equal feeds per animal. One portion was fed at 09.00 hours and the other stored at 4° and fed later at 16.30 hours.

Refusals, if any, were removed daily at 08.30 hours, weighed and, during measurement periods only, dried at 100° for 24 h to determine actual DM intake.

Animals

A total of twenty-seven castrated Friesian male calves aged 4–5 months and weighing approximately 130 kg at the start of the experiment were used. They were kept unrestrained indoors in individual pens and had free access to water. Twelve calves were each prepared with a rumen cannula (PVC, 13 mm i.d.) and a T-piece cannula (PVC, 20 mm i.d.) in the proximal duodenum anterior to the bile duct (digestion group). A further nine calves were used for digestibility and balance trials (balance group), whilst the remaining six calves were used for measurement of methane production in open-circuit respiration chambers (calorimetry group).

When the calves were fed on white clover they were dosed orally each day at 09.00 and 16.00 hours with 5 ml poloxalene (980 ml/l; Smith, Kline & French) to prevent legume bloat. All calves were weighed weekly.

Experimental design and sampling

The experiment comprised five periods (corresponding to forages) in the sequence described previously. The digestion and balance groups were each divided into three subgroups of four and three calves respectively, and fed at three levels of DM intake: viz. (L) 18·0, (M) 22·0, and (H) 26·0 per kg mean subgroup body-weight. These calves were re-randomized to different intake levels whenever periods/diets changed. The six calves destined for measurement of CH_4 production were fed at 22 g/kg body-weight only.

The measurement sequence within each experimental period is given in Table 1. The final 13 d of each period were used for sampling and all previous days were regarded as a preliminary feeding period.

A digestibility trial was conducted with the nine calves in the balance group for a duration of 10 d during each period (Table 1). Total faeces were collected and weighed daily from all calves (Thomson *et al.* 1981) and held frozen daily before bulking (10% fresh weight) over the collection period. At this stage appropriate samples were freeze-dried for subsequent analysis and three 400 g samples were oven-dried for determination of DM content. Urine collections were only made from the group at the 22 g/kg intake level. Urine was collected via a rubber funnel attached to the harness, and a time-controlled peristaltic pump (Watson Marlow) was used to drain the funnel for 30 s in every minute into a collection vessel, into which 200 ml of 10 M-sulphuric acid/d was added as a urine preservative before urine collection. Urine was weighed daily, thoroughly mixed, and bulked over the collection period on the basis of 1% of the daily excretion.

CH_4 production was measured in the calorimetry group of cattle by means of open-circuit respiration chambers during the period shown in Table 1, using the techniques described by Cammell *et al.* (1981). All calves were allowed 1 d of acclimatization to the chambers

Table 1. *Sequence of procedures within each experimental period*

Day of week...	F	S	S	M	T	W	T	F	S	S	M	T	W
Animal group													
Balance	—	D	D	D	D	D	D	D	D	D	D	—	—
Calorimetry	—	—	M_p	M_m	M_m	M_p	M_m	M_m	—	—	—	—	—
Digestion													
Subgroup A	I_s	—	—	—	—	C	C	I_f	—	—	—	—	—
Subgroup B	—	—	—	—	—	I_s	—	—	—	—	C	C	I_f

D, digestibility measurement period; M, methane measurement indicating preliminary (p) and main (m) periods of estimation; I, rumen infusion started (s) and finished (f); C, collection of duodenal digesta.

(M_p) before 2 d of measurement (M_m). For each period a total of four calves, each for 2 d, was used.

For the determination of duodenal flow, two indigestible markers, as proposed by Faichney (1975), were used. Rumen infusions of these markers to the calves in the digestion group commenced 5 d before duodenal sampling and continued for the whole collection period. During the infusions the calves were tethered and infusion rates of approximately 20 ml/h were established by means of a multi-channel peristaltic pump with an infusate concentation sufficient to supply 120 mg chromium/kg DM intake (DMI) as CrEDTA and 12 mg ruthenium/kg DMI as Ru-phenanthroline (Faichney, 1975). In each period all duodenal digesta collections were undertaken during a 7 d time interval, in two groups each of six calves (comprising two calves from each intake level) on days 1 and 2, and 6 and 7 respectively as illustrated in Table 1, to maximize the use of a limited number of duodenal digesta samples (Evans *et al.* 1981). During each sampling day duodenal digesta collections were made at regular intervals (approximately 15 min) using the automatic sampler, commencing at 09.00 hours on each day. Samples of accumulated digesta were removed from the sampler at approximately 16.00 hours and again at 09.00 hours on the next day to provide a 24 h (09.00–09.00 hours) bulked sample. Between 2 and 6 litres duodenal digesta were collected each day, with the intention of not exceeding 5% of total flow. The bulked samples for each day were then thoroughly mixed and 800 ml were centrifuged for 15 min at 15000 ***g***, followed by separation of the supernatant and solid fractions. Subsequently, the supernatant fraction was frozen and the solid fraction (centrifuged digesta) frozen and freeze-dried. Additionally, 1 l of representative digesta was frozen directly and freeze-dried to give a sample of whole digesta.

