Estimating rumen degradability of forages from semi-natural grasslands, using nylon bag and gas production techniques

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Abstract

To obtain insight into ruminal digestion of forages from extensively managed semi-natural grasslands, degradation characteristics and kinetics of silages of three different forages in the rumen of lactating dairy cows were estimated *in vitro*, using the gas production technique (GPT), and *in situ*, using the nylon bag technique. Silages originated from intensively managed grassland (IMG), extensively managed species-poor grassland (SPP) and extensively managed species-rich grassland (SPR). Some individual species originating from extensively managed species-poor and species-rich grassland were used to estimate their degradability with GPT, in order to obtain insight into the differences between the main species occurring on these two types of grassland. All samples were also analysed for *in vitro* organic matter digestibility. *In situ* degradability was estimated by nylon bag incubation in the rumen of three dairy cows in two different periods. Rate of organic matter degradation was highest for IMG (4.93 and 4.54% h^{-1}), intermediate for SPR (3.50 and 4.11% h^{-1}) and lowest for SPP (2.62 and 2.72% h^{-1}). Also the rates of degradation for protein and neutral detergent fibre were highest for IMG. The undegradable fraction was the same for SPP and SPR. Highest cell wall fermentation was observed for IMG and lowest for SPP, but SPP and SPR did not differ statistically in this respect. Cell wall degradability of the individual species from the species-poor and species-rich grasslands was highest for *Lolium perenne* and *Dactylis glomerata* and lowest for *Lathyrus pratensis* and *Anthriscus sylvestris*. Based on the *in situ* and *in vivo* degradation characteristics it was concluded that SPR appears to have more potential as a component of the ration of dairy cows than SPP.

Additional keywords: grass, *in situ* degradation, silages

Introduction

Forage from extensively managed semi-natural grasslands differs from forage from intensively managed grasslands: the former is often harvested later and may include many different forage species, including dicotyledonous species (dicots) and a diversity of grasses. This difference in composition makes it difficult to estimate the nutritional value of the forage from semi-natural grasslands, and reduces the likelihood of integrating it into the ration of dairy cows (Bruinenberg *et al.*, 2002).

In an advanced stage of maturity most species occurring on semi-natural grasslands are less readily digested than young, intensively managed grass (Bruinenberg *et al.*, 2002), but dicots may be better digestible than grass species cut at the same date (Duru, 1997). In sheep, dicots have also been shown to have a faster *in situ* degradation rate than grasses of the same age (Lopez *et al.*, 1991). For an efficient integration of forages from semi-natural grasslands in the ration of dairy cows, further information on rumen degradation characteristics of less common forage species is needed.

This paper describes the rumen degradation characteristics of forages from extensively managed, lowland semi-natural grasslands, using the *in vitro* gas production technique (GPT[1]) and the *in situ* nylon bag technique. To investigate the differences among plant species, silages of three different types of forage and some fresh (not ensiled) individual species were tested. The silages originated from intensively managed grassland (IMG), from extensively managed species-rich grassland containing grasses and dicots (SPR) and from extensively managed species-poor grassland containing mainly grasses (SPP). The individual species originated from the species-rich and species-poor grasslands. As degradation rate has been shown to be higher in dicots than in grasses of the same age (Lopez *et al.*, 1991), degradation rates were expected to be higher for dicots and for the mixture containing dicots than for grass harvested at approximately the same date. Furthermore, because it was expected that the rumen microbial population would adapt to the diet (Grubb & Dehority, 1975), influence of the diet on degradation characteristics was expected, and therefore degradation was measured with (donor) dairy cows that had been fed different diets. Degradation characteristics of the silages were determined both *in vitro*, with GPT, and *in situ*, using the nylon bag technique. Degradation characteristics of the individual species were only determined *in vitro*.

Materials and methods

Forage samples

IMG was harvested on 5 May 2000 from a fertilized sward used for intensive production and consisting mainly of *Lolium perenne*. SPP was harvested on 7 June 2000 from a sward managed for meadow-nesting birds and consisting of 96% grasses and 4%

[1] For the abbreviations used in this paper see Appendix.

herbs. SPR was harvested on 21 June 2000 from semi-natural grassland with a high proportion of herbs, managed by a non-governmental organization for nature conservation and consisting of 53% grasses, 11% legumes and 36% non-leguminous herbs. For further details on the botanical composition and management of these grasslands see Bruinenberg (2003).

The types of silage were ensiled individually in 400 to 500-kg bales of pre-wilted forage wrapped in plastic. For *in situ* degradation composite samples were taken from several bales. The study was carried out in two different periods, each period with samples from different bales. The samples were cut manually with a paper-guillotine to a length of about 1 cm and an equivalent of approximately 5 g dry matter (DM) was weighed in nylon (polyamide) bags (19 x 10 cm, pore size 41 µm, porosity 30%; Nybolt, Switzerland). The bagged samples were stored at –20 °C until required for incubation or analysis. During weighing sub-samples were taken, dried at 70 °C and ground over a 1-mm sieve for chemical analysis.

