Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage *ad lib.*

BY J. GASA*, K. HOLTENIUS†, J. D. SUTTON‡, M. S. DHANOA AND D. J. NAPPER§

AFRC Institute of Grassland and Environmental Research, Hurley, Maidenhead SL6 5LR

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Four lactating Friesian cows with permanent cannulas in the rumen and proximal duodenum were given early (EC)- or late (LC)-cut grass silage *ad lib.*, each with either 3 or 9 kg concentrate dry matter (DM)/d in a 4 × 4 Latin square design starting about 10 weeks after calving. Feed was offered twice daily at 08.30 hours and 15.30 hours. Periods lasted 5 weeks and measurements were made in the last 15 d. The higher amount of concentrates increased total DM intake but reduced silage DM intake and the fractional rate of degradation of silage-fibre DM. Later cutting date of silage had no effect on DM intake or the fractional rate of degradation of silage-fibre DM but reduced potential degradability of silage fibre. Dilution rate of CoEDTA in rumen fluid was greater during the day (eating period) than during the night (resting period). Dilution rates measured at the duodenum were lower than those measured in the rumen, but neither was affected by diet. Silage-particle passage rates were measured by use of ytterbium-labelled silage fibre (Yb-fibre) and chromium-mordanted faecal particles (Cr-faeces) and samples were taken at the duodenum and in the faeces. Values for slower rate constant (k_1) and transit time were higher and for faster rate constant (k_2) were lower for Yb-fibre than for Cr-faeces, but differences in total mean retention time were inconsistent. Values for k_1 for both markers and k_2 for Yb-fibre only were similar at both sampling sites, but values for k_2 for Cr-faeces were lower in the faeces. No diet effects were established with Yb-fibre but, with Cr-faeces, k_1 was reduced by more concentrates and EC-silage. Daily mean weights of wet digesta, liquid, neutral-detergent fibre (NDF) and indigestible NDF in the rumen were greater with LC-silage but were unaffected by the amount of concentrates whereas weight of rumen DM was increased by the amount of concentrates only. Maximum rumen fill occurred at 18.00 hours with all diets. Particle-size distribution of rumen contents did not vary markedly during the day. Mean particle size was generally greater with LC-silage than EC-silage. Very small particles, passing through the 0·3 mm screen, constituted about half the rumen DM. It is concluded that rumen fill could have limited intake of LC-silage but not EC-silage. The reduced silage intake with greater concentrate intake was associated with a reduction in fibre degradation rate and an increase in rumen DM fill but no other consistent effects on weight or kinetics of rumen fractions were established.

Digesta kinetics: Rumen fill: Voluntary intake

Despite the increasing importance of grass silage in the diet of dairy cows in the UK, little is known about the factors limiting its intake (Gill *et al.* 1988); date of cutting of forage and fermentation during ensiling undoubtedly contribute. Increasing the amount of concentrates increases total feed intake but reduces silage intake, possibly by reducing rate of

Present addresses: * Departamento de Producción Animal, Facultad de Veterinaria, M. Servet 177, Zaragoza, Spain; † Department of Animal Physiology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden; § Selborne Biological Services Ltd, Goleigh Farm, Selborne, Alton GU34 3SE.

‡ For reprints.

passage and digestion of the forage (Blaxter *et al.* 1961). It is probable that both physical and chemical factors contribute to controlling feed intake by ruminants, with the importance of physical factors being greater with higher-forage diets. Despite the importance of physical factors, remarkably few estimates of rumen fill or rates of digestion and outflow in the rumen of lactating dairy cows have been reported. The purpose of the present experiment was to concentrate on physical regulation of intake, particularly rumen fill, digesta kinetics and particle distribution, as affected by date of cutting of grass silage and amount of concentrates in the total diet of Friesian cows in mid-lactation.

MATERIALS AND METHODS

Animals and diets

Four multiparous lactating Friesian cows were used. Each had been fitted with a large rumen cannula, 100 mm internal diameter, and a simple duodenal cannula within about 50 mm of the pylorus at least 3 months before the start of the experiment. From calving until the experiment began, all the cows were treated similarly. They were given 7 kg dairy concentrates daily with grass silage *ad lib*. They were kept in individual standings and bedded on rubber mats and sawdust. They had free access to water and a mineral block. Starting at about week 10 of lactation, the cows were allocated to one of the four experimental treatments. These consisted of either early (EC)- or late (LC)-cut grass silage, each with either 3 or 9 kg dairy concentrates dry matter (DM) daily in a 4×4 Latin square design, giving four treatments: EC-3, EC-9, LC-3 and LC-9.

Silages were prepared from a single sward of perennial ryegrass (*Lolium perenne*) cut at two stages of maturity with a chop length of 15 mm. The dates of harvesting were 4–7 June (EC) and 30 June–1 July (LC) respectively. The ingredients of the concentrates (kg/tonne) were: 370 barley, 183 wheat, 139 wheat middlings, 139 maize gluten feed, 96 soya-bean meal, 34 fishmeal, 24 Megalac (calcium soaps of long-chain fatty acids, Volac Ltd, Royston), and 15 minerals and vitamins. Silage was given in two meals/d, at 08.30 and 15.30 hours. Concentrates were also offered twice daily at 09.00 and 16.30 hours in equal meals. Uneaten feed was removed, weighed and recorded at 07.45 hours every day.

Experimental procedure

The experiment was based on a 4×4 Latin square design. Starting in the 9th or 10th week of lactation, each period lasted for 5 weeks of which the first 20 d were for changeover and adaptation, and experimental measurements were carried out during the last 15 d. Digesta kinetics, rates of digestion and rumen metabolism were studied during the first 5 d and rumen emptyings and particle size analysis were performed during the last 9 d. Feed intake and milk yield were measured daily during days 21–35 and milk composition was measured on a daily pooled sample on four well separated days over the period.

Rumen fluid sampling

Rumen samples were obtained on day 21 of each period by vacuum through a filter kept in the ventral rumen sac. They were taken just before morning feeding and 1·5, 3·5, 5·5, 7·5, 9·5, 11·5, 13·5, 15·5 and 19·5 h after the feed was offered. Rumen pH was measured immediately and about 20 ml were acidified with two or three drops of concentrated sulphuric acid and stored at $-20°$ for subsequent analysis of volatile fatty acids (VFA) and ammonia-nitrogen concentrations. On centrifuged samples of the acidified extract, VFA were determined by gas–liquid chromatography using a Packard model 437s gas–liquid

chromatograph with a column of Chromosorb 101 at 160°; NH_3-N was analysed by an automated colorimetric analysis (Davidson *et al.* 1970).

Rate of passage measurements

At 06.30 hours on day 21 of each period, each cow received a single dose of 1·38 g CoEDTA/kg daily DM intake diluted in 500–600 ml distilled water via the rumen cannula as a fluid-phase marker. In addition, a further single dose of chromium-mordanted faecal residues and ytterbium-labelled silage 'fibre', equivalent to 0·5 g Cr and 0·2 g Yb/kg daily DM intake, were given 15 min before silage was offered in the morning. Markers not consumed were added to the rumen through the cannula just before feeding.