Analyses

Samples of the frozen feeds were thawed slowly overnight and botanical separations, as outlined in Table 2 (p. 768) were carried out in triplicate.

All freeze-dried samples of feed, duodenal digesta and faeces were ground to pass through a 1 mm sieve before chemical analysis, and the duodenal samples required for Ru analysis were further ball milled and finally pelleted (Beever *et al.* 1978).

In vitro DM and organic matter (OM) digestibilities of the feeds were determined by the method of Tilley & Terry (1963) and water-soluble carbohydrate contents by an automated ferricyanide procedure (Technicon Instruments Co. Ltd, 1969). OM contents of all feed, digesta and faecal samples were determined by ashing at 550° for 16 h, and gross energy (GE) contents determined by means of adiabatic bomb calorimetry. Urine energy values were similarly estimated after freeze-drying in order to permit satisfactory combustion. N

content of all samples was determined by microKjeldahl and ammonia content of duodenal digesta by an alkaline phenate method (Gehrke *et al.* 1968). Cellulose contents of feed, digesta and faecal samples were estimated according to the method of Crampton & Maynard (1938), neutral- and acid-detergent fibre contents of the feeds by the method of Van Soest & Wine (1967), and pectin contents by the method of McComb & McReady (1952).

Ru contents of infusates and digesta were estimated by X-ray fluorescence as described by Evans *et al.* (1977) and Cr content of the same samples by a modified autoanalyser technique based on the method of Christian & Coup (1954).

Calculation of results

Estimates of intake relating to individual daily duodenal collections were obtained as the mean of the intakes recorded during the day of the collection and the preceding 2 d. Duodenal flows were estimated by the dual phase marker technique proposed by Faichney (1975) and measurements of CH_4 production were corrected to the intakes observed on the animals in the balance trials on the basis of the intakes measured during the days when CH_4 estimates were made in the calorimeters.

Statistical analysis

The results were subjected to analysis of variance to compare forages (grass *v.* clover), levels of intake, stages of growth and interactions. Further comparisons were made using regressional analysis of nutrient flow on nutrient intake.

RESULTS

The botanical and chemical compositions of the perennial-ryegrass and white-clover swards as harvested and fed to the cattle indoors are presented in Table 2.

The swards were relatively weed-free and percentage purity was in excess of 97%. The pre-trimming treatment of the perennial ryegrass was designed to produce a range of leaf:stem values at approximately the same digestibility. The ratios of 4·2:1, 1·5:1, 2·8:1 obtained for periods 1, 2 and 3 respectively (together with the digestibility values in Table 2) indicate that this was achieved. The in vitro DM and OM digestibilities of the grasses did not differ significantly between the three periods, but the white clover harvested in period 4 was more mature than intended with a high proportion of flowering-stems and seed heads. This was associated with a lower in vitro digestibility than both the other clover (W2) and the three grasses. The grasses were characterized by higher water-soluble carbohydrate and neutral-detergent fibre contents, but lower N contents than the clovers, with the exception of the primary growth harvested in period 1, which had a high content of N. The cellulose contents were similar between the species, with the possible exception of a higher value for the grass in period 2, whilst the two clover diets had marginally higher ash contents. As expected, diet W1 had a high content of lignin, and pectin levels in both clover diets were higher than those found in the grasses.

Live-weight changes and feed consumption

Values in Table 3 indicate that over the experimental feeding period the animals gained on average 48 kg, equivalent to approximately 0·44 kg/d. At the two lowest levels of intake, feed refusals were small and the intakes shown in Table 3, which were on average 1·0–2·0 g DM/kg live weight below the intended levels, are a reflection of the actual allocations being somewhat lower than planned, due to the accepted difficulty of accurately estimating relative DM content at the time of feeding. On the other hand, at the highest level of feeding, the primary growth and the trimmed primary growth of perennial ryegrass

Table 2. *The botanical and chemical composition of fresh perennial ryegrass* (Lolium perenne *L. cv. Melle*) (*R*) *and white clover* (Trifolium repens *L. cv. Blanca*) (*W*) *fed to Friesian steers*

