

Ruminal and total plant cell-wall digestibility estimated by a combined *in situ* method utilizing mathematical models

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Three ruminally and duodenally cannulated non-lactating Finnish Ayrshire cows were used to investigate ruminal and intestinal digestion of cell-wall carbohydrates by a combined *in situ* method. Five grasses cut at 10 d intervals were incubated in the rumen for 0, 6, 12, 24, 48, 72 and 96 h, and the undegraded residues were exposed to intestinal digestion. With advancing maturity of grass both the rate and extent of cell-wall digestion decreased. At early stages of growth the decreases were faster for the rate of digestion and at late stages of growth for the extent of digestion. Applying a passage rate of 0.02/h in one compartmental rumen model resulted in digestibility values markedly lower than typically observed *in vivo*. However, applying a rumen model incorporating a selective retention of particles and time-dependent release of particles from the non-escapable pool resulted in much higher digestibility values. Recovery of lignin after 96 h ruminal incubation with a subsequent mobile-bag incubation was very low (from 244 to 460 mg/g). Intestinal disappearance of neutral-detergent fibre (NDF) and hemicellulose decreased with advancing maturity of grass and with increasing length of preceding ruminal incubation period, i.e. with decreasing potential digestibility of the material. Disappearance of hemicellulose was much greater than that of cellulose for intact grasses but the difference diminished with increasing length of preceding rumen incubation period. On average, 195 mg/g of potentially digestible NDF disappeared from the mobile bags in the intestines. The post-ruminal digestion as a proportion of the total NDF digestibility varied between 0.034 and 0.058. Despite methodological problems both in ruminal *in situ* and intestinal mobile bag techniques, these methods can be used to investigate ruminal and intestinal cell-wall digestion and to partition cell-wall digestibility between ruminal and post-ruminal digestion providing that appropriate rumen models are used.

Ruminant: Cell wall: Digestion kinetics: Mobile-bag method

A variable proportion of plant cell-wall carbohydrates is digested in the caecum and proximal colon by microbial fermentation (Ulyatt *et al.* 1975). Several factors can shift the site of cell-wall digestion from the rumen to the hindgut. These factors include physical processing, increased level of feed intake and supplementation with rapidly fermentable carbohydrates. The contribution of the hindgut to the total cell-wall digestion was shown to be especially large in sheep receiving fat supplements which markedly depressed ruminal cell-wall digestibility (Igwuegbu & Sutton, 1982).

To determine the digestibility of cell-wall fractions in the large intestine, measurements of digesta flow in the ileum are required. Duodenal flow may also be used because no significant digestion of cell-wall carbohydrates occurs in the small intestine (Hoover, 1978). Digesta flow measurements are relatively inaccurate considering that a relatively small proportion of cell-wall digestion takes place in the hindgut, and consequently the variability in post-ruminal cell-wall digestion calculated as the difference between either duodenal or ileal flow and faecal output can sometimes be large. The

mobile-bag method, which was originally introduced to study protein digestibility in pigs (Sauer *et al.* 1983) and later applied to investigate intestinal digestion of ruminally undegraded feed protein (Hvelplund, 1985; Voigt *et al.* 1985; de Boer *et al.* 1987), is an alternative technique for the investigation of the extent of post-ruminal cell-wall digestion. Varvikko & Vanhatalo (1990) found large differences between feeds when the intestinal digestibility of neutral-detergent fibre (NDF) was determined from intact feed samples, i.e. from samples that had not been exposed to rumen digestion before introducing them into the duodenum. They also used a combined nylon bag method to estimate true digestibility of the nutrients of grass silage (Varvikko & Vanhatalo, 1992). Vanhatalo & Ketoja (1995) observed that the recovery site (ileum *v.* faeces), bag cloth type and ruminal pre-incubation of the samples had significant effects on NDF disappearance from mobile bags.

The objective of the present study was to evaluate the nylon-bag method for estimating ruminal and post-ruminal digestion of cell-wall components of dried grass. The potential digestibility of the samples introduced into the duodenum was modified by cutting the grass at five different stages of maturity and varying the length of the preceding ruminal incubation period. The data obtained from ruminal and post-ruminal cell-wall digestion studies were utilized in digestion models to estimate ruminal and total digestibility of cell-wall carbohydrates. The results pertaining to degradation of N constituents have been published previously (Vanhatalo *et al.* 1996).

EXPERIMENTAL METHODS

Primary growth of timothy (*Phleum pratense*) sward was cut at five different stages of growth at 10 d intervals from 3 June onwards. The experimental grass samples were oven-dried at 60 ° for 48 h and milled to pass a 2 mm screen. Three non-lactating Finnish Ayrshire cows (live weight 550 kg) were used to investigate ruminal and post-ruminal degradation of grass cell walls using the nylon-bag technique. The cows were fitted with a permanent rumen cannula and a simple T-piece duodenal cannula. The animals were fed twice daily at maintenance level with grass silage and barley (70:30 on a DM basis). The crude protein content of the diet was 170 g/kg DM. The grasses were incubated in the rumen for 0, 6, 12, 24, 48, 72 and 96 h. The residues from each incubation period were pooled per grass and cow. Part of each residue was used for chemical analyses and part for subsequent mobile-bag studies. Grass residues and intact grass (0.8 g) were weighed into mobile bags (*n* 6–10 per sample). The bags were made of the same material as rumen nylon bags (free surface area 33 % and pore size 41 µm). NDF, acid-detergent fibre (ADF) and permanganate lignin were determined according to the methods of Robertson & Van Soest (1981). Cellulose was calculated as the difference between ADF and lignin, and hemicellulose as the difference between NDF and ADF.

The disappearance of different cell-wall fractions in the rumen was fitted to the equations of McDonald (1981) by using non-linear procedures:

$$P_1 = a' \quad \text{up to time } t_0,$$

$$P_2 = a + b(1 - e^{-kdt}) \quad \text{from time } t_0 \text{ onwards.}$$

The potential extent of degradation (PD) was calculated as the sum of instantly degraded (*a*) and slowly degraded (*b*) cell-wall fractions. Ruminal digestibility (RD) of cell-wall fractions was calculated according to Allen & Mertens (1988) using the simple model: $RD = PD \times k_d / (k_d + k_p)$, in which k_d and k_p are the rates of digestion and passage for cell-wall fractions. The values 0.01 and 0.02 were used for k_p . These values are based on

passage rates of NDF and digestible NDF determined by the rumen evacuation technique (Huhtanen & Kukkonen, 1995). Total disappearance and the proportion of ruminal disappearance after each rumen incubation period were also computed for each cell-wall fraction.

