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Fermentation and degradation in the rumen of dairy cows fed on diets consisting of silage from an intensively managed sward and silages from semi-natural grasslands

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SUMMARY

To assess the effect of grassland management on the ruminal digestion of silages, four lactating dairy cows, fitted with a rumen cannula, were fed diets consisting of concentrates and different grass silages. The grass silages consisted of intensively managed grass (IM) in variable proportions replaced by silages harvested from a 'species-poor' grassland managed to stimulate nesting of birds (SPP) or from a grassland managed to increase plant species diversity (SPR). The roughage part of the diets was composed completely of IM (100IM), or 200 g/kg (in dry matter) of IM replaced by SPP (20SPP) or 600 g/kg of IM replaced by SPP (60SPP), or SPR (60SPR). The pH in the rumen was highest on 60SPR and lowest on 100IM and 20SPP ($P<0{\cdot}05$), whereas volatile fatty acids (VFA) concentrations were lowest on 60SPP and 60SPR and highest on 100IM ($P<0{\cdot}05$). No differences in the ratio non-glucogenic:glucogenic volatile fatty acids were observed among the diets. The NH_3 concentration was highest on 100IM and 20SPP and lowest on 60SPR ($P<0{\cdot}05$), reflecting differences in CP intake. The concentration of uric acid in the urine (mg per kg metabolic body weight) was highest on 100IM ($P<0{\cdot}05$). Rumen pool size of OM and DM did not differ among treatments, but pool size of NDF and IADF were highest on 60SPR ($P<0{\cdot}05$). Passage rate was high on 100IM and 60SPR, but no significant differences with the other treatments were established. Also, no significant differences were observed in rates of degradation. Clearance rate of large particles was highest on 60SPP and differed significantly from 60SPR ($P<0{\cdot}05$) only. No differences were observed in clearance rate of small particles. In conclusion, for most rumen fermentation characteristics measured in the study, no noticeably aberrant behaviour of the silages from semi-natural grassland was observed.

INTRODUCTION

Semi-natural grasslands are different from intensively managed grasslands used to feed dairy cows in temperate regions. First, many different unusual forage species and dicotyledons may occur compared with an intensively managed grassland (Tallowin & Jefferson 1998). Those forage species often have a higher cell wall or lignin concentration and a lower digestibility than the grass species occurring in intensively managed grasslands (mainly *Lolium perenne*). Second, semi-natural grasslands are usually cut or grazed in a more advanced stage of maturity than the intensively managed grasslands (Korevaar 1986; Bruinenberg *et al.* 2002). Both aspects, the higher diversity of species and the more advanced stage at cutting, are associated with a reduced digestibility and thus a lower net energy value of forages (Armstrong *et al.* 1986). Korevaar & Van der Wel (1997) also observed a reduced intake of silage of a mixture of mainly *Agrostis stolonifera*, *Lolium perenne*, *Poa trivialis*

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and *Alopecurus geniculatus* compared with silages consisting mainly of *L. perenne*. However, in that study the impact of differences in botanical composition on milk production was small (Korevaar & Van der Wel 1997). In a performance trial with dairy cows a decrease in fat and protein-corrected milk did not occur until 400 g/kg of silage from intensively managed grassland was replaced by silage from ('species-poor') semi-natural grasslands containing mostly grasses in an advanced stage of maturity (Bruinenberg 2003). A decrease in intake only occurred if as much as 600 g/kg of the intensively managed silage was replaced (Bruinenberg 2003). Such a decrease in intake was not observed if 600 g of the silage/kg was replaced by silage from 'species-rich' semi-natural grassland containing approximately 500 g herbs (plants with soft stems, which die down after flowering)/kg (Bruinenberg 2003). These contrasting results may be explained if more is known about rumen fermentation and kinetics on those diets. Rumen fermentation characteristics (pH, volatile fatty acids (VFA), NH_3) give an indication of the availability of energy and protein in the rumen, and differences in intake may be explained by differences in rumen degradation and passage rate. Also studying the amount of uric acids excreted in the urine is important, as this gives an indication of microbial protein synthesis in the rumen (Chen *et al.* 1992; Johnson *et al.* 1998).

Information on rumen kinetics and fermentation of forages from semi-natural grasslands in dairy cows, in comparison with forages from intensively managed grasslands, is scarce. *In situ* or *in vitro*, herb-rich forage of an advanced stage of maturity may have higher degradation rates than grass of the same age (Lopez *et al.* 1991; Bruinenberg *et al.* 2004). To obtain insight into rumen kinetics and rumen fermentation patterns in the rumen of dairy cows, two forages originating from different semi-natural grasslands were combined with intensively managed grass and concentrates, and fed to lactating dairy cows fitted with a rumen cannula. It was expected that the degradation and fermentation characteristics of the forages from semi-natural grasslands would differ from those of intensively managed grasslands, and that the combination of the forages from semi-natural grasslands with grass from intensively managed grasslands would result in a positive response on rumen fermentation patterns and rumen kinetics by reducing rate of degradation and passage through the rumen. Furthermore, as the surplus of nitrogen of diets with intensively managed grass will be reduced when some of the intensively managed grass is replaced by low protein silage, less ammonia will lower rumen [NH_3] as reduced NH_3 liberation from micro-organisms on the lower CP direct, and thus positive effects are expected on utilization of protein.