Diet...	R1	R2	R3	W1	W2
Botanical composition (g/kg DM)					
Leaf*	79·2	57·7	70·9	59·7	87·6
Stem	18·8	37·3	25·0	—	—
Dead	0·8	2·7	1·8	3·0	3·4
Flower head	—	—	—	36·7	7·0
Purity	98·8	97·7	97·7	99·4	98·0
Chemical composition (g/kg DM)					
Ash	96	90	87	105	110
Water-soluble carbohydrate	135	143	215	75	79
Cellulose	225	264	225	238	213
Neutral-detergent fibre	440	505	438	330	252
Acid-detergent fibre	242	286	248	296	219
Lignin	17	22	23	58	6
Pectin	0·5	0·7	1·3	3·8	5·1
Total nitrogen	34	24	22	38	43
Gross energy (MJ/kg DM)	19·37	18·90	18·81	18·75	18·80
DM digestibility (in vitro)	810	780	795	709	767
OM digestibility (in vitro) (g/kg OM)	781	744	774	681	734

DM, dry matter; OM, organic matter.
* Includes petiole for clover.

Table 3. *The live weight* (*kg*) *and dry-matter intake* (*g/kg live weight*) *of Friesian steers fed on fresh perennial ryegrass* (Lolium perenne *L. cv. Melle*) (*R*) *and white clover* (Trifolium repens *L. cv. Blanca*) (*W*) *at three planes of nutrition*

Experimental period...	1	2	3	4	5
Diet*...	R1	R2	R3	W1	W2
Daily intake level					
		Live wt			
L	156	163	184	189	209
M	154	178	180	200	210
H	170	174	177	201	206
		Dry-matter intake			
L	16·1	16·9	16·9	17·8	17·2
M	19·1	19·4	20·3	20·8	21·0
H	22·6	23·8	25·1	25·2	24·9

L, 18 g/kg live wt; M, 22 g/kg live wt; H, 26 g/kg live wt.
* For details, see Table 2 and p. 764.

Table 4. *The apparent digestibility of the organic matter (OM), energy, cellulose and nitrogen of fresh perennial ryegrass* (Lolium perenne *L. cv. Melle*) *(R) and white clover* (Trifolium repens *L. cv. Blanca*) *(W) fed at three planes of nutrition to Friesian steers*

Daily intake level	Diet*					
	R1	R2	R3	W1	W2	SEM
			OM digestibility			
L	0·841	0·801	0·821	0·741	0·785	
M	0·834	0·811	0·828	0·747	0·778	0·0068
H	0·825	0·796	0·800	0·725	0·773	
			Energy digestibility			
L	0·800	0·751	0·771	0·697	0·734	
M	0·787	0·770	0·782	0·703	0·726	0·0093
H	0·778†	0·756	0·749	0·681	0·719	
			Cellulose digestibility			
L	0·895	0·837	0·847	0·741	0·810	
M	0·892	0·863	0·855	0·744	0·815	0·0061
H	0·889	0·859	0·827	0·722	0·807	
			N digestibility			
L	0·797	0·721	0·704	0·778	0·813	
M	0·782	0·735	0·715	0·778	0·808	0·0082
H	0·763	0·717	0·689	0·741	0·794	

L, 18 g/kg live wt; M, 22 g/kg live wt; H,26 g/kg live wt.
* For details, see Table 2 and p. 764.
† Mean of two animals only.

were consumed at approximately 23 g/kg body-weight, indicating feed refusals equivalent to between 5 and 10% of total feed offered. On diet R3 and the two clover diets, feed consumption levels were almost 10% higher (mean 25 g/kg) and feed refusals were negligible.

Digestibility

The digestiblities of the OM, energy, N, and cellulose fractions at the three planes of nutrition are presented in Table 4.

The effect of plane of nutrition was evident with respect to OM, energy and N digestibilities on all diets, but overall digestibilities of level H were only 1·8–2·8% lower than the values noted on levels L and M ($P < 0·05$). No statistically significant differences between intake levels were detected for cellulose. Within the grasses, OM, energy and cellulose digestibilities tended to be highest on R1 ($P < 0·05$) compared with R2 and R3 which did not significantly differ from each other, whereas overall N digestibility declined from 0·78 (R1) to 0·72 (R2) and 0·70 (R3) ($P < 0·05$), presumably as a result of the lower contents of N present in diets R2 and R3. The primary-growth clover (W1) had considerably lower OM, energy and cellulose digestibilities than all other diets and all values (including N) were significantly lower than those observed for W2 ($P < 0·01$). Diet W2 had OM, energy and cellulose digestibilities less than those found on the three grass feeds, but a higher N digestibility (0·81) than all other diets.