The ruminal, post-ruminal and total digestibility of cell-wall fractions were also calculated using a modelling approach. A two-compartment model (Allen & Mertens, 1988) incorporating a selective retention of particles in the rumen (Fig. 1) was used. Cell-wall fractions can disappear from the first compartment (non-escapable pool) either by digestion (k_d) and/or release to the second compartment (escapable pool). The rate of the release (k_r) of particles is a function of the changes both in the specific gravity and size of particles. From the escapable pool cell-wall fractions disappear either by digestion or passage (k_p) to the lower tract. By definition k_d for indigestible cell-wall fractions is zero. The k_r has been shown to be time-dependent (Pond *et al.* 1988), i.e. the probability of the particles escaping increases with time spent in the system. In cattle, the best fit of duodenal Yb concentration data was found with a model including the third degree of gamma time-dependency in the first compartment (Huhtanen & Kukkonen, 1995). The values of 20 and 30 h were used as mean retention times (MRT) in the non-escapable and escapable pools. These values were obtained from cattle fed at a level of 80 g DM/kg metabolic weight (Huhtanen & Kukkonen, 1995). The k_r at time t is a derivative of the dose (D) remaining in the system. In the model with the third order of gamma time dependency in the non-escapable pool, the dose remaining in the system is $De^{-\lambda t}(1 + \lambda t + 0.5(\lambda t)^2)$ and its derivative $(0.5\lambda^3 + t^2)/(1 + \lambda t + 0.5(\lambda t)^2)$, in which λ is $3/\text{MRT}$ (Pond *et al.* 1988). The amount of each cell-wall fraction digested in the lower tract was computed using, at any time, polynomial interpolation of the disappearance values determined by the mobile-bag technique. Simulations were made by POWERSIM[®] software (version 2.0; Bergen, Norway). Simulation step of 0.125 h was used.

Intestinal digestion time (IT) was calculated using the following equation:

$$IT = [\ln((a + b - \text{NDF}_{\text{total}})/b) / -k_d] - [\ln(a + b - \text{NDF}_{\text{rumen}}) / b] / -k_d,$$

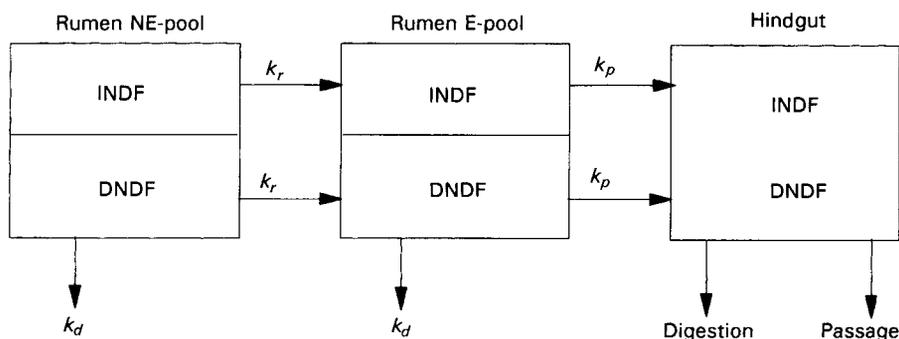


Fig. 1. Digestion model used to calculate neutral-detergent fibre (NDF) digestion in the rumen, hindgut and the total digestive tract. NE-pool, rumen non-escapable pool, from which NDF can disappear by digestion (k_d) or by release (k_r) to rumen escapable pool (E-pool). From E-pool NDF can disappear by k_d or by passage (k_p) to the hindgut (Allen & Mertens, 1988). From the hindgut NDF disappears either by digestion or passage. Digestibility in the hindgut was interpolated at any time from the disappearance values determined by the mobile-bag method. DNDF, potentially digestible NDF; INDF, indigestible NDF.

where $\text{NDF}_{\text{rumen}}$ and $\text{NDF}_{\text{total}}$ refer to disappearance of NDF from bags in the rumen and total tract respectively. This equation is based on the assumption that the rate of digestion (k_d) in the lower-gut fermentation pool is similar to that in the rumen.

The data were subjected to a two-way ANOVA; cow (df 2) and grass (df 4) were the main sources of variation. The sums of squares of the grass effects were further separated into single degree polynomial orthogonal contrasts to estimate linear and quadratic effects of grass maturity. To evaluate the effects of rumen incubation time and interaction between grass maturity and rumen incubation time on subsequent post-ruminal digestion the data were analysed as a split-plot design using the following model: $y = A_i + G_j + e_{ij} + T_k + (AT)_{ik} + (AG)_{jk} + e_{ijk}$, where A, G and T are the effects of animal, grass and rumen incubation time with their corresponding interactions.

RESULTS

The chemical composition of the grasses is presented in Table 1. As expected, the content of cell-wall fractions increased and N content declined with advancing maturity of grass. However, the changes were not linear. NDF content increased more rapidly during the early phase of growth than later. The cellulose content increased much more with the maturity of grass than the hemicellulose content which remained rather constant.

Both the extent (PD) and rate of digestion (k_d) of all cell-wall fractions calculated according to McDonald (1981) decreased (at least $P < 0.01$) with maturity (Table 2). The changes were quadratic with a greater depression for k_d occurring during the early stage of growth and for PD during the late stage of growth. The effects of maturity on k_d of cellulose and hemicellulose were similar but the extent of hemicellulose digestion decreased more than that of cellulose. The estimated lag time of NDF digestion varied quadratically ($P < 0.05$) and for cellulose cubically ($P < 0.05$) reaching a minimum with grass IV. The lag time of hemicellulose digestion was negative for all grasses. Because of the reverse changes in kinetic parameters with advancing maturity of grass, the decreases in calculated RD of cell-wall constituents were rather linear ($P < 0.001$). The regression analyses showed that the average decrease in NDF digestibility was 6.3 mg/g per d. As expected from the greater decrease in PD for hemicellulose than for cellulose digestion, hemicellulose digestibility decreased more with advancing maturity of grass (6.7 v. 5.3 mg/g per d).

There was a considerable loss of lignin from the bags during ruminal incubations (Fig. 2). The disappearance of lignin decreased with advancing maturity of grass and increased

Table 1. Chemical composition of timothy grass cut at different stages of growth*

Component (g/kg DM)	Grass				
	I	II	III	IV	V
Organic matter	918	923	930	934	944
Nitrogen	29	24	19	14	12
Neutral-detergent fibre	509	564	664	703	671
Acid-detergent fibre	239	292	371	410	401
Lignin	49	39	60	69	80
Cellulose	190	253	311	342	321
Hemicellulose	269	272	293	293	271

* Grasses I–V were cut from a timothy sward at five different stages of growth at 10 d intervals.