Table 1. *The amount of different silages used in the four treatments diets and the chemical composition of the silages. The roughage part of the 100IM diet consisted of silage from intensively managed grassland; in 20SPP and 60SPP, 200 and 600 g/kg DM of the intensively managed silage was replaced by 'species-poor' silage, and in 60SPR, 600 g/kg DM of the intensively managed silage was replaced by 'species-rich' silage*

Diet	100IM	20SPP	60SPP	60SPR
Silage composition (g/kg)				
Intensively managed silage	1000	800	400	400
'Species-poor' silage	0	200	600	0
'Species-rich' silage	0	0	0	600
Chemical composition (g/kg DM)				
Ash	115	111	103	103
Organic matter	885	889	897	897
Crude protein	191	181	159	139
Sugars	32	39	56	42
Neutral detergent fibre	524	540	568	547
Fermentable organic matter*	561	531	471	464
Nutritive quality				
d_{OM} (proportion of OM)	0·757	0·721	0·646	0·628
Net energy (MJ/kg DM)†	5·9	5·6	5·0	4·7

* Calculated based on *in situ* degradation characteristics (Bruinenberg *et al.* 2003*b*).
† Net energy was calculated according to CVB (2003).
d_{OM} = digestibility of the organic matter.

MATERIAL AND METHODS

Cows and experimental design

In the experiment, four lactating dairy cows, fitted with a rumen cannula (internal diameter 10 cm; Bar Diamond, Parma, ID) (Dougherty 1981) and with a milk production of 20–25 kg per day, were used. Cows were housed in tied stalls on rubber mats and had free access to water. The experiment was carried out from May to August 2001 and was designed as a Latin square, with four periods, each of 3 weeks. The first 2 weeks of each period were used for adaptation of the cows to the diet and in the third week the measurements were carried out. The protocol of the experiment was approved by the Ethical Committee for Animal Experiments of ID-Lelystad.

Treatments

Treatments were four different diets, consisting of one or combinations of two out of three different silages

Table 2. *Ingredients and composition of concentrates*

Ingredients	g/kg product	Chemical composition	(g/kg DM)
Rape seed, extracted	83	Ash	93
Wheat	74	Organic matter	907
Molasses, cane	50	Crude protein	247
Vinasses (beet)	50	Neutral detergent fibre	311
Palm oil fatty acids	2		
Premix minerals/ vitamins	8	Nutritive value	
Chalk ($CaCO_3$)	6·2	d_{OM} (proportion OM)	0·826
Salt (NaCl)	1·9	Net energy (MJ/kg)	7·4
Magnesium oxide 80 % MgO	4		
Coconut expeller	133		
Maize gluten meal	293·9		
Palm kernel expeller	150		
Soya bean meal	18		
Soya bean meal (mervobest)	125		
Mono calciumphosphate	1		

(Table 1). The silages were obtained by cutting intensively managed grassland (IM) and two semi-natural grasslands. One semi-natural grassland ('species-poor' grassland; SPP) consisted of mainly mature grasses and the other semi-natural grassland ('species-rich' grassland; SPR) consisted of 0·34 non-leguminous herbs, 0·11 legumes and 0·55 grasses. Grass from SPP was cut on 7 June 2000, grass from SPR on 21 June 2000 and grass from IM on 5 May 2000. All three types of grass were prewilted (wilting period <72 h) and subsequently ensiled in big bales (400–600 kg). For more details on the botanical composition and the management of the three grasslands, we refer to Bruinenberg (2003).

In addition to the silage, cows received 4·5 kg dry matter (DM) concentrates per day (composition in Table 2), offered during milking in two portions of 2·25 kg DM each. The silage was fed restricted (16 kg DM/day) in two portions a day, offered directly after the concentrates were eaten. In the morning the animals received 40 % and in the evening they received 60 % of the daily silage offer. Once every 2 weeks the four silage mixtures were prepared, weighed into individual plastic bags per cow per feeding time, and stored at -18 °C until 1 day before feeding. Silages were thawed for 24 h at environmental temperature, resulting in thoroughly thawed palatable silage at feeding.