Partition of ingested energy

Values obtained at the 22 g/kg body-weight level of feeding for the partition of ingested energy are presented in Table 5. With increased body-weight, total GE intake increased from

Table 5. *The contents of digestible energy (DE) and metabolizable energy (ME) in the perennial ryegrass* (Lolium perenne *L. cv. Melle*) *(R) and white clover* (Trifolium repens *L. cv. Blanca*) *(W) diets offered at an intake level of 22 g/kg per d to Friesian steers*

Diet*	Gross energy (GE) intake (MJ/d)	Faecal energy (MJ/d)	Urine energy (MJ/d)	Methane energy† (MJ/d)	Percentage of DE as Methane	Percentage of DE as Urine	ME/MJ GE	ME/MJ DE	ME/kg DM
R1	56·74	12·08	5·21	3·68	8·2	11·7	0·630	0·801	12·20
R2	64·97	14·98	4·59	5·14	10·3	9·2	0·620	0·805	11·72
R3	66·27	14·41	5·10	5·41	10·4	11·6	0·611	0·780	11·49
W1	79·03	23·49	5·82	5·65	10·2	10·5	0·558	0·793	10·46
W2	81·01	22·18	7·02	5·97	10·1	11·9	0·566	0·779	10·64
SEM		0·426	0·686						

* For details, see Table 2 and p. 764.
† Measurements derived from calorimetry group of animals.

Table 6. *The mean quantities of organic matter (OM) consumed and entering the small intestine of Friesian steers fed on fresh perennial ryegrass* (Lolium perenne *L. cv. Melle*) *(R) and white clover* (Trifolium repens *L. cv. Blanca*) *(W) at three planes of nutrition, along with the proportional digestion of ingested OM in the rumen*

Daily intake level	Diet* R1	R2	R3	W1	W2	SEM
			OM intake (kg/d)			
L	2·28	2·51	2·83	3·01	3·20	
M	2·70	3·15	3·33	3·73	3·92	0·032
H	3·47	3·77	4·06	4·54	4·56	
			OM entering the duodenum (kg/d)			
L	0·92	1·07	1·21	1·72	1·87	
M	1·11	1·32	1·48	2·16	2·20	0·087
H	1·33	1·46	1·92	2·84	2·97	
			OM apparently digested before the duodenum (kg/kg intake)			
L	0·60	0·57	0·57	0·43	0·42	
M	0·59	0·58	0·56	0·42	0·44	0·024
H	0·62	0·61	0·53	0·37	0·35	

L, 18 g/kg live wt; M, 22 g/kg live wt; H, 26 g/kg live wt.
* For details, see Table 2 and p. 764.

57 (period 1) to 81 (period 5) MJ/d, and faecal, CH_4 and urine energy losses increased accordingly. On diets 2–5, CH_4 energy mounted to 10·3% of digestible energy (DE), compared with a value of only 8·2% for primary growth ryegrass (period 1), while urine energy values for diets 1, 3 and 5 varied little between 11·6 and 11·9% of DE, compared with only 9·2 and 10·5% on diets 2 and 4 respectively. Overall metabolizable energy (ME):DE varied only between 0·78 and 0·80 with the lower values being on the regrowth grass and the two clover diets. The primary-growth grass was found to have an ME value of 12·2 MJ/kg DM with an estimated metabolizability (q = ME/GE) of 0·63. Advancing

Table 7. *The mean quantities (g/d) of nitrogen consumed and of non-ammonia-N (NAN) entering the small intestine of Friesian steers fed on fresh perennial ryegrass* (Lolium perenne *L. cv. Melle*) *(R) and white clover* (Trifolium repens *L. cv. Blanca*) *(W) at three planes of nutrition*

Daily intake level	Diet*					
	R1	R2	R3	W1	W2	SEM
			N intake			
L	84·9	65·8	69·2	126·3	156·2	
M	100·4	82·6	81·3	157·2	191·2	1·37
H	129·4	99·1	99·3	191·1	222·3	
			NAN flow at duodenum			
L	59·2	62·4	67·8	92·8	110·7	
M	75·2	81·4	89·6	112·6	132·9	6·36
H	93·1	86·1	114·0	160·7	181·6	
			N intake less NAN flow at duodenum (g/g N intake)			
L	0·30	0·05	0·02	0·27	0·29	
M	0·25	0·01	−0·10	0·28	0·30	0·058
H	0·28	0·13	−0·15	0·16	0·18	

L, 18 g/kg live wt; M, 22 g/kg live wt; H, 26 g/kg live wt.
* For details, see Table 2 and p. 764.

season gave ME values of 11·7 and 11·5 for the two grass diets, with negligible effects on *q*. The mature clover (W1) had lower values for both ME (10·5) and *q* (0·56) whilst, rather surprisingly, the values for the later-cut clover were similar (10·6 and 0·57).