Table 2. The effects of grass maturity on the parameters of ruminal digestion kinetics and calculated ruminal digestibility of cell-wall fractions

	Grass					SEM	Statistical significance‡	
	I	II	III	IV	V		Lin	Quad
NDF								
PD (mg/g)	884	901	853	809	712	10.6	***	***
k_d (1/h)	0.0686	0.0425	0.0345	0.0278	0.0325	0.00398	***	**
Lag time (h)	0.8	-0.3	-0.6	-2.0	1.9	0.79	NS	*
RD§ (mg/g)	681	609	539	467	440	10.4	***	†
RD (mg/g)	770	726	660	591	543	5.2	***	†
Cellulose								
PD (mg/g)	891	931	892	855	759	12.1	***	***
k_d (1/h)	0.0627	0.0422	0.0344	0.0289	0.0326	0.00389	***	***
Lag time (h)	2.2	2.5	2.9	0.4	3.5	0.62	NS	NS
RD§ (mg/g)	672	627	563	501	469	10.9	***	NS
RD (mg/g)	766	749	690	632	580	5.1	***	**
Hemicellulose								
PD (mg/g)	905	901	849	790	708	5.6	***	***
k_d (1/h)	0.0750	0.0448	0.0366	0.0314	0.0344	0.00347	***	***
Lag time (h)	-0.4	-2.7	-1.2	-4.5	-1.3	1.00	NS	NS
RD§ (mg/g)	712	619	549	481	446	9.0	***	**
RD (mg/g)	797	733	667	597	547	5.7	***	NS

k_d , rate of digestion; PD, potential digestibility; RD, ruminal digestibility; NDF, neutral-detergent fibre.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, † $P < 0.10$.

‡ Linear and quadratic effect of the maturity of the grass.

§ Passage rate 0.02/h.

|| Passage rate 0.01/h.

with increasing rumen incubation period. Ruminal lignin 'digestibility' calculated from kinetic parameters using a passage rate of 0.02/h decreased linearly ($P < 0.001$) from 648 (grass I) to 366 mg/g (grass V) with advancing maturity.

Intestinal disappearance of NDF for the intact grasses and rumen undegraded residues is shown in Fig. 3(a). Post-ruminal NDF digestion decreased ($P < 0.001$) with increasing rumen incubation period, the depression being much greater for early- than late-cut grasses (interaction grass \times time, $P < 0.001$).

Post-ruminal cellulose digestion only tended ($P < 0.06$) to decrease with increasing maturity of grass (Fig. 3(b)). Except for the intact grass, intestinal digestibility of cellulose was not dependent on the length of rumen incubation period. Much more hemicellulose than cellulose was digested in the lower gut (Fig. 3(c)). Hemicellulose digestibility decreased with advancing maturity of grass in a linear ($P < 0.001$) and quadratic ($P < 0.001$) manner, and with increasing rumen incubation period ($P < 0.001$). The decrease in the intestinal hemicellulose digestibility with increasing rumen incubation period was much greater for early-cut grasses than for late-cut grasses (time \times grass interaction, $P < 0.01$). The disappearance of lignin from the mobile bags was on average greater than for other cell-wall constituents (mean 228 mg/g). When averaged over grasses, lignin disappearance decreased from 279 (intact grass) to 188 mg/g (96 h rumen incubation).

Grass maturity had no effect on the proportion of total NDF digestion occurring in the rumen (Fig. 4(a)). The post-ruminal digestion accounted for a decreasing proportion of total NDF digestion as rumen incubation period increased but the changes with time did not

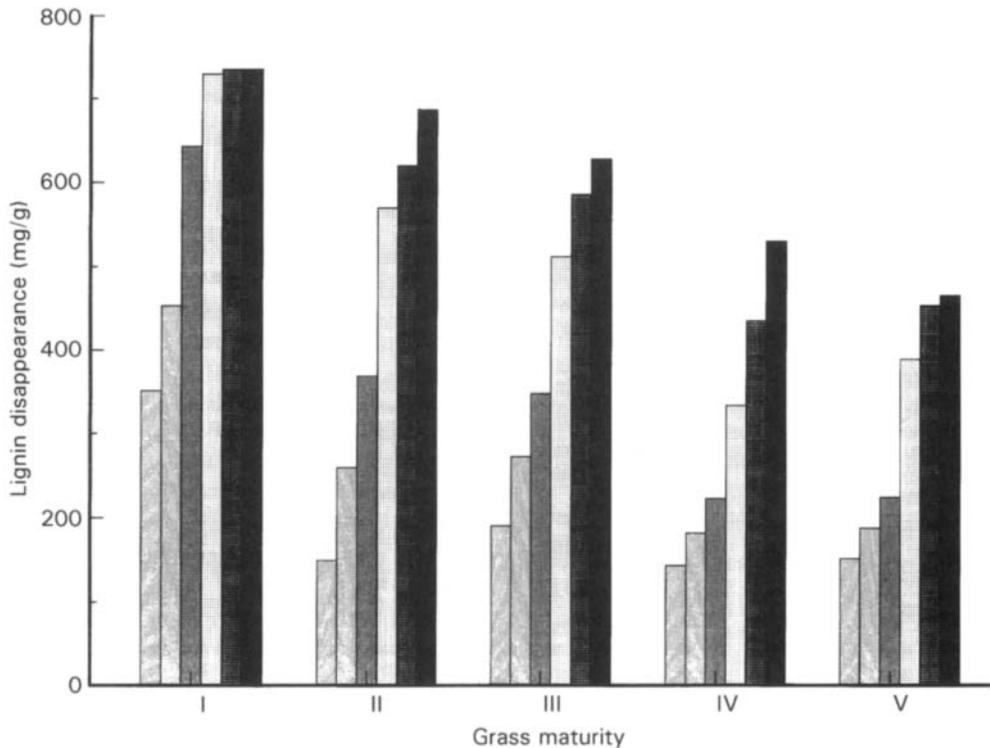


Fig. 2. The effects of grass maturity (I, earliest; V, latest) and rumen incubation period (6–96 h) on disappearance of lignin from the rumen bags in cattle. (□), 6 h; (▨), 12 h; (▩), 24 h; (▧), 48 h; (▦), 72 h; (■), 96 h.

depend on the maturity of grass. The ruminal digestion as a proportion of total cellulose digestion decreased linearly ($P < 0.01$) with advancing maturity of grass (Fig. 4(b)), mainly because of reduced ruminal digestibility and not because of quantitatively increased post-ruminal digestion. Grass maturity had no effect on the site of hemicellulose digestion (Fig. 4(c)). Thus, the contribution of the lower-gut to the total disappearance of cell-wall constituents was relatively small, especially when the preceding rumen incubation period was 24 h or longer.