Samples for *in vitro* digestion (GPT) were taken from the same silage samples as those incubated *in situ* in the rumen in the first period. In addition, 15 dried samples of individual species and dried samples from not ensiled SPP and SPR were incubated *in vitro*. The individual species analysed were the main species found on extensively managed species-poor and species-rich grassland (Bruinenberg, 2003). Five grass species from species-poor grassland were analysed: *Lolium perenne, Alopecurus geniculatus, Poa trivialis, Agrostis stolonifera* and *Holcus lanatus*. From species-rich grassland 4 grass species (*Lolium perenne, Dactylis glomerata, Arrhenaterum eliatus* and *Alopecurus pratensis*), 5 dicotyledonous species (*Cirsium arvense, Galium mollugo, Ranunculus acris, Crepis biennis* and *Anthriscus sylvestris*) and 1 leguminous species (*Lathyrus pratensis*) were analysed. The samples of the species used for measuring gas production characteristics were analysed for dry matter (DM), ash and *in vitro* digestibility of organic matter (d_{OM}).

Nylon bag incubation

In situ incubation was carried out in the rumen of three lactating dairy cows fitted with a large rumen cannula (ID 100 mm, Bar Diamond Inc., Parma, Idaho, USA). The incubations were performed twice, in two different periods, with each cow offered one of the following three rations: (1) 100IMG, consisting of 4.5 kg DM concentrates and 15 kg DM silage from intensively managed grassland per day, (2) 60SPP, consisting of 4.5 kg DM concentrates, 6 kg DM silage from intensively managed grassland and 9 kg DM from species-poor grassland per day, and (3) 60SPR, consisting of 4.5 kg DM concentrates, 6 kg DM silage from intensively managed grassland and 9 kg DM from species-rich grassland per day. To avoid differences between the cows to be attributed to the diets, the diet per cow changed between the two periods.

The nylon bags were taken from the freezer one hour before incubation. Each bag was attached to a polypropylene block weighing 750–800 g, which in turn was attached to the cannula by a 75-cm nylon cord. Incubations were performed with 24 bags per feed in each cow. The bags were incubated for 3 (n = 3), 6 (n = 3), 12 (n = 6), 24 (n = 3), 48 (n = 4) or 264 (n = 5) hours. Three bags per silage were washed without

incubation to measure the fraction that disappears during washing in a washing machine (W). The residue after 264 hours of incubation was considered as the undegradable fraction (U). The degradable fraction (D) was calculated as D = 100 – (W + U).

After removal from the rumen, the nylon bags with incubation residues were rinsed with tap water to remove excess material from the outer surface, after which they were placed in ice water to stop microbial activity. Subsequently, the bags were washed in a washing machine with cold tap water for 30 minutes, oven-dried at 70 °C for 24 hours and weighed. The residues were pooled per feed, animal and incubation time, ground over a 1 mm sieve and analysed for DM, ash, nitrogen (N) and neutral detergent fibre (NDF).

Gas production technique

Gas production analysis with the dried samples of the ensiled and not ensiled forages and the individual species, was performed in two periods as described by Cone *et al.* (1996). During each period, incubation was performed for 72 hours in two series, on different days, and each sample was incubated in duplicate. Rumen fluid required for the incubations was obtained from a lactating dairy cow fitted with a rumen cannula. The ration of the cow changed between the two periods, but the rations were the same as two of the rations offered to the cows with the *in situ* experiment, i.e., 100IMG in the first and 60SPP in the second period.

Gas production curves were fitted with a three-phasic-model as described by Cone *et al.* (1996) and Groot *et al.* (1996). The cumulative gas production profiles were mathematically divided into three sub-curves as described by Cone *et al.* (1997): (1) fermentation of soluble, readily degradable components, (2) fermentation of non-soluble components of the sample, which in grass mainly consist of the cell wall fraction, and (3) gas production caused by microbial turnover. The model is described by:

$$\text{ml gas} = A1/[1+(B1/t)^{C1}] + A2/[1+(B2/t)^{C2}] + A3/[1+(B3/t)^{C3}]$$

where
An = the maximum gas production in ml per g organic matter in phase n (1, 2, 3),
Bn = the time (h) needed to reach 50% of the maximum gas production (An),
Cn = a constant determining the shape of the curve; and
t = incubation time in hours.

From the parameters Bn and Cn the fractional rates of digestion (R) can be calculated (Groot *et al.*, 1996). The maximum R (Rmax) is reached when the size of the microbial population no longer limits fermentation of the forage. The time interval between the moment incubation started and the moment Rmax is reached (tRmax) characterizes the growth of the micro-organisms and the colonization of the feed component (Groot *et al.*, 1996).

In a second experiment with *in vitro* digestion (GPT) the three silages were incubated in rumen fluid obtained from three different cows fed on each of the three rations described (100IMG, 60SPP and 60SPR) to determine the influence of the

ration on rumen microbial activity. The donor cows were the same cows used for the *in situ* technique in the second period.

Chemical analysis and *in vitro* digestibility

The DM content of the oven-dried samples was determined after drying for 4 h at 103°C and ash was determined after 3 h at 550°C. The N content was determined using the Dumas method (Merz, 1979) and crude protein (CP) was calculated as 6.25 × N-Dumas. The NDF and lignin contents were determined according to Robertson & Van Soest (1981) and sugar was measured as described by Van Vuuren *et al.* (1993). *In vitro* digestibility of OM (d_{OM}) was determined as described by Tilley & Terry (1963) and energy contents were calculated according to Anon. (2001).

