# Influence of stage of maturity of grass silages on digestion processes in dairy cows

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Proefschrift
ter verkrijging van de graad van
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op gezag avn de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op vrijdag 10 mei 1991
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

# Stellingen

1. Het vloeistofvolume in de pens van koeien is sterker gerelateerd aan het lichaamsgewicht van de dieren dan aan het lactatiestadium of de voersamenstelling.
(Dit proefschrift)

2. Afname in pensvulling van met grassilage gevoerde koeien wordt niet in de eerste plaats beperkt door verkleining van lange voerdeeltjes, maar door de afvoer van deeltjes die een zeefopening van 1,25 mm wel en één van 0,071 mm niet kunnen passeren.
(Dit proefschrift)

3. Wanneer koeien per etmaal 9 uur of langer herkauwen, resulteert een verhoging van het celwandgehalte van het rantsoen niet in een verlenging van de herkauwtijd, maar in een verlaging van de opname.
(Dit proefschrift)

4. Voor het bepalen van de hoeveelheid microbiëel eiwit in de pens is DAPA geen goede indicator.
(Dit proefschrift)

5. De hoeveelheid droge stof in de pens van koeien, uitgedrukt in g per kg lichaamsgewicht, wordt voor meer dan 75% verklaard door de melkproduktie (g FCM/$kg^{0.75}$).
(Dit proefschrift)

6. De verklaring van Himmelsbach *et al.* dat een stijging van het N-gehalte in het residu van grassilages tussen 48 en 336 uur pensincubatie waarschijnlijk veroorzaakt wordt door microbiële contaminatie, berust op een foutieve interpretatie van de resultaten. (Himmelsbach *et al.*, 1988; In: *Analytical Applications of Spectroscopy:* C.S. Creaser and A.M.C. Davies [Eds.], The Royal Society of Chemistry, 410-413).

7. Het berekenen van de DVE-waarde van een voedermiddel is een ingewikkelde zaak, het vaststellen ervan vooralsnog onmogelijk.

8. Het begrip ileaal verteerbare aminozuren voor varkens wordt in de CVB-tabel ten onrechte aangeduid met de term darmverteerbare aminozuren voor varkens.

9. De uitspraak van de politicoloog R. Andeweg (NOS-laat, 19 maart 1991), dat kamerleden meer geïnteresseerd zijn in onze stem dan in onze mening, geeft te denken over het functioneren van onze democratie.

10. Het opstellen van een tussenbalans kan een regeringscoalitie langdurig uit evenwicht brengen.

11. De termen OIO en AIO suggereren ten onrechte dat de eerste wordt opgeleid tot onderzoeker, de tweede slechts tot assistent.

12. Positieve discriminatie bij sollicitaties is in strijd met de wet gelijke behandeling.

Proefschrift van M.W. Bosch.
Influence of stage of maturity of grass silages on digestion processes in dairy cows.
Wageningen, 10 mei, 1991.

*Aan mijn ouders*

*Aan Peter*

**Voorwoord**

Het in dit proefschrift beschreven onderzoek is uitgevoerd bij de vakgroep Fysiologie van Mens en Dier in nauwe samenwerking met de vakgroep Veevoeding, beiden van de Landbouw Universiteit Wageningen. Vanaf deze plaats wil ik een woord van dank richten aan iedereen die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift.

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Vele (ex)studenten hebben een deel van het onderzoek

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**Contents**

Chapter I

# Introduction

A suitable climate, combined with a fertile soil has made the Netherlands an attractive area for dairy production. At present dairy production in the Netherlands is partly (50 - 55%) based on homegrown forages (grass, grass silage, corn silage) and partly on largely imported concentrates. Because of the large number of dairy cows (2 million) and the limited area available for grass production, forage production systems are highly intensified by using high levels of N-fertilisation.
High doses of N-fertilizer result in high outputs of dry matter per acre. To ensure a high quality, the grass is harvested in a young stage of growth, resulting in grass silages with a high nitrogen content and a relatively low cell wall content. The digestibility of this cell wall fraction is high, allowing high ad libitum intakes. A disadvantage of harvesting at this stage is the low utilization of nitrogen. When the grass is harvested at a later growth stage, the protein:energy ratio is more favourable, but digestibility of the cell wall fraction decreases (Reid *et al.*, 1988).

In 1984 a milk quota system was introduced in the Netherlands. The reaction of the farmers was to sell their least productive animals and produce their smaller amount of milk with less animals. As a result, the number of dairy cows has decreased with about 25% since 1984. On an increasing number of farms this has resulted in a surplus of grass and grass silage. Increasing the proportion of roughage in the diet seems therefore interesting. However, roughage intake by dairy cows in early lactation is limited. The mechanisms controlling roughage intake are still not well understood.

In early lactation, nutrient requirements are high and when the energy content of the ration is relatively low, rumen capacity can be the limiting factor (Weston, 1982). Possible factors which limit roughage intake could be the volume of the feed, the rate of size reduction, the rate of degradation in the rumen, passage rate of undigested feed particles to the lower gut and removal of fermentation end products (Tamminga & van Vuuren, 1988).

If intake is not limited physically, physiological status and type and amount of digestion products may be controlling feed

intake.

**Ruminal degradation rate.**

Major components of cell walls are cellulose and hemicellulose, which may be encrustrated with lignin. Lignin is hardly digestible and acts as a barrier for rumen microbes. Energy present in cell walls, can only be made available after microbial degradation of the cellulose and hemicellulose. The rate of degradation of the potentially digestible fraction of the cell wall constituents of grass silages is negatively related to the lignin content (Van Soest, 1982).
Maturing increases the undegradable cell wall fraction and the lignin content, resulting in a decreased degradation rate. Bacteria have to attach to the cell walls before they can digest them. This means that a bigger surface of the particles probably results in a faster rate of degradation.

Next to energy, protein is an important component of high quality roughages. Of the protein, the soluble fraction is believed to have a very high rate of degradation and to be fully degraded in the rumen. With an increase in cell wall content of the grass, degradation rate of the potentially digestible but non-soluble protein decreases, mainly due to the lower digestion rate of the surrounding cell walls.

**Particle size reduction and passage from the rumen.**

Before large feed particles can leave the rumen to the lower tract, they have to be reduced in size, which is achieved by chewing and rumination. The probability of passage of particles is inversely related to their size (Poppi *et al.*, 1980), but even the finest particles have a lower rate of passage than the fluid (Faichney, 1986).

Microbial degradation decreases rumen dry matter content, but it has relatively little effect on particle size and thus on

reduction in rumen fill (Van Soest, 1982; Welch, 1982; Ulyatt *et al.*, 1986). According to McLeod & Minson (1988a, 1988b), microbial digestion plus rumen contractions would be responsible for only about 20% of particle size reduction. The main factor reducing particle size is chewing during eating and rumination.
Rumination time per kg dry matter ingested increases with cell wall content of the diet (Welch & Smith, 1969a, 1969b; Murphy *et al.*, 1983; Ulyatt *et al.*, 1986). Because rumination time has a maximum of 9 to 10 h a day (Bae *et al.*, 1979; Welch, 1982), for high cell wall silages the time required to reduce particle size through rumination to a size below the CPS can be the limiting factor for silage intake.
Particles smaller than the critical particle size (CPS), for cattle reported to be 1.18 mm (Kennedy & Poppi, 1984), have a high probability of passing out of the rumen.
More than half of the rumen dry matter is in the small particle pool, with a size smaller than the CPS (Poppi *et al.*, 1980).

Not only the size of particles, but also their functional specific gravity (FSG) modifies their chance of leaving the rumen (Welch, 1986; Sutherland, 1987). Fermentation gas, which lowers the FSG of particles, is removed by rumination and rumen contractions, resulting in an increase in FSG. As a result, the FSG of particles which are further degraded is higher, increasing their probability of passage. Maximal rates of passage were found for plastic particles with a specific gravity of 1.2-1.4 (Welch, 1986).

**Fermentation products.**

Microbial degradation of the carbohydrates and the protein ingested with the feed results in a production of volatile fatty acids (VFA) and ammonia ($NH_3$). One of the factors determining microbial growth rate is the rate of ATP generation from the degradation of carbohydrates. When energy becomes available for the microbes spread over the day and other essential nutrients (VFA, $NH_3$, amino acids, minerals and vitamins) are available as

well, efficiency of biomass production will be highest.

The concentrations and the ratio of VFA, as well as the $NH_3$ concentration, are the result of microbial activity on one hand and rate of clearance from the rumen, by absorption through the rumen wall and by passage to the omasum, on the other (Satter & Slyter, 1974; Hoover, 1986).

The pH of rumen fluid is negatively related to the concentration of VFA (van Soest, 1982; Hoover, 1986; Tamminga & Van Vuuren, 1988). When the degradation rate of the carbohydrates is very high, a rapid increase in VFA concentration will take place, resulting in a decline in pH. A pH below 6 is less favourable for cellulolytic microbes (Ørskov, 1982) and can result in a decrease in cell wall digestion.

Ammonia is toxic to animal cells and high concentrations of ammonia and other non protein nitrogen (NPN) compounds, which can be found feeding high-nitrogen silages, can limit intake (Van Soest, 1982).

Most of the factors influencing rumen capacity discussed above were studied in isolation. Not only that for a large number of questions no unequivocal answer is given, very little attempts have been made sofar to study the several aspects as an integrated system.

The objective of this study was to quantify in an integrated approach, the effect of the stage of maturity of grass silages on intake, digestibility, rumen fermentation pattern, rumination activity, passage rate from the rumen, digestion rate of the potentially digestible fractions and composition of rumen contents. Therefore four experiments were conducted, in which dairy cows were fed grass silages harvested at different growth stages ad libitum and a fixed amount of concentrates, 1 or 7 kg depending on the stage of lactation.

The influence of maturing on chemical composition of the silages, their nylon bag degradation rates (Mehrez & Ørskov, 1977) and the overall digestibility are described in Chapter II. Multiple regression analysis was used to analyse the influence

of the different parameters on ad lib silage intake.

The fractional passage rates of the fluid and particulate phases from the rumen, using CoEDTA and Cr mordanted Neutral Detergent Fibre (Cr-NDF) as markers (Uden *et al.*, 1980) were measured (Chapter III). Differences in total rumen contents (kg), kg dry matter (DM), organic matter (OM), crude protein (CP) and cell wall components for the different growth stages of the silages and stages of lactation of the animals are also described.

Diurnal patterns of pH and $NH_3$ concentrations were measured in the four experiments. The average daily VFA concentrations were measured in Exps. 1, 2 and 3, and the diurnal pattern of VFA concentrations in Exp. 4. The results are discussed in Chapter IV.

Eating and rumination times were also measured and distribution of rumen and faecal particle sizes was determined. The results are also discussed in Chapter IV.

The high losses of nitrogen (N) occuring when grass products are fed to dairy cows can be reduced by lowering the CP content of the grass (Van Vuuren & Meijs, 1987). With an increase in cell wall content usually the CP content of the grass decreases. Changes in the rate of protein degradation in the rumen and microbial protein synthesis were therefore measured for the different stages of maturity and results are reported and discussed in Chapter V.

In a separate experiment, the influence of size of particles mordanted with Cr on the fractional passage rate from the rumen was determined. The results and the consequences of using Cr-NDF of different particle sizes and the differences between silages are reported and discussed in Chapter VI.

In a last experiment, in which dry cows were fed grass silages of two different growth stages, a simplified rumen model was used to determine the rate limiting step in reduction in rumen fill. Results are reported and discussed in Chapter VII.

In the General Discussion (Chapter VIII), an attempt is made to integrate the results of all the experiments reported in the preceding chapters.

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Chapter II

# Influence of stage of maturity of grass silages on digestion processes in dairy cows. 1. Composition, nylon bag degradation rates, digestibility and intake.

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**Abstract.**

In four change-over experiments, dairy cows were fed wilted grass silages (G1, G2, G3, G4 and G5) ad libitum. In early lactation, silage was supplemented with 7 kg of concentrates and in late lactation with 1 kg. The silages were harvested at different growth stages, resulting in different cell wall contents (G1 44.6%, G2 54.7%, G3 54.8%, G4 64.1% and G5 with 67.3% Neutral Detergent Fibre, NDF). With an increase in cell wall content, crude protein (CP) content decreased (21.3% CP for G1 to 11.2% CP for G5), and organic matter (OM) digestibility decreased ($P<0.001$) (76.2 for G1 to 63.7 for G5).

The degradation rates of dry matter (DM), NDF and acid detergent fibre (ADF) were measured using nylon bags incubated in the rumen. The soluble fraction ($f_S$, %) decreased, the undegradable fraction ($f_R$, %) increased, and the degradation rate of the non-soluble, degradable fraction ($k_d$, %/h) decreased with an increase in cell wall content of the silages. High correlation coefficients between the $k_d$'s of DM ($r=-0.91$, $P<0.001$), NDF ($r=-0.90$, $P<0.001$), ADF ($r=-0.93$, $P<0.001$) and the NDF content of the silages were found. The in-vivo DM digestibility was positively related to $f_S$ ($r=0.88$, $P<0.01$) and $k_d$ ($r=0.84$, $P<0.01$) and negatively to $f_R$ ($r=-0.97$, $P<0.001$).

Multiple regression analysis showed that the amount of concentrates consumed, the level of milk production, the nitrogen (N)/OM ratio of the silages and the $f_R$, could explain almost 70% of the variation in silage DM intake.

**Introduction.**

One of the barriers that prevents a substantial increase of the roughage proportion in dairy diets in early lactation is its limited ad libitum intake. Changing this situation seems only possible if our, as yet inadequate, understanding of the regulation of feed intake, especially the regulation of roughage intake, is improved.