Measurements, sampling and analysis

Intake was measured at four consecutive days in the third week of the experimental period. Samples of the silage mixtures were taken directly after mixing and stored at -18 °C. Production of urine, the pH of the rumen fluid, and VFA and NH_3 concentrations in the rumen, as well as the rumen contents were measured during the third week. Starting at 13:00 h on day 1, the urine was quantitatively collected for 48 h, using a bladder catheter. Urine was collected under 5·12 M sulphuric acid to maintain a pH <3. After 48 h, samples of the acidified urine were stored at -18 °C until analysis for uric acid. Furthermore, starting on day 1, the rumen fluid was sampled at 16:00, 18:00, 20:00, 22:00, 24:00, 03:00, 06:00, 08:00, 10:00 and 13:00 h. The pH of the rumen fluid was measured directly and a subsample was taken, mixed with 0·89 M phosphoric acid in a ratio of rumen fluid : phosphoric acid of 5 : 1, and frozen at -18 °C until analysis of NH_3 and VFA. Rumen contents were evacuated manually at 04:00, 10:00 and 20:00 h on day 4, and at 09:00 h on day 5. Cows were deprived of food between 20:00 h, day 4, and 09:00 h, day 5. Rumen contents were weighed and two samples were taken, one for the analysis of the chemical composition and the other for the analysis of the particle size distribution in the rumen. Approximately 2 h before the sampling of 20:00 h, 30 g Cobalt Ethylenediaminetetraacetate (Co-EDTA), dissolved in water, was added in the rumen. Concentrations of Co-EDTA were determined in the rumen samples collected at 20:00 and 09:00 h, to estimate the passage rate of the rumen fluid.

After freeze-drying the samples of feed and rumen contents, DM, ash, neutral detergent fibre (NDF), sugars (only of feed) and Kjeldahl N were determined as described by Van Vuuren *et al*. (1993) and *in vitro* organic matter digestibility was determined according to Tilley & Terry (1963). The indigestible acid-detergent fibre (IADF) content was determined as described by Penning & Johnson (1983). The VFAs and uric acid were analysed by high performance liquid chromatography, using a Merck polyspher OA-HY 51272 column. For VFA, the mobile phase was 0·0025 M sulphuric acid followed by RI-detection. For uric acid, the eluent was 0·005 M sulphuric acid

and the detection method was with ultraviolet light at λ 283 nm. The NH_3 was determined with a modified Berthelot method (Robinson *et al*. 1986). Organic matter (OM; g per kg) was calculated as 1000 – ash, and crude protein (CP) was calculated as $6{\cdot}25 \times N$. Fermentable organic matter (FOM) was calculated based on *in situ* degradation characteristics (Bruinenberg *et al*. 2004).

From the fresh sample of the rumen contents, two subsamples were taken, one for the analysis of the DM content, and the other for the analysis of the distribution of the particle size of the rumen contents. The particle size analysis was carried out by wet sieving of a fresh sample of the rumen contents, using four sieves with mesh sizes of 4, 2·5, 1 and 0·045 mm. The sieves were placed on top of each other, the one with the widest mesh on top. The subsample was placed in the top sieve, tap water was added, enough to cover the subsamples, the sieves with rumen contents were shaken for 15 min, and then water was removed. This procedure was repeated twice before the sieves were emptied and the different fractions were oven-dried and weighed. The fraction of each particle size in the total rumen contents was then calculated based on their oven-dried weight. Fractions were divided into large ($>2{\cdot}5$ mm) and small ($<2{\cdot}5$ mm) particle size.

Calculations and statistics

Rumen turnover rates of different fractions were described by rate of intake, passage rate and degradation rate of OM and NDF.

Rate of intake (%/h) of OM (k_{iOM}) and NDF (k_{iNDF}) were calculated as:

$$\frac{\text{(kg OM or NDF intake per day/kg OM or NDF in the rumen/24 h)}}{\text{kg OM or NDF in the rumen}} \times 100\%.$$

The passage rate (k_p, %/h) was calculated as:

$$\frac{\text{(kg IADF intake per day/kg IADF in the rumen/24 h)}}{\text{kg IADF in the rumen}} \times 100\%.$$

where kg OM, NDF or IADF in the rumen is the average of the rumen evacuations at 04:00, 10:00 and 20:00 h on day 4.

The fractional degradability (%/h) of OM (k_{dOM}) or NDF (k_{dNDF}) was calculated as:

$$k_i - k_p.$$

Furthermore, based on the disappearance of rumen contents after 13 h of fasting, the rates of clearance (%/h) of rumen OM (k_{cOM}), NDF (k_{cNDF}), particles $>2{\cdot}5$ mm (k_{cL}) and particles $<2{\cdot}5$ mm (k_{cS}) were calculated as:

$$[(\ln(\text{rumen contents at } t = 20{:}00) - \ln(\text{rumen contents at } t = 9{:}00)13\text{ h})] \times 100\%.$$

Results were statistically analysed with the analysis of variance (ANOVA) procedure for a Latin square design using Genstat 5 (Genstat 5 Committee 1993), with cows × periods as the block structure and diets as the treatment. Treatment means were compared with the Student's *t*-test. Significance was determined at $P<0{\cdot}05$. The pH, VFA and NH3 patterns were analysed with a repeated measurement analysis of variance (REML) with Genstat (VSN International Ltd 2002). Differences in patterns were determined with the Wald-test.