CoEDTA and Cr-mordanted faecal residues were prepared according to the procedures of Uden *et al.* (1980). Faecal residues were produced by thoroughly washing fresh faeces with running tap water through a 1 mm sieve. Silage 'fibre' was prepared by washing fresh silage with tap water and soaking overnight in a solution of sodium lauryl phosphate (30 g/l) at about 40°; the following morning the material was thoroughly washed with tap water until no more detergent remained and then dried at 60° for 40 h. The 'fibre' was labelled with Yb by being soaked overnight in a buffered solution of ytterbium acetate (0·1 M-acetic acid to pH 6·0 with ammonium hydroxide) at the rate of 50 g Yb/kg DM. The labelled fibre was washed several times with deionized water, soaked for 3 h in dilute hydrochloric acid solution (pH 2·0) and finally washed again several times with deionized water and allowed to dry.

The particle size distribution of the markers for mesh sizes of > 2·4, 1·2–2·4, 0·3–1·2 and < 0·3 mm respectively were: Cr–faeces 0·20, 0·15, 0·53, 0·12; Yb–'fibre' (EC-silage) 0·86, 0·06, 0·02, 0·06; Yb–'fibre' (LC-silage) 0·64, 0·24, 0·07, 0·05.

For each animal, rumen fluid samples were taken before dosing, 1·5 and 3 h after dosing, then at 2 h intervals to 17 h after dosing, then 21 and 25 h after dosing. Duodenal samples were taken at the following times after dosing: 30 min, 2 h, then every 2 h to 16 h, every 4 h to 56 h, every 8 h to 88 h and every 12 h to 148 h; grab samples of faeces were taken from the rectum at the same times starting 6 h after dosing. Rumen samples were frozen immediately until required for Co analysis. Duodenal and faecal samples were dried at 100° for 48 h and ground to pass through a 1 mm screen. Marker concentrations were measured by atomic absorption spectroscopy: Co in rumen and duodenal fluid by direct aspiration and duodenal and faecal DM after extraction with 0·1 M-H_2SO_4, Cr after digestion of ashed samples with a mixture of orthophosphoric acid and H_2SO_4, and Yb after extraction of ashed samples with nitric acid (20 ml/l) (Siddons *et al.* 1985).

Fluid dilution rates from rumen and duodenal samples were calculated by regression of the natural log of marker concentration in the fluid *v.* time after dosing (single-exponential (SE) method). Based on results of patterns of eating and general feeding behaviour found by Gill *et al.* (1987) with the same diets and similar animals, a modification of the procedure was introduced (two-exponential (TE) method) by considering the fluid dilution rate to be the weighted mean (using the time period of each rate as the weights) of two partial daily rates, a faster rate (k_f) during the day being coincident with the maximum intake activity period, and a slower rate (k_s) later in the evening and at night reflecting mainly the period of resting and ruminating. The time when the two regression lines met, expressed in hours after the morning meal of silage was offered, was defined as the 'break-point' and was estimated by fitting linear supline curves using non-linear fitting package (Ross, 1987), which was judged to be the most appropriate for the data.

Estimates of particulate passage rates (Cr and Yb) from duodenal and faecal samples and Co outflow rate measured in the faeces were calculated according to Dhanoa *et al.*

(1985). The slower rate constant (k_1) in this report was based on the main period of declining marker concentration while the faster rate constant (k_2) was determined from the period of increasing concentration. Transit time (TT) and total mean retention time (TMRT) were also obtained (Dhanoa *et al.* 1985).

Rate of digestion

Polyester bags containing weighed quantities (approximately 5 g DM) of the dried silage 'fibre' were used to estimate in situ kinetics of digestion of the silages in the rumen. The bags used measured 150 mm × 60 mm, with pore size of approximately 40 μm. One pair of bags was soaked in water for 10 min for determination of '0 h wash' values for each silage and period. Pairs of bags containing silage 'fibre', prepared from the same silage type that each animal was consuming, were inserted into the rumen for each animal and period immediately before feeding and incubated for 2, 6, 10, 16, 24, 48, 72 or 96 h. After withdrawal from the rumen, bags were gently washed out with tap water and stored at 4°. When all the set was out they were washed in cold water in a domestic washing machine for 55 min. After washing the bags were dried at 60° for 48 h for DM determinations and the residues were ground through a 1 mm screen and stored for neutral-detergent fibre (NDF) analysis.

The fractional rates of digestion, lag times and maximal disappearance were estimated by applying the Ørskov & McDonald (1979) model, fitting the lag parameter (t_0) following Dhanoa (1988):

$$Y = a + b(1 - e^{-kt}), t > t_0,$$

where Y is DM or NDF disappearance from the bag, a is loss at zero time, b is potentially degradable insoluble fraction, k is fractional rate of digestion (per h) of DM or NDF, t is period of incubation, and t_0 is lag time.

Rumen contents and particle size measurements

During the last 10 d of each experimental period all the rumen contents were manually removed, weighed, sampled and returned to the rumen on six occasions. In order to overcome any effects of emptying on subsequent measurements, a minimum interval of 38 h was allowed between each emptying. Measurements were made at 08.00, 10.00, 14.00, 18.00, 22.00 and 03.00 hours on days 27, 34, 32, 30, 28 and 35 respectively. Each required about 30 min. One of every twenty digesta handfuls or filled jars removed from the rumen was retained in a bulk sample for analysis. Each digesta sample was divided into four subsamples. Two subsamples were kept for determination of DM content, a third subsample was freeze-dried for subsequent NDF analysis, and the fourth subsample was kept for particle size analysis.

DM content of all samples was determined on fresh material. The freeze-dried samples of digesta and feeds offered were ground to pass through a 1 mm screen and stored. The content of indigestible DM in feed and digesta was determined by in situ digestion in polyester bags for 96 h at the end of the experiment in the rumen of two of the cows given 3 kg concentrates and a mixture of the EC- and LC-silages *ad lib*. Indigestible NDF (INDF) was measured by conventional NDF analysis of the indigestible DM residue of the bags.

Particle size measurements were made by the wet-sieving technique described by Evans *et al.* (1973). Three screens were used with apertures of 2·4, 1·2 and 0·3 mm. All particle measurements were made in duplicate.

Table 1. *Composition and particle size distribution of the feeds*

Feed...	Early-cut silage	Late-cut silage	Concentrates
Dry matter (DM) (g/kg)	200	258	856
Indigestible DM (g/kg DM)	143	252	49
Neutral-detergent fibre (NDF) (g/kg DM)	471	575	221
Indigestible NDF (g/kg DM)	120	216	40
Total nitrogen (g/kg DM)	25·2	19·1	31·0
Ammonia-N (g/kg total N)	159	122	ND
pH	4·6	4·4	ND
Particle size (mm)			
> 2·4	0·65	0·65	0·03
1·2–2·4	0·04	0·07	0·12
0·3–1·2	0·03	0·04	0·27
< 0·3	0·28	0·24	0·58

ND, not determined.

Chemical analysis

DM content of feeds and digesta were determined by drying at 100° or by freeze-drying as appropriate. Total N was determined as described by Siddons *et al.* (1985). NDF and acid-detergent fibre (ADF) were measured on freeze-dried samples according to the method of Goering & Van Soest (1970).

Statistical analysis

The statistical package GENSTAT 5 (1987) was used to perform analysis of variance of the Latin square design in which animal, period and diet (two dates of cutting × two concentrate amounts) effects were evaluated. The general linear models procedure (SAS Institute Inc. 1985) was used for the analysis of the particle measurements.