Calculation methods *in situ*

The degradation rate (kd, % h^{-1}) of the insoluble but potentially degradable fraction [D, calculated as 100 – (W + U)] was calculated according to the first order model of Robinson *et al.* (1986) including U, D and kd, e.g. residue at time t = D * $e^{(-kd)\,(t)}$ + U. Data were calculated for OM, CP and NDF.

Statistical analysis

Results of the different estimates of the three silages were statistically analysed with analysis of variance (Anon., 1993), using the model: $Y_{ij} = u + C_i + S_j + E_{ij}$ (C = cow, S = silage, E = standard error). Analysis of variance (Anon., 1993) was also used to decide on a statistically significant ($P < 0.05$) sample × ration interaction. Treatment means were separated using a Student's t-test.

Results

Degradation characteristics of the three types of silage

In vitro OM digestibility was lower for SPP and SPR than for IMG (Table 1). The low d_{OM} of SPP was accompanied by higher NDF and lignin contents than for IMG. Due to the later stage of harvesting and the unfertilized sward, the CP content was lower for SPP and SPR than for IMG. The D-fractions of organic matter (D_{OM}), crude protein (D_{CP}) and NDF (D_{NDF}) were higher in IMG than in SPP and SPR and degraded faster (higher value of kd), although the differences in degradability between IMG and SPR in the second period were not statistically significant (Table 2). The undegradable fractions of organic matter (U_{OM}), crude protein (U_{CP}) and NDF (U_{NDF}) were significantly lower in IMG than in the other silages.

The silages from the two semi-natural grasslands also differed from each other. The rate of degradation of organic matter (kd_{OM}) was higher in SPR ($P < 0.05$), although not statistically significant in the first period, but the potentially degradable

Table 1. *In vitro* organic matter digestibility (d_{OM}), energy (NE) content, dry matter (DM) content and chemical composition of silage from intensively managed (IMG), from species-poor (SPP) and from species-rich (SPR) grassland, in two different periods.

Diet characteristics[1]	Silage					
	IMG		SPP		SPR	
	period 1	period 2	period 1	period 2	period 1	period 2
d_{OM}	0.75	0.73	0.54	0.57	0.54	0.52
NE (MJ kg^{-1})	6.0	5.8	4.3	4.6	4.4	4.3
DM (g per kg silage)	571	610	716	751	589	548
Chemical composition (g per kg DM)						
Ash	116	120	117	101	101	92
OM	884	880	883	899	899	908
CP	180	177	125	131	99	99
NDF	470	463	553	547	489	475
Lignin	14.6	13.0	40.8	35.7	57.0	54.3
Sugars	31.1	40.0	44.5	59.5	42.4	24.3

[1] For abbreviations see Appendix.

fraction of organic matter (D_{OM}) did not differ between SPP and SPR ($P > 0.05$). For SPR in the second period the washout fraction of organic matter (W_{OM}) seemed to be higher than for SPP. However, the W fractions could not be analysed for significance. Furthermore, the rate of degradation of crude protein (kd_{CP}) was also higher for SPR ($P < 0.05$) and the potentially degradable fraction of crude protein (D_{CP}) did not differ significantly between SPP and SPR ($P > 0.05$), except that D_{CP} for SPR in the second period was significantly higher than for SPP and SPR in the first period ($P < 0.05$). SPP had a significantly higher potentially degradable fraction of NDF (D_{NDF}) than SPR, but the rate of degradation of NDF (kd_{NDF}) did not differ significantly between SPP and SPR ($P > 0.05$). The undegradable fraction of NDF (U_{NDF}) was significantly lower in SPP than in SPR ($P < 0.05$).

Gas production and digestibility of the silages

The differences among the three silages in cumulative gas production and in gas production rate during incubation are presented in Figures 1 and 2. *In vitro* digestibility of OM (d_{OM}), gas production of the gradually degradable phase (A2), gas production after 72 hours (GP72) and maximum rate of degradation (Rmax) were higher for IMG than for SPP and SPR ($P < 0.05$; Table 3). However, for IMG and SPR the amounts of

Table 2. *In situ* degradation characteristics[1] of silage from intensively managed (IMG), from species-poor (SPP) and from species-rich (SPR) grassland, in two different periods.

	Silage						SED[2]
	IMG		SPP		SPR		
	period 1	period 2	period 1	period 2	period 1	period 2	
Organic matter							
W	21.1	21.1	15.4	15.9	20.3	17.9	nd[3]
U	9.7b[4]	10.5b	30.4a	28.0a	28.6a	29.8a	1.44
D[5]	69.9a	66.9a	52.9bc	55.2b	49.9c	52.7bc	1.44
kd	4.9a	4.5a	2.6c	2.7c	3.5bc	4.1ab	0.42
Crude protein							
W	41.5	41.1	32.4	32.7	35.7	27.5	nd
U	9.6b	11.6b	37.9a	32.8a	35.4a	32.6a	3.31
D[5]	46.8a	44.6a	26.4b	32.6b	30.6b	42.3a	2.94
kd	6.4a	5.2ab	1.5c	1.3c	3.7b	4.2b	0.80
Non-detergent fibre							
U	8.8e	9.4e	28.9c	26.9d	35.5b	38.3a	0.82
D[5]	91.9a	88.5b	66.6c	66.7c	57.5d	57.4d	1.22
kd	4.6a	4.1a	2.7b	2.9b	2.7b	3.1ab	0.46

[1] W = soluble fraction (%); U = undegradable fraction (%); D = degradable fraction (%), calculated as D = 100 – (U + W); kd = rate of degradation (% h^{-1}).