Partition of OM digestion

The results for OM intake, flow at the duodenum and apparent digestion before the intestines are presented in Table 6. Between periods 1 and 5, OM intake increased from 2·28 to 3·20 kg/d on level L, and from 3·47 to 4·56 g/d on level H. As a consequence of these changes, estimates of OM flow to the small intestine varied between 0·92 (R1L) and 2·97 (W2H) kg/d, indicating an apparent digestion of OM in the rumen equivalent to 8·7, 10·3 and 12·5 g/kg live weight for levels L, M and H respectively. Corresponding mean values of 6·6, 8·0 and 8·1 g/kg live weight for the two clover diets were all significantly ($P < 0·01$) lower.

On the grass diets, OM apparently digested in the rumen amounted to between 0·53 and 0·62 of OM intake. Effects due to level of feeding (0·58, 0·58 and 0·59 for levels L, M and H respectively) and stage of maturity (0·60, 0·59 and 0·55) were small and not significantly different from the mean overall value of 0·58. On the other hand, values on the clover diets were all significantly ($P < 0·001$) lower (mean value 0·40) and, although the effect of stage of maturity was not detectable (0·41 and 0·40 respectively; $P > 0·05$), a marked reduction at the highest level of feeding was observed (0·42, 0·43 and 0·36 respectively; $P < 0·01$).

N digestion

Values presented in Table 7 relate to N intake and the flow of non-ammonia-N (NAN) to the small intestine. N intakes showed a threefold range across all diets, with the lowest and highest values being observed on the 2 regrowth grasses and W2 respectively. On diets R1, W1 and W2, NAN flows at the duodenum were all substantially less than the respective intake of N, indicating apparent losses of N between the diet and the duodenum. The

Table 8. *Regression equations describing the amounts of organic matter (OM;Y_{OM}) and non-ammonia-nitrogen (NAN;Y_{NAN}) entering the small intestine from OM intakes (X_{OM}) and N intakes (X_N) in Friesian steers fed on perennial ryegrass* (Lolium perenne *L. cv. Melle*) *(R) and white clover* (Trifolium repens *L. cv. Blanca*) *(W) (g/kg live weight per d)*

Experimental period	Diet		Statistical significance	r	SE_Y
		OM			
1	R1	$Y_{OM} = 0{\cdot}331(SE\ 0{\cdot}049)\ X_{OM} + 1{\cdot}182(SE\ 0{\cdot}879)$	***	0·904	0·131
2	R2	$Y_{OM} = 0{\cdot}298(SE\ 0{\cdot}072)\ X_{OM} + 2{\cdot}009(SE\ 1{\cdot}336)$	**	0·810	0·208
3	R3	$Y_{OM} = 0{\cdot}568(SE\ 0{\cdot}113)\ X_{OM} - 2{\cdot}192(SE\ 2{\cdot}098)$	***	0·858	0·304
4	W1	$Y_{OM} = 0{\cdot}741(SE\ 0{\cdot}108)\ X_{OM} - 2{\cdot}781(SE\ 2{\cdot}089)$	***	0·908	0·310
5	W2	$Y_{OM} = 0{\cdot}754(SE\ 0{\cdot}113)\ X_{OM} - 2{\cdot}830(SE\ 2{\cdot}143)$	***	0·904	0·331
		NAN			
1	R1	$Y_{NAN} = 0{\cdot}773(SE\ 0{\cdot}106)\ X_N - 0{\cdot}033(SE\ 0{\cdot}070)$	***	0·917	0·010
2	R2	$Y_{NAN} = 0{\cdot}628(SE\ 0{\cdot}277)\ X_N + 0{\cdot}144(SE\ 0{\cdot}136)$	*	0·603	0·021
3	R3	$Y_{NAN} = 1{\cdot}406(SE\ 0{\cdot}344)\ X_N - 0{\cdot}146(SE\ 0{\cdot}155)$	**	0·806	0·023
4	W1	$Y_{NAN} = 1{\cdot}090(SE\ 0{\cdot}193)\ X_N - 0{\cdot}256(SE\ 0{\cdot}157)$	***	0·872	0·023
5	W2	$Y_{NAN} = 0{\cdot}983(SE\ 0{\cdot}159)\ X_N - 0{\cdot}217(SE\ 0{\cdot}138)$	***	0·902	0·021