The results of simulation of ruminal, post-ruminal and total cell-wall digestion using a two-compartment model (Allen & Mertens, 1988) are shown in Table 3. Ruminal NDF digestibility decreased with maturity from 789 to 509 mg/g and total digestibility from 815 to 541 mg/g. The decrease in total digestibility was 7.2 mg/g per d. Post-ruminal NDF digestibility remained fairly stable with increasing maturity of grass. Ruminal digestion as a proportion of total NDF digestion averaged 953 mg/g, and it decreased linearly ($P < 0.05$) with the maturity of grass. The effect of the maturity of grass on ruminal and total digestibility was greater for hemicellulose than for cellulose. The ruminal digestion accounted for, on average, 971 and 967 mg/g of the total digestibility of cellulose and hemicellulose. Grass maturity had no consistent effect on post-ruminal digestion of cellulose and hemicellulose.

Intestinal digestion time for NDF corresponded to 4.9 (SE 0.29) h assuming a similar rate of digestion in the lower tract to that in the rumen. The values ranged from 4.3 (grass II) to 6.6 h (grass I) and from 3.6 (24 h ruminal incubation) to 7.7 h (48 h ruminal incubation).

DISCUSSION

Ruminal digestion, one-compartment model

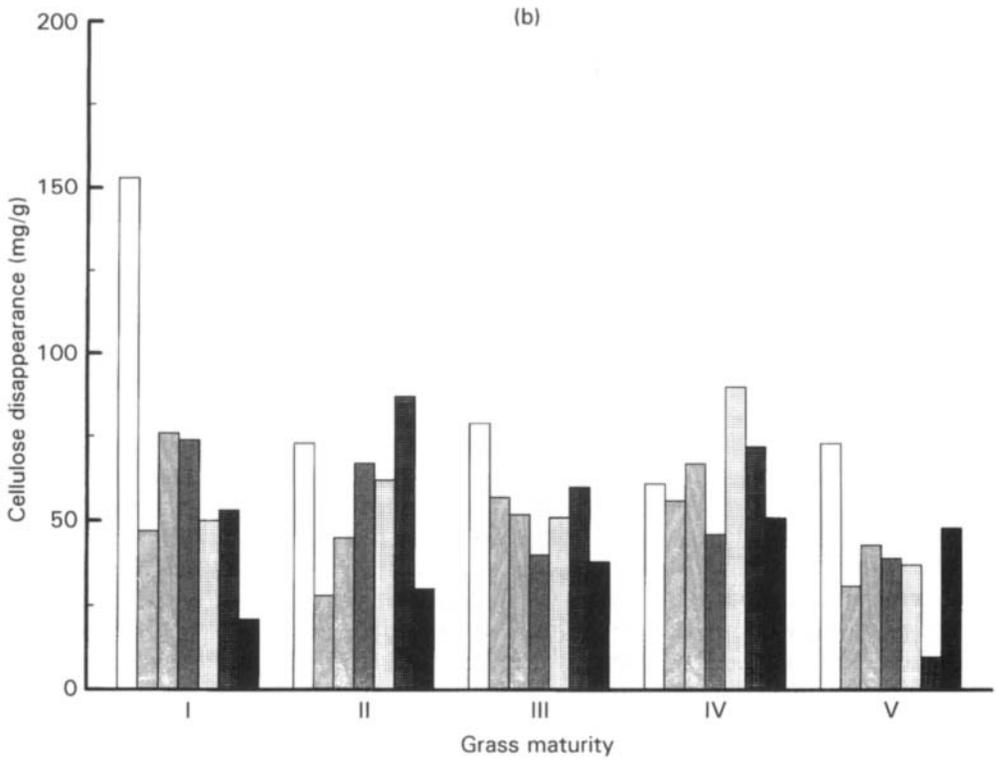
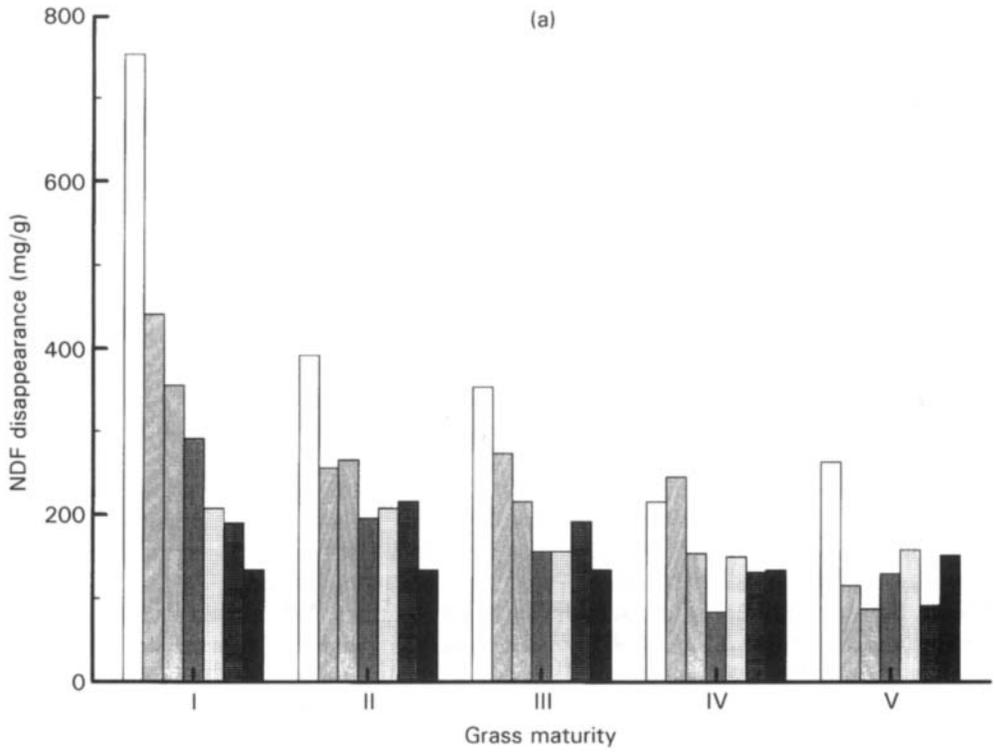
In agreement with the observations of Smith *et al.* (1972) *in vitro*, and Lindberg & Lindgren (1988), van Vuuren *et al.* (1989), Bosch *et al.* (1992) and Huhtanen & Jaakkola (1994) *in situ*, both the k_d and PD of cell-wall degradation decreased with advancing grass maturity. The curvilinear changes in kinetic parameters were similar to those reported by Huhtanen & Jaakkola (1994). The decrease in k_d became slower with advancing maturity while the decrease in PD became faster. These changes may be associated with the changes in plant anatomy and cell-wall architecture.