RESULTS

The composition of the silages and concentrates and particle size distribution are given in Table 1.

Intake and milk production

Mean daily intakes of the four diets are given in Table 2. Increasing concentrate intake from 3 to 9 kg DM/d reduced silage DM intake but increased total DM intake ($P < 0·001$). The proportion of silage in the total diet fell from 0·76 to 0·45 (LC-silage) or 0·43 (EC-silage). Silage cutting date had no effect on DM intake but intakes of indigestible DM, NDF and indigestible NDF were greater with the LC-silage ($P < 0·001$).

Milk production results are presented in Table 3. The higher level of concentrates increased milk yield ($P < 0·01$) and milk protein concentration ($P < 0·05$) but tended to reduce milk fat concentration ($P < 0·10$), particularly with the EC-silage. The EC-silage increased milk yield but reduced milk fat concentration; milk protein concentration was unaffected. Diet effects on lactose concentration, though statistically significant, were trivial.

Rumen fermentation

Mean daily values for pH and VFA and NH_3 concentration are given in Table 4. Mean daily rumen pH and total VFA concentration were unaffected by the treatments. Mean NH_3 concentration in the rumen was greater with the EC-silage but was unaffected by level

Table 2. *Intake (kg/d) of dry matter (DM), indigestible DM, neutral-detergent fibre (NDF) and indigestible NDF in cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage† ...	EC		LC			Main effects‡	
Level of concentrates (kg/d) ...	3	9	3	9	SEM	Silage	Concentrates
Total DM	12·26	15·59	12·51	16·36	0·264	—	***
Silage DM	9·28	6·63	9·53	7·41	0·260	—	***
Total indigestible DM	1·33	1·34	2·40	2·25	0·079	***	—
Neutral-detergent fibre (NDF)	5·86	5·95	6·62	6·87	0·102	***	—
Indigestible NDF	1·06	1·06	2·04	1·92	0·069	***	—

*** $P < 0·001$.
† For details of composition, see Table 1.
‡ Interactions were not significant.

Table 3. *Milk yield and composition in cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage† ...	EC		LC			Main effects‡	
Level of concentrates (kg/d) ...	3	9	3	9	SEM	Silage	Concentrates
Milk yield (kg/d)	19·0	21·9	16·7	20·7	0·70	*	**
Composition (g/kg)							
Fat	38·6	33·0	40·0	38·9	2·09	*	—
Protein	30·8	32·2	31·3	32·4	0·63	—	*
Lactose	45·1	45·1	45·8	45·3	0·16	***	*

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see Table 1.
‡ Interactions were not significant.

Table 4. *Mean daily pH, concentrations of ammonia-nitrogen and total volatile fatty acids (VFA) and molar proportions of acetic, propionic and butyric acids in the rumen of cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage† ...	EC		LC			Main effects		
Level of concentrates (kg/d) ...	3	9	3	9	SEM	Silage	Concentrates	Interaction
Mean pH	6·48	6·35	6·53	6·29	0·087	—	—	—
NH_3-N (mg/l)	139·5	125·2	92·0	104·4	7·97	**	—	—
Total VFA (mmol/l)	112·8	118·4	107·1	115·3	8·37	—	—	—
Acetic acid (mmol/mol)	682	586	650	610	7·0	—	***	**
Propionic acid (mmol/mol)	147	216	169	170	8·5	—	**	**
Butyric acid (mmol/mol)	113	121	143	169	8·9	**	—	—

** $P < 0·01$; *** $P < 0·001$.
† For details of composition, see Table 1.

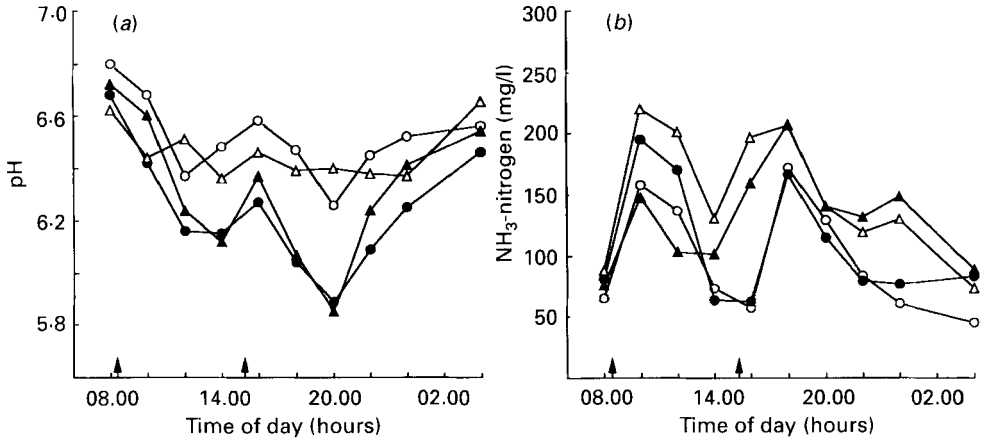

Fig. 1. Changes during the day in (*a*) pH and (*b*) ammonia concentration in the rumen fluid of four cows given 3 or 9 kg concentrates/d with early-cut (3 kg/d △, 9 kg/d ▲) or late-cut (3 kg/d ○, 9 kg/d ●) silage *ad lib.* ↑, Time point when silage was offered. For details of composition of silage, see Table 1.

Table 5. *Polyester bag degradability of dry matter (DM) and neutral-detergent fibre (NDF) of silage 'fibre' incubated in the rumen of cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage† ...	EC		LC			Main effects‡	
Level of concentrates (kg/d) ...	3	9	3	9	SEM	Silage	Concentrates
DM							
Potential degradability	0·825	0·797	0·723	0·700	0·0138	***	—
Degradation rate-constant (/h)	0·025	0·019	0·026	0·020	0·0021	—	*
Lag time (h)	7·8	13·5	5·0	10·1	2·93	—	—
NDF							
Potential degradability	0·756	0·760	0·684	0·675	0·0239	*	—
Degradation rate-constant (/h)	0·030	0·024	0·030	0·025	0·0029	—	—
Lag time (h)	14·8	15·0	12·1	15·7	2·75	—	—

* $P < 0·05$, *** $P < 0·001$.
‡ Interactions were not significant.
† For details of composition, see Table 1.

of concentrates. The higher level of concentrates reduced the proportion of acetic acid and increased the proportion of propionic acid, the responses being greater with the EC-silage. The proportion of butyric acid was greater with the LC-silage.