In the discussion, results of the different variables (e.g. OM, FOM and CP intake, pH, VFA, NH_3 and NDF pool) were related. Relationships between the variables, such as OM intake and pH, FOM and VFA, CP intake and NDF pool in the rumen, etc. were calculated by regression analysis using Genstat 5 (Genstat 5 Committee 1993).

RESULTS

Diet composition, quality and intake

The four silage mixtures differed in concentrations of CP, NDF, fermentable organic matter (FOM), sugars and in the values for *in vitro* digestibility and net energy (Table 1). The digestibility of OM and the CP, FOM and net energy (NE) concentrations were highest in 100IM and lowest in 60SPR, whereas the NDF and sugar (SU) concentrations were lowest in 100IM and highest in 60SPP.

Concentrate intake was maximal, and therefore differences in total intake were caused by differences in silage intake (Table 3). Intakes of DM and OM were highest on 100IM and 20SPP and lowest on 60SPP ($P<0{\cdot}05$; Table 3). CP intake was highest on 100IM, and it was equally low on 60SPP and 60SPR. No significant differences were observed in intake of NDF. Sugar intake was highest on 60SPP ($P<0{\cdot}05$). Intake of IADF was highest on 60SPR and lowest on 100IM and 20SPP ($P<0{\cdot}05$).

Fermentation characteristics

The pH was highest for 60SPR, and lowest for 100IM and 20SPP ($P<0{\cdot}05$; Table 4). This was also reflected by the VFA concentrations in the rumen, which increased with a decrease in pH ($R^2=0{\cdot}70$, $P<0{\cdot}001$; $n=160$). Type of silage had no effect on the molar proportions of acetate or propionate although the proportion of butyrate on 60SPP was significantly higher than on 100IM ($P<0{\cdot}05$). No significant differences were found for the ratio of nonglucogenic : glucogenic volatile fatty acids (NGGR). The average concentration of NH_3 over the day reflected CP intake and was significantly highest in 100IM and 20SPP, and lowest in 60SPR ($P<0{\cdot}05$; Table 4). The NH_3 concentration in 60SPP was also significantly lower than

Table 3. *Intake of dry matter, organic matter, crude protein, neutral detergent fibre, cellulose-indigestible acid detergent fibre, fermentable organic matter and particles larger or smaller than 2·5 mm on the four treatments. Abbreviations of the treatments as in Table 1. All values in kg per day*

	100IM	20SPP	60SPP	60SPR	S.E.D.
Diet					
Silage	14·0[a]	13·6[a]	12·2[b]	13·0[ab]	0·49
Concentrates	4·5	4·5	4·5	4·5	0
Total intake					
Dry matter	18·5[a]	18·1[a]	16·7[b]	17·5[ab]	0·49
Organic matter	16·4[a]	16·1[a]	15·0[b]	15·7[ab]	0·46
Crude protein	3·8[a]	3·6[b]	3·1[c]	2·9[c]	0·06
Sugars	0·80[b]	0·87[b]	1·02[a]	0·88[b]	0·041
Neutral detergent fibre	8·7	8·7	8·3	8·5	0·31
Indigestible acid detergent fibre	0·7[a]	0·8[a]	1·0[b]	1·4[c]	0·11
Fermentable organic matter	10·3	9·7	8·2	8·5	*
Particles >2·5 mm	10·3	10·2	9·6	10·2	0·40
Particles <2·5 mm	8·2[a]	7·9[b]	7·2[c]	7·3[c]	0·10

[a,b,c] Within a row, means not sharing common superscripts differ significantly ($P<0·05$).
* Calculated based on averages (this table and Bruinenberg *et al.* 2004) and assuming fermentable organic matter of concentrates was 520 g/kg, so no S.E.D. could be calculated.

Table 4. *The pH and the concentration (mmol/l) of volatile fatty acids and ammonia in the rumen for different silages. Numbers are the average over 10 times within 24 h*

Diet	100IM	20SPP	60SPP	60SPR	S.E.D.
pH	6·2[a]	6·2[a]	6·3[b]	6·5[c]	0·04
Total VFA	125[a]	119[a]	106[b]	104[b]	2·65
Acetate, mol proportion	0·710	0·709	0·709	0·711	0·0038
Propionate, mol proportion	0·179	0·178	0·174	0·175	0·0041
Butyrate, mol proportion	0·112[a]	0·113[ab]	0·118[b]	0·115[ab]	0·0022
NGGR	5·3	5·3	5·5	5·4	0·16
NH_3	12·9[a]	12·3[a]	10·5[b]	8·3[c]	0·37

[a,b,c] Within a row, means not sharing common superscripts differ significantly ($P<0·05$).
NGGR = (acetate + 2 × butyrate)/propionate. Abbreviations of the treatments as in Table 1.

in 100IM and 20SPP ($P<0·05$). Over the day, the pH, VFA and NH_3 fluctuated, and for pH and VFA the daily patterns were not significantly different among the different diets, but for NH_3 there were significant differences in daily pattern among the diets. Especially shortly after feeding the pH decreased (Fig. 1) and the concentrations of NH_3 increased (Fig. 2).