It is known, that the volume of the feed and the capacity of the rumen to hold and turnover feed play an important role. When the demand for nutrients is high (early lactation), and the energy content of the feed is low, the capacity of the rumen to turnover feed can be the limiting factor (Weston, 1982). The maximum intake of a roughage then not only depends on the volume of the feed, but is also related to the rate of degradation in the rumen and the rate of passage of undigested feed particles to the lower gut. The latter may depend on the rate of particle size reduction through chewing during eating and rumination. If intake is not limited physically, the type and amount of digestion products may be controlling feed intake.

For high fibre silages maximum intake by lactating dairy cows may be limited by the capacity of the rumen (Mertens, 1987). Rumen dry matter (DM) content is reduced by passage of particles to the lower gut and digestion by rumen microbes (Ulyatt *et al.*, 1986; Kennedy & Murphy, 1988), although the latter has relatively little effect on reduction in rumen fill (Welch, 1982; Ulyatt *et al.*, 1986). However, microbial digestion weakens the cell wall structure, and thus facilitates particle breakdown during rumination (Chai *et al.*, 1984).

A dynamic model simulating fibre disappearance from the digestive tract from ruminants was developed by Mertens & Ely (1979). They concluded that maximum intake of digestible DM is influenced more by the proportion of fibre that is undegradable and the rate of particle passage to the lower gut than by the rate of fibre digestion. A 1% increase in the undegradable fraction ($f_R$) resulted in a 1% decrease in maximum digestible DM intake, and a 1% increase in digestion rate ($k_d$) resulted in a 0.6% increase in maximum digestible DM intake. Within species however, the $f_R$ and the $k_d$ are negatively correlated.

The objective of this study was to quantify the effect of the stage of maturity of grass silages on intake, digestibility, rumen fermentation pattern, degradation rate of the potentially digestible fractions, rumination activity, size and composition of rumen contents and passage rate from the rumen.

This paper presents the nylon bag degradation characteristics,

overall apparent digestibility and ad libitum intake of organic matter and cell wall components of five grass silages, harvested at different growth stages.

**Materials and methods.**

Experimental silages.

Five wilted grass silages (G1, G2, G3, G4 and G5) were harvested at different growth stages, resulting in different chemical compositions. The silages were fed ad libitum to six (Exps. 1, 2 and 3) or four (Exp. 4) dairy cows in combination with either 1 (low, L) or 7 (high, H) kg of concentrates, depending on the stage of lactation. The chemical composition of the silages and the concentrates, and the ingredients (g/kg) of the concentrates are given in Table 1.

Experimental designs.

Four experiments were conducted, whereby in each experiment two silages were fed ad libitum to dairy cows according to a change-over design. All cows were fitted with a rumen cannula, and, except for two animals in Exp. 4, also fitted with a T-cannula in the proximal duodenum. Each experiment consisted of two experimental periods of five weeks, preceded by adaptation periods of three weeks. An outline of the four experiments is given in Table 2.

In all four experiments, the silages were fed at 7.00, 15.00 and 23.00 h, in such quantities that approximately 10% of what was offered was not consumed. When the animals were fed 1 kg of concentrates, this was offered at 14.45h. In early lactation the 7 kg of concentrates was supplied in two equal portions at 6.45 and at 14.45h. The concentrates were always consumed completely. Left-overs from the silages were removed before feeding at 7.00 and at 15.00h. Left-overs were weighed, sampled and dried, so dry matter (DM) intake during the experimental periods was recorded per day. The left-overs were pooled per week and analysed for ash content, so that organic matter (OM) intake per cow per week

could be calculated. Water was available ad lib. The animals were milked twice a day and the milk yield per day was recorded.

The animals were weighed before and after each experimental period. The intake per kg body weight was calculated, based on the average live weights during the experimental periods.

The measurements done in the four experiments are presented in Tables 3A (Exps. 1, 2 and 3) and 3B (Exp. 4).

Table 1. Chemical composition of the five grass silages and the concentrate fed (mean of four experiments).

| | G1 | G2 | G3 | G4 | G5 | concentrates |
|---|---|---|---|---|---|---|
| DM | 59.4 | 54.3 | 60.8 | 38.7 | 55.0 | 88.1 |
| in DM | | | | | | |
| OM | 86.8 | 90.8 | 89.8 | 92.5 | 92.6 | 90.4 |
| CP | 21.3 | 19.6 | 20.9 | 11.9 | 11.2 | 18.2 |
| NDF | 44.6 | 54.7 | 54.8 | 64.1 | 67.3 | 28.6 |
| cellulose | 23.4 | 29.3 | 25.8 | 33.6 | 32.9 | 12.4 |
| hemicellulose | 19.1 | 22.0 | 26.1 | 25.9 | 26.9 | 14.5 |
| lignin | 2.1 | 3.4 | 2.9 | 4.6 | 7.5 | 1.7 |
| $NH_3$[1] | 4 | 6 | 2 | 10 | 5 | |
| harvesting date | 7-2 '85 | 5-28 '85 | 8-12 '86 | 6-23 '86 | 7-6 '87 | |

ingredients (g/kg) in the concentrate

| | |
|---|---|
| corn gluten feed | 250 |
| sugarbeet pulp | 200 |
| citrus pulp | 200 |
| cane molasses | 26 |
| soyabean meal solvent extracted | 107 |
| lineseed | 38 |
| wheat middlings | 138 |
| tallow | 18 |
| lime | 10 |
| NaCl | 8 |
| vitamin/mineral premix | 5 |

[1] $NH_3$-N as a % of total N

Table 2. Outline of the four experiments.

| | Exp.1 | Exp.2 | Exp.3 | Exp.4 |
|---|---|---|---|---|
| number of cows | 6 | 6 | 6 | 4 |
| concentrates (kg/day) | 1 | 7 | 1 | 7 |
| silage | | | | |
| G1 | x | x | | |
| G2 | x | x | | |
| G3 | | | x | x |
| G4 | | | x | |
| G5 | | | | x |

The degradation rates of the different components of the silages were measured by means of nylon bag incubations (Mehrez & Ørskov, 1977). Nylon bags (9 * 18 cm) were sewn with nylon thread, and needle holes were sealed with waterproof glue. Pore size of the bags was 41 $\mu$m (Nybolt, Switzerland). Fresh samples (circa 5 g dry matter) of the silages (chopped at approximately 6 mm) were weighed into the bags and the bags were closed and tied with a nylon string. Up to 23 bags were tied to a stainless steel ring of approximately 600 g. The ring was tied to the rumen cannula with a nylon cord of about 70 cm. Bags were incubated in the rumen for 0, 3, 5, 8, 16, 24, 48, 72 and 336 hours, the latter for determination of the undegradable fraction. The number of bags per incubation period and the incubation scheme are given in Table 4. After incubation the bags were washed twice with cold water in a domestic washing machine, shortly spinned and dried at 70 $^{0}$C. The airdry samples were pooled per incubation time per cow, ground at 1 mm and stored for further analysis. The 0 h incubation samples, as well as the 336 hour incubation samples were pooled per silage, so one soluble and one undegradable fraction was determined per silage. The samples were analysed for dry matter (DM), Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF).

Table 3A. Scheme of measurements done in experiments 1, 2 and 3.

| | cow number 1, 2, 3 | cow number 4, 5, 6 |
|---|---|---|
| week 1 | * rumen fermentation characteristics (pH, $NH_3$, VFA)<br>* rumination activity and rumen motility<br>* rumen turnover rates | * degradation rates by means of nylon bag incubations (Mehres & Ørskov, 1977)<br>* overall digestibility |
| week 2 | * degradation rates by means of nylon bag incubations (Mehres & Ørskov, 1977)<br>* overall digestibility | * rumen fermentation characteristics (pH, $NH_3$, VFA)<br>* rumination activity and rumen motility<br>* rumen turnover rates |
| week 3 | as week 1 | as week 1 |
| week 4 | as week 2 | as week 2 |
| week 5 | * rumen evacuations | * rumen evacuations |

Table 3B. Scheme of measurements done in experiment 4.

| | cow number 1, 2 | cow number 3, 4 |
|---|---|---|
| week 1 | * rumen turnover rates<br>* rumen fermentation characteristics (pH, $NH_3$, VFA)<br>* degradation rates by means of nylon bag incubations (Mehres & Ørskov, 1977) | * rumen turnover rates<br>* rumen fermentation characteristics (pH, $NH_3$, VFA)<br>* rumination activity and rumen motility |
| week 2 | * overall digestibility<br>* rumination activity and rumen motility | * overall digestibility<br>* degradation rates by means of nylon bag incubations (Mehres & Ørskov, 1977) |
| week 3 | as week 1 | as week 1 |
| week 4 | as week 2 | as week 2 |
| week 5 | * rumen evacuations | * rumen evacuations |

Table 4. Number of bags per incubation time and incubation scheme.

| Incubation time (h) | number of bags | In | Out |
|---|---|---|---|
| 3 | 6 | Fri 8.00 h | Fri 11.00 h |
| 5 | 7 | Fri 8.00 h | Fri 13.00 h |
| 8 | 8 | Tue 16.00 h | Tue 24.00 h |
| 16 | 9 | Thu 16.00 h | Fri 8.00 h |
| 24 | 15 | Mon 16.00 h | Tue 16.00 h |
| 48 | 15 | Tue 16.00 h | Thu 16.00 h |
| 72 | 18 | Fri 16.00 h | Mon 16.00 h |

DM, NDF, ADF and hemicellulose degradation curves were determined twice for each silage in each cow according to the model (Robinson *et al.*, 1986):

$$f_t = f_R + (1-f_S-f_R)*e^{-kd(t-t')}$$

in which:

$f_t$ = residue (%) at time t
$f_R$ = undegradable fraction (%), as derived from the 336 h incubation time
$f_S$ = soluble fraction (%), as determined by washing after 0 h incubation
$k_d$ = fractional rate of degradation (%/h) of the potentially degradable, but water insoluble fraction
$t'$ = lag-time (h)

The curves were fitted using the NLIN procedure of the SAS package (SAS, 1985).

To determine the overall digestibility of the different components, faeces were collected quantitatively from Monday 8.00 h till Thursday 8.00 h, weighed and sampled per two hours. A pooled sample per cow was preserved with formaldehyde and kept at $4^0$C till further analysis.

Chemical analysis.

The silages were analysed for DM, ash, crude protein (CP), ammonia fraction, NDF, ADF and Acid Detergent Lignin (ADL). Fresh faecal samples were analysed for DM and N content. Oven dried (70 $^0$ C) faecal samples were analysed for DM, ash, NDF, ADF and ADL. Dry matter was determined by drying to constant weight at 103 $^0$C, ash in an oven at 550 $^0$C and N by the Kjeldahl method with $K_2SO_4$ and HgO as catalysts. NDF, ADF and ADL were measured according to Goering & van Soest (1970). Hemicellulose was calculated as NDF-ADF, and cellulose as ADF-ADL. To determine the ammonia fraction in the silages, 450 ml water was added to 50 g of silage. After standing one night at 4$^0$C, the ammonia concentration in the filtrate was determined according to the method described by Scheiner (1976).

Statistics.

The data were analysed statistically for each experiment using the manova and anova procedures of the spsspc+ statistical package (SPSS Inc, 1988).

Because there was no significant effect of time within the experimental periods, the mean values of weeks 1 and 3, respectively weeks 2 and 4 were used in the statistical analysis. The order in which the silages were fed to the animals was different for the two groups of cows in each experiment. To check whether the silage fed in the first experimental period influenced the results of the second experimental period, first the manova procedure was used with the animals nested within order of silages fed. Because no such effect was found for any of the dependent variables, the anova procedure was used with cow, experimental period and silage fed as factors, according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + S_k + e_{ijkl}$$

$Y_{ijkl}$ = dependent variable
$\mu$ = overall mean
$C_i$ = cow effect (i=1-6)
$P_j$ = effect of the experimental period (j=1-2)
$S_k$ = effect of the silage fed (k=1-2)
$e_{ijkl}$ = error

Correlations among DM intake (g/kg Body Weight), silage composition, $k_d$, $f_S$, $f_R$, the amount of concentrates consumed and the level of milk production were determined by the Pearson correlation technique using the SPSSPC+ statistical package (SPSS Inc, 1988).

Subsequently stepwise multiple regression analysis (SPSS Inc, 1988) was used to identify variables by which dry matter intake from silages was influenced. Variables included in this analysis were the same as used in the Pearson correlation matrix.

**Results.**

Composition of the silages.

Silage composition changed with maturity (Table 1), particularly with regard to the cell wall, crude protein and lignin contents. Maturing increased cell wall and lignin contents, but decreased crude protein content. Within cell walls, lignin content increased with an increased cell wall content ($R^2$=0.73), but the ratio between cellulose and hemicellulose was not affected. The ratio cellulose to hemicellulose was 1.22, 1.33, 0.99, 1.30 and 1.22 for respectively G1, G2, G3, G4 and G5.

In silages G2 and G3, with the same NDF content, the lignin content was lower for G3 than for G2 (2.9 vs 3.4), and the CP content was slightly higher (20.9 vs 19.6). In silages G4 and G5, the difference in NDF content was almost completely attributable to the difference in lignin content.

Degradation characteristics.

The soluble ($f_S$) and undegradable ($f_R$) fractions of the different components of the five silages are given in Table 5. With an increase in cell wall content of the silages, the $f_S$-DM decreased linearly ($r$=-0.94, $P$<0.001), whereas the $f_R$-DM increased linearly ($r$=0.95, $P$<0.001) (Fig. 1). The soluble part of the cell wall fraction was for all silages negligible, and the $f_R$-NDF increased with cell wall content.