[2] SED = standard error of difference between means.

[3] nd = SED could not be determined.

[4] Means in the same row, followed by the same letter are not statistically different ($P < 0.05$).

[5] The sum of W, U and D does not always add up to 100 because of statistical calculations.

gas produced in the first (soluble) phase (A1) were similar but the time taken to reach Rmax (tRmax) was shorter for SPR than for IMG.

A1 and Rmax were significantly higher for SPR than for SPP, but B2 and tRmax were significantly lower ($P < 0.05$). However, between SPR and SPP there were no statistical differences in d_{OM}, A2 or GP72.

Gas production of the individual species

The cumulative gas production, the gas production rate and the gas production rate of the second phase for the six different forage species are shown in Figures 3–5. Of the individual grass species, *Lolium perenne* had the highest and *Holcus lanatus* and

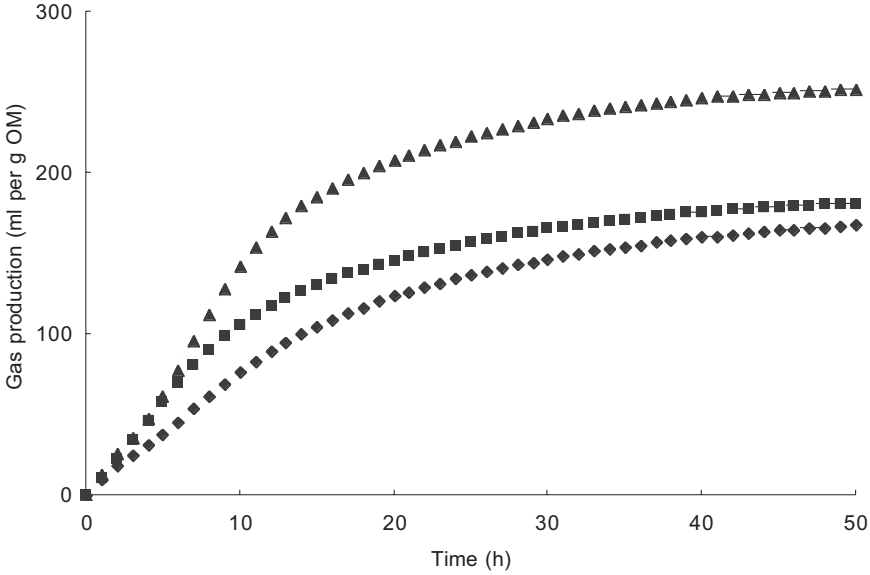

Figure 1. Cumulative gas production of silages digested *in vitro*. ◆ = silage from extensively managed, species-poor grassland; ■ = silage from extensively managed, species-rich grassland; ▲ = silage from intensively managed grassland.

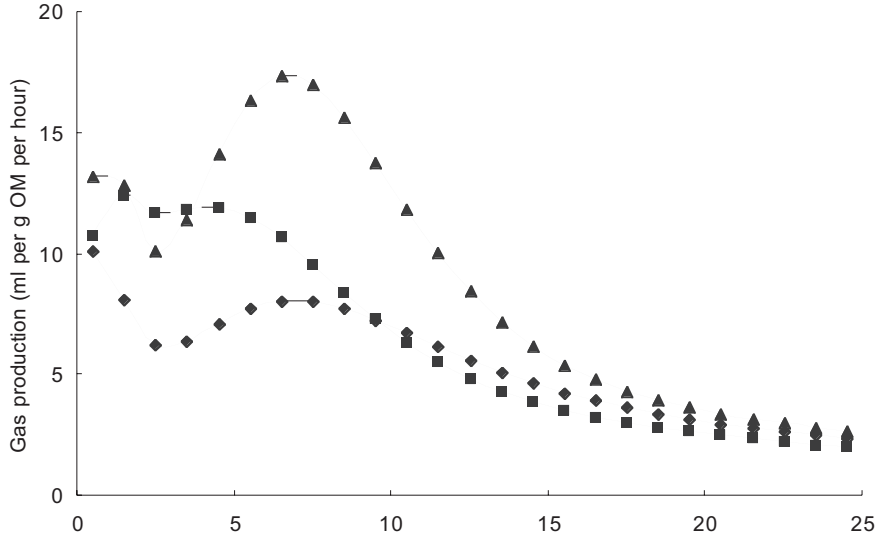

Figure 2. Gas production rate of silages digested *in vitro*. ◆ = silage from extensively managed, species-poor grassland; ■ = silage from extensively managed, species-rich grassland; ▲ = silage from intensively managed grassland.