Rumen pH fell rapidly after the morning meal with all diets (Fig. 1(*a*)). The fall was greater with the higher level of concentrates, and a further fall to minimum values below 6·0 occurred about 2·5 h after the afternoon meal. With the lower level of concentrates the

Table 6. *Fractional dilution rates (/h) of CoEDTA introduced into the rumen and measured in (a) rumen and (b) duodenal fluids by the single-exponential (SE) or two-exponential (TE) methods† or (c) in the faeces of cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage†...	EC		LC				
Level of concentrates (kg/d)...	3	9	3	9	SEM	Concentrate effects§	Interaction
(a) Rumen samples							
SE							
Dilution rate (k_r)	0·167	0·176	0·148	0·173	0·0104	—	—
TE							
Dilution rate $k_{r(f)}$	0·175	0·191	0·158	0·180	0·0067	*	—
Dilution rate $k_{r(s)}$	0·134	0·102	0·102	0·105	0·0060	—	*
Break-point‖	9·70	10·18	10·52	11·33	0·883	—	—
$k_{r(f)}+k_{r(s)}$¶	0·150	0·139	0·127	0·140	0·0046	—	—
(b) Duodenal samples							
SE							
Dilution rate (k_d)	0·170	0·132	0·121	0·160	0·0135	—	—
TE							
Break-point‖	12·03	13·38	13·54	13·60	1·473	—	—
$k_{d(f)}+k_{d(s)}$¶	0·131	0·109	0·108	0·114	0·0036	—	**
(c) Faecal samples							
Dilution rate (k_1)	0·127	0·117	0·118	0·114	0·0035	—	—

$k_{r(f)}$, $k_{r(s)}$, $k_{d(f)}$, $k_{d(s)}$, faster and slower dilution rates in the rumen and duodenum respectively.
* $P < 0·05$, ** $P < 0·01$.
† For details, see p. 383.
‡ For details of composition, see Table 1.
‖ Break-point between both rates (h after morning feeding).
¶ Weighted mean.
§ No silage effects were significant.

pattern was less clear-cut. Rumen NH_3 concentration increased after both daily meals and for all diets (Fig. 1(*b*)).

Kinetics of digestion and passage

Degradability estimates of DM and NDF are given in Table 5. As expected, the potential degradability of the DM and NDF in silage 'fibre' was greater with the EC-silage. The higher level of concentrates reduced the fractional rate of digestion of silage 'fibre' DM ($P < 0·05$) and tended to reduce that of NDF ($P < 0·10$). Lag time tended to be longer with the larger amount of concentrates but not significantly so.

Fractional dilution rates of CoEDTA measured in the rumen, duodenum and faeces are presented in Table 6. Analysis of the rates of change of concentration of Co in rumen and duodenal fluid showed that the residual mean squares were less for the TE method than for the SE method (0·0087 (SE 0·0015) *v.* 0·0268 (SE 0·0143) in the rumen and 0·0323 (SE 0·0233) *v.* 0·0549 (SE 0·0286) at the duodenum for the TE and SE methods respectively). Mean time of the break-point (h after the morning feed) was 10·4 (SE 0·88) h in the rumen and 13·1 (SE 1·47) h at the duodenum and was unaffected by diet. In the faeces, the multi-compartmental analysis of Dhanoa *et al.* (1985) was used to calculate the results. Only the slow exponential (k_1) could be calculated as there were insufficient samples for calculation of k_2.

Table 7. *Fractional passage rates* (k_1 *and* k_2; */h*), *transit time* (*TT; h*) *and total mean retention time* (*TMRT; h*) *of chromium-mordanted faecal residues estimated at the duodenum and in the faeces of cows offered diets with early-cut* (*EC*) *or late-cut* (*LC*) *silage supplemented with 3 or 9 kg concentrates/d*†

(Mean values for four cows)

Silage‡...	EC		LC			Main effects		
Level of concentrates (kg/d)...	3	9	3	9	SEM	Silage	Concentrates	Interaction
Duodenum								
k_1	0·027	0·021	0·031	0·028	0·0010	**	**	—
k_2	0·204	0·153	0·202	0·206	0·0143	—	—	—
TT	8·8	8·8	11·4	8·4	0·88	—	—	—
TMRT	51·2	64·1	49·7	51·1	0·89	***	***	***
Faeces								
k_1	0·025	0·022	0·033	0·029	0·0009	***	**	—
k_2	0·165	0·157	0·160	0·174	0·0149	—	—	—
TT	16·5	16·6	20·0	16·7	1·00	—	—	—
TMRT	64·9	70·4	57·5	58·1	1·37	**	—	—

** $P < 0·01$, *** $P < 0·001$.
† For details of procedures, see pp. 383–384.
‡ For details of composition, see Table 1.

The weighted mean estimates of dilution rates based on the TE method in the rumen and the duodenum were 16% (rumen) and 21% (duodenum) lower than the equivalent values based on the SE analysis and values measured at the duodenum tended to be lower than those measured in the rumen. The values for k_1 measured in faecal samples were not directly comparable with either the SE or TE values measured at the other two sites because the method of calculation was different, but the resulting rate constants were very similar to the weighted mean rate constants measured at the duodenum.

Whatever method of calculation or site was used, there were no significant diet effects on either the SE rate constants or the weighted mean of the TE rate constants.

The variables of the passage of undigested solids residues measured at the duodenum and in the faeces are given in Tables 7 and 8. Mean fractional passage rates (k_1) of Cr-mordanted faecal residues were lower with the higher level of concentrates and the EC-silage whether the measurements were made at the duodenum or in the faeces (Table 7). There was no diet effect on k_2 or on TT. TMRT was greater with the EC-silage, particularly with the higher level of concentrates. Comparing duodenal and faecal results, values for k_1 were on average identical, but values for k_2 at the duodenum were significantly greater ($P < 0·05$) than those in the faeces (0·191 *v.* 0·163). Values for TT and TMRT were inevitably greater in the faeces.

Measurements of fractional rates of passage obtained by the use of Yb-labelled silage 'fibre' (Table 8) differed from those found with Cr-mordanted faecal particles. Values for k_1 and TT were, on average, higher ($P < 0·01$) and values for k_2 and TMRT were lower ($P < 0·01$) with the Yb-labelled 'fibre', though the differences in estimates of TMRT were not consistent. There were no significant diet effects on any of the parameters of passage of Yb-labelled 'fibre' measured, though there was a tendency ($P < 0·10$), consistent with the results for Cr-mordanted faecal particles, for k_1 measured in the faeces to be smaller with the EC-silage.

Values for k_1 and k_2 were similar at the two sampling sites using this method.

Table 8. *Fractional passage rates* (k_1 *and* k_2; */h*), *transit time* (*TT; h*) *and total mean retention time* (*TMRT; h*) *of ytterbium-labelled silage 'fibre' estimated at the duodenum and in the faeces of cows offered diets with early-cut* (*EC*) *or late-cut* (*LC*) *silage supplemented with 3 or 9 kg concentrates/d*†

(Mean values for four cows)

Silage‡...	EC		LC			
Level of concentrates (kg/d)...	3	9	3	9	SEM	Interaction§
Duodenum						
k_1	0·036	0·036	0·037	0·040	0·0023	—
k_2	0·128	0·115	0·134	0·131	0·0093	—
TT	12·3	14·5	14·9	13·2	1·53	—
TMRT	48·8	52·2	50·7	47·5	1·26	*
Faeces						
k_1	0·036	0·035	0·039	0·039	0·0015	—
k_2	0·128	0·115	0·130	0·127	0·0093	—
TT	20·1	21·3	24·3	20·1	0·75	*
TMRT	57·5	59·0	58·2	54·5	1·33	—

* $P < 0·05$.
† For details of procedures, see pp. 383–384.
‡ For details of composition, see Table 1.
§ No main effects were significant.