Urine production was highest on 100IM and 20SPP and lowest on 60SPP and 60SPR (Table 5, $P<0·05$). The total amount of uric acid produced in the urine per kg metabolic body weight were highest in 100IM ($P<0·05$).

Rumen kinetics

Rumen pool sizes of DM and OM did not differ significantly between diets (Table 6). The pool size of NDF was significantly smaller on 100IM than on 60SPP or 60SPR. The pool size of IADF was lowest on 100IM and 20SPP and highest on 60SPR.

No significant differences in k_p among the different diets were observed, but the k_i of OM and NDF was significantly higher on 100IM compared with 60SPP (k_{iOM}) or compared with 20SPP, 60SPP and 60SPR (k_{iNDF}) (Table 7; $P<0·05$). The k_d of NDF was significantly lower on 60SPR than on 100IM ($P<0·05$). No differences in clearance rate after 13 h of fasting among the different treatments were observed.

Before as well as after fasting, the highest proportion of large particles was observed on 60SPR and the lowest proportion for 20SPP (before fasting) and 60SPP (after fasting) ($P<0·05$; Table 8). Rumen clearance rate of the particles >2·5 mm was highest on 60SPP and lowest on 60SPR ($P<0·05$), but clearance

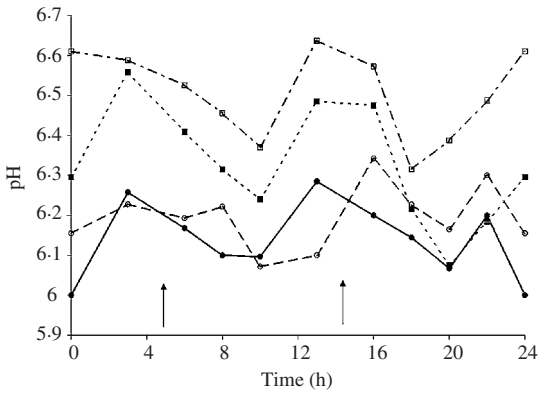

Fig. 1. The pH pattern over the day of the four different diets. Arrows indicate the time of feeding. Standard error of difference was approximately 0·11 for all levels (● = 100IM; ○ = 20SPP; ■ = 60SPP; □ = 60SPR).

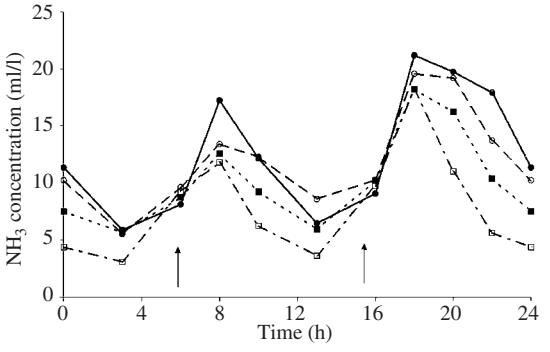

Fig. 2. The NH_3 pattern on the four diets over the day. Arrows indicate the time of feeding. Standard error of difference was 2·13 on average, but higher (maximum 3·45) for the high levels, and lower (minimum 0·69) for the low levels. (● = 100IM; ○ = 20SPP; ■ = 60SPP; □ = 60SPR).

rate of particles $<2{\cdot}5$ mm were not statistically different between diets.

DISCUSSION

To obtain insight in rumen kinetics and rumen fermentation patterns of forages from semi-natural grasslands, four diets with differing proportions of semi-natural grassland silage in the diet were fed to lactating dairy cows fitted with a rumen cannula. Compared with diets consisting solely of intensively managed grass silage and concentrates, the inclusion of forages from semi-natural grasslands in the diet was expected to result in differences in rumen fermentation and kinetics due to a reduction of degradation rate, passage rate and nitrogen surplus in the rumen. This discussion consists of four sections. First, the intake of the different diets, as intake determines rumen fermentation processes and flows of digesta. Second, the rumen fermentation patterns, subsequently the kinetics of nutrients and particles in the rumen, and finally conclusions are drawn about the behaviour of forages from semi-natural grasslands in the rumen of dairy cows.