Table 3. *In vitro* organic matter digestibility (d_{OM}) and gas-production characteristics of some 15 forage species and 5 mixed silage samples (SPP = silage from species-poor grassland; SPR = silage from species-rich grassland; IMG = silage from intensively managed grassland).

		d_{OM}	Gas-production characteristics[1]					
			A1	A2	B2	GP72	Rmax	tRmax
Mixed samples								
SPP not ensiled		0.63	42	109	9.4	195	13.7	10.9
SPP ensiled		0.54	25	99	9.9	173	12.7	11.5
SPR not ensiled		0.61	51	107	7.9	196	14.6	8.7
SPR ensiled		0.54	36	110	7.7	183	15.3	8.6
IMG ensiled		0.75	36	171	8.6	253	18.4	10.7
Species-poor grassland								
Lolium perenne	G[2]	0.74	59	153	8.9	257	15.8	10.8
Alopecurus geniculatus	G	0.67	66	116	8.9	243	12.8	9.9
Poa trivialus	G	0.65	60	121	9.8	245	11.6	10.7
Agrostis stolonifera	G	0.64	43	102	10.0	191	12.0	11.3
Holcus lanatus	G	0.62	45	109	10.1	205	11.6	11.3
Species-rich grassland								
Lolium perenne	G	0.66	66	124	8.8	233	13.9	10.1
Dactylis glomerata	G	0.65	55	150	7.7	254	19.4	9.6
Arrhenaterum eliatus	G	0.65	65	114	9.1	230	11.7	9.4
Alopecurus pratensis	G	0.60	43	108	10.5	213	11.0	11.7
Cirsium arvense	D[2]	0.71	63	138	6.8	224	17.1	7.7
Galium mollugo	D	0.69	61	135	7.5	218	15.7	8.5
Ranunculus acris	D	0.61	55	142	6.8	229	20.1	8.1
Crepis biennis	D	0.61	48	126	6.9	200	19.8	8.3
Anthriscus sylvestris	D	0.56	54	95	6.3	169	20.1	7.3
Lathyrus pratensis	L[2]	0.55	43	104	8.3	176	12.7	8.6
SED[3]		nd[4]	5.1	9.3	0.21	16.0	0.63	0.24

[1] A1 = gas production (ml per g organic matter) of the soluble phase; A2 = gas production (ml per g organic matter) of the non-soluble phase; B2 = time (h) needed to reach 50% of A2; GP72 = gas production (ml per g organic matter) after 72 hours; Rmax = maximum relative rate of degradation (ml per g organic matter h^{-1}) of the non-soluble phase; tRmax = time (h) at which Rmax is reached.

[2] G = grass species; D = dicotyledonous (non-leguminous) species; L = leguminous species.

[3] SED = standard error of difference between means.

[4] nd = SED could not be determined.

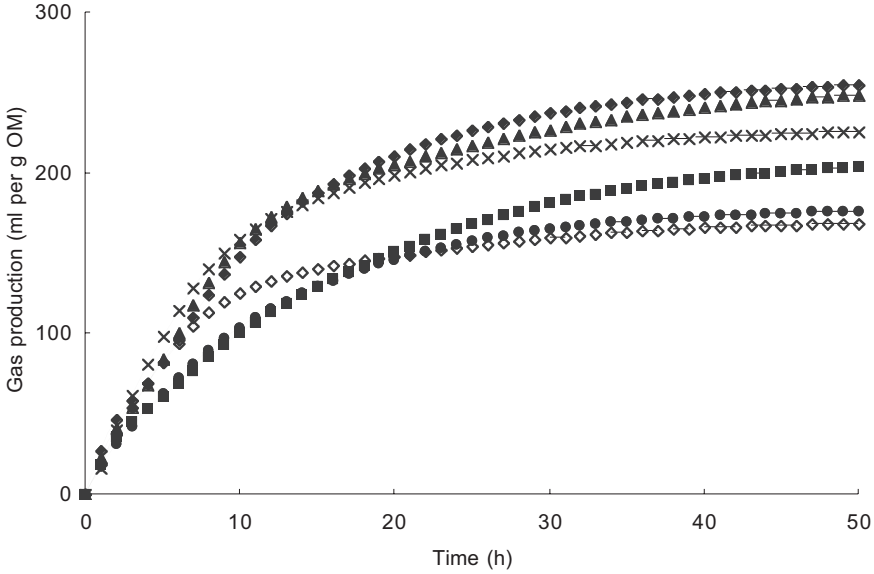

Figure 3. Cumulative gas production of forage species digested *in vitro*. ◆ = *Lolium perenne;* ■ *Holcus lanatus;* ▲ = *Dactylis glomerata;* × = *Cirsium arvense;* ◇ = *Anthriscus sylvestris;* ● = *Lathyrus pratensis.*