The values for RD of NDF calculated using a value of 0.02/h for k_p were markedly lower than those observed *in vivo* but the values were similar to those based on an *in situ* technique (Huhtanen & Jaakkola, 1994). With early-cut grasses both ruminal and total digestibility of cellulose and hemicellulose *in vivo* have often exceeded 800 g/kg (Beever *et al.* 1972, 1986; Khalili & Huhtanen, 1991; Tuori *et al.* 1992).

Lower cell-wall digestibility *in situ* than *in vivo* is partly related to underestimation of k_d by the *in situ* method (Tamminga *et al.* 1989; Stensig *et al.* 1994; Huhtanen *et al.* 1995). The lower number of cellulolytic bacteria (Mackie & Meyer, 1986) and lower fibrolytic activities (Huhtanen & Khalili, 1992; Nozière & Michalet-Doreau, 1996) within the bags than in the surrounding digesta support these observations. When used in rumen models, the lower rate of degradation of NDF *in situ* than *in vivo* can lead to a considerable bias in ruminal digestibility (Huhtanen *et al.* 1995). Even using a value of 0.01/h for k_p resulted in values of ruminal NDF digestibility which are likely to be smaller than the corresponding *in vivo* digestibility. Although the digestibility values based on *in situ* data are biased, they still provide useful information about the changes in digestibility with advancing maturity of grass. The decrease in NDF digestibility (6.3 mg/g per d) corresponds to the values of 5.3 and 6.0 derived from *in vivo* digestibility of timothy (Lindberg & Lindgren, 1988) and meadow fescue (*Festuca pratensis*; Tuori *et al.* 1992). The digestibility of the potentially digestible fraction (96 h ruminal incubation + mobile bag) of NDF decreased from 767 (grass I) to 600 (grass IV) and 628 mg/g (grass V) with advancing maturity when a value of 0.02/h was used for k_p (results not shown). However, this fraction seems to be independent of forage quality because 850–900 mg/g of potentially digestible fraction was actually digested (Tamminga, 1993). On that basis, the *in situ* method may underestimate the digestibility of late-cut grass more than that of early-cut grass.

Ruminal digestion, two-compartment model

When a model of two ruminal compartments (Allen & Mertens, 1988) and a time-dependent release from the first compartment (Pond *et al.* 1988) was applied, the values for ruminal and total cell-wall digestibility were much higher than those obtained using a one-compartment model despite similar total rumen retention time (50 h) being used. In a previous study (Huhtanen *et al.* 1995), using the two-compartment model resulted in digestibility values which were much closer to *in vivo* data than the values obtained by the one-compartment model. Our values also agree with *in vivo* digestibility values for cell fractions of early-cut fresh (Beever *et al.* 1986), dried (Beever *et al.* 1972) and ensiled (Khalili & Huhtanen, 1991; Tuori *et al.* 1992) grasses. It appears that the model estimated a greater depression in the digestibility with advancing maturity than typically found *in vivo*. This may be related to the distribution of compartmental retention time between the non-escapable and the escapable pool. Irrespective of grass maturity, values of 20 and 30 h