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

differences between total N intake and duodenal NAN flows were on average 29, 36 and 48 g/d for diets R1, W1 and W2 respectively, i.e. 0·28, 0·24 and 0·26 of total N intakes. In contrast, on diet R2, whilst NAN flows to the small intestine were all less than N intake, the differences between total N intake and duodenal NAN flow amounted to between 0·01 and 0·13 of N intake. On diet R3, NAN flows were on average 0·08 greater (range −0·02 to +0·15) than N intake, this effect being most pronounced at intake levels M and H. Consequently, the rumen losses of N, expressed per unit N intake, and including duodenal NH_3-N, were significantly greater in diets R1, W1 and W2 compared with diets R2 and R3 ($P < 0{\cdot}01$) whilst, overall, the losses seen with clover were significantly greater than those seen with the grass diets ($P < 0{\cdot}05$).

DISCUSSION

The major aim of this experiment was to investigate the digestion and nutrient supply achieved from two contrasting forage species, each examined at different stages of growth. Within this framework, it was the intention to provide forages of similar OM digestibilities but with contrasting chemical and morphological compositions. This was achieved with the three grass diets as reflected in the compositional (Table 2) and OM (Table 4) digestibility values. Unfortunately, attempts were less successful with the white-clover diets. The clover sward was in its first year of establishment after sowing during the previous summer, and early growth in the year of the experiment was disappointing such that adequate ground cover was not evident until early June. At this stage, all the white cover was lightly topped and a vigorous period of growth ensued but by the time of the first measurement period (July) extensive flower formation was observed. This resulted in a significant decline in OM digestibility as noted in Table 4. The problem was not experienced with the later-cut clover.

The experiment lasted approximately 4 months and there were no significant problems with animal health or diet consumption. All the animals grew at satisfactory rates,

considering that during the experiment each animal received at least one period of quite severely restricted intake. The levels of intake recorded on the highest level of allowance for diets R1 and R2 were significantly less than those noted for the other three diets. Reduced intakes of ryegrass in early season has been observed in other experiments (D. E. Beever, unpublished results) but as yet no satisfactory explanation is available.

From these studies it appeared that forage species had a major effect on the site of digestion of the ingested OM, whilst both forage species and stage of growth influenced NAN flow to the small intestine. On the grass diets, approximately 0·58 of ingested OM appeared to be digested in the rumen. Using estimates of OM digestibility presented in Table 4 to compute digestible OM intakes (DOMI) relating to the values presented in Table 6, it can be calculated that rumen digestion of OM accounted for 0·72, 0·74 and 0·68 of DOMI respectively for diets R1, R2 and R3. These values are in general agreement with those produced earlier for similar ryegrass diets offered to sheep (Beever *et al.* 1976) but somewhat higher than those reported by Corbett *et al.* (1982) for sheep grazing Phalaris or native pasture. On the other hand, similar calculations for the two white-clover diets revealed mean values of 0·56 and 0·51 respectively, for the proportion of DOMI apparently digested in the rumen. These results are contrary to the findings of Ulyatt & MacRae (1974), who reported mean values of 0·64 and 0·65 for Ruanui perennial ryegrass and white clover respectively when fed to sheep, compared with a value of 0·55 for Manawa ryegrass. They are also contrary to the general relation produced by Thomson & Beever (1980) who, for a series of fresh forages fed to sheep, estimated a mean value of 0·60 and were unable to detect any differences which could be attributed to either forage species or level of feeding.

If this difference in OM digestion between grass and clover is real, it must be considered to reflect either differences in passage of undegraded dietary OM from the rumen, or differences in synthesis of microbial biomass within the rumen. Some support for the first suggestion does exist (Moseley & Jones, 1984) but in a recent review Beever & Siddons (1985) were unable to demonstrate, on the basis of experiments where net microbial synthesis had been measured, any support for the suggestion of a reduced rumen digestion of fresh legumes. For the range of diets considered, the proportion of DOMI truly digested in the rumen exceeded 0·85 in every instance, and the apparent reduction in the proportion of DOMI apparently digested in the rumen observed for some of the white-clover diets could be totally accounted for by an elevated duodenal OM flow arising from an increased outflow of microbial OM. As yet, direct evidence to support this suggestion is not available, but it does appear compatible with the high rates of rumen digestion noted for white-clover diets by Cammell *et al.* (1983) and an extensive rumen digestion of readily fermentable and structural carbohydrates reported by Ulyatt & MacRae (1974) for both perennial ryegrass and white clover fed to sheep.