Table 9. *Mean daily weight* (*kg*) *of various fractions of rumen digesta in cows offered diets with early-cut* (*EC*) *or late-cut* (*LC*) *silage supplemented with 3 or 9 kg concentrates/d*

(Mean values for four cows)

Silage†...	EC		LC			Main effects‡	
Level of concentrates (kg/d)...	3	9	3	9	SEM	Silage	Concentrates
Wet digesta	84·4	84·4	96·8	93·1	2·07	**	—
Liquid	74·1	72·5	84·1	80·5	1·80	**	—
Dry matter (DM)	10·3	11·8	11·5	12·6	0·45	—	**
Indigestible DM	3·0	3·2	5·1	5·0	0·21	***	—
Neutral-detergent fibre (NDF)	6·2	7·0	7·7	8·0	0·24	**	—
Indigestible NDF	2·4	2·5	4·4	4·7	0·27	***	—

** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see Table 1.
‡ Interactions were not significant.

Rumen pool and particle sizes

The mean daily weights of various fractions of rumen contents in the cows are shown in Table 9 and daily patterns of weight changes are given in Fig. 2. The weight of wet digesta and fluid in the rumen was significantly greater in the cows given the LC-silage at all sampling times. In contrast, the mean weight of DM in the rumen was unaffected by silage type, although it was significantly greater with LC-silage at 14.00 and 18.00 hours. The amount of concentrates had no effect on weights of wet digesta or fluid but significantly increased the mean weight of DM in the rumen and the weight at all sampling times except 08.00 and 03.00 hours.

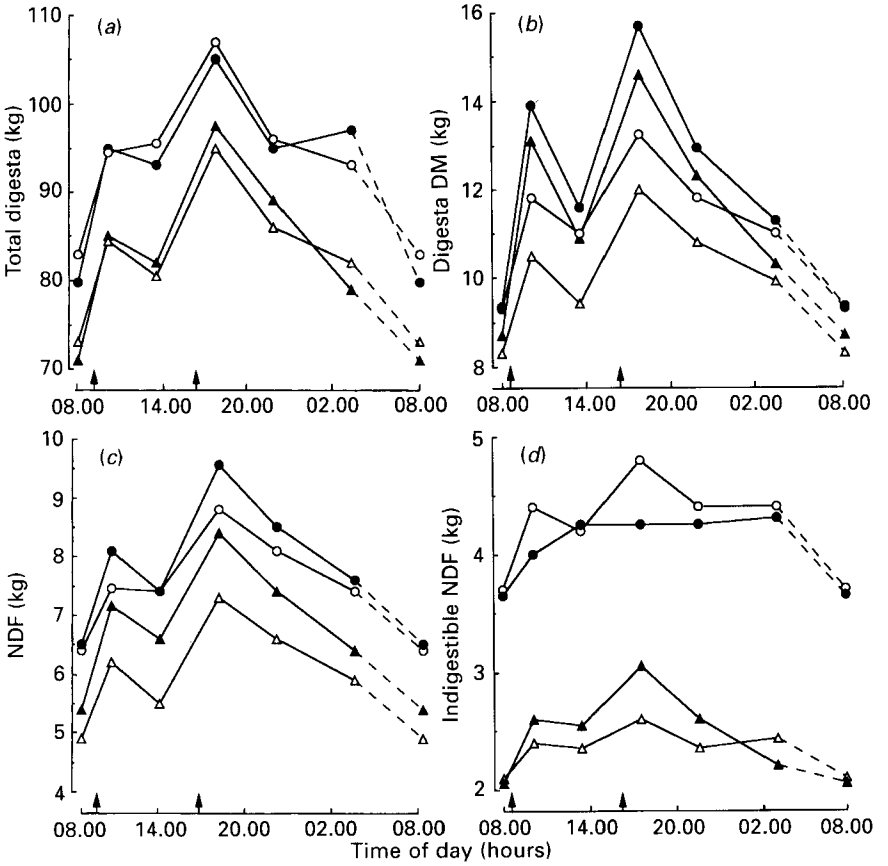

Fig. 2. Weight in the rumen of (*a*) total digesta, (*b*) digesta dry matter (DM), (*c*) neutral-detergent fibre (NDF) and (*d*) indigestible NDF in four cows given 3 or 9 kg concentrates/d with early-cut (3 kg/d △, 9 kg/d ▲) or late-cut (3 kg/d ○, 9 kg/d ●) silage *ad lib.* ↑, Time-point when silage was offered. The values at 08.00 hours on the second day are a repeat of those at 08.00 hours on the first day. For details of composition of silage, see Table 1.

At all sampling times the weights of NDF and indigestible NDF (Fig. 2) and indigestible DM were greater with LC-silage than EC-silage but they were unaffected by amount of concentrates.

There were no large differences in particle size distribution at different times of sampling within treatments (Table 10). Very small particles and soluble DM passing through the 0·3 mm screen constituted about half the rumen DM. Cows given EC-silage had a higher proportion of the smallest particles and a smaller proportion of the medium-size particles. The higher level of concentrates significantly reduced the proportion of the largest particles in the period after feeding (10.00–18.00 hours) but a tendency for an associated increase in the proportion of smaller particles only reached significance ($P < 0·05$) at 14.00 hours for the fraction < 0·3 mm.

DISCUSSION

Marker kinetics in the digestive tract

The rumen is a complex continuous-flow system and mathematical treatment of events within the system, based on marker behaviour studies, has usually been restricted to hypothetical steady-state conditions implying the assumptions of constant volume, ideal

Table 10. *Distribution (%) of dry matter in various particle groups determined by wet sieving of rumen digesta from cows offered diets with early-cut (EC) or late-cut (LC) silage supplemented with 3 or 9 kg concentrates/d*†

(Mean values for four cows)

Silage‡...		EC		LC			Main effects§	
Level of concentrates (kg/d)...		3	9	3	9	SEM	Silage	Concentrates
Time of day (hours)	Screen mesh size (mm)							
08.00	< 0·3	50·4	46·8	43·6	45·4	1·26	—	—
	0·3–1·2	15·8	22·7	25·0	24·8	1·24	**	—
	> 1·2	33·6	29·7	31·4	29·8	1·16	—	—
10.00	< 0·3	56·0	55·8	46·5	50·3	1·40	***	—
	0·3–1·2	14·3	19·3	20·3	21·2	0·76	***	—
	> 1·2	30·9	25·0	33·2	28·6	1·12	*	***
14.00	< 0·3	54·3	54·3	44·9	49·3	1·15	***	*
	0·3–1·2	14·7	19·7	22·5	21·6	0·80	***	—
	> 1·2	31·1	26·1	32·7	29·3	0·77	***	***
18.00	< 0·3	50·8	55·2	43·6	48·0	1·38	**	—
	0·3–1·2	13·2	16·8	18·2	19·2	0·74	*	—
	> 1·2	36·1	28·1	37·3	32·9	1·17	—	***
22.00	< 0·3	52·0	51·3	44·6	45·1	1·16	**	—
	0·3–1·2	12·5	17·5	20·7	20·0	0·90	***	—
	> 1·2	26·0	31·3	34·8	35·2	0·76	—	—
03.00	< 0·3	52·3	49·4	47·2	45·4	1·09	*	—
	0·3–1·2	15·1	18·7	22·5	22·2	0·85	***	—
	> 1·2	32·1	32·0	33·3	31·7	0·82	—	—

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of procedures, see p. 384.
‡ For details of composition, see Table 1.
§ Interactions were not significant.

mixing and continuous, constant and equal inflow and outflow in the system (Faichney, 1975; Warner, 1981). Since none of these assumptions is properly fulfilled in our experiment, deviations from the ideal system and their implications need to be appreciated.