Table 5. Soluble ($f_S$) and indigestible $f_R$) fractions of the different components of the five silages as determined by washing after 0 and 336 h incubations, respectively.

| | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|
| dry matter | | | | | |
| $f_S$ | 35.6 | 28.6 | 26.6 | 25.0 | 20.6 |
| $f_R$ | 8.8 | 14.4 | 13.9 | 20.4 | 27.2 |
| neutral detergent fibre | | | | | |
| $f_S$ | 0.8 | 5.3 | 0.0 | 0.0 | 1.6 |
| $f_R$ | 10.6 | 16.6 | 16.0 | 24.4 | 29.7 |
| acid detergent fibre | | | | | |
| $f_S$ | 1.6 | 4.6 | 0.0 | 0.0 | 1.7 |
| $f_R$ | 10.7 | 16.0 | 19.4 | 24.2 | 30.2 |

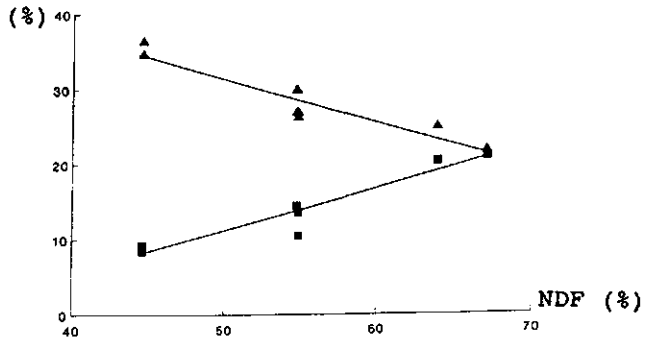


Figure 1. The $f_S$-DM (▲) and the $f_R$-DM (■) as related to the NDF fraction of the silages.

The fractional degradation rates ($k_d$) and the lag-times (t') are given in Table 6. In general, the degradation rates were decreasing with an increase in cell wall content. Differences in $k_d$ between silages were comparable for the various components, and the $k_d$'s of the various fractions were highly correlated with the cell wall content of the silages ($r$=-0.91 for DM ($P$<0.001), $r$=-0.90 for NDF ($P$<0.001) and $r$=-0.93 for the ADF fraction ($P$<0.001)). The $k_d$ was for all silages higher for the ADF fraction than for the NDF fraction. Hence, for the hemicellulose fraction the $k_d$ was lower than for the ADF fraction (Table 6). The t' hardly showed any significant differences.

Table 6. Degradation rate ($k_d$, %/h) and lag-time (t', h) of dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose (NDF-ADF) of the five silages.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| DM | | | | | | | | | | | | |
| $k_d$ | 5.91 | 4.15 | 0.18 ** | 4.88 | 4.10 | 0.13 * | 6.74 | 4.39 | 0.17 ** | 4.14 | 2.91 | 0.10 * |
| t' | 2.98 | 2.89 | 0.12 NS | 3.33 | 3.70 | 0.10 NS | 1.56 | 2.50 | 0.19 NS | 1.57 | 1.36 | 0.25 NS |
| NDF | | | | | | | | | | | | |
| $k_d$ | 5.91 | 3.75 | 0.23 ** | 4.60 | 3.90 | 0.12 * | 6.35 | 3.99 | 0.16 ** | 3.79 | 2.71 | 0.10 * |
| t' | 2.85 | 4.15 | 0.20 * | 3.01 | 3.16 | 0.13 NS | 2.48 | 4.60 | 0.12 ** | 1.31 | 2.05 | 0.12 NS |
| ADF | | | | | | | | | | | | |
| $k_d$ | 6.74 | 4.51 | 0.27 * | 4.70 | 4.11 | 0.12 NS | 6.79 | 4.19 | 0.19 ** | 4.17 | 2.81 | 0.09 * |
| t' | 2.53 | 2.29 | 0.19 NS | 3.62 | 3.32 | 0.14 NS | 4.12 | 4.86 | 0.17 NS | 3.55 | 2.97 | 0.23 NS |
| NDF-ADF | | | | | | | | | | | | |
| $k_d$ | 5.47 | 3.20 | 0.20 ** | 4.56 | 3.59 | 0.13 * | 6.36 | 3.89 | 0.14 ** | 3.69 | 2.63 | 0.10 * |
| t' | 2.56 | 5.54 | 0.17 ** | 2.52 | 2.78 | 0.11 NS | 0.83 | 4.26 | 0.10 *** | 0.14 | 1.11 | 0.22 NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

Table 7. Digestibility coefficients of the different chemical components of the rations in the four experiments.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| dm | 73.0 | 68.2 | 0.22 *** | 68.6 | 62.5 | 0.14 *** | 72.0 | 68.8 | 0.15 *** | 69.0 | 61.5 | 0.26 ** |
| om | 76.2 | 70.5 | 0.19 *** | 70.4 | 64.0 | 0.08 *** | 75.2 | 70.9 | 0.18 *** | 71.5 | 63.7 | 0.34 ** |
| cp | 69.7 | 68.4 | 0.21 * | 68.0 | 59.3 | 0.21 *** | 67.5 | 66.4 | 0.19 * | 67.2 | 61.6 | 0.20 ** |
| NDF | 78.0 | 70.9 | 0.17 *** | 73.8 | 62.9 | 0.05 *** | 74.7 | 66.4 | 0.19 *** | 71.4 | 56.8 | 0.73 ** |
| cellulose | 84.6 | 78.6 | 0.19 *** | 74.5 | 69.3 | 0.07 *** | 81.4 | 74.4 | 0.22 *** | 75.7 | 65.8 | 0.90 * |
| hemi-cellulose | 79.0 | 72.3 | 0.28 *** | 80.2 | 65.8 | 0.17 *** | 74.3 | 65.5 | 0.26 *** | 76.1 | 59.9 | 0.74 ** |

*** P<0.001, ** P<0.01, * P<0.05

Digestibility.

The overall digestibility coefficients, based on total faecal excretion are presented in Table 7.

Organic matter digestibility was higher for silages with a lower cell wall content. Cell wall digestibility decreased with an increase in cell wall content, except for G3, which had the same cell wall content as G2 (55% NDF), but a slightly better NDF digestibility. Cellulose digestibility was in all but silage G3 higher than hemicellulose digestibility. Apparent crude protein digestibility was in all four experiments highest for the silage with the lowest cell wall content ($P<0.05$ in Exps. 1 and 2; $P<0.001$ in Exp. 3 and $P<0.01$ in Exp. 4).

Intake.

The daily OM, N and NDF intake of the silages (kg as well as g/kg BW), daily milk yields (kg) and the weight of the cows are given in Table 8.

The OM intakes of the grass silages with the lower cell wall contents were significantly higher in Exps. 3 ($P<0.001$) and 4 ($P<0.05$), but not in Exps. 1 and 2. The intake of the silages was lower if supplemented with 7 kg of concentrates as compared to 1 kg of concentrates. For Exps. 1 and 2 the NDF intake (kg/day) was higher ($P<0.05$) for the silages with the higher NDF contents.

NDF intake of the silages (g/kg BW) increased and, except for G3, OM intake (g/kg BW) decreased with an increase in cell wall content.

Milk yields were only significantly lower for diets containing the least digestible silages if fed in combination with 1 kg of concentrates in late lactation ($P<0.05$ in Exp. 1, and $P<0.001$ in Exp. 3).

No significant correlations between silage DM intake (g/kg BW) and $f_s$, $f_R$ or $k_d$ were found (Table 9). Silage DM intake was negatively correlated with the ADF and ADL content of the silages, and positively with the N/OM ratio of the silages. The highest correlation was found between the level of concentrates consumed (g/kg BW) and silage DM intake ($r=-0.52$, $P<0.001$).

Regression analysis based on the nitrogen content in the organic

Table 8. Daily intake of OM, N and NDF (kg and g/kg BW), and daily intake of cellulose and hemicellulose (kg) for the grass silages and for the total diets, daily milk yield (kg), fat corrected milk (FCM, kg), and mean live weight of the cows (kg).

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | | | Exp. 3 | | | Exp. 2 | | | Exp. 4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| Intake silage | | | | | | | | | | | | |
| OM (kg) | 10.7 | 10.3 | 0.17 | 12.8 | 9.9 | 0.10 *** | 9.8 | 9.5 | 0.18 | 11.0 | 9.1 | 0.22 * |
| N (kg) | 0.42 | 0.35 | 0.01 ** | 0.47 | 0.20 | 0.01 *** | 0.39 | 0.33 | 0.01 * | 0.41 | 0.17 | 0.01 ** |
| NDF (kg) | 5.4 | 6.2 | 0.10 * | 7.8 | 6.9 | 0.07 ** | 5.1 | 5.7 | 0.10 * | 6.7 | 6.6 | 0.15 |
| cellulose (kg) | 2.8 | 3.3 | 0.05 ** | 3.7 | 3.6 | 0.03 | 2.7 | 3.1 | 0.06 * | 3.2 | 3.2 | 0.07 |
| hemicellulose (kg) | 2.3 | 2.5 | 0.04 | 3.7 | 2.8 | 0.03 *** | 2.2 | 2.3 | 0.04 | 3.2 | 2.7 | 0.06 |
| OM (g/kg BW) | 19.5 | 18.8 | 0.34 | 23.0 | 18.6 | 0.25 ** | 17.4 | 16.6 | 0.28 | 19.0 | 15.9 | 0.30 * |
| N (g/kg BW) | 0.76 | 0.64 | 0.01 ** | 0.85 | 0.38 | 0.01 *** | 0.68 | 0.58 | 0.01 * | 0.71 | 0.30 | 0.01 ** |
| NDF (g/kg BW) | 9.8 | 11.3 | 0.20 * | 14.0 | 13.0 | 0.16 * | 9.0 | 9.9 | 0.15 * | 11.6 | 11.7 | 0.20 |
| Total intake | | | | | | | | | | | | |
| OM (kg) | 11.5 | 11.1 | 0.17 | 13.6 | 10.7 | 0.10 *** | 15.5 | 15.1 | 0.18 | 16.5 | 14.6 | 0.22 * |
| N (kg) | 0.44 | 0.37 | 0.01 ** | 0.50 | 0.23 | 0.01 *** | 0.57 | 0.52 | 0.01 * | 0.58 | 0.35 | 0.01 ** |
| NDF (kg) | 5.6 | 6.5 | 0.10 * | 8.0 | 7.1 | 0.07 ** | 6.8 | 7.4 | 0.10 * | 8.6 | 8.5 | 0.15 |
| cellulose (kg) | 2.9 | 3.4 | 0.05 ** | 3.8 | 3.7 | 0.03 | 3.5 | 3.9 | 0.06 * | 4.0 | 4.1 | 0.07 |
| hemicellulose (kg) | 2.4 | 2.6 | 0.04 | 3.9 | 2.9 | 0.03 *** | 3.0 | 3.1 | 0.04 | 4.1 | 3.6 | 0.06 |
| OM (g/kg BW) | 21.0 | 20.0 | 0.34 | 24.4 | 20.1 | 0.25 ** | 27.3 | 26.4 | 0.29 | 28.5 | 25.6 | 0.25 * |
| N (g/kg BW) | 0.81 | 0.68 | 0.01 ** | 0.90 | 0.43 | 0.01 *** | 1.02 | 0.91 | 0.01 * | 1.01 | 0.61 | 0.01 ** |
| NDF (g/kg BW) | 10.3 | 11.8 | 0.20 * | 14.4 | 13.4 | 0.16 * | 11.9 | 12.8 | 0.15 * | 14.8 | 14.9 | 0.18 |
| milk yield (kg) | 11.0 | 9.3 | 0.23 * | 16.8 | 11.2 | 0.21 *** | 25.0 | 24.1 | 0.31 | 22.6 | 18.6 | 0.49 |
| FCM (kg) | 12.9 | 10.9 | 0.26 * | 19.2 | 12.1 | 0.13 *** | 26.4 | 24.8 | 0.64 | 25.6 | 20.7 | 0.77 |
| weight cows (kg) | 546 | 550 | 1.99 | 560 | 534 | 2.29 ** | 569 | 576 | 2.19 | 579 | 570 | 2.00 |

*** P<0.001, ** P<0.01, * P<0.05

Table 9. Coefficients of correlation within and between dry matter degradation characteristics, the cell wall constituents of the silages, concentrates consumed (C, g/kg BW), milk production (FCM, g/kg MBW) and silage DM intake (g/kg BW).

| | silage composition silages | | | | degradation characteristics | | | C | FCM |
|---|---|---|---|---|---|---|---|---|---|
| | DM intake | NDF% | ADF% | ADL% | N/OM | $f_S$-DM | $f_R$-DM | $k_d$-DM | | |
| intake | | | | | | | | | | |
| DM | -- | -0.19 | -0.37 * | -0.38 * | 0.34 * | 0.07 | -0.29 | 0.15 | -0.52 ** | -0.08 |
| cell wall contents | | | | | | | | | | |
| NDF % | | - | 0.95 ** | 0.89 ** | -0.90 ** | -0.96 ** | 0.97 ** | -0.78 ** | -0.03 | -0.18 |
| ADF % | | | - | 0.91 ** | -0.95 ** | -0.85 ** | 0.95 ** | -0.74 ** | 0.00 | -0.25 |
| ADL % | | | | - | -0.90 ** | -0.84 ** | 0.98 ** | -0.70 ** | 0.16 | -0.11 |
| N/OM | | | | | - | 0.76 ** | -0.93 ** | 0.61 ** | 0.04 | 0.26 |
| $f_S$-DM | | | | | | - | -0.92 ** | 0.80 ** | -0.02 | 0.08 |
| $f_R$-DM | | | | | | | - | -0.75 ** | 0.07 | -0.14 |
| $k_d$-DM | | | | | | | | - | 0.01 | 0.19 |
| concentrates g/kg BW | | | | | | | | | - | 0.82 ** |
| FCM/kg MBW | | | | | | | | | | - |

** P<0.001, * P<0.01

matter of the silages (N/OM), the residu after long term nylon bag incubation ($f_R$), or the level of concentrates consumed (g/kg BW) alone, showed that each of these factors explained a significant part of the variation in silage DM intake (Table 10). Multiple regression analysis showed that in combination, the N/OM ratio, the $f_R$, the amount of concentrates consumed (g/kg BW), and the level of milk production (FCM/kg MBW), could explain 68% of the total variation in dry matter intake.