For three of the diets examined (R1, W1 and W2), significant rumen losses of N were observed, amounting to almost 0·3 of N intake. These occurred on the highest-N diets, all of which had in excess of 35 g N/kg OM, whereas for diets R2 and R3, which had N contents of 26 and 24 g N/kg OM respectively, either negligible losses or small gains of N across the rumen were detected. Similar results have been obtained with fresh forages fed to sheep. MacRae & Ulyatt (1974) reported rumen losses equivalent to 0·24 and 0·19 of N intake for Ruanui ryegrass and white clover respectively, whilst for Manawa ryegrass, no significant rumen loss of N was observed.

All these studies provide an overall balance of rumen digestion but from them it is only possible to speculate on causal mechanisms. As stated previously, diets R1, W1 and W2 were all characterized by high contents of N, and on the basis of studies by Mangan (1982) and Ulyatt *et al.* (1985) it appears reasonable to conclude that much of this N would be rapidly released during mastication and thus readily degraded in the rumen. From the

present results it may be estimated that degraded N:degraded OM values varied between 45 g/kg (R1) and 62 g/kg (white-clover diets), all of which would on the basis of current knowledge supply adequate degraded N to support active and efficient microbial growth. Equally, on the basis of these estimates, it would appear that significant quantities of dietary N may not be used for microbial synthesis and would be absorbed as ammonia, so contributing to the considerable rumen loss of N observed on the high-N diets used in the present study.

The estimates of energy partition of the grass diets were, on the basis of the composition of the forages examined, very much as expected, with ME values declining with season from 12·2 to 11·5 MJ/kg DM. The small decline in energy digestibility noted on R2 and R3 compared with R1 contributed to this, but diets R2 and R3 both had notably lower GE values, presumably due to the much lower levels of protein found in these diets. The lower value noted on diet W1 could be largely attributed to the advanced stage of maturity of this crop at the time of feeding, but the similarity in estimates of ME between W1 and W2 were surprising in relation to the higher contents of protein and DE found on W2. For all diets, combined losses of energy as urine and CH_4 amounted to between 0·195 and 0·22 of DE and no systematic effects with respect to variations in ME content were detected. However, these values were higher than the mean value of 0·163 reported by Ekern *et al.* (1965) for fresh ryegrass fed to sheep.

From the values for the flows of NAN into the small intestine at the medium level of feeding and the measured ME contents of the diets, and assuming an average availability of NAN in the small intestine of 0·63 (MacRae & Ulyatt, 1974), the quantities of absorbed NAN/MJ ME were calculated. For the grass diets the values ranged from 1·27 (R1) to 1·20 (R2) and 1·30 (R3) g/MJ ME with no major deviations from the overall mean of 1·26 g/MJ ME. These contrast with estimates of apparently digested N of 2·2, 1·5 and 1·4 g/MJ ME and highlight the consequence that inefficiencies of rumen digestion can have on nutrient supply. On the two clover diets, values for NAN absorption were 1·74 (W1) and 1·90 (W2) g/MJ ME and, whilst these were considerably lower than digestible values of 2·8 and 3·3 g/MJ ME respectively, they illustrate the marked superiority (+44%) of white clover over ryegrass with respect to protein supply.

From the values obtained within each forage at the different levels of feeding, relations between the flows of duodenal OM and NAN with respect to the intakes of OM and N were developed (Table 8). There was a strong correlation between increased OM and N intakes and duodenal OM and NAN flows respectively, and all relations were best described by linear functions, indicating no major interactions with respect to nutrient flow in response to the feeding levels adopted in the present study. Furthermore, attempts to combine some of the relations for individual forages were not successful, indicating a marked heterogeneity within the diets examined with respect to nutrient flow.

In conclusion, the present study which used cattle has clearly confirmed some of the earlier findings relating to the digestion of fresh forages by sheep (Beever *et al.* 1971, 1974, 1976; MacRae & Ulyatt, 1974; Ulyatt & MacRae, 1974; Ulyatt & Egan, 1979; Egan & Ulyatt, 1980). It has demonstrated that considerable differences in both nutrient digestion and supply can occur between different forage species and within forage species at different stages of growth. Additionally, it has established that extensive protein breakdown in the rumen of animals given high-N diets can have a major quantitative consequence on nutrient supply. The restricted nature of the study precluded examination of causal mechanisms, but the reasons for the substantial losses of ingested N across the stomach, and the implications of the reduced supply of protein to the small intestine, both merit further examination.