In a parallel experiment to the present one, Gill *et al.* (1987) recorded the diurnal pattern of feed intake for cows which were treated the same way and fed on the same diets as the cows in our study. During the first 12 h after the morning feeding, their cows consumed 70–85% of total daily DM intake and, therefore, in terms of input of feed to the reticulo-rumen, the day could be divided into two main periods, one with a high rate of eating or 'eating period' and the other with a low rate of eating or 'resting period'.

In the present experiment, the rate of feed ingestion varied markedly over the day, being concentrated mainly in two periods, each of 4–5 h following a meal. Associated with this, the weight of digesta in the reticulo-rumen changed considerably through the day, increasing during the eating period and decreasing later during resting period (Fig. 2). Therefore, the behaviour of markers in the reticulo-rumen might be expected to vary considerably during the daily cycle. The effects of this variation on estimates of marker kinetics are likely to be greater with markers with a relatively short turnover time of less than 24 h than with markers with a turnover time extending over longer periods.

The deviation of the dilution of CoEDTA in the rumen fluid from linearity on a semilog scale and the improvement achieved by a two-phase linear model are illustrated in Fig. 3.

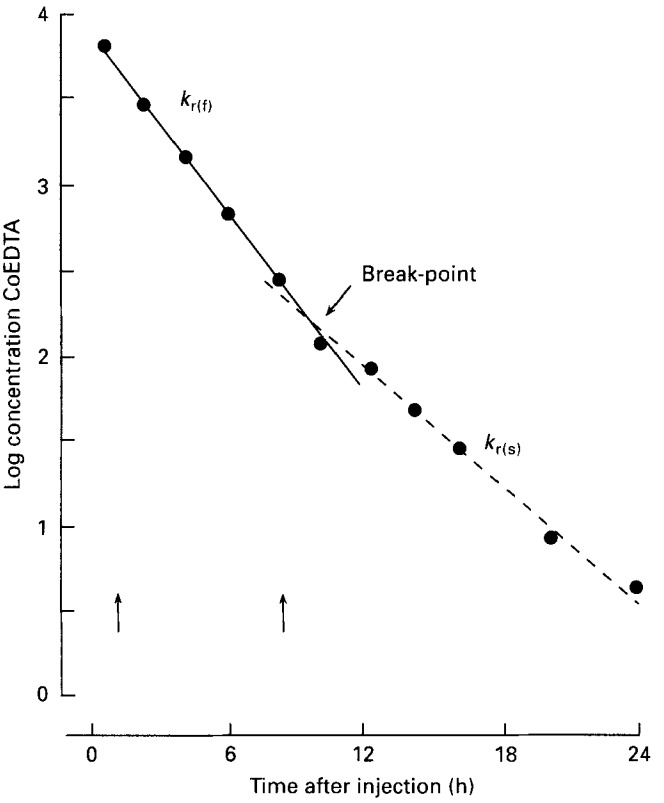

Fig. 3. Example of fitting a fast exponential ($k_{r(f)}$,——), a slow exponential ($k_{r(s)}$,– – –) and a break-point to the concentration curve (log) of CoEDTA in the rumen of a cow fed on grass silage and concentrates twice daily. Injection of CoEDTA occurred 2 h before the first meal. For details of procedures, see p. 383.

Similar results were obtained at the duodenum. This non-linearity does not seem to be related to the non-ideal behaviour of markers discussed by Teeter & Owen (1983) but rather to the diurnal variation in rumen fluid volume as was found by rumen emptying.

Applying the TE model to the rumen data, the mean break-point was found at 10·4 (SE 0·88) h after the morning feeding (about 18.30 hours) which coincided with the maximum weight of wet digesta in the rumen (18.00 hours). Several authors (Balch, 1958; Bueno, 1975) have proposed that rumen dilution rate and outflow to the abomasum are greater during eating than during ruminating or resting and the higher values for rumen fluid dilution rates during the eating period ($k_{r(f)}$) than during the resting period overnight $k_{r(s)}$ in the present experiment support this. The weighted mean of these two rate constants therefore appears to provide a better estimate of the daily mean that the value obtained by the use of an SE.

Estimates of rumen-fluid dilution rates measured by the TE method were lower when based on rumen samples than on duodenal samples, but were not further affected when measurements were based on faecal samples (Table 6). These results are similar to others reported for dairy cattle. Estimates of rumen-fluid dilution rates based on rumen samples have been reported to be higher than estimates based on faecal samples for adult dairy cows (Hartnell & Satter, 1979; Robinson & Sniffen, 1983) and young, non-lactating heifers (Goetsch & Owens, 1985) given a variety of diets, whereas values based on duodenal and faecal samples have been reported to be similar (Robinson & Sniffen, 1983; Goetsch &

Owens, 1985). No clear reason for the difference between rumen and post-rumen samples has been identified but the results emphasize the importance of sampling from the primary pool, i.e. the rumen.

Because of the longer retention times of solid particles, estimates of parameters of their passage are likely to be less affected by the pattern of eating within the day than are the parameters of fluid passage. The rate of passage of feed solids is most frequently described in terms of a slow and a fast rate constant and a time delay but the biological interpretation of these variables is widely disputed. It has been suggested that each of the two rate constants is located in a different mixing site within the digestive tract, most commonly the reticulo-rumen and the caecum, although there is disagreement over the identification of rate constants with sites (Blaxter *et al.* 1956; Grovum & Williams, 1973). Alternatively both have been considered to occur within the reticulo-rumen and to represent a pool based on mixing and particle breakdown (k_2) and a passage pool (k_1) (Ellis *et al.* 1979). Pond *et al.* (1988) have recently suggested, on the basis of results with dry cows, that both the rumen and the caecum contribute to k_2 as measured in the faeces, and results by Gasa & Sutton (1991) with lactating cows appear to support that view.

In the present experiment, estimates of the two rate constants with Yb-labelled 'fibre' were similar at the duodenum and in the faeces, implying that both pools occur anterior to the duodenum and that, if the caecum does act as a mixing pool, its effects could not be detected. With Cr-mordanted faecal particles, values for k_1 were the same at the two sites but mean values for k_2 measured in the faeces were 15% lower than those measured at the duodenum. The difference was significant ($P < 0{\cdot}05$) but not consistent as the values on diet EC-9 were almost identical at the two sites. In a separate experiment with lactating cows given mixed diets based on grass silage (Gasa & Sutton 1991), k_2 was about 20% lower in the faeces than at the duodenum with Cr-mordanted and Yb-labelled silage, while values for k_1 were similar at the two sites when the results were analysed according to the model of Grovum & Williams (1973). However, when they were analysed according to the model used in the present paper (Dhanoa *et al.* 1985), there were no consistent differences between faecal and duodenal values for k_1 or k_2.

The similarity of values for k_1 at the duodenum and in the faeces of both dry (Pond *et al.* 1988) and lactating (Gasa & Sutton, 1991 and present experiment) cattle given a variety of diets and markers can be interpreted as evidence supporting the view that k_1 describes passage out of the rumen. However, there is less consistency in the results for k_2 in this same series of experiments, with values sometimes being the same at the two sampling sites and sometimes lower in the faeces. It seems reasonable to conclude that real differences between duodenal and faecal values for k_2 do exist under certain conditions which have yet to be properly defined.