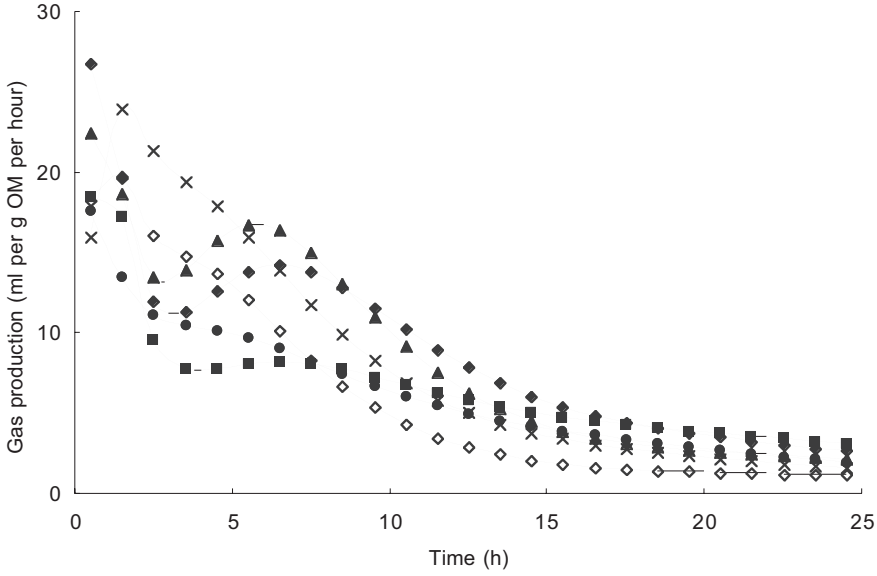

Figure 4. Gas production rate of forage species digested *in vitro*. ◆ = *Lolium perenne;* ■ *Holcus lanatus;* ▲ = *Dactylis glomerata;* × = *Cirsium arvense;* ◇ = *Anthriscus sylvestris;* ● = *Lathyrus pratensis.*

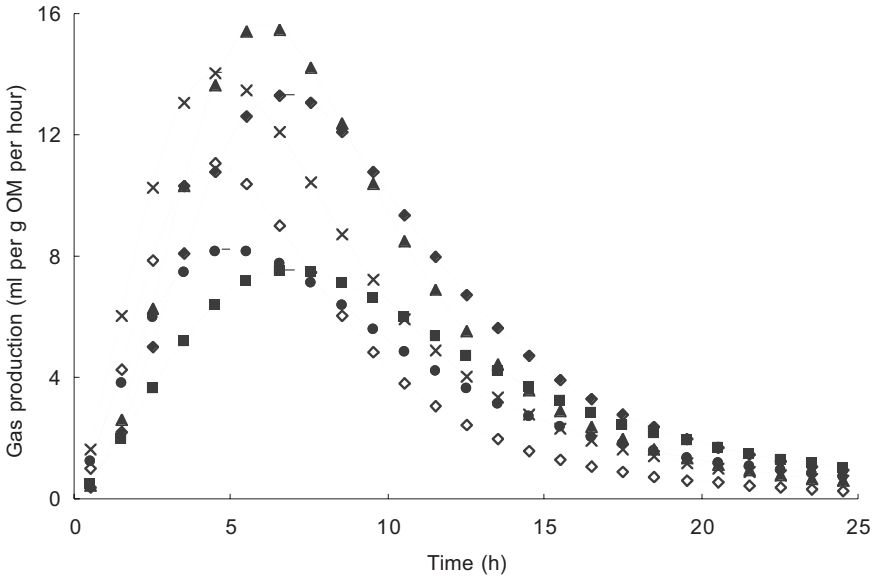

Figure 5. Gas production rate of the insoluble phase of forage species digested *in vitro*. ◆ = *Lolium perenne;* ■ *Holcus lanatus;* ▲ = *Dactylis glomerata;* × = *Cirsium arvense;* ◇ = *Anthriscus sylvestris;* ● = *Lathyrus pratensis.*

Alopecurus pratensis had the lowest d_{OM}, A2 and GP72. Of the dicots, *Cirsium arvense* had the highest d_{OM} but A2 and GP72 were similar for *Cirsium arvense* and *Ranunculus acris* (Table 3). *Anthriscus sylvestris* and *Lathyrus pratensis* were lowest for d_{OM} and GP72, but *Anthriscus sylvestris* and *Ranunculus acris* had the highest Rmax. The herbs seemed to decompose more rapidly than the grasses, i.e., they had a lower B2 and tRmax than the grasses, and some of the dicots also had a high Rmax (e.g. *Ranunculus acris, Crepis biennis* and *Anthriscus sylvestris*). Of the dicots, *Lathyrus pratensis*, the only legume studied, had the lowest d_{OM}, the highest B2 and the lowest Rmax.

Influence of ration

Statistically significant interactions between ration and sample were observed for A2, B2 and Rmax (Table 4), and a trend towards interaction was observed for GP72. No statistically significant interactions were observed for A1 and tRmax. Initially, gas production for IMG was rapid, whereas SPP started slowly (Figures 1 and 2). Maximum rate of degradation (Rmax) was higher with 100IMG using rumen fluid from the cow offered the IMG diet, and with 60SPR using rumen fluid from cows offered SPP and SPR diets.

Table 4. Gas production characteristics of 3 rations composed of silage from species-poor (SPP), from species-rich (SPR) and from intensively managed (IMG) grassland, for cows that had been fed on different diets.