Intake

On 60SPP, intake was lower than on 100IM, which confirmed earlier results (Bruinenberg 2003). This reduction was attributed to the low degradation rate of SPP as has been observed *in vitro*, using the gas production technique, as well as *in situ*, using the nylon bag technique (Bruinenberg *et al.* 2004). Compared with 60SPP, the intake of 60SPR was numerically higher but not significantly different, which was attributed to the presence of herbs, having higher *in vitro* and *in situ* degradation rates than grasses (Lopez *et al.* 1991; Bruinenberg *et al.* 2004). Rumen pool sizes and *in vivo* degradation and their relationships with intake will be discussed later in this paper.

Fermentation characteristics

No relationships were found between rumen pH and any of the intakes of chemical components. However, when 60SPR was excluded from calculations, there was a good relation between rumen pH and OM or sugar intake. On 60SPR, rumen pH was higher than was expected from OM or sugar intake, or even from VFA concentrations in the rumen. The reason for this high pH was not found, but it could have been caused by some nutritional factor that was not analysed, such as concentration of tannins, which can be present in herbs (Mertens 1998; Rezvani Moghaddam & Wilman 1998; Scehovic 2000), and thus in 60SPR. The presence of tannins may increase saliva production (Van Soest 1994), which results in the adding of more buffer into the rumen, and thus in a higher pH.

As expected, the pH and the VFA concentration were inversely correlated ($R^2 = 0{\cdot}70$) and the highest VFA concentrations were observed on 100IM and 20SPP, reflecting the relatively high *in vitro* digestibility. The VFA concentration on 100IM was comparable with values in the literature (e.g. Bosch *et al.* 1992*a*; De Visser *et al.* 1993). As expected, the relationship between FOM and VFA was approximately linear ($R^2 = 0{\cdot}96$). The R^2 of the relationship between CP and VFA concentration was even higher, 0·98, which was attributed to the relationship between protein concentration of the silages and their OM degradability.

Cell walls in the diet are known to favour acetate over propionate and butyrate production (Miller 1979; Bannink *et al.* 2000), but no differences were observed between diets. Also the proportion of propionate was rather consistent over the diets in this study. The

Table 5. *The effect of treatment on urine production and uric acid. Abbreviations of the treatments as in Table 1*

Diet	100IM	20SPP	60SPP	60SPR	S.E.D.
Urine production, kg/day	37·1[a]	35·2[a]	26·7[b]	23·8[b]	2·27
Uric acid, mg/kg $BW^{3/4}$	22·7[a]	15·8[b]	13·9[b]	14·7[b]	2·77

[a,b] Within a row, means not sharing common superscripts differ significantly ($P<0·05$).

Table 6. *Average pool size of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and cellulose-indigestible acid detergent fibre (IADF)*

	100IM	20SPP	60SPP	60SPR	S.E.D.
Rumen pool size (kg)					
DM	13·9	14·2	14·0	13·8	0·50
OM	12·3	12·7	12·5	12·3	0·47
NDF	6·1[a]	6·7[ab]	7·0[b]	7·1[b]	0·31
IADF	1·2[a]	1·5[a]	1·8[ab]	2·4[b]	0·30

[a,b] Within a row, means not sharing common superscripts differ significantly ($P<0·05$).
Abbreviations of the treatments as in Table 1.

Table 7. *Passage rate of indigestible acid detergent fibre (IADF) and turnover of organic matter and neutral detergent fibre (NDF), calculated from the rumen evacuation data.* k_p *is passage rate,* k_i *is rate of intake,* k_d *is rate of degradation (calculated from* k_i *and* k_p*) and* k_c *is clearance rate after 13 h of fasting (rates in %/h)*

	100IM	20SPP	60SPP	60SPR	S.E.D.
IADF					
k_p*	2·7	2·3	2·5	2·8	0·50
Organic matter					
k_{iOM}	5·7[a]	5·4[ab]	5·1[b]	5·5[ab]	0·22
k_{dOM}	3·0	3·0	2·7	2·7	0·33
k_{cOM}	5·7	5·2	5·2	5·0	0·52
NDF					
k_{iNDF}	6·1[a]	5·5[b]	5·1[b]	5·0[b]	0·26
k_{dNDF}	3·4[a]	3·2[ab]	2·6[ab]	2·3[b]	0·40
k_{cNDF}	6·3	5·7	5·0	4·7	0·66
Fluid (Co-EDTA)					
k_c	12·8	12·4	11·2	12·5	0·65

[a,b] Means within a row with a different superscript are significantly different ($P<0·05$).
* Calculated as $k_p=k_i$ IADF = (intake IADF/rumen IADF content I/24h) × 100 % $k_d=k_i-k_p$, k_p used in this Table based on intake.
$k_c = 100 \times [\ln(\text{Co-EDTA in rumen fluid at } t=20) - \ln(\text{Co-EDTA in rumen fluid at } t=9)]/13$.

proportion of butyrate was significantly higher on 60SPP, but the difference was small.