Table 10. Stepwise multiple regression analysis on factors responsible for variation in silage intake (g/kg BW).

| Constant | FCM (g/kg MBW) | Concentrates (g/kg BW) | N/OM (%) | $f_R$ | $R^2$ |
|---|---|---|---|---|---|
| 21.0 | -0.004 NS | | | | 0.01 |
| 22.2 | | -0.32 *** | | | 0.27 |
| 16.1 | | | 1.30 * | | 0.12 |
| 22.6 | | | | -0.15 NS | 0.08 |
| 17.7 | | -0.32 *** | 1.38 ** | | 0.40 |
| 16.5 | 0.054 *** | -0.83 *** | | | 0.64 |
| 4.9 | | -0.37 *** | 3.75 ** | 0.35 NS | 0.45 |
| 5.3 | 0.047 *** | -0.81 *** | 2.44 * | 0.28 * | 0.68 |

NS non significant, *** $P<0.001$, ** $P<0.01$, * $P<0.05$

**Discussion.**

For all silages, $NH_3$-N as a fraction of total N was low. The $NH_3$ fraction of silage G4 was highest, but because of the low CP content, absolute amount of $NH_3$ was still low.
The soluble non protein organic matter was 14.8, 12.0, 11.5, 13.2 and 9.0 for silages G1, G2, G3, G4 and G5, respectively. This fraction is likely to be rapidly and fully degraded in the rumen, and can therefore give a fast decline in pH. No relation between this fraction and DM content of the silages was found, but this fraction was negatively correlated ($r$ = -0.73) to the NDF content of the silages.
The CP, cellulose and lignin content of G3, would suggest a NDF

content between that of silages G1 and G2. Because of the high hemicellulose content however, the total cell wall content was the same as for silage G2, but the ratio cellulose to hemicellulose was lower for G3. Jones *et al.* (1988) reported a ratio of 0.58 in Bermuda grass hay and of 1.07 in Orchard grass hay, indicating that big differences between grasses may occur. Bailey (1973) also reports big differences in the ratio cellulose to hemicellulose between grasses, but also within grasses between growth stages and growing seasons. Differences due to different growth stages and/or differences in growing seasons may be due to changes in leaf to stem ratios. The silages in these experiments were harvested in different seasons and the weather conditions during growth varied as well.
Based on the chemical parameters CP, lignin and cellulose, however, despite the slightly higher NDF content of silage G3, the silages can be ranked in the order of G1, G3, G2, G4 and G5.

When the plant matures, cell wall content increases and lignin content of the cell walls also increases. The lignin is bound to the cellulose-hemicellulose fraction of the cell wall, and acts as a barrier for the enzymatic degradation by rumen microorganisms, because microbes can only ferment the cell walls if they come into direct contact with them (Engels, 1987). Therefore, the degradation rate of the cell wall fraction decreases with maturity.

Negative correlations between the rumen undegradable fraction and the rate of degradation of the digestible fraction for DM as well as for NDF were found ($r$ = -0.87 and -0.84, respectively). With respect to the composition, contents of cell wall components (NDF, ADF, lignin) were negatively correlated with $f_s$-DM and $k_d$-DM, but positively with $f_R$-DM. This can be explained as a result of maturation and lignification. As far as the lignin content concerns, this relation appears to be restricted to within species. In comparing grasses, legumes and clover, Nocek & Grant (1987) as well as Seoane (1982) also report negative correlations between $f_s$ and the NDF and ADF contents, but not between $f_s$ and the lignin content of the feeds.

The level of concentrates fed had a variable effect on nylon bag degradation rates of the silages. The increase in degradation rate with an increase in concentrate level for silages G1 and G2 may have resulted from a better energy supply for the rumen microorganisms. Robinson *et al.* (1986), in their experiments, fed the same basal diet at low and high intake level. They found no effect of level of intake on degradation rate, but the lag time was higher ($P<0.05$) at the low intake level. In our experiments lag time for DM disappearance was higher for the low intake level, but lag times for the other components showed no consistent relation with level of intake.

Cell walls or NDF can be looked upon as a mixture of cellulose, hemicellulose and lignin, of which particularly hemicellulose is encrustrated with lignin (Van Soest, 1982). This explains why ADF, of which the degradable part is mainly cellulose, is degraded at a faster rate than NDF. The difference in rate of degradation between cellulose and hemicellulose is also reflected in the apparent digestibility, except with silage G3.

It is also of interest to note that the undegradable proportion of hemicellulose relates fairly well to the lignin/hemicellulose ratio.

OM digestibility and NDF digestibility decrease within forages with stage of growth (Panditharatne, 1988) and with an increase in cell wall content and lignification (Robles, 1981; Reid *et al.*, 1988; Tamminga & Van Vuuren, 1988; Van Soest, 1982). In our experiments, a linear relationship between NDF content and OM digestibility ($r=-0.99$) as well as NDF digestibility ($r=-0.96$) was found.

Based on undegradable cell wall contents of different concentrate ingredients as given by Tamminga *et al.* (1990), the $f_{R\text{-}NDF}$ of the concentrates was estimated to be approximately 15%. Total cell wall intake can be divided in rumen undegradable and rumen degradable material. The fraction of rumen degradable NDF, excreted undigested with the faeces was calculated and increased with an increase in cell wall content and with an increase in concentrate level (G1L, 12.6; G3L, 12.2; G2L, 15.0; G4L, 17.2;

G1H, 15.4; G3H, 15.2; G2H, 20.7 and G5H, 22.7). The fraction of the digestible cell walls that is digested in the large intestines increases with a decrease in apparent cell wall digestibility (Ulyatt *et al.*, 1975) and thus with an increase in cell wall content. This seems, however, not enough to compensate for the lower rumen degradability due to a lower degradation rate of the cell wall fraction and a higher rate of passage from the rumen (Bosch *et al.*, submitted). Concentrate particles have a higher rate of passage from the rumen than roughage particles (Owens & Goetsch, 1986). The probably higher rate of degradation (the individual ingredients have a higher $k_{d\text{-}NDF}$ (Tamminga *et al.*, 1990) than the silage cell wall fractions), does not seem to compensate for the shorter rumen retention time, resulting in a bigger degradable NDF fraction that is excreted undigested for the high concentrate diets.

The apparent crude protein digestibility decreased with stage of maturity ($P<0.05$). Two aspects are important to explain this. 1. The true protein digestibility decreases because of an increase of protein incorporated in the cell wall fraction with increasing cell wall content (Krishnamoorthy *et al.*, 1982; Van Soest, 1982). 2. The quantity of endogenous protein increases with increasing cell wall content of the diet as induced by higher DM passages in the intestines (Van Bruchem *et al.*, 1989), resulting in a higher faecal excretion.

A linear negative relationship ($r=-0.58$) between dry matter intake and dietary NDF concentrations was reported by Hoover (1986), in a review of studies with lactating dairy cows. In our experiments a non significant decrease in DM intake (g/kg BW) was found, with an increase in NDF content, although intake of silage G3 was higher as would be expected. Intake of G3 was significantly ($P<0.001$) higher as compared to the other four silages, resulting in an even higher NDF intake as compared to silages G4 and G5. For silages G1, G2, G4 and G5 total NDF intake (g/kg BW) significantly increased ($P<0.01$) with an increase in NDF content.

The difference in intake of the silages in combination with 7 kg of concentrates as compared to 1 kg, was biggest for silage G3. This is in agreement with the positive relation between ingestibility of a forage (voluntary dry matter intake as a sole feed) and substitution rate of forages by concentrates as reported by Jarrige *et al.* (1986).

About half of the variation in silage DM intake can be explained by the amount of concentrates consumed and the level of milk production. Thus, physiological status of the animal appears to be one of the main factors controlling feed intake. After correction for concentrate intake and milk production, the N/OM ratio in the silages and the $f_R$ of the silages, together, explain almost half of the variation in DM intake.
Our results agree with the results of Mertens & Ely (1979), who concluded from their simulation model, that the digestible DM intake is influenced more by the proportion of undegradable fibre than by the rate of degradation. In our experiments, it is clear that ad libitum silage DM intake is influenced by silage characteristics. It is, however, not yet possible to identify a restricted number of components, which can be determined accurately, and can be used to predict silage intake at a given concentrate level and an expected milk production.

According to Forbes (1988), who compared different equations to predict voluntary intake by dairy cows, most equations found in literature can only be used under the same circumstances as under which the data from which they were calculated were collected. He concluded that the simple equation:
Total DM intake = 0.025(Body Weight, kg) + 0.1(Milk Yield, kg/d), was not significantly worse than more complex ones. For our experiments, this equation as well as most other equations given by Forbes (1988), overestimate DM intake. It seems correct that equations to predict voluntary intake can only be used under, at least comparable, circumstances. It is therefore useful to develop these equations per type of diet and type of animal.

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Chapter III

# Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents and ruminal passage rates.

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**Abstract.**

In four change-over experiments, wilted grass silages, differing in cell wall content (Neutral Detergent Fiber (NDF) content ranging from 44.6% to 67.3% of dry matter (DM)), were fed ad libitum to dairy cows in early and late lactation.

The influence of cell wall content of the grass silages on rumen contents and on fractional passage rates of particulate ($k_p$, %/h) and liquid ($k_l$, %/h) phases, was determined using Cr-NDF and Tritium (T) labelled hay as particulate markers and Co-EDTA as a soluble marker.

Rumen contents (total as well as DM, kg) increased with an increasing proportion of concentrates in the diet, but did not show any significant relation with silage cell wall content.

The $k_p$ increased with an increase in NDF content of the diet, and with an increase in intake level, whereas the $k_l$ was not affected by diet composition.

**Introduction.**

For lactating dairy cows fed rations with a high roughage proportion, intake can be limited by the capacity of the rumen (Mertens, 1987). Intake then depends on rumen fill and its reduction by microbial digestion of the feed, particle size reduction through rumination and rate of particle passage to the lower gut (Ulyatt *et al.*, 1986; Kennedy & Murphy, 1988).

Since microbial degradation hardly influences particle size, rate of passage of small particles to the lower gut is the main factor reducing rumen fill, and therefore considered an important factor in feed intake regulation (Van Soest, 1982).

The effect of roughage characteristics, like chemical composition and degradation characteristics, on their behaviour in the rumen is not well documented.

In four experiments, in which the effect of cell wall content of grass silages on intake and digestion in dairy cattle is investigated (Bosch *et al.*, submitted), rumen contents were

determined. In addition, the rate of passage of the fluid and particulate phases to the lower gut were measured using soluble and particulate markers.

**Materials and methods.**

Five wilted grass silages (G1 to G5), differing in cell wall content were fed ad libitum to dairy cows, supplemented in early and late lactation with 7 and 1 kg of concentrates, respectively. In each of the four change-over experiments, two silages were fed to six (Exps. 1, 2 and 3) or four (Exp. 4) rumen fistulated dairy cows. In Exps. 1 and 3 the cows were in late lactation, so 1 kg of concentrates was included in the diet, whereas in Exps. 2 and 4, when the cows were in early lactation, 7 kg of concentrates was added to the diet. Each experiment consisted of two experimental periods of five weeks, preceded by adaptation periods of three weeks. Silage was offered three times a day, at 7.00, 15.00 and 23.00 h. The concentrates were fed at 14.45 h (1 kg) or at 6.45 and 14.45 h (two portions of 3.5 kg each). The experimental designs were as described in more detail by Bosch *et al.* (submitted). The composition of the five silages, their intake and the apparent digestibility of the total diet are given in Table 1.

Rumen evacuations were done in the fifth week of each experimental period, at approximately 2 and 4 h after the end of the morning meal, which is assumed to be 2 h after feeding time, in Exps. 1, 2 and 3, and at approximately 0, ½, 3, 4½ and 6 h after the end of the morning meal in Exp. 4.

Rumen contents were weighed, sampled and returned into the rumen. This procedure took about 25-30 minutes per cow. Rumen samples (1% of rumen contents) were dried at 70°C, ground and stored till further chemical analysis.

Dried rumen samples were analysed for dry matter (DM), ash, crude protein (CP, N (nitrogen) * 6.25), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). DM was determined by drying to constant weight at 103°C,

Table 1. Chemical composition of the silages (dry matter (DM), organic matter (OM) and cell wall content (NDF)), DM and NDF intake and apparent digestibility of the total diets in the four experiments.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1 | G2 | SEM | G3 | G4 | SEM | G1 | G2 | SEM | G3 | G5 | SEM |
| chemical composition | | | | | | | | | | | | |
| DM | 59.4 | 54.3 | | 60.8 | 38.7 | | 59.4 | 54.3 | | 60.8 | 55.0 | |
| OM | 86.8 | 90.8 | | 89.8 | 92.5 | | 86.8 | 90.8 | | 89.8 | 92.6 | |
| NDF | 44.6 | 54.7 | | 54.8 | 64.1 | | 44.6 | 54.7 | | 54.8 | 67.3 | |
| silage intake | | | | | | | | | | | | |
| DM | 12.0 | 11.1 | 0.17 NS | 14.1 | 10.7 | 0.10 *** | 10.8 | 10.5 | 0.18 NS | 12.1 | 9.9 | 0.23 * |
| NDF | 5.4 | 6.2 | 0.10 * | 7.8 | 6.9 | 0.07 ** | 5.1 | 5.7 | 0.10 * | 6.7 | 6.6 | 0.15 NS |
| total intake | | | | | | | | | | | | |
| DM | 12.8 | 12.0 | 0.17 NS | 15.0 | 11.5 | 0.10 *** | 17.0 | 16.7 | 0.18 NS | 18.2 | 16.0 | 0.23 * |
| NDF | 5.6 | 6.5 | 0.10 * | 8.0 | 7.1 | 0.07 ** | 6.8 | 7.4 | 0.10 * | 8.5 | 8.6 | 0.15 |
| apparent digestibility | | | | | | | | | | | | |
| DM | 73.0 | 68.2 | 0.22 *** | 68.6 | 62.5 | 0.14 *** | 72.0 | 68.8 | 0.15 *** | 69.0 | 61.5 | 0.26 *** |
| NDF | 78.0 | 70.9 | 0.17 *** | 73.8 | 62.9 | 0.05 *** | 74.7 | 66.4 | 0.19 *** | 71.4 | 56.8 | 0.73 ** |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

ash in an oven at 550°C and N by the Kjeldahl method with $K_2SO_4$ and HgO as catalysts. NDF, ADF and ADL were measured according to Goering & Van Soest (1970). Hemicellulose was calculated as NDF minus ADF and cellulose as ADF minus ADL.