Diet	Ration[1]	Gas production characteristics[2]					
		A1	A2	B2	GP72	Rmax	tRmax
IMG	100IMG	39	152	7.8	254	18.9	9.6
	60SPP	33	185	8.9	256	17.7	11.3
	60SPR	25	182	9.2	246	16.1	11.4
SPP	100IMG	42	102	8.7	192	12.9	9.5
	60SPP	30	115	10.3	211	11.9	11.8
	60SPR	20	86	9.8	167	13.6	11.7
SPR	100IMG	43	89	6.6	166	15.3	6.7
	60SPP	35	118	7.9	188	15.9	9.2
	60SPR	31	113	7.3	188	16.2	8.3
SED[3]		3.9	9.8	0.2	12.2	0.9	0.3
P-value[4]		0.38	0.04	0.01	0.09	0.03	0.13

[1] 100IMG = 15 kg IMG silage + 4.5 kg concentrates; 60SPP = 6 kg IMG silage + 9 kg SPP silage + 4.5 kg concentrates; 60SPR = 6 kg IMG silage + 9 kg SPR + 4.5 kg concentrates. Weights on a dry matter basis.

[2] For abbreviations and units see Table 2.

[3] SED = standard error of difference between means.

[4] *P* calculated with F-test. $P < 0.05$ indicates statistically significant interaction between sample and ration.

Discussion

Samples from intensively managed grassland

The grass that had been harvested in an immature stage caused the high D and kd found for IMG degraded *in situ*. The low content of undegradable fibre in immature grass resulted in low U fractions of OM, CP and NDF and in high D fractions. The characteristics of IMG are more or less similar to data for other grass silages reported in the literature (e.g. Van Vuuren *et al.*, 1989; Bosch *et al.*, 1992; Valk *et al.*, 1996). IMG is therefore thought to provide a good reference for comparison with silages from the semi-natural grasslands.

Samples from species-poor grassland

As expected, *in situ* degradation characteristics were inferior for SPP compared with IMG, because the forage was harvested later (Bruinenberg, 2003) and most of the grasses were in a mature stage and already had produced inflorescences. This resulted in contents of cell wall material – as shown by the NDF content – that were higher than for IMG (Table 1). Considering the advanced stage of maturity, the lignin content was also expected to be higher for SPP than for IMG, which was confirmed by chemical analysis (Table 1). Furthermore, SPP consisted mainly of grass species, like *Holcus lanatus* and *Agrostis stolonifera*, that are assumed to be inferior to *Lolium perenne* (Bruinenberg *et al.*, 2001; 2002). This was confirmed by the relatively low degradation rates of OM for SPP found *in vitro*, with the GPT. This is shown by relatively low values for Rmax and high values for B2 (Table 3). As expected, relatively low degradation rates were also found for the individual forage species from species-poor grassland. Based on the d_{OM} and the gas production characteristics and as expected, *Lolium perenne* – which is known to be highly digestible (Bruinenberg *et al.*, 2002) – was the best species. However, only 6% of the total botanical composition of species-poor grassland was formed by *Lolium perenne* (Bruinenberg, 2003). The other grass species had low gas production rates, and low total gas productions, especially *Holcus lanatus*, which was the most frequently found species on species-poor grassland (35% of the botanical composition; Bruinenberg, 2003). *Holcus lanatus* is highly digestible in the vegetative stage, but digestibility decreases rapidly during maturing (Korevaar, 1986). As *Holcus lanatus* already had produced inflorescences at the time of harvesting, its stage of maturity could be limiting for degradability. The digestibility of the other grass species from species-poor grassland was consistent with observations of Korevaar & Van Der Wel (1997). The slow fermentation could be caused by the structure of the grasses: because of their parallel venation (Wilson, 1985) and high NDF content (Table 1) microbes will probably have difficulties penetrating grass tissues.

The results of the *in situ* degradability study and the *in vitro* gas production test indicate that SPP is difficult to degrade in the rumen of dairy cows, which is probably due to its high cell wall content. A high NDF content combined with a low degradation rate of NDF, is probably responsible for a reduction in feed intake *in vivo* (Forbes, 1995; Bruinenberg, 2003) because cows have a limited rumen content (De Visser *et al.*, 1998). As the energy value of SPP was also low (Table 1), total energy intake would be limited too, affecting milk production. Therefore, this type of forage should not be included in the ration of lactating dairy cows in too high proportions.

Samples from species-rich grassland

The higher kd_{OM} and kd_{CP} observed for SPR compared with SPP was in agreement with Lopez *et al.* (1991), who found a higher rumen degradation rate for weeds than for grasses of the same age. The higher kd_{OM} can be explained by the higher ratio between cell contents and cell walls in SPR. However, kd_{NDF} was similar for SPP and SPR, and D_{NDF} was lower for SPR than for SPP. As the species-rich grassland consisted for 45% of legumes and other dicots, differences in degradation characteristics