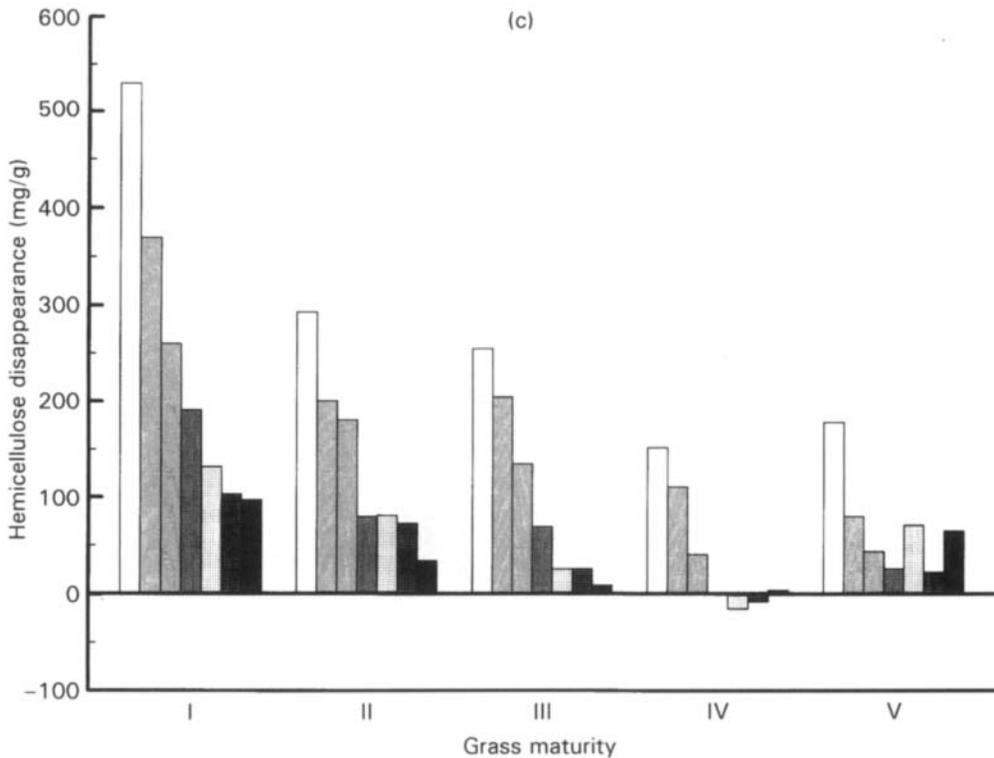
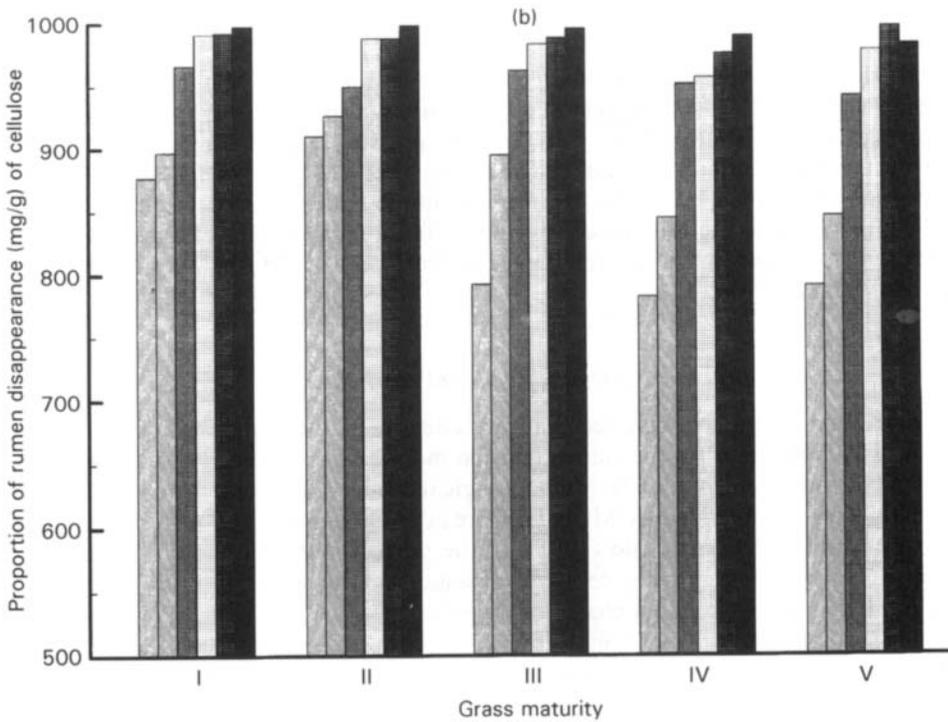
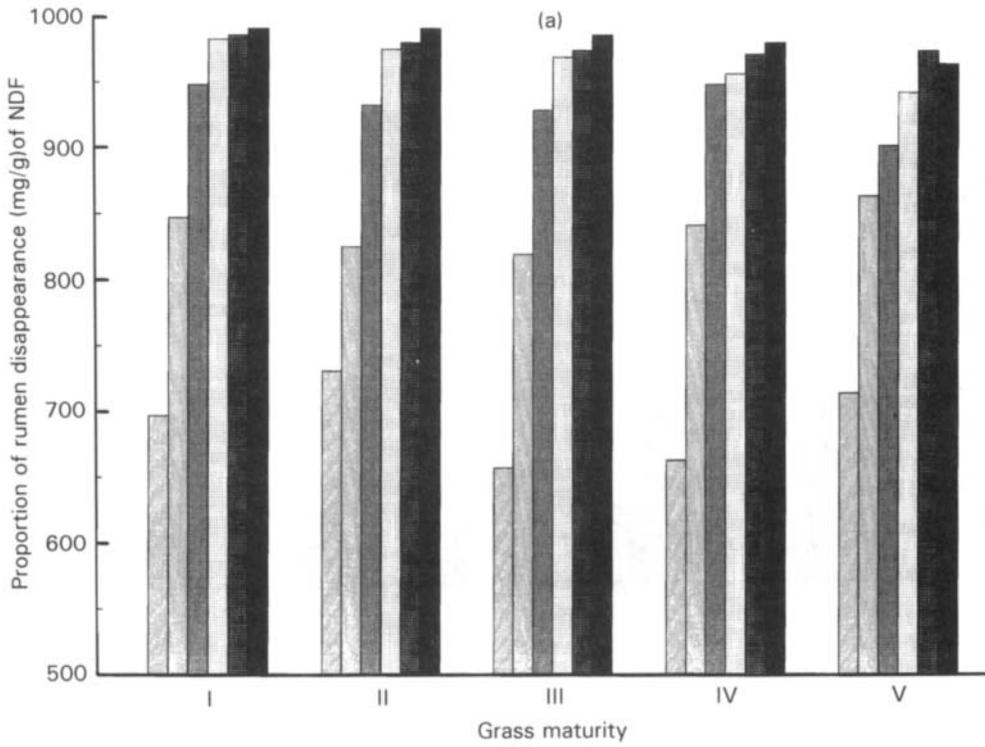


Fig. 3. The effects of grass maturity (I, earliest; V, latest) and rumen incubation period (0–96 h) on disappearance of (a) neutral-detergent fibre (NDF), (b) cellulose and (c) hemicellulose from mobile bags in cattle. (□), Intact; (▨), 6 h; (▩), 12 h; (▤), 24 h; (▥), 48 h; (▦), 72 h; (▧), 96 h.

were used. However, the data from studies of Pond *et al.* (1987), Mambrini & Peuraud (1994) and Rinne *et al.* (1997) suggest that with early-cut grass the retention time in the non-escapable pool is shorter than with late-cut grasses. Easier comminution of the particles during rumination and a faster increase in specific gravity of the particles from early-cut grass make them available for passage faster than the particles from late-cut grass. The effect of grass maturity on cell-wall digestibility may also be exaggerated by the model because total compartmental retention can increase with advancing grass maturity (see e.g. Pond *et al.* 1987).

Intestinal digestion of cell-wall carbohydrates

The *in situ* method has gained wide acceptance for determination of digestion kinetics of N and cell-wall carbohydrates in the rumen and the mobile-bag method is used to estimate digestion of ruminally undegraded N. Methodological details of the rumen *in situ* technique have recently been summarized by Michalet-Doreau & Ould-Bah (1993) and those of the mobile bag technique by Vanhatalo (1995). In the present study the mobile-bag method was used to evaluate post-ruminal cell-wall digestion and to partition total digestibility between ruminal and post-ruminal phases of digestion. The use of the mobile-bag method for studying post-ruminal cell-wall digestion can be criticized for the same reasons as the ruminal *in situ* method. Since microbial access to cell walls within the bags may be limited, as in the *in situ* method (Mackie & Meyer, 1986; Huhtanen & Khalili, 1992), the values