Per cow per diet, fractional outflow rates of the rumen particulate and fluid phases were measured twice for every diet, using Cr mordanted Neutral Detergent Fibre (Cr-NDF) and Co-Ethylene Diamino Tetra Acetate (Co-EDTA) as markers (Uden *et al.*, 1980). In addition to Cr-NDF, tritium (T) labeled hay was used as a marker for the particulate phase in Exp. 1 for all six cows and in Exps. 2 and 3 for three of the cows. The T-labeled hay was obtained by spraying young growing grass at regular intervals with tritiated water (THO), as described by Van den Hoek *et al.* (1985).

On Monday (6.00 a.m.) 100 g Cr-NDF (ca. 5% Cr, 0.2-1 mm), 30 g Co-EDTA (ca. 15% Co) and 40 g T-labeled hay (10-20 mm, 2000 pCi/g), the latter only in the previously indicated experiments, were administered into the ventral rumen sac.

Samples of rumen fluid were taken every two hours between Monday 9.00 a.m. and Tuesday 7.00 a.m.. Faeces were collected from Monday 10.00 till Tuesday 24.00 h and sampled per 2 hours, and on Wednesday and Thursday from 6.00 till 22.00 h and sampled per 4 hours. Co concentrations in the rumen fluid were determined using the standard addition method, with an atomic absorption spectrophotometer (Perkin Elmer 360), at a wavelenght of 251.0 nm. Faeces were dried (70°C), ground (1 mm) and analysed for Cr and Co by atomic absorption using the concentration method after wet destruction at wavelenghts of 357.9 and 251.0 nm, respectively.

Tritium activity in the dried faecal samples was determined by liquid scintillation counting in a Packard 1900 CA tri-carb liquid scintillation analyzer after converting the organically bound tritium into THO by combustion in a Packard Sample Oxidizer, Tricarb 306 (Van den Hoek *et al.*, 1983).

Fractional passage rate from the rumen of the liquid phase ($k_l$, %/h) was calculated from the logarithmic decline in Co concentration in the rumen fluid. Fractional passage rate of the

Table 2. Average determined rumen pools (total, dry matter (DM), organic matter (OM) and crude protein (CP), kg), rumen content (g DM/kg body weight (BW)), rumen content (DM) as a fraction of DM intake (DMR/DMI) and the total clearance rate of rumen DM ($k_c$, %/h) in the four experiments.

| | late lactation | | | | | | | early lactation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | | Exp.3 | | | Exp.2 | | | | Exp.4 | | |
| | G1 | G2 | SEM | | G3 | G4 | SEM | G1 | G2 | SEM | | G3 | G5 | SEM | |
| total | 88.9 | 89.7 | 0.34 | NS | 93.9 | 82.3 | 1.81 | NS | 91.2 | 101.4 | 1.98 | NS | 94.8 | 104.7 | 3.76 | NS |
| DM | 8.9 | 9.3 | 0.28 | NS | 11.1 | 8.9 | 0.22 | ** | 10.3 | 12.0 | 0.19 | * | 12.2 | 12.6 | 0.40 | NS |
| OM | 7.7 | 8.3 | 0.25 | NS | 9.9 | 8.0 | 0.19 | ** | 9.0 | 10.8 | 0.17 | ** | 10.9 | 11.5 | 0.37 | NS |
| CP | 2.4 | 1.8 | 0.06 | * | 2.7 | 1.2 | 0.05 | *** | 2.7 | 2.5 | 0.03 | NS | 2.9 | 1.8 | 0.06 | * |
| rumen content gDM/kgBW | 16.2 | 16.9 | 0.50 | NS | 19.9 | 16.8 | 0.29 | ** | 18.1 | 20.7 | 0.24 | ** | 21.0 | 22.1 | 1.79 | NS |
| DMR/DMI | 0.69 | 0.77 | 0.01 | * | 0.74 | 0.77 | 0.02 | NS | 0.60 | 0.71 | 0.01 | ** | 0.69 | 0.76 | 0.03 | NS |
| $k_c$ | 6.05 | 5.55 | 0.08 | * | 5.62 | 5.43 | 0.10 | NS | 6.96 | 5.89 | 0.05 | *** | 6.16 | 5.50 | 0.32 | NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

particulate phase from the rumen ($k_p$, %/h) as well as $k_l$ were calculated from the logarithmic decline in marker concentration (Cr, T and Co) in the faeces.

Total daily rumen clearance rate ($k_c$) was calculated from the ratio DM intake/average rumen DM pool size. From this, the hourly rate (%/h) was calculated.

Data were analysed statistically using the Anova procedure of the spsspc+ statistical package (SPSS Inc., 1988) as described by Bosch *et al.*, (submitted).

**Results.**

Average total rumen contents, rumen DM, OM and CP pools (kg), g DM in the rumen per kg body weight (g DM/kg BW) and the total clearance rate of rumen DM ($k_c$, %/h), calculated as DM intake/rumen DM content/24h, are given in Table 2. Rumen contents, total as well as DM, OM and CP pools were higher for the high concentrate level in early lactation (Exps. 2 and 4) than for the low concentrate level in late lactation (Exps. 1 and 3). No clear relation between rumen DM, OM or CP content (kg) and cell wall content of the silages was found. Rumen contents (g DM/kg BW) were higher for the high concentrate level in early lactation. DMR/DMI was lower when the silages were supplemented with 7 kg of concentrates than when supplemented with 1 kg, and increased with stage of maturity of the silages, up to a maximum of approximately 0.77. Thus, the $k_c$, which is the sum of disappearance due to degradation and disappearance due to passage to the omasum, decreased with stage of maturity. At the high concentrate level, $k_c$ was higher than at the low concentrate level.

Average rumen NDF, cellulose, hemicellulose and lignin contents (kg) are given in Table 3. Rumen cell wall contents increased with cell wall content of the silages. As for DM, OM and CP, rumen cell wall contents were higher for the high concentrate level than for the low concentrate level.

Differences in $k_l$ between diets, determined directly in the

Table 3. Average daily rumen contents (kg) of neutral detergent fibre (NDF), cellulose, hemicellulose, lignin and indigestible acid detergent fibre (iADF) and the ratio of iADF to ADF.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| NDF | 4.7 | 5.9 | 0.19 * | 6.9 | 6.3 | 0.13 NS | 5.4 | 7.4 | 0.15 ** | 5.9 | 7.8 | 0.23 NS |
| cellulose | 1.9 | 2.5 | 0.08 * | 2.8 | 2.9 | 0.06 NS | 2.1 | 3.0 | 0.08 ** | 2.7 | 3.8 | 0.11 * |
| hemicellulose | 2.5 | 2.8 | 0.10 NS | 3.4 | 2.7 | 0.06 ** | 2.9 | 3.6 | 0.07 ** | 2.6 | 3.1 | 0.08 NS |
| lignin | 0.4 | 0.6 | 0.02 ** | 0.7 | 0.7 | 0.02 NS | 0.5 | 0.7 | 0.01 *** | 0.6 | 0.9 | 0.04 * |

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Table 4. Fractional passage rates of Cr and T ($k_pCr$ and $k_pT$, respectively) measured in the faeces, and fractional passage rate of Co, measured in the faeces and in the rumen ($k_lCo$-faeces and $k_lCo$-rumen, respectively), rumen liquid volume calculated from the intercept of the Co curve measured in the rumen fluid (V, l), the flow of rumen liquid (l/h) to the omasum and rumen liquid volume, measured by rumen evacuations ($V_m$, l).

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1 | G2 | SEM | G3 | G4 | SEM | G1 | G2 | SEM | G3 | G5 | SEM |
| $k_pCr$ | 4.07 | 5.16 | 0.15 * | 3.67 | 4.96 | 0.13 ** | 3.61 | 4.22 | 0.08 * | 3.65 | 4.25 | 0.11 NS |
| $k_pT$ | 3.14 | 3.77 | 0.09 * | 4.40 | 4.19 | 0.19 NS | 3.87 | 4.19 | 0.08 NS | | | |
| $k_lCo$-faeces | 9.91 | 9.51 | 0.14 NS | 10.34 | 9.69 | 0.15 NS | 10.47 | 10.25 | 0.13 NS | 9.03 | 8.40 | 0.13 NS |
| $k_lCo$-rumen | 13.80 | 13.02 | 0.16 NS | 14.07 | 13.02 | 0.35 NS | 14.14 | 13.90 | 0.14 NS | 12.12 | 11.21 | 0.47 NS |
| V | 88.7 | 93.0 | 1.02 NS | 89.2 | 77.8 | 2.17 NS | 80.9 | 79.3 | 3.74 NS | 100.2 | 102.1 | 4.66 NS |
| flow | 12.22 | 12.09 | 3.46 NS | 12.54 | 10.00 | 0.18 ** | 11.33 | 10.90 | 0.40 NS | 12.12 | 11.13 | 0.33 NS |
| $V_m$ | 80.0 | 80.4 | 0.57 NS | 82.8 | 73.5 | 1.86 NS | 81.0 | 89.5 | 2.49 NS | 82.6 | 92.0 | 3.36 NS |

NS not significant, ** P<0.01, * P<0.05

rumen as well as determined from the faecal excretion curves were not significant in all four experiments ($P>0.05$) (Table 4). However, the $k_l$ values derived from the faecal excretion curves were significantly lower ($P<0.001$, paired Student t) than those determined directly in the rumen (9.7 vs 13.2%$h^{-1}$). Rumen liquid volume (l), calculated from the intercept of the curve describing logarithmic marker decline in the rumen, was not significantly ($P>0.05$) influenced by diet in all four experiments. Though rumen liquid volume and $k_l$ did not differ significantly, the flow of rumen liquid to the omasum (l/h) differed significantly ($P<0.01$) between diets in experiment 3, giving a higher flow for silage G3 than for silage G4 (12.5 vs 10.0 l/h). The rumen volume, measured by rumen evacuations ($V_m$, l) was significantly lower than the rumen volume calculated from the Co-curve in Exp. 1 ($P<0.001$), Exp. 3 ($P<0.05$) and in Exp. 4 ($P<0.01$), but not in Exp. 2.

The $k_p$ was, except for the $k_p$ in Exp. 3 based on the T-excretion curves, higher for the silages containing the higher cell wall content ($P<0.05$ in Exps. 1 and 2, $P<0.01$ in Exp. 3, N.S. in Exp. 4 for the Cr-excretion curves, and $P<0.05$ in Exp. 1, N.S. in Exps. 2 and 3 for the T-labeled hay).

In Exp. 1, Cr-NDF gave significantly higher ($P<0.001$) $k_p$ values as compared to the T-labeled hay (4.61 vs 3.46 %$h^{-1}$). However, in Exps. 2 and 3, there were no significant differences ($P>0.05$) between the Cr and T ruminal passage rates (4.16 vs 4.03 %$h^{-1}$ in Exp. 2 and 4.40 vs 4.19 in Exp. 3).

With an increase in intake level (Exp.2 vs Exp.1), $k_p$-Cr decreased, whereas $k_p$-T increased.

**Discussion.**

Ad libitum intake of roughages can be limited by the capacity of the rumen (Van Soest, 1982; Mertens, 1987). Total rumen contents were higher for the high concentrate level in early lactation than for the low concentrate level in late lactation. Higher rumen DM contents whith an increased concentrate level,

are probably not only due to the higher density of the concentrate particles, but according to Baile and Forbes (1974) also to a higher rumen capacity during lactation.
For the low concentrate level, G3 had the highest rumen DM content, which may be due to a higher density of this silage. The lower rumen DM content for silage G1 supplemented with 7 kg, indicates that, for this diet, intake is not limited by the capacity of the rumen. As a consequence, DMR/DMI was lowest for G1H.
Rumen contents (g DM/kg BW) are within the range of approximately 14 for dry cows to 26 in early lactation, reported by Hartnell & Satter (1979). Although Robinson *et al.* (1987a, 1987b) fed concentrate rich diets in early lactation (2/3 concentrates, 1/3 long hay), rumen contents of approximately 20 g DM/kg BW were comparable to those for the roughage rich diets in these experiments, both somewhat lower than the early lactation data reported by Hartnell & Satter (1979).

Mostly, external markers, such as Cr-NDF, are used to determine the fractional passage rate of the particulate phase from the rumen. In Exp.1, an average rumen content of 9.3 kg DM for silage G2 was found. A $k_p$ of 5.16 %/h, as determined with Cr-NDF, would give a rumen outflow of 11.5 kg DM per day. This would mean that with a DM intake of 12 kg/day, only 0.5 kg DM was degraded in the rumen, showing that Cr-NDF does not represent total rumen DM pool. Even if it is assumed that Cr-NDF may be regarded only as being representative for small particles, it would not correct this. In these experiments, between 75 and 80% of the total rumen dry matter was in particles small enough to pass a sieve with a pore size of 1.25 mm, meaning that Cr-NDF even overestimates passage rate of small particles.
Small particles, however, can get trapped in the floating material, and their gas content influences their functional specific gravity (FSG) (Sutherland, 1987). The specific gravity of Cr-NDF is higher as compared to most rumen particles (Ehle, 1984). Specific gravity of the Cr-NDF used in these experiments was about 1.35. This is within the range of densities found by Welch (1986) and Ehle (1984) with the highest passage rates,

indicating that Cr-NDF could only be representative for small particles with an optimal specific gravity for passage.