On all diets a decrease in pH was observed after feeding, with a slight increase in pH several hours later (Fig. 1). However, after feeding in the afternoon the pH started to increase earlier on 60SPR than on the other diets, although differences in the overall patterns among diets were not significant. The early increase of pH was caused by the lower production of VFAs on this diet, due to a lower supply of available fermentable material, and thus a more rapid exhaustion of degradable energy in the rumen. As mentioned earlier, perhaps also an increase in saliva production played a role here.

The NH_3 concentration in the rumen increased with higher CP intake and with higher ratio CP intake/OM intake. After feeding, the NH_3 concentration in the rumen increased, and within 2 h the NH_3 concentration began to decline. This indicates that degradation of protein from the diet occurs directly after feeding. The decline in NH_3 concentration occurred faster after feeding on SPR60 than on the other treatments. This is attributed to the low CP concentration and to a low rumen availability of CP. Part of the protein may be intertwined with lignin or other cell wall material (Iiyama *et al.* 1993), and SPP and SPR contained larger proportions of lignin and cell walls than IM (Table 1; Bruinenberg *et al.* 2004). Just before feeding, the NH_3 concentration on 60SPR was relatively low compared with the other diets, but concentrations (>2 mmol/l) were probably still sufficient for efficient microbial degradation and protein synthesis in the rumen (Satter & Slyter 1974). An inhibited microbial protein synthesis would be shown in a reduced uric acid excretion in the urine, as uric acid is an indicator of the production of microbial protein in the rumen (Chen *et al.* 1992; Johnson *et al.* 1998). Indeed, a reduction in uric acid excretion in the urine was not observed (Table 5).

The quantity of uric acids in the urine is usually influenced by FOM intake. This was also observed in this study, although the proportion of uric acids in the urine on 20SPP was low, compared with 100IM, 60SPP and 60SPR. The low quantity of uric acids on 20SPP could not satisfactorily be explained by the available feed and intake characteristics. Maybe attachment of microbes to large forage particles delayed the passage rate of the microbes, thus increasing recycling of energy and N in the rumen and thereby

Table 8. *Distribution of particle size in the rumen (kg small and large particles before and after fasting) and rumen clearance rate (%/h) of the different size particles. Distribution in the rumen before* (t = *20*) *and after fasting* (t = *9*)

Diet	100IM	20SPP	60SPP	60SPR	S.E.D.
Rumen pool size (kg; before fasting)					
>2·5 mm	5·2^{ab}	4·3^{b}	4·6^{ab}	5·8^{a}	0·53
<2·5 mm	10·6	11·0	11·4	10·4	0·60
Rumen pool size (kg; after fasting)					
>2·5 mm	2·0^{ab}	1·9^{ab}	1·4^{b}	2·9^{a}	0·43
<2·5 mm	5·6	5·9	7·0	5·8	0·75
Rumen clearance rate (%/h) of different sized particles					
>2·5 mm	7·3^{ab}	5·6^{ab}	9·3^{a}	5·3^{b}	1·6
<2·5 mm	5·0	4·7	4·0	5·0	0·9

^{a,b} Figures with a different superscript are significantly different ($P < 0{\cdot}05$).
Abbreviations of the treatments as in Table 1.

causing a larger quantity of energy and nitrogen to be used for maintenance of the microbial population rather than for growth (Clark *et al*. 1992). Indeed, the k_p was observed to be lower on 20SPP than on the other treatments, although differences were not significant (Table 7).

Rumen kinetics

Pool sizes

The NDF pool size was in accordance with NDF pool sizes observed by Bosch *et al*. (1992*b*). The NDF pool in the rumen is probably a factor limiting intake (De Visser *et al*. 1998). A lower CP intake resulted in a larger NDF pool. This is due to a decrease of CP concentration with advancing stage of maturity (Beever *et al*. 2000), resulting in a positive relationship between CP concentration (and thus between CP intake) and degradability of NDF. Intake of NDF had no effect on the NDF pool, as similar NDF intakes of the four diets (Table 3) was accompanied with significantly increased NDF pools (Table 6).

Nutrient flows

In the study IADF was used to determine the k_p (Tamminga *et al*. 1989). The k_ps of IADF on the different treatments were calculated to be between 2·3 and 2·8 %/h (Table 7). Passage rates of OM in rumen and duodenum fistulated cows fed fresh grass have been observed to be between 3·2 and 5·1 %/h (Van Vuuren *et al*. 1993), and passage rates for OM were higher than the passage rates for NDF (Van Vuuren *et al*. 1993). Therefore, it is likely that k_p differs among nutrients and thus passage rates of OM and NDF may have been higher or lower than k_p of IADF measured in this study. Based on the *in situ* degradation of the silages (Bruinenberg *et al*. 2004), passage rates of OM and NDF were calculated to be lower than the k_p of IADF (k_p of OM: 1·0–1·6 %/h, k_p of NDF 1·5–1·8 %/h).