According to passage parameters measured at the duodenum, which are likely to describe rumen processes more accurately than those measured in the faeces, the values for TMRT measured by the two types of marker were quite similar except for diet EC-9, which gave 23% longer retention times with Cr-faeces than with Yb-'fibre'. However, with all four diets values for k_1 were lower and values for k_2 and TT were higher with Cr-faeces than with Yb-'fibre'. Based on the interpretation that k_2 measured at the duodenum represents breakdown of large particles to small particles and k_1 the passage of small particles from the rumen, these results imply that Cr-faeces, which are derived from particles that have already passed through the digestive tract, move from the large particle pool to the small-particle pool more rapidly than Yb-'fibre', which must be broken down to a smaller size before entering the small-particle pool. However, once they have entered the small-particle pool, the Yb-'fibre' particles leave the rumen more rapidly than the Cr-faeces particles, possibly because of the high specific gravity of Cr-mordanted particles (Ehle, 1984) which

has been shown to reduce particle outflow from the rumen (Campling & Freer, 1962; DesBordes & Welch, 1984).

Feed particle movement in the reticulo-rumen

Factors controlling the passage of particles out of the rumen are poorly defined. Few particles larger than 1·2 mm leave the rumen (Poppi *et al.* 1980) even though the opening of the reticulo-omasal orifice in cattle is about 35 mm (Bueno, 1975). In order to pass out of the rumen, particles must be transferred to the floor of the reticulum (Ehrlein, 1979). The density of the particles must be sufficiently high to allow them to sediment. Newly ingested feed particles have low functional density due to chemical composition, trapped air and gas formed by microbial fermentation. The probability of such newly ingested particles passing out of the rumen ought to be low. Thus, particles have different probabilities of passing out of the rumen according to the length of time they have spent there, as has been proposed by Matis (1972). In our experiment there was virtually no net outflow of indigestible NDF between 22.00 and 03.00 hours except with diet EC-9. However, there was a substantial net disappearance between 03.00 and 08.00 hours. Based on the results of Gill *et al.* (1987) it is reasonable to assume that the feed intake in the present study was low from 22.00 hours until feeding the next morning at 08.30 hours. The higher net disappearance of indigestible NDF after 03.00 hours could thus be explained by increasing age of the digesta. The digesta flow through the reticulo-omasal orifice has been reported to be 30% higher during eating and ruminating than during resting (Bueno, 1975). Since no behaviour studies were done in the present experiment it is not known to what extent rumination pattern differed between 22.00–03.00 hours and 03.00–08.00 hours and whether it could partly explain the marked differences of rumen net INDF movements between the periods.

The diurnal variation in particle size distribution was quite small, although there was a larger proportion of very small particles 2 h after the morning feeding. There were no obvious differences in particle size distribution between concentrate levels. The pattern found in cows fed on hay *ad lib.* twice daily with no concentrate (Waghorn, 1986) was different from that in the present experiment, with a significantly increased proportion of particles longer than 1 mm 2 h after the hay was offered. This difference may partly be explained by the fact that the concentrates in the present experiment consisted of a large amount of very small particles and soluble material.

Animals fed on EC-silage always had a larger proportion of very small particles in the rumen than those fed on LC-silage, possibly because EC-silage caused a more complex structure of the raft which became more effective in trapping those particles, thereby retaining a higher concentration in the rumen.

In the present study about 50% of the DM contents of the reticulo-rumen passed through a 0·3 mm screen. Thus, a large part of the rumen digesta consists of very small particles which theoretically would be able to escape from the rumen with the liquid phase (Hungate, 1966; Owens & Goetsch, 1986), although possibly not at the same rate (Dixon & Milligan, 1985). The high proportion of very small particles in the rumen indicates that the genesis of that fraction was neither the only nor the rate-limiting factor in clearing the particulate matter from the rumen and, thereby, limiting food intake. Similar results have been reported by Ellis *et al.* (1982).

Factors affecting feed intake of silage–concentrate diets

Rumen distension caused by digesta load is generally considered to limit intake of certain diets, particularly those with large amounts of forage. Blaxter *et al.* (1956) defined gut fill in terms of DM but other descriptions related to total digesta weight or volume may be

more useful. Digesta volume is particularly difficult to measure, so digesta weight offers the most practical estimate of rumen fill. In the present experiment, the mean daily rumen fill of all fractions measured except DM was higher with LC-silage than with EC-silage, indicating that rumen fill did not limit intake of EC-silage.

Further, although silage was available to the animals at all times, rumen fill varied widely over the day and in a very similar pattern for the four diets. The maximum fill measured was always at 18.00 hours, after the afternoon meal, and, in terms of total digesta, was 29–38 % greater than the minimum which was always at the empyting at 08.00 hours. Thus, the cows did not eat to maintain a constant rumen fill, and the day could be divided into a period from about 08.30 hours to about 18.00 hours when rumen fill was increasing to a maximum and which broadly coincided with the eating period, and the remaining period when rumen fill was decreasing which broadly coincided with the resting period. A very similar pattern was found by Gill *et al.* (1988) with young cattle given free access to grass hay or grass silage.

From the results of the present experiment, the only occasion when rumen fill might have been limiting intake was at about 18.00 hours with the LC-silage diets, although the results do not provide direct evidence that rumen fill was the limiting factor even then. Unless there is some interaction between silage type and rumen fill capacity, the intake of EC-silage must have been limited by other factor(s). Indeed other workers have concluded that, while rumen fill may limit the meal size and total intake of some dried forages, other factors are probably more important with grass silage (Waldo *et al.* 1965; Campling, 1966; Gill *et al.* 1988). Among many factors proposed are fermentation products such as VFA and NH_3 produced in the rumen (Baile & Forbes, 1974). In the present experiment, mean and maximum concentrations of rumen NH_3 were higher with the EC-silage than with LC-silage and so could have contributed to the limitation of EC-silage intake. Although VFA concentrations in the rumen undoubtedly contribute to intake control, the effects are complex (Gill *et al.* 1988) and the result of the present experiment did not allow their role to be critically evaluated.

The effects of increasing the amount of concentrates were to increase total DM intake and reduce silage intake. The silage intake was depressed to an extent which resulted in the same total intake of indigestible DM, NDF and INDF at both concentrate levels. The mean reduction in silage DM intake (substitution rate) was 0·44 kg/kg concentrate DM with EC-silage and 0·35 kg/kg concentrate DM with LC-silage. The higher level of concentrates reduced the degradation rate and tended to increase the lag time of both silages but the effects on rumen retention time and rate of passage out of the rumen were not clear from the present results.

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REFERENCES

Baile, C. A. & Forbes, J. M. (1974). Control of feed intake and regulation of energy balance in ruminants. *Physiological Reviews* **54**, 160–214.

Balch, C. C. (1958). Observations on the act of eating in cattle. *British Journal of Nutrition* **12**, 330–345.

Blaxter, K. L., Graham, N. McC. & Wainman, F. W. (1956). Some observations on the digestibility of food by sheep, and on related problems. *British Journal of Nutrition* **10**, 69–91.