The T-labeled hay, used in these experiments, is not indigestible, one of the criteria markers used for studying digestion kinetics, have to meet. An advantage of the T-labeled hay, however, is its participation in the fermentation process, resulting in a change in FSG of the particles during the period they are retained in the rumen. Disappearance of T from the rumen is not restricted to passage to the lower gut only, but microbial degradation of the organically bound T may contribute as well. According to Van Bruchem *et al.* (1990), who used the same type of T-labeled hay as a marker in sheep, the digestible fraction of the hay recovered in the faeces, is negligible at the part of the curve used to calculate $k_p$. They simulated marker excretion curves, assuming rumen degradation rates for the T-labeled hay of 4 and 6%$h^{-1}$ and an undegradable fraction of 15%. For a degradation rate of 6%$h^{-1}$ they only found small differences between the total excretion curve of T and the excretion curve of the indigestible organically bound T. They concluded that, with this type of T-labeled hay, the $k_p$ of T is measured, not a total rumen clearance (passage plus digestion). Compared to the undegradable fractions of our silages (Bosch *et al.*, submitted), however, un undegradable fraction of 15% for this hay seems too high. It is therefore possible that with the T-labeled hay, the $k_p$ is overestimated.

In Exp. 3, when the silages, as in Exp. 1, were supplemented with 1 kg of concentrates, the difference between $k_p$-Cr and $k_p$-T for silage G4 was the same as the differences found in Exp. 1. For silages G1, G2 and G4, supplemented with 1 kg of concentrates, a linear relation ($r$=0.99) between $k_p$-T and the NDF content of the silages was found, whereas the correlation between $k_p$-Cr and the NDF content of the silages was much lower ($r$=0.78). However, the results for silage G3 in Exp. 3 deviated from the results found for the other silages. The $k_p$-Cr of silage G3 was lower than expected from the results on the $k_p$-Cr of G1L, G2L and G4L, while the $k_p$-T for silage G3L was higher than expected from the results on the $k_p$-T of G1L, G2L and G4L.

Both markers, except for T in Exp. 3, gave the same differences between diets, namely a higher passage rate for the silage with the higher cell wall content. Because of the lower digestibilities of the high cell wall diets, more undegraded feed particles, even as a fraction of total rumen DM contents, had to leave the rumen by passage, explaining the higher $k_p$ for both markers for the higher cell wall diets.

Increasing the level of intake in dairy cows fed concentrate rich diets with a constant roughage to concentrate ratio, was reported to increase the rate of passage of Cr mordanted NDF (Robinson *et al.*, 1987b). In the present experiments level of intake was increased by increasing the amount of concentrates fed, concomitantly resulting in a somewhat reduced intake of silage. In this case, rate of passage of Cr mordanted NDF decreased, suggesting that the behaviour of Cr mordanted NDF varies with ration composition. An explanation could be the higher dry matter % in the rumen at the higher intake, possibly increasing the probability of an external marker to get entrapped and therefore slowing down its rate of passage. It is of interest in this respect that the increase in rate of passage as reported by Robinson *et al.* (1987b) was not linear; between the one but highest and the highest level of intake even a decrease was observed. Average rumen dry matter content at both levels of intake in the experiments of Robinson *et al.* (1987b) was 12.0 and 13.8%, respectively. At the high concentrate level, rumen DM% in our experiments varied between 11.3 and 12.9%, whereas for the low concentrate level, rumen DM% varied between 10.0 and 11.8%. The reason for the differences in behaviour between the Cr-NDF particles and the T-labeled hay particles with an increase in intake is not clear.

Owens & Goetsch (1986) reviewed literature on passage of particles from the rumen and derived regression equations which related the rate of passage of fluid, forage particles and concentrate particles to intake of forages and concentrates. Applying these equations to the experiments reported here, results in the figures shown in Table 5. It should be realised however, that most of the results used to derive the regression

equations were from experiments with intakes below ad libitum. Besides, the equations of Owens & Goetsch explained only 38 and 61 % of the total variation of passage of forage and concentrate particles, respectively.

Table 5. Passage rates of concentrates ($k_p$-concentrate, %/h) and silages ($k_p$-roughage, %/h) in the four experiments, calculated using the equations of Owens & Goetsch (1986).

| Diet | $k_p$-concentrate | $k_p$-roughage |
|---|---|---|
| G1L | 6.08 | 3.87 |
| G2L | 6.15 | 3.65 |
| G3L | 5.74 | 4.25 |
| G4L | 6.15 | 3.62 |
| G1H | 6.72 | 4.76 |
| G2H | 6.70 | 4.64 |
| G3H | 6.68 | 4.94 |
| G5H | 6.66 | 4.53 |

Another phenomenon which may interfere, and apparently not taken into account by Owens & Goetsch (1986) is the difference in digestibility, as applied in the experiments reported here. Despite the lower intakes for the high cell wall silages, intakes of undegradable material, which has to be cleared fom the rumen by passage, are still higher. According to the equations of Owens & Goetsch (1986), this decrease in intake would always result in a decrease in $k_p$, which does not seem correct. A comparison of intake levels shows that a higher intake will result in a considerably higher $k_p$, which is in agreement with the T-results in Exps. 1 and 2. Comparing the levels of the calculated $k_p$'s with the levels of $k_p$-Cr and $k_p$-T, however, shows considerably higher calculated $k_p$'s for the higher intake levels. It seems therefore that the T-labeled hay, though the fraction of rumen particles represented by the T-labeled hay was not defined, can reliably be used as a marker to rank the silages for passage

rates from the rumen.

An increased cell wall content increases rumen fill, mainly by an increased % of dry matter. The reduced rate of degradation, as shown with nylon bag incubation studies (Bosch *et al.*, submitted), was partly compensated for by an increased rate of passage. This compensation is however not enough to prevent a reduction in total clearance rate, resulting in a decreased intake.

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Chapter IV

# Influence of stage of maturity of grass silages on digestion processes in dairy cows. 3. Fermentation characteristics, rumination activity and distribution of rumen and faecal particles.

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**Abstract.**

In four change-over experiments, wilted grass silages, differing in cell wall content (Neutral Detergent Fiber (NDF) content ranging from 44.6% to 67.3% of dry matter (DM) were fed ad libitum to dairy cows in early and late lactation.

When supplemented with 7 kg of concentrates (early lactation) rumen pH was lower, and VFA concentrations were higher as compared to supplementation with 1 kg (late lactation). Diurnal variations in ammonia concentrations were higher for the high concentrate level. On average, the ammonia concentrations were lower in combination with 7 kg of concentrates.

Total chewing time (CT), rumination time (RT) and eating time (ET) were recorded for six 24 h periods per cow. When the animals ruminated for more than 9 h a day, an increase in cell wall content of the diet did not further increase RT, but resulted in a decreased intake. CT, RT and ET per kg DM tended to increase with an increase in cell wall content, whereas CT, RT and ET per kg ingested NDF tended to decrease.

The pool of rumen dry matter (DM) able to flow to the omasum (passing a 1.25 mm sieve), was not influenced by cell wall content of the silages. The pool of rumen DM passing a 0.071 mm sieve, however, decreased with increasing cell wall content. The mean faecal particle size (MFP) significantly increased with cell wall content, and was negatively correlated ($r$ = -0.90, $P$<0.001) with rumen pool of particles passing a 0.071 mm sieve.

**Introduction.**

Intake of roughage diets by lactating dairy cows may be limited by the capacity of the rumen (Mertens, 1987). Dry matter (DM) is cleared from the rumen by microbial fermentation and by passage of the undigested material to the omasum. Passage from the rumen depends on the probability to escape, which increases with decreasing particle size (Poppi *et al.*, 1980). As a practical approach it is often assumed that this probability becomes

sufficiently high when the size of a particle is reduced to a size smaller than the critical particle size (CPS), by Kennedy & Poppi (1984) defined as the nominal sieve aperture, which retains the top 5% of the faecal particulate dry matter. They reported a CPS for cattle of 1.18 mm.

Microbial digestion decreases rumen dry matter (DM) content, but has relatively little effect on particle size reduction (Welch, 1982; Ulyatt *et al.*, 1986). It may however weaken the cell wall structure, so that particle breakdown during rumination is facilitated (Chai *et al.*, 1984). According to Ulyatt *et al.* (1986), also rumen contractions do hardly contribute to particle size reduction. Microbial digestion plus rumen contractions would account for only about 20 % of particle size reduction (McLeod & Minson, 1988a and 1988b). That leaves chewing during eating and rumination as the main factors involved in particle size reduction. Rumination time (RT) per kg DM ingested differs between diets, and increases with an increase in cell wall content (Murphy *et al.*, 1983; Ulyatt *et al.*, 1986). A high correlation ($r > 0.9$) between RT and cell wall content of the diet was found by Welch & Smith (1969a, 1969b). A grass silage with a higher cell wall content thus requires a longer rumination time per kg DM. Because rumination time is limited to a maximum of 9 to 10 h per day (Bae *et al.*, 1979; Welch, 1982), RT required to reduce particle size to a size below the CPS can presumably limit forage intake.

If intake is not limited physically, the type and amount of digestion products may control feed intake. The pH of the rumen fluid is mainly determined by the VFA concentrations. The concentrations and the ratio of volatile fatty acids (VFA) and the ammonia ($NH_3$) concentration (Satter & Slyter, 1974; Hoover, 1986) are the result of microbial activity on one hand and the rate of clearance from the rumen on the other. The animal's physiological status largely controls the latter.

In four experiments, the effect of stage of maturity of grass silages on intake and digestion in dairy cattle is investigated (Bosch *et al.*, submitted[a]). The present paper describes fermentation characteristics, eating and rumination behaviour and rumen

contractions. In addition, the results of rumen and faecal particle size distributions are reported.

**Materials and Methods.**

Five wilted grass silages (G1 to G5) differing in cell wall content were fed ad libitum to dairy cows, supplemented with 7 or 1 kg of concentrates, in early and late lactation, respectively. In each of the four change-over experiments, two silages were fed to six (Exps.1, 2 and 3) or four (Exp.4) rumen fistulated dairy cows. In Exps. 1 and 3 the cows were in late lactation, so 1 kg of concentrates, and in Exps. 2 and 4, when the cows were in early lactation, 7 kg of concentrates was included in the diet. Each experiment consisted of two experimental periods of five weeks, preceded by adaptation periods of three weeks. The animals were offered silage three times a day, at 7.00, 15.00 and 23.00 h. The concentrates were fed at 14.45 h (1 kg) or at 6.45 and 14.45 h (two portions of 3.5 kg each). The experimental design was as described in more detail by Bosch *et al.* (submitted[a]). Dry matter (DM), nitrogen (N) and cell wall (NDF) content, intake and apparent digestibility of the silages in the four experiments are given in Table 1.

To determine end products of rumen fermentation, samples of rumen fluid were taken every two hours between Monday 9.00 and Tuesday 7.00 h. The pH and the ammonia-N concentration ($NH_3$-N) were measured in each sample, and the average VFA concentrations were determined in a pooled sample per cow (Exps. 1, 2 and 3), or in each sample (Exp. 4). The pH was deterimed immediately, whereas the $NH_3$-N was determined after storage for one night with Tri Chloro Acetic acid at 4°C. The samples to determine the VFA concentrations were preserved with 5% phosphoric acid and stored at -20°C untill analysis.

Per experimental period, eating time, rumination activity and rumen motility were recorded for six 24-hour periods per animal. For recording eating and rumination, the animals were provided

Table 1. Chemical composition of the silages (dry matter (DM), nitrogen (N) and cell wall content (NDF)), DM, N and NDF intake and apparent digestibility and the mean weight of the cows.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1 | G2 | SEM | G3 | G4 | SEM | G1 | G2 | SEM | G3 | G5 | SEM |
| chemical composition | | | | | | | | | | | | |
| DM | 59.4 | 54.3 | | 60.8 | 38.7 | | 59.4 | 54.3 | | 60.8 | 55.0 | |
| N | 3.41 | 3.14 | | 3.34 | 1.90 | | 3.41 | 3.14 | | 3.34 | 1.79 | |
| NDF | 44.6 | 54.7 | | 54.8 | 64.1 | | 44.6 | 54.7 | | 54.8 | 67.3 | |
| silage intake | | | | | | | | | | | | |
| DM | 12.0 | 11.1 | 0.17 NS | 14.1 | 10.7 | 0.10 *** | 10.8 | 10.5 | 0.18 NS | 12.1 | 9.9 | 0.23 * |
| N | 0.42 | 0.35 | 0.01 ** | 0.47 | 0.20 | 0.01 *** | 0.39 | 0.33 | 0.01 * | 0.41 | 0.17 | 0.01 ** |
| NDF | 5.4 | 6.2 | 0.10 * | 7.8 | 6.9 | 0.07 ** | 5.1 | 5.7 | 0.10 * | 6.7 | 6.6 | 0.15 NS |
| total intake | | | | | | | | | | | | |
| DM | 12.8 | 12.0 | 0.17 NS | 15.0 | 11.5 | 0.10 *** | 17.0 | 16.7 | 0.18 NS | 18.2 | 16.0 | 0.23 * |
| N | 0.44 | 0.37 | 0.01 ** | 0.50 | 0.23 | 0.01 *** | 0.57 | 0.52 | 0.01 * | 0.58 | 0.35 | 0.01 ** |
| NDF | 5.6 | 6.5 | 0.10 * | 8.0 | 7.1 | 0.07 ** | 6.8 | 7.4 | 0.10 * | 8.5 | 8.6 | 0.15 |
| apparent digestibility | | | | | | | | | | | | |
| DM | 73.0 | 68.2 | 0.22 *** | 68.6 | 62.5 | 0.14 *** | 72.0 | 68.8 | 0.15 *** | 69.0 | 61.5 | 0.26 *** |
| N | 69.7 | 68.4 | 0.21 * | 68.0 | 59.3 | 0.21 *** | 67.5 | 66.4 | 0.19 * | 67.2 | 61.6 | 0.20 ** |
| NDF | 78.0 | 70.9 | 0.17 *** | 73.8 | 62.9 | 0.05 *** | 74.7 | 66.4 | 0.19 *** | 71.4 | 56.8 | 0.73 ** |
| weight of cows | 546 | 550 | 1.99 NS | 560 | 534 | 2.29 ** | 569 | 576 | 2.19 NS | 579 | 570 | 2.50 NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

with a halter with a microswitch. Jaw movements were recorded on paper and number of chews was recorded by a counter. Rumen contractions were recorded using an open tip catheter. Contractions were transformed into electrical signals by a pressure transducer. Specifications were as follows: frequency band 0.01-0.10 Hz, duration pressure peak > 2 sec., pressure > 5 mm Hg. The number of rumen contractions was recorded by a counter.