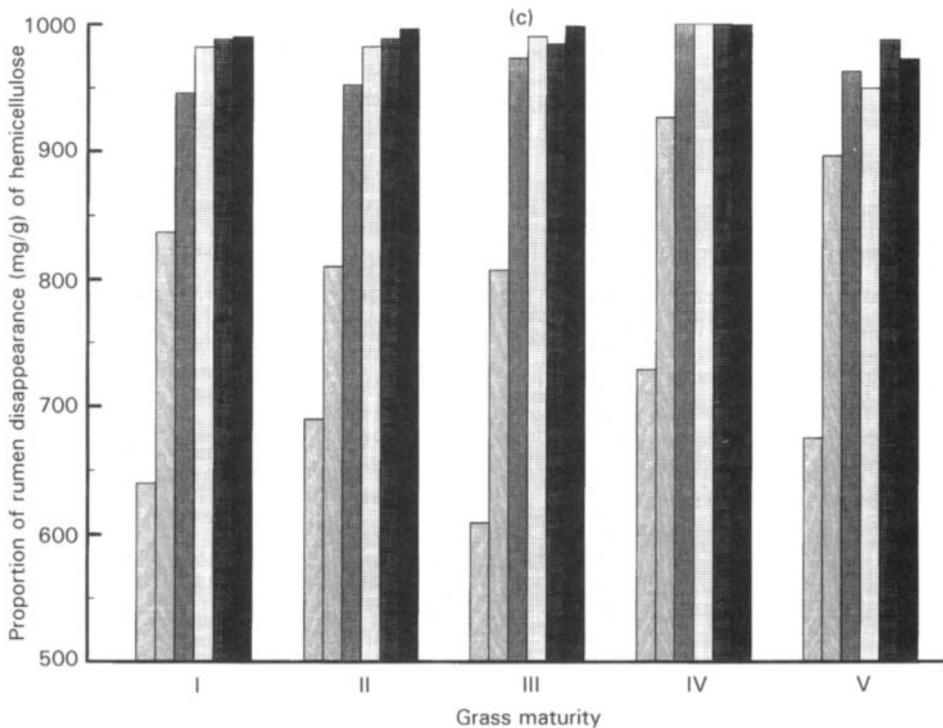


Fig. 4. The effects of grass maturity (I, earliest; V, latest) and rumen incubation period (6–96 h) on the proportion of ruminal disappearance of total disappearance of (a) neutral-detergent fibre (NDF), (b) cellulose and (c) hemicellulose. (□), 6 h; (▤), 12 h; (▥), 24 h; (▦), 48 h; (▧), 72 h; (▨), 96 h.

may be biased downwards. Moreover, disappearance of small particles can occur without microbial degradation. NDF loss from 0 h rumen bags varied between 35 (grass V) and 100 mg/g (grass I). Particle loss partly explains the greater disappearance of intact grass compared with grasses which were pre-incubated in the rumen. Low recovery of lignin after 96 h ruminal incubation and a subsequent intestinal digestion (from 244 (grass I) to 460 mg/g (grass V)) suggest that the nylon-bag method, like other methods based on insoluble gravimetric residues, will overestimate potential cell-wall digestibility. Based on the lignin data of Giger (1985), Van Soest (1995) recalculated that the recovery of lignin in faeces was 860 mg/g. This value is much higher than observed in the present study even with the latest-cut grass. Lower molecular mass fragments of lignin are absorbed and excreted in the urine (Van Soest, 1995), which partly explains why the recovery is not complete. However, solubilization of lignin polymers rather than absorption is a more likely explanation for the large difference in lignin recovery in the present study and in the data reviewed by Van Soest (1995). According to Van Soest (1995), using permanganate oxidation to determine lignin will lead to systematic apparent digestibility, which partly explains our high values for total lignin digestibility.

Pore size and open surface area of the bags used and sample size are also critical aspects in nylon-bag techniques. Varvikko & Vanhatalo (1990) and Vanhatalo & Ketoja (1995) demonstrated the importance of cloth type on the intestinal digestibility values of N and also of NDF. In the present study the sample size was about 32 mg/cm² for the rumen bags and 22 mg/cm² for the mobile bags. However, when the sample size was calculated in terms of NDF per cm², the values were rather similar for the rumen and mobile bags. Because similar cloth type and sample size were used for ruminal and intestinal

Table 3. The effects of grass maturity on ruminal, intestinal and total digestibility of cell-wall fractions estimated by digestion model† using a two-compartment model (Allen & Mertens, 1988)

	Grass					SEM	Statistical significance§	
	I	II	III	IV	V		Lin	Quad
NDF digestibility (mg/g)								
Rumen	789	708	626	538	509	12.2	***	†
Intestines	28	31	31	29	31	3.1	NS	NS
Total	815	739	657	567	541	10.5	***	*
Rumen/total	966	957	953	949	942	4.8	**	NS
Cellulose digestibility (mg/g)								
Rumen	778	729	653	579	544	14.0	***	NS
Intestines	14	16	21	28	16	4.5	NS	NS
Total	791	745	673	606	561	13.6	***	NS
Rumen/total	983	978	970	954	971	6.6	NS	NS
Hemicellulose digestibility (mg/g)								
Rumen	819	720	638	557	517	10.0	***	*
Intestines	31	29	24	8	21	3.2	**	†
Total	851	749	662	564	539	9.5	***	**
Rumen/total	963	960	964	986	960	5.1	NS	†

NDF, neutral-detergent fibre.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, † $P < 0.10$.

† For modelling approach used, see detailed explanation given on pp. 584–586.

§ Linear and quadratic effect of the maturity of the grass.

|| mg/g intake.

incubations, the mobile-bag method may still be valid for comparing the capacity of ruminal and post-ruminal cell-wall digestion and partitioning of the digestion, in spite of the methodological limitations discussed earlier.

Longer retention time of the bags than that of digesta is a major concern when the mobile-bag method is applied to study post-ruminal cell-wall digestion. Vanhatalo & Ketoja (1995) reported that the retention time of bags was markedly longer than the retention time of Yb-labelled feed particles. Retention time of the bags does not seem to have any effect on N disappearance (Hvelplund, 1985; Voigt *et al.* 1985; Vanhatalo, 1995; Vanhatalo & Ketoja, 1995). However, the longer retention time of bags than digesta is more critical for NDF digestion. Vanhatalo & Ketoja (1995) found that increased retention time at lower level of feeding was reflected in higher values of intestinal NDF disappearance when compared with the values obtained at a higher level of feeding. Voigt *et al.* (1985) reported a positive correlation between retention time of the bags and DM disappearance. This may increase NDF digestion in the hindgut. As a result of the longer retention time of bags than digesta and of the positive relationship between retention time and digestibility, the mobile-bag method is more likely to overestimate than underestimate the contribution of the post-ruminal phase to cell-wall digestion.