Blaxter, K. L., Wainman, F. W. & Wilson, R. S. (1961). The regulation of food intake by sheep. *Animal Production* **3**, 51–61.

Bueno, L. (1975). Les fonctions motrices et digestives du feuillet. (The mechanical and digestive function of the omasum) PhD Thesis, University of Toulouse.

Campling, R. C. (1966). The intake of hay and silage by cows. *Journal of the British Grassland Society* **21**, 41–48.

Campling, R. C. & Freer, M. (1962). The effect of specific gravity and size on the mean time of retention of inert particles in the alimentary tract of the cow. *British Journal of Nutrition* **16**, 507–518.
Davidson, J., Mathieson, J. & Boyne, A. W. (1970). The use of automation in determining nitrogen by the Kjeldahl method, with final calculation by computer. *Analyst* **95**, 181–193.
DesBordes, C. K. & Welch, J. G. (1984). Influence of specific gravity on rumination and passage of indigestible particles. *Journal of Animal Science* **59**, 470–475.
Dhanoa, M. S. (1988). On the analysis of dacron bag data for low degradability feeds. *Grass and Forage Science* **43**, 441–444.
Dhanoa, M. S., Siddons, R. C., France, J. & Gale, D. L. (1985). A multicompartmental model to describe marker excretion patterns in ruminant faeces. *British Journal of Nutrition* **53**, 663–671.
Dixon, R. M. & Milligan, L. P. (1985). Removal of digesta components from the rumen of steers determined by sieving techniques and fluid, particulate and microbial markers. *British Journal of Nutrition* **53**, 347–362.
Ehle, F. R. (1984). Influence of feed particle density on particulate passage from rumen of Holstein cow. *Journal of Dairy Science* **67**, 693–697.
Ehrlein, H. J. (1979). Motility of the forestomachs in goats. *Annales de Recherches Vétérinaires* **10**, 173–175.
Ellis, W. C., Lascano, C., Guerrero, J., Pond, K. & Matis, J. H. (1982). Particle size degradation in and escape from the rumen. *Federation Proceedings* **41**, 342.
Ellis, W. C., Matis, J. H. & Lascano, C. (1979). Quantitating ruminal turnover. *Federation Proceedings* **38**, 2702–2706.
Evans, E. W., Pearce, G. R., Burnett, J. & Pillinger, S. L. (1973). Changes in some physical characteristics of the digesta in the reticulo-rumen of cows fed once daily. *British Journal of Nutrition* **29**, 357–376.
Faichney, G. J. (1975). The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In *Digestion and Metabolism in the Ruminant*, pp. 277–291 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: The University of New England Publishing Unit.
Gasa, J. & Sutton, J. D. (1991). Empleo de marcadores en estudios de cinética de paso a través del tracto digestivo de vacas lecheras (Use of markers in studies of the kinetics of rates of passage through the digestive tract of lactating cows). *Investigación Agraria: Producción y Sanidad Animal* **6**, 39–50.
GENSTAT 5 (1987). *Genstat 5 Reference Manual*. Oxford: Clarendon Press.
Gill, M., Rook, A. J. & Thiago, L. R. S. (1988). Factors affecting the voluntary intake of roughages by the dairy cow. In *Nutrition and Lactation in the Dairy Cow*, pp. 262–279 [P. C. Garnsworthy, editor]. London: Butterworths.
Gill, M., Sargeant, A. & Evans, R. T. (1987). The effect of type of silage and level of concentrate on pattern of eating in dairy cows. In *Proceedings of the Eighth Silage Conference*, pp. 63–64. Hurley, Berks.: AFRC Institute for Grassland and Animal Production.
Goering, H. K. & Van Soest, P. J. (1970). Forage fibre analysis. *Agricultural Handbook* no. 379. Washington, D.C.: US Department of Agriculture.
Goetsch, A. L. & Owens, F. N. (1985). Effects of sampling site on passage rate estimates in heifers fed alfalfa hay or high concentrate diet. *Journal of Dairy Science* **68**, 914–922.
Grovum, W. L. & Williams, V. J. (1973). Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces. *British Journal of Nutrition* **30**, 313–329.
Hartnell, G. F. & Satter, L. D. (1979). Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. *Journal of Animal Science* **48**, 381–392.
Hungate, R. E. (1966). *The Rumen and its Microbes*. New York: Academic Press.
Matis, J. H. (1972). Gamma time-dependency in Blaxter's compartmental model. *Biometrics* **28**, 597–602.
Ørskov, E. R. & McDonald, I. (1979). The estimation of protein degradability from incubation measurements weighted according to the rate of passage. *Journal of Agricultural Science, Cambridge* **92**, 499–503.
Owens, F. N. & Goetsch, A. L. (1986). Digesta passage and microbial protein synthesis. In *Control of Digestion and Metabolism in Ruminants*, pp. 196–233 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.
Pond, K. R., Ellis, W. C., Matis, J. H., Ferreiro, H. M. & Sutton, J. D. (1988). Compartment models for estimating attributes of digesta flow in cattle. *British Journal of Nutrition* **60**, 571–595.
Poppi, D. P., Norton, B. W., Minson, D. J. & Hendricksen, R. W. (1980). The validity of the critical size theory for particles leaving the rumen. *Journal of Agricultural Science, Cambridge* **94**, 275–280.
Robinson, P. M. & Sniffen, C. J. (1983). Comparison of rumen, duodenal and faecal sampling sites to estimate rumen turnover rate of markers in cows. *Journal of Dairy Science* **66**, Suppl. 1, 187.
Ross, G. J. S. (1987). *MLP, Maximum Likelihood Program, Version 3.08*, Oxford: Numerical Algorithms Group.
SAS Institute Inc. (1985). *Statistical Analysis System User's Guide: Statistics*. Cary, North Carolina: SAS Institute Inc.
Siddons, R. C., Arricastres, C., Gale, D. L. & Beever, D. E. (1984). The effect of formaldehyde or glutaraldehyde application to lucerne before ensiling on silage fermentation and silage N digestion in sheep. *British Journal of Nutrition* **52**, 391–401.
Siddons, R. C., Paradine, J., Beever, D. E. & Cornell, P. R. (1985). Ytterbium acetate as a particulate-phase digesta flow marker. *British Journal of Nutrition* **54**, 509–519.

Teeter, R. G. & Owen, F. N. (1983). Characteristics of water soluble markers for measuring rumen liquor volume and dilution rate. *Journal of Animal Science* **56**, 717–728.
Uden, P., Colucci, P. E. & Van Soest, P. J. (1980). Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. *Journal of the Science of Food and Agriculture* **31**, 625–632.
Waghorn, C. C. (1986). Changes in rumen digesta of cows during a restricted feeding period when offered fresh red clover, lucerne, or lucerne hay. *New Zealand Journal of Agricultural Research* **29**, 233–241.
Waldo, D. R., Miller, R. W., Okamoto, M. & Moore, L. A. (1965). Ruminant utilization of silage in relation to hay, pellets, and hay plus grain. 1. Composition, digestion, nitrogen balance, intake and growth. *Journal of Dairy Science* **48**, 910–916.
Warner, A. C. I. (1981). Rate of passage of digesta through the gut of mammals and birds. *Nutrition Abstracts and Reviews* **51**, 789–820.

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