Faeces were collected quantitatively from Monday 8.00 h till Thursday 8.00 h, weighed and sampled per two hours. A pooled sample per cow was preserved with formaldehyde and kept at $4^0$C till the end of the collection period. After the collection period the faeces were subsampled and stored at $-20^0$C till further analysis. This was done twice per cow for the silages fed. Distribution of the faecal particle size was determined in subsamples by wet sieving.

Rumen evacuations were done in the fifth week of the experimental periods, at two times after feeding in Exps. 1, 2 and 3, and at five times after feeding in Exp. 4.

Rumen contents were weighed, sampled (1%) and returned into the rumen. This procedure took about 25-30 min. per cow. Rumen samples were stored at -20°C till further analysis.

Chemical analysis.

The $NH_3$-concentration in the rumen fluid was determined by the indophenol method as described by Scheiner (1976). The VFA concentrations in rumen fluid were determined by gas liquid chromatography (Packard 419, glass column filled with chromosorb 101, carrier gas ($N_2$) saturated with formic acid, 190 $^0$C, isocapronic acid as internal standard).

Sieve analyses.

Faecal and rumen samples were wet sieved with a Fritsch Analysette 3. The lid was equiped with a shower, and at the bottom there was a water outlet.

*Faecal samples*. Faecal samples of 100.0 g were soaked in water and stirred till the suspension was free of lumps. The sieves used

had a mesh aperture of 1.25, 0.63, 0.315, 0.16 and 0.071 mm. The intermediate water supply ring was placed just above the smallest sieve, to prevent the 0.071 mm sieve from silting up. The sieving was done with a continuous maximum vibration amplitude. The water flow was about 4 l/minute.

The suspension was quantitatively spread out on the top sieve. The water was turned on. After 5 min. of sieving, the water outlet was closed till the water level had risen till 2 cm above the top sieve. The water outlet was opened to lower the water level till below the smallest sieve. This procedure was repeated twice. After the third time the water outlet was opened and sieving was continued for another five minutes. Then the water was turned off, and the Analysette switched off. The fractions retained on the sieves, were quantitatively collected in a dried and weighed glass filter crucible number 2 (pore size 40-100 $\mu$m). The crucibles were dried at 103 ± 2 °C during one night. The dry matter retained on each sieve, was expressed as a percentage of total dry matter. The mean faecal particle size (MFP) was calculated using the log-normal distribution as described by Waldo *et al.* (1971).

*Rumen samples*. Rumen samples of 50.0 g were soaked in water and stirred till the suspension was free of lumps. For the rumen samples three sieves were used, with sieve apertures of 5.0, 1.25 and 0.071 mm. After removing the showerheads, the intermediate water supply ring was placed above the top sieve, just underneath the main water supply. The samples were sieved for 30 minutes, with a maximal vibration with minimum intervals, and a water flow of about 10 l/minute.

The suspension was quantitatively spread on the top sieve. The water was turned on and the outlet tube was lifted till above the top sieve. When the water level had risen to 2 cm above the top sieve, the water was turned off. The water level was slowly lowered till under the top sieve. The outlet tube was lifted again and the water turned on till the level had risen till 2 cm above the top sieve. This was done for 7 times during about 7.5 min. Then the water level was kept 2 cm above the top sieve for about 7.5 minutes, while the water flow stayed on. After 15

minutes sieving time, the whole procedure was repeated once. Then the water was turned off and the Analysette switched off. The material retained on the sieves was collected and dried as described for the faecal samples. The material retained on the 5.0 and the 1.25 mm sieve was designated as the material not able to leave the rumen (Large Particles), while the material passing the 1.25 mm sieve was designated as the material able to flow to the omasum (Small Particles).

Statistics.

The data were analysed statistically using the anova procedure of the spsspc+ statistical package (SPSS Inc., 1988) according to the model as described by Bosch *et al.* (submitted[a]).

**Results.**

Chemical composition (DM, N, NDF), intake and digestibility are shown in Table 1. Apparent digestibility and, apart from G3, intake decreased with an increase in cell wall content of the silages. Only in Exp. 3 silage DM intake was significantly ($P<0.001$) different between silages. The cows were gaining weight when they were fed silage G3 and loosing weight when they were fed silage G4.

Fermentation characteristics are given in Table 2. The mean pH was only significantly related to the cell wall content of the silages if fed with 1 kg of concentrates ($P<0.001$ in Exp.1 and $P<0.05$ in Exp.3). Then a higher cell wall content coincided with a slightly higher rumen pH. The rumen pH was negatively influenced by the amount of concentrates in the diet. For the high concentrate diets the rumen ammonia-N concentrations were not significantly different between silages (Exps. 2 and 4). The amount of concentrates in the diet varied in these experiments from 31 to 41% of the dry matter intake. A higher amount of concentrates leveled down the differences between silages. Contradictory to what was found in Exp.3, the $NH_3$-concentration in Exp.1 was higher for the silage with the lower crude protein

Table 2. Rumen fermentation characteristics pH, $NH_3$ (mg $NH_3$-N/l), acetic acid (HAc, mmol/l), propionic acid (HP, mmol/l), isobutyric acid (HiBu, mmol/l), butyric acid (HBu, mmol/l), isovaleric acid (HiV, mmol/l), valeric acid (HV, mmol/l), total fatty acids (mmol/l) and the ratio glucogenic versus non-glucogenic fatty acids (NGR).

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| pH | | | | | | | | | | | | |
| mean | 6.21 | 6.40 | 0.01 *** | 6.40 | 6.51 | 0.02 * | 6.14 | 6.21 | 0.02 | 6.07 | 6.34 | 0.04 |
| min | 5.94 | 6.13 | 0.01 *** | 6.15 | 6.28 | 0.02 | 5.75 | 5.88 | 0.02 * | 5.76 | 6.02 | 0.03 |
| max | 6.52 | 6.70 | 0.01 *** | 6.71 | 6.71 | 0.03 | 6.61 | 6.58 | 0.03 | 6.42 | 6.71 | 0.05 |
| $NH_3$ | | | | | | | | | | | | |
| mean | 199 | 239 | 2.81 ** | 186 | 125 | 6.31 ** | 195 | 198 | 4.22 | 150 | 104 | 7.30 |
| min | 87 | 122 | 4.13 * | 86 | 49 | 4.83 * | 71 | 86 | 5.23 | 43 | 33 | 4.93 |
| max | 360 | 405 | 6.55 * | 322 | 257 | 11.17 * | 362 | 385 | 8.90 | 290 | 219 | 6.27 * |
| HAc | 66.1 | 55.9 | 1.89 | 79.2 | 66.2 | 1.40 ** | 94.8 | 83.4 | 1.04 ** | 96.4 | 80.2 | 2.14 |
| HP | 16.7 | 13.6 | 0.50 * | 17.5 | 17.4 | 0.46 | 24.9 | 21.7 | 0.17 *** | 25.0 | 20.5 | 0.46 * |
| HiBu | 1.03 | 0.96 | 0.01 * | 0.97 | 0.76 | 0.02 ** | 1.12 | 0.95 | 0.02 ** | 1.01 | 0.76 | 0.01 ** |
| HBu | 11.35 | 8.57 | 0.18 ** | 11.82 | 14.20 | 0.14 *** | 18.12 | 15.48 | 0.22 ** | 14.99 | 11.93 | 0.32 * |
| HiV | 1.33 | 1.14 | 0.02 ** | 1.44 | 1.01 | 0.01 *** | 1.75 | 1.26 | 0.07 * | 2.06 | 1.55 | 0.06 * |
| HV | 1.63 | 1.10 | 0.03 *** | 1.56 | 1.20 | 0.03 ** | 2.44 | 1.77 | 0.02 *** | 2.00 | 1.21 | 0.07 * |
| total VFA | 98.2 | 81.2 | 2.59 * | 112.5 | 100.7 | 1.97 * | 143.1 | 124.6 | 1.46 ** | 141.4 | 116.2 | 2.92 * |
| NGR | 4.77 | 4.91 | 0.03 | 5.28 | 5.03 | 0.06 | 4.75 | 4.84 | 0.03 | 4.56 | 4.66 | 0.02 |

*** P<0.001 ** P<0.01 * P<0.05

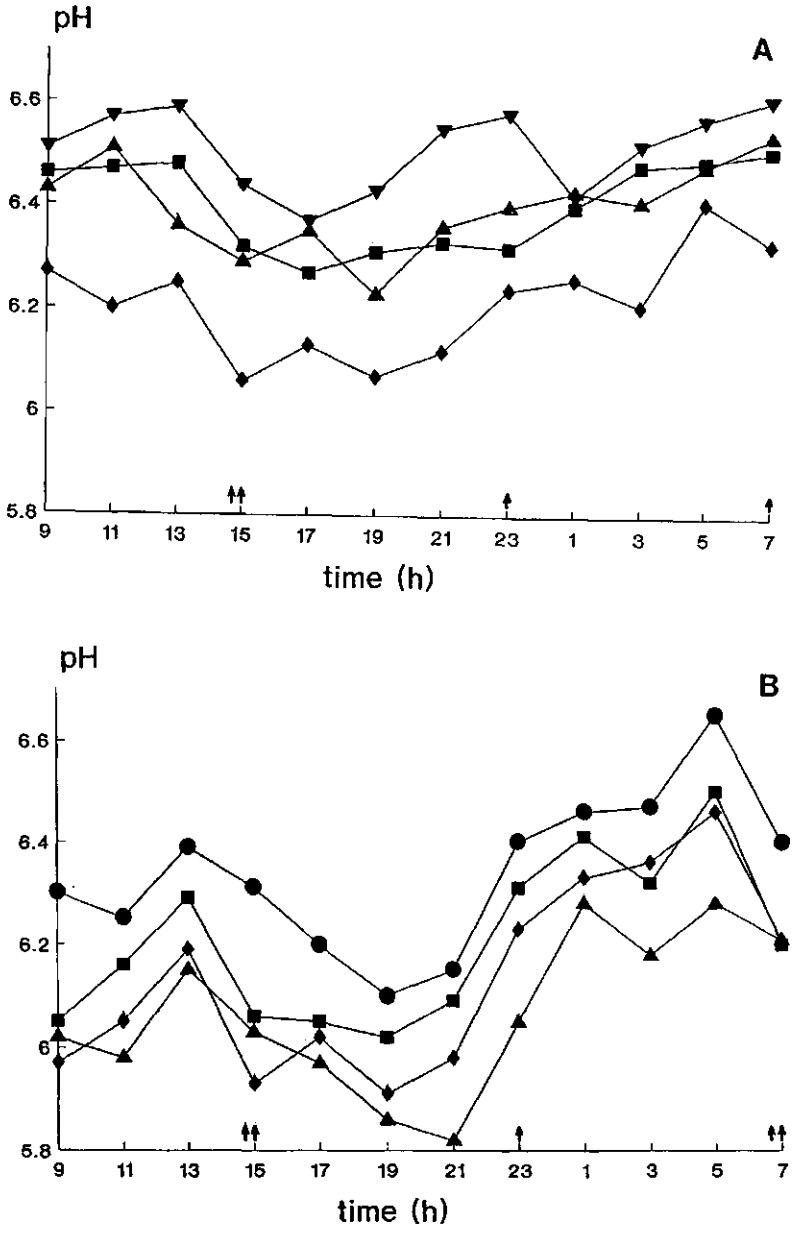


Fig 1. Patterns of pH values for the silages in combination with 1 (A) and with 7 (B) kg of concentrates. ( ◆ G1, ■ G2, ▲ G3, ▼ G4 and ● G5 , two arrows indicate time of feeding concentrates plus silage, one arrow time of feeding silage).

and the higher cell wall content.

Patterns of pH values for the silages fed with 1 or 7 kg of concentrates are shown in Fig. 1. The decline in pH after feeding concentrates increased with the amount of concentrates fed. The variations in $NH_3$-N concentrations over the day are given in Fig. 2, for the silages fed in combination with the low (1 kg) as well as the high (7 kg) level of concentrates. After feeding concentrates plus silage the $NH_3$-N concentration rose more than after feeding silage only. With 7 kg of concentrates (twice a day 3.5 kg) ammonia-N concentrations (average as well as minimum concentrations per day) were lower than with 1 kg of concentrates.

VFA concentrations were higher for the better digestible silages, and higher in combination with 7 kg than with 1 kg of concentrate. The ratio non-glucogenic versus glucogenic fatty acids (NGR) decreased with an increasing concentrate proportion in the diet. Though not significantly, the NGR was higher for the silages with the higher cell wall content and the lower total VFA concentrations, except for Exp. 3. The daily variation in VFA concentrations in Exp. 4 is given in Fig. 3. The VFA concentrations differed between G3H and G5H ($P<0.05$), but the patterns were comparable.