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REFERENCES

Beever, D. E., Cammell, S. B. & Wallace, A. S. (1974). *Proceedings of the Nutrition Society* **33**, 73A.
Beever, D. E., Kellaway, R. C., Thomson, D. J., MacRae, J. C., Evans, C. C. & Wallace, A. S. (1978). *Journal of Agricultural Science, Cambridge* **90**, 157–163.
Beever, D. E. & Siddons, R. C. (1985). In *Proceedings of VIth International Symposium on Ruminant Physiology, Banff* (In the Press).
Beever, D. E., Terry, R. A., Cammell, S. B. & Wallace, A. S. (1978). *Journal of Agricultural Science, Cambridge* **90**, 463–470.
Beever, D. E., Thompson, D. J. & Cammell, S. B. (1976). *Journal of Agricultural Science, Cambridge* **88**, 61–70.
Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1971). *British Journal of Nutrition* **26**, 123–134.
Beever, D . E., Ulyatt, M. J., Thomson, D. J., Cammell, S. B., Austin, A. R. & Spooner, M. C. (1981). *Proceedings of the Nutrition Society* **39**, 66A.
Cammell, S. B., Beever, D. E., Skelton, K. V. & Spooner, M. C. (1981). *Laboratory Practice* **30**, 115–119.
Cammell, S. B., Beever, D. E., Thomson, D. J., Austin, A. R., Losada, H. R., Evans, R. T., Spooner, M. C. & Terry, R. A. (1983). *Animal Production* **36**, 501.
Christian, K. R. & Coup, M. R. (1954). *New Zealand Journal of Science and Technology* **A36**, 328–330.
Corbett, J. L., Furnival, E. P., Inskip, M. W. & Pickering, F. S. (1982). In *Forage Protein in Ruminant Animal Production*, pp. 141–143. [D. J. Thomson, D. E. Beever and R. G. Gunn, editors]. Occasional Publication of the British Society of Animal Production no. 6.
Crampton, E. W. & Maynard, R. A. (1938). *Journal of Nutrition* **15**, 387–395.
Egan, A. R. & Ulyatt, M. J. (1980). *Journal of Agricultural Science, Cambridge* **94**, 47–56.
Ekern, A., Blaxter, K. L. & Sawers, D. (1965). In *Energy Metabolism* pp. 217–224 [K. L. Blaxter, editor]. European Association of Animal Production Publication no. 11. London and New York: Academic Press.
Evans, C. C., MacRae, J. C. & Wilson, S. (1977). *Journal of Agricultural Science, Cambridge* **89**, 17–22.
Evans, R. T., Skelton, K. V. & Beever, D. E. (1981). *Laboratory Practice* **30**, 997–1000.
Faichney, G. J. (1975). In *Digestion and Metabolism in the Ruminant*, pp. 277–291 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, Australia: University of New England Publishing Unit.
Gehrke, G. W., Kaiser, F. E. & Ussary, J. P. (1968). *Journal of the Association of Official Analytical Chemists* **51**, 200–211.
McComb, E. A. & McReady, R. M. (1952). *Analytical Chemistry* **24**, 1630–1632.
MacRae, J. C. & Ulyatt, M. J. (1974). *New Zealand Journal of Agricultural Research* **82**, 309–319.
Mangan, J. L. (1982). In *Forage Protein in Ruminant Animal Production*, pp. 25–40 [D. J. Thomson, D. E. Beever and R. G. Gunn, editors]. *Occasonal Publication of British Society of Animal Production* no. 6.
Moseley, G. & Jones, J. R. (1984). *British Journal of Nutrition* **52**, 381–390.
Technicon Instrument Co. Ltd (1969). *Technical Methodology Sheet* N9B. Basingstoke: Technicon Instruments Co. Ltd.
Thomson, D. J. & Beever, D. E. (1980). In *Digestive Physiology & Metabolism in Ruminants*, pp. 291–308. [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press.
Thomson, D. J., Beever, D. E., Lonsdale, C. R., Haines, M. J., Cammell, S. B. & Austin, A. R. (1981). *British Journal of Nutrition* **46**, 193–207.
Tilley, J. M. A. & Terry, R. A. (1963). *Journal of the British Grassland Society* **18**, 104–111.
Ulyatt, M. J., Dellow, D. W., John, A., Reid, C. S. W. & Waghorn, G. C. (1985). In *Proceedings of VIth International Symposium on Ruminant Physiology, Banff* (In the Press).
Ulyatt, M. J. & Egan, A. R. (1979). *Journal of Agricultural Science, Cambridge* **92**, 605–616.
Ulyatt, M. J. & MacRae, J. C. (1974). *New Zealand Journal of Agricultural Research* **82**, 295–307.
Van Soest, P. J. & Wine, R. H. (1967). *Journal of the Association of Official Analytical Chemists* **50**, 50–55.
Verite, R., Remond, B. & Journet, M. (1984). *Canadian Journal of Animal Science* **64**, Suppl. 328–329.

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