between the two silages could be explained by differences in degradability between grasses and dicots. SPR contained more lignin than SPP, decreasing degradability. A higher lignin content in weeds combined with a lower cell wall content was also observed by Lopez *et al.* (1991). The higher rate of gas production observed *in vitro* also indicated a more rapid breakdown of OM in SPR than in SPP. The Rmax was higher for SPR than for SPP, but somewhat lower than for IMG, probably because IMG only contained highly degradable grasses, whereas the grasses in SPR were mostly in a more mature stage, and therefore less readily degradable. GP72 and A2 of SPR were low and comparable with those of SPP. This was expected as D_{OM} and d_{OM} were also comparable between SPR and SPP, even though D_{NDF} was lower for SPR. The GPT data for the individual forage species showed clearly that the high Rmax of SPR could indeed be attributed to the presence of dicots, because the highest Rmax was reached with the dicots *Anthriscus sylvestris* and *Ranunculus acris*. Although Rmax and kd_{OM} were higher for SPR than for SPP, d_{OM} was similar or even lower for SPR. The low d_{OM} for SPR is probably due to the high lignin content (Table 1), whereas the difference in degradation rate between SPP and SPR indicates differences in accessibility of the cell contents, or differences in rate of degradation of cell wall material. The rapid breakdown of dicots was probably caused by several factors. The first factor could be that dicots have a reticulate venation, which might lead to less vascular tissue per unit volume. Furthermore, because of the junctions between cells, the fibre is more easily degraded into small particles than the veins in grass leaves, which have a parallel girder system of vascular bundles running through the full length of the leaves (Wilson, 1985). A second explanation for the rapid breakdown of dicots might be related to the fact that the distribution of lignin in the stems from legumes is different from that in grasses. The lignin in the mature stems of legumes is mainly concentrated in the xylem (Wilson & Hatfield, 1997), which is therefore highly indigestible. Pith and cortex are not lignified and therefore rapidly digestible, explaining the high rate of stem digestion in legumes. In grasses, lignin is present in most cell types (Wilson, 1993) and thus affects the rate of digestion of all cell types. In the literature no information was found on the distribution of lignin in other dicots, but it is assumed that these are probably more comparable with legumes than with grasses. A third explanation for kd_{OM} being higher for SPR than for SPP could be the higher amount of pectins in leguminous dicots than in grasses (Wilson, 1994). Pectins are (almost) completely degradable in the rumen (Tamminga, 1993). A high amount of pectins in SPR would be in agreement with the high content of OM components other than NDF and CP [rest components = OM – (NDF + CP)], which were 311–334 g in SPR. In IMG and SPP, rest components, including crude fat, soluble carbohydrates and organic acids were between 205 and 240 g. The higher A1 for SPR confirms this hypothesis.

The different results of degradation rate and digestibility indicate that an analysis of d_{OM} alone is not sufficient to characterize forages from semi-natural grasslands, and that such an analysis should be complemented with the *in situ* technique or GPT.

The relatively high rate of degradation, *in vitro* (OM) as well as *in situ* (OM, CP), of SPR suggests that the intake of SPR could be higher than the intake of SPP. The similar kd_{NDF} of SPR and SPP might suggest the opposite, but the proportion of NDF was lower in SPR than in SPP (Table 1). A disadvantage of SPR degradation *in vivo* could

be that dicots are known to contain certain anti-nutritional factors, such as the presence of thorns (*Cirsium arvense*), a low palatability (not ensiled *Rumex obtusifolius*; Derrick *et al.*, 1993), or the presence of secondary metabolites inhibiting enzymatic or microbial activity in the rumen (Scehovic, 1995). However, in earlier research the intake of a mixed ration consisting of SPR, IMG, maize silage and concentrates (ratio SPR:IMG = 6:4) was similar to the intake of a mixed ration consisting of IMG, maize silage and concentrates (Bruinenberg, 2003). So in that experiment anti-nutritional factors did not appear to cause a reduction in intake.

Influence of ration of the donor animals

Since the microbial population in the rumen will adapt to available nutrients (Grubb & Dehority, 1975), effects were expected of the ration of the donor animals. Indeed, interactions were found between ration of the donor animal and samples in both the experiment with the individual grass species and the experiment with the three different cows on different rations. In the latter experiment only one animal per ration was used, and therefore it remained uncertain whether the observed effect was a result of ration or animal. No interactions were found for GP72 and A2, but B2 was higher on 60SPP. This was not expected, because the microbial population on that ration should be adapted to it, and therefore able to 'attack' earlier. However, because the energy content of this ration was lower, the number of microbes per ml rumen fluid was probably lower than on IMG, which could reduce the rate of fermentation. Total gas production was highest for SPP when the donor animal consumed 60SPP.

Conclusions

Considering the characteristics of degradation *in situ* and degradation *in vitro*, silages from species-rich grassland containing grasses and dicots appear to have more potential for use on dairy farms than silages from species-poor grassland containing mainly grasses. It became clear that d_{OM} by itself is insufficient for estimating the nutritional value of such types of forage because of differences in rate of degradability.

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Appendix

Abbreviations used

An	maximum gas production caused by fermentation of fraction n
Bn	time needed to reach 50% of An
CP	crude protein
DM	dry matter
d_X	digestibility of chemical component x
D_X	potentially degradable fraction of chemical component x
GP72	gas production after 72 h of incubation
IMG	silage from intensively managed grassland
kd_X	degradation rate of chemical component x
NDF	neutral detergent fibre
NE	nett energy
OM	organic matter
Rmax	maximum relative rate of degradation of the non-soluble fraction
SPP	silage from extensively managed species-poor grassland
SPR	silage from extensively managed species-rich grassland
tRmax	time to reach Rmax
U_X	undegradable fraction of chemical component x
W_X	washout fraction of chemical component x