Intestinal NDF digestibility decreased with the decreasing potential digestibility of material exposed to the duodenum, i.e. with advancing maturity of grass and increasing length of the preceding rumen incubation period. As a proportion of potentially digestible

NDF (96 h rumen incubation + mobile bag) 195 mg/g was digested in the intestines. The values were higher for grass I than for the other grasses (352 v. 156 mg/g), and for the intact grass than for rumen-undegraded residues (237 v. 150 mg/g (mean for incubation period of 6–24 h)). The high post-ruminal digestibility of hemicellulose compared with cellulose (210 v. 95 mg/g) can partly be attributed to high intestinal digestibility of NDF-bound N (Vanhatalo *et al.* 1996) and partly to a greater susceptibility of hemicellulose to mild acid hydrolysis in the abomasum and duodenum (Merchen & Bourquin, 1994; Van Soest, 1995). Also, comparing the values of 6 h ruminal incubation and intestinal digestion of intact grass displays a greater capacity of digestion of hemicellulose than cellulose in the intestines. Considerably more cellulose was digested during 6 h ruminal incubation than during intestinal digestion (182 v. 88 mg/g) while the reverse was true for hemicellulose (260 v. 282 mg/g). Ulyatt *et al.* (1975) concluded, based on a literature review, that more hemicellulose than cellulose was digested post-ruminally in sheep.

Contribution of post-ruminal digestion to the total cell-wall digestibility

In the present study, the amount of NDF digested post-ruminally was not related to grass maturity but when expressed as a proportion of total digestibility post-ruminal digestion increased with advancing maturity. Similar trends have been observed *in vivo* by Hogan *et al.* (1969) in sheep and by Südekum (1989) in cows. The importance of hindgut digestion appears to increase with decreasing total tract digestibility (Ulyatt *et al.* 1975; Tamminga, 1993).

Our values for the proportion of total NDF digestion occurring in the rumen are in reasonable agreement with values reviewed by Tamminga (1993). Our own experiments in duodenally cannulated cattle *in vivo* suggest very limited post-ruminal cell-wall digestion (e.g. Khalili & Huhtanen, 1991). When post-ruminal NDF digestibility was estimated from digesta passage parameters assuming the same rate for digestion in the hindgut as in the rumen, the proportion of NDF digested in the rumen was 950 mg/g (Huhtanen *et al.* 1995). Using indigestible NDF as a marker Huhtanen *et al.* (1993) estimated that 100–160 mg/g NDF entering the duodenum was digested in the intestines, which proportionally corresponded to 0.03–0.05 of the total NDF digestion. Using a combined *in situ* method, Varvikko & Vanhatalo (1992) estimated that approximately 900 mg/g of the total NDF digestibility occurred in the rumen. They used a value of 0.0625 for ruminal passage rate, which probably underestimated ruminal and total NDF digestibility. All these different approaches suggest the limited importance of post-ruminal cell-wall digestion in cattle.

The contribution of the hindgut to cell-wall digestion depends on the potential digestibility of fibre entering the caecum, microbial activity and digesta retention time in the hindgut. Factors which influence the amount of potentially digestible fibre depend largely on the efficiency of ruminal digestion. When ruminal cellulolysis is depressed, reduced ruminal digestibility can partially be compensated in the hindgut. However, the simulation model used suggests that not more than 0.20 of the depression in ruminal digestibility can be compensated by increased post-ruminal digestion. Digestibility of potentially digestible NDF in the intestines (195 mg/g) is consistent with this value. It is unlikely that the rate of cell-wall digestion is markedly faster in the large intestine than in the rumen. Viable bacterial counts are considerably lower in the caecum than in the rumen (Ulyatt *et al.* 1975), and the physico-chemical environment of the hindgut is similar to that of the rumen (Merchen & Bourquin, 1994). Also the rate of breakdown of cellulose was similar in the rumen and caecum of sheep (Hecker, 1971).

Digesta retention time in the hindgut is relatively short in cattle, and it decreases as feed intake is increased (Huhtanen & Kukkonen, 1995; Vanhatalo & Ketoja, 1995). In dairy cows, retention time in the intestines can be less than 10 h when calculated as the difference between total retention time and pre-duodenal retention time (Poore *et al.* 1991; Udén & Sutton, 1994). Reduced retention time with increased feed intake suggests that a lower ruminal cell-wall digestibility at a high level of feed intake cannot be compensated by increased digestion in the hindgut. Similar retention times of liquid and solid markers in the intestines (e.g. Huhtanen & Kukkonen, 1995) indicate that there is no mechanism for selective retention in ruminant caecum and proximal colon. In sheep, digesta retention time in the hindgut appears to be longer than in cattle (see Ulyatt *et al.* 1975), which may explain the greater contribution of the hindgut to fibre digestion in sheep (Tamminga, 1993). While the post-duodenal retention time in cattle is often less than 10–12 h or 0.20 of the total retention time (Poore *et al.* 1990; Udén & Sutton, 1994; Huhtanen & Kukkonen, 1995), in sheep the corresponding values can be more than 20 h or more than 0.30 (Faichney & Barry, 1986). Longer ruminal retention time in cattle than in sheep (see Tamminga, 1993) together with a shorter post-duodenal retention time may explain the smaller contribution of the hindgut to total cell-wall digestion.

In summary, both the rate and extent of cell-wall digestion decreased with advancing maturity of grass. The changes were curvilinear, being greater at early stage of growth for the rate and at late stage of growth for the extent of digestion. As a result of these opposite curvilinear changes in kinetic parameters the calculated ruminal and total digestibility of cell-wall fractions decreased with maturity rather linearly. Intestinal digestibility of cell-wall fractions decreased with advancing maturity of grass and with increasing length of preceding ruminal incubation. For the intact grass intestinal disappearance of hemicellulose was markedly greater than that of cellulose, partly due to high digestibility of NDF-bound N. The contribution of the hindgut to total cell-wall digestibility was small, the values being consistent with *in vivo* measurements and the relatively short retention time of digesta in the hindgut in cattle. Despite certain methodological shortcomings, the mobile-bag method can be used to study post-ruminal cell-wall digestion. Combined with the ruminal *in situ* technique it can be used to partition total cell-wall digestion between ruminal and post-ruminal digestion providing that appropriate rumen models are used.

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