Because intake and chemical composition of the four diets differed, differences in k_p were expected. Indeed, differences for k_p among the four diets were observed, but difference remained statistically insignificant. Intake of DM (DMI) is often related to rumen clearance rate, which is the sum of k_d and k_p. The animal can achieve higher DMI by either an increase of k_d or k_p or both. In this experiment, for three of the four treatments, DMI was related to k_d of OM and NDF, and not to k_p. For 60SPR, DMI was higher in relationship to k_{dNDF}. In this treatment, the relatively high k_p (compared to e.g. 20SPP or 60SPP) resulted in maintaining a certain level of DMI.

Two methods were used to estimate disappearance from the rumen, i.e. the k_i and the k_c. On most diets, k_{iOM} was approximately equal to k_{cOM}, and k_{iNDF} was approximately equal to k_{cNDF}. Small differences between k_i and k_c were attributed to differences in the normal and fasting situation. Sometimes, in the fasting situation k_{cOM} and k_{cNDF} were smaller than k_{iOM} and k_{iNDF} (60SPR). For a better explanation of this phenomenon, more measurements during fasting should have been taken. However, this could have led to other disturbances in rumen kinetics.

The k_{iNDF} increased with higher CP intakes, due to the interrelationship of CP concentration of the silage with degradability of the NDF, and thus on rumen NDF pool, as discussed earlier. The k_{iOM} also had relationships with CP intake, but on 60SPR the k_{iOM} was higher than expected from CP intake.

Particle size

The relatively low intake of large particles on 60SPP was due to the low DM intake of the diet, which resulted in a relatively high proportion of concentrates in the diet.

No relationship was observed between k_c of large particles (k_{cL}) and NDF pool size in the rumen before fasting, and differences in k_{cL} between the different diets could therefore not be attributed to nutrients in the rumen. The high k_{cL} on 60SPP was not expected, as the diet consisted mostly of mature grasses, causing a more difficult reduction of particles. However, on tropical, less digestible grasses, more time is spent on chewing, resulting in greater breakage (Mtengeti *et al.* 1996). The vascular structure of the forage in 60SPP probably also stimulated chewing and rumination, resulting in the high k_{cL}. More time spent on mechanical reduction through rumination would also explain the relatively low intake of the diet. On 20SPP, the k_{cL} was lower than on both 100IM and 60SPP, although differences were not significant. It was expected that 20SPP would show values between 100IM and 60SPP. The reason why this did not occur was not clear – maybe the quantity of SPP ingested was not large enough to stimulate rumination, but large enough to reduce degradation. The k_{cL} of 60SPR was low compared with 100IM and 60SPP. This was not expected, because usually fibre in dicotyledons is more easily degraded into small particles than fibre in grasses. This may be due to junctions between cells, instead of the parallel girder system of vascular bundles found in grasses (Wilson 1985). However, probably 60SPR does not stimulate rumination, and k_{cL} was reduced because of the high IADF pool in the rumen on 60SPR (Table 6). Plant parts containing IADF, e.g. the lignified xylem tissue (Wilson & Hatfield 1997), are difficult to degrade, resulting in a long retention time in the rumen. The low k_{cL} on 60SPR (Table 8) is contradictory to the relatively low rumen pool size (Table 6) and high k_p (Table 7) on 60SPR. The contrasting results could be due to differences in rumen behaviour with fasting, compared with the normal situation.

The high k_{cL} on 60SPP resulted in the low k_c of small particles in this ration (k_{cS}), as reduced large particles shift towards the small particle pool, whereas the low k_{cL} on 60SPR resulted in a relatively high k_{cS}. High values of k_{cS} indicate a fast degradation or passage, but the quantity of small particles should be corrected for the flow from the large particle pool. If the pool size of small particles is corrected for the flow from the large particles, clearance rate of small particles increases, but the same trends are observed for the different treatments (k_{cS}: 100IM: 7·8, 20SPP: 7·4, 60SPP 6·4, 60SPR 7·8).

CONCLUSION

Based on rumen fermentation and kinetics, it can be concluded that forages from semi-natural grasslands behave in a similar way as intensively managed forages. The concentration of VFA and NH_3 in the rumen and the ratio between the different VFAs were not remarkably different from expectations based on FOM and CP intake. However, the k_p and k_i of herb-rich forage were higher than expected, which could explain why a reduction of intake on 60SPR did not occur, despite the low digestibility and high IADF concentration. Furthermore, the rate of clearance of large particles on 60SPP was higher than expected, probably indicating more rumination activity. However, for most rumen fermentation characteristics measured in this study, no remarkably aberrant behaviour of the silages from semi-natural grassland was observed.

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