Rumination and eating parameters and number of rumen contractions per day are given in Table 3. Total chewing times (CT) only differed significantly ($P<0.001$) between silages in Exp. 1. Rumination times (RT, min/day) and eating times (ET, min/day) were only slightly different between the silages, both within and between experiments. RT did not differ significantly between the concentrate levels. Because of the lower silage intake with the high concentrate level, RT per kg silage DM ingested was higher in Exps. 2 and 4 than in Exps. 1 and 3. ET (min/day) was only in Exp.4 higher for the silage with the higher cell wall content ($P<0.05$). Per kg NDF ingested CT and RT only differed between silages in Exp.3 (Table 3). Number of rumination boli per day differed significantly ($P<0.01$) between silages in Exp. 3. Number of chews during rumination (RC) differed between silages in Exp. 1 ($P<0.01$) and number of chews during eating (EC)

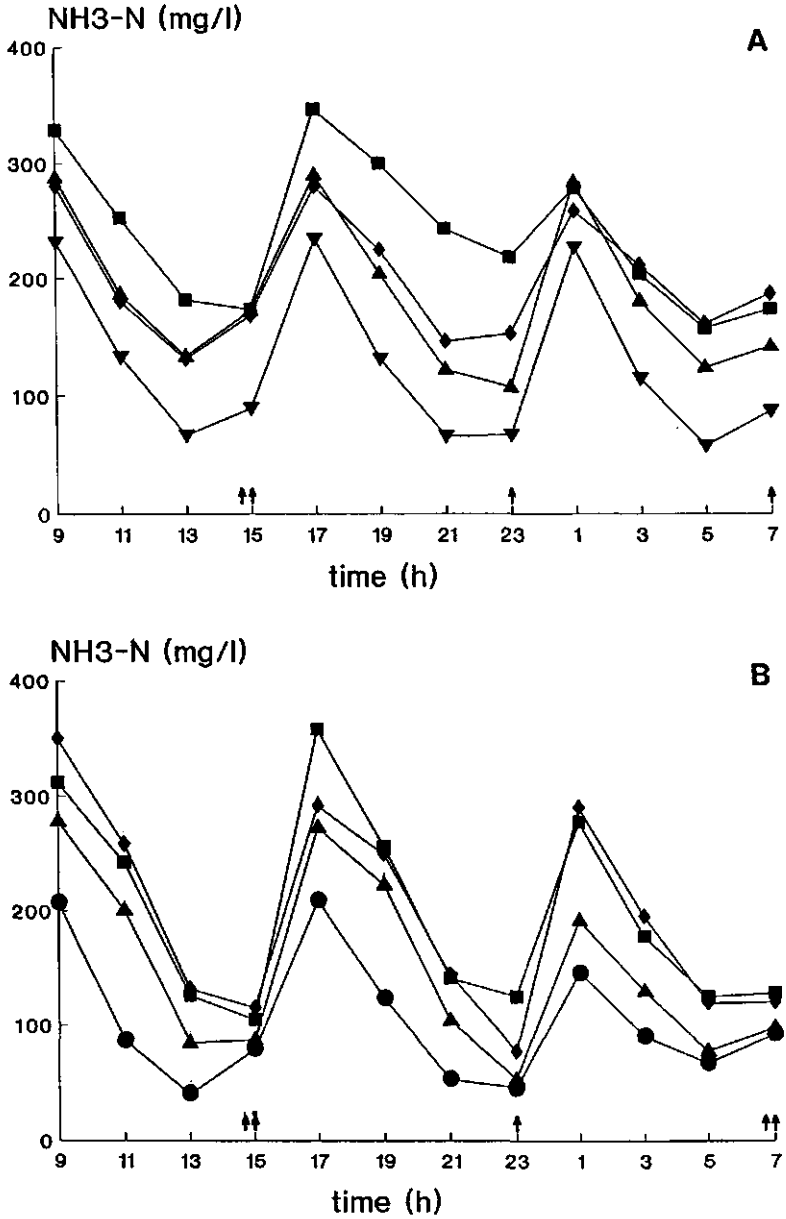


Fig 2. Patterns of $NH_3$-N concentrations for the silages in combination with 1 (A) and with 7 (B) kg of concentrates. ( ◆ G1, ■ G2, ▲ G3, ▼ G4 and ● G5, two arrows indicate time of feeding silage plus concentrates, one arrow time of feeding silage).

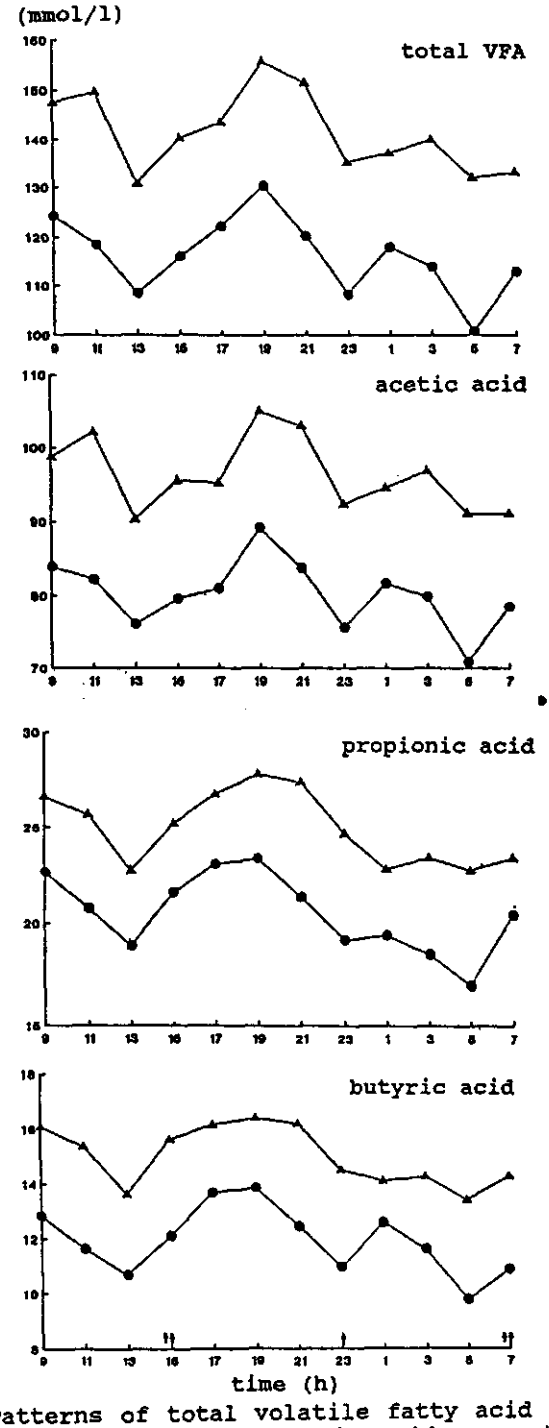


Fig 3. Patterns of total volatile fatty acid (VFA), acetic acid, propionic acid and butyric acid concentrations for silages G3 (▲) and G5 (●) in Exp. 4, fed in combination with 7 kg of concentrates. (two arrows indicate time of feeding silage plus concentrates, one arrow time of feeding silage).

Table 3. Total chewing time (CT, min/day), ruminating time (RT, min/day), eating time (ET, min/day), CT, RT and ET per kg silage dry matter (DM) ingested, and per kg silage cell wall constituents (NDF) ingested, number of boli per day, number of chews during ruminating (RC) and during eating (EC) and the number of rumen contractions per day.

| | late lactation | | | | | | | early lactation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | | Exp.3 | | | Exp.2 | | | | Exp.4 | | |
| | G1 | G2 | SEM | | G3 | G4 | SEM | G1 | G2 | SEM | | G3 | G5 | SEM | |
| silage NDF% | 44.6 | 54.7 | | | 54.8 | 64.1 | | 44.6 | 54.7 | | | 54.8 | 67.3 | | |
| CT | 790 | 894 | 6.11 | *** | 829 | 870 | 15.6 | NS | 775 | 831 | 14.6 | NS | 869 | 928 | 9.11 | NS |
| RT | 498 | 568 | 4.51 | NS | 523 | 558 | 4.46 | * | 518 | 573 | 6.56 | * | 559 | 554 | 9.65 | NS |
| ET | 292 | 326 | 7.32 | NS | 306 | 312 | 12.7 | NS | 257 | 258 | 14.5 | NS | 310 | 374 | 4.20 | * |
| /kg DM | | | | | | | | | | | | | | | | |
| CT | 67.0 | 79.0 | 1.01 | ** | 58.3 | 81.8 | 1.38 | *** | 71.3 | 78.4 | 1.50 | NS | 71.1 | 95.6 | 2.83 | * |
| RT | 42.4 | 50.5 | 0.53 | ** | 36.9 | 52.5 | 0.56 | *** | 47.5 | 54.0 | 0.74 | * | 45.7 | 57.3 | 1.99 | NS |
| ET | 24.7 | 28.5 | 0.77 | NS | 21.5 | 29.3 | 1.19 | * | 23.8 | 24.4 | 1.33 | NS | 25.3 | 38.3 | 1.12 | * |
| /kg NDF | | | | | | | | | | | | | | | | |
| CT | 146.1 | 140.6 | 1.95 | NS | 107.1 | 131.0 | 2.18 | ** | 151.3 | 143.9 | 2.85 | NS | 127.8 | 140.0 | 5.10 | NS |
| RT | 92.3 | 89.8 | 0.64 | NS | 67.7 | 84.1 | 0.84 | *** | 100.9 | 99.1 | 1.21 | NS | 82.3 | 84.0 | 3.46 | NS |
| ET | 53.8 | 50.7 | 1.56 | NS | 39.4 | 46.9 | 1.88 | NS | 50.4 | 44.8 | 2.66 | NS | 45.5 | 56.0 | 1.87 | NS |
| boli | 601 | 654 | 12.0 | NS | 593 | 660 | 6.22 | ** | 623 | 632 | 4.28 | NS | 568 | 599 | 16.0 | NS |
| RC | 30824 | 36357 | 522 | ** | 30149 | 32432 | 407 | NS | 31424 | 31769 | 1341 | NS | 34652 | 35066 | 932 | NS |
| EC | 20634 | 24188 | 710 | NS | 21412 | 23288 | 1164 | NS | 18854 | 17370 | 1553 | NS | 20796 | 27542 | 263 | ** |
| rumen contr. | 2899 | 2822 | 38.8 | NS | 2780 | 2723 | 39.8 | NS | 2900 | 3036 | 53.9 | NS | 2826 | 2836 | 33.6 | NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

differed between silages in Exp. 4 ($P<0.01$). Number of rumen contractions was not significantly influenced by diet in all four experiments. Though during eating frequency of rumen contractions increased , differences in eating times did not result in different numbers of rumen contractions per day.

The MFP ($\mu$m) and the particle size distribution of rumen particles, the latter as kg DM of the different sizes in the rumen, are given in Table 4. The MFP was lower when the silages were supplemented with 7 than when supplemented with 1 kg of concentrates. Within level of concentrates fed, the MFP increased with stage of maturity of the silages. The fraction of faecal particles retained on the top sieve (1.25 mm) varied between 2% and 5%.

In the rumen, the fraction of small particles, able to pass to the omasum considering their size (<1.25 mm), ranged from 74 to 81% of rumen DM. The pool of particles <1.25 mm was not significantly different between silages in experiments 1 and 4. In Exp. 2, the fraction of rumen particles smaller than 1.25 mm was somewhat higher for silage G1. The pool of particles <1.25 mm, however, was higher for silage G2, due to the higher rumen DM content. In Exp. 3, the fractions of rumen particles <1.25 mm were the same for both silages, resulting in 8.5 kg DM able to leave the rumen for silage G3 and 6.6 kg DM for silage G4 ($P<0.01$).

Within the fraction of small particles (<1.25 mm), the amount of particles <0.071 mm was highest for the silage with the lower cell wall content in all four experiments. The pool of particles >5 mm was also highest for the low cell wall silages in all four experiments. A negative correlation ($r = -0.90$, $P<0.001$) between pool sizes of particles smaller than 0.071 mm and MFP was found.

**Discussion.**

In Exps. 2 and 4, ammonia concentrations were not significantly ($P>0.05$) influenced by diet, probably because the protein ingested consisted of 30-50% of concentrate protein.

Table 4. Mean faecal particle size (MFP, μm) and ruminal particle size distribution (kg DM of the different particle sizes) for the silages in the four experiments.

| | late lactation | | | | | | | early lactation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | | Exp.3 | | | Exp.2 | | | | Exp.4 | | |
| | G1 | G2 | SEM | | G3 | G4 | SEM | G1 | G2 | SEM | | G3 | G5 | SEM | |
| MFP | 25.5 | 53.5 | 1.71 | ** | 27.0 | 102.3 | 1.06 | *** | 22.8 | 43.5 | 1.22 | ** | 20.5 | 85.2 | 0.67 | *** |
| Rumen particles | | | | | | | | | | | | | | | | |
| >1.25mm | 1.70 | 2.43 | 0.11 | * | 2.64 | 2.33 | 0.08 | NS | 1.96 | 2.59 | 0.04 | ** | 2.68 | 2.70 | 0.11 | NS |
| <1.25mm | 7.17 | 6.88 | 0.21 | NS | 8.49 | 6.55 | 0.16 | ** | 8.30 | 9.37 | 0.16 | * | 9.53 | 9.94 | 0.29 | NS |
| >5mm | 0.81 | 0.77 | 0.04 | NS | 0.77 | 0.53 | 0.04 | * | 1.25 | 0.79 | 0.04 | ** | 1.05 | 0.67 | 0.07 | NS |
| >1.25mm, <5mm | 0.89 | 1.67 | 0.08 | ** | 1.86 | 1.81 | 0.05 | NS | 0.71 | 1.79 | 0.07 | ** | 1.63 | 2.03 | 0.06 | NS |
| >0.071mm, <1.25mm | 1.99 | 3.17 | 0.11 | ** | 3.69 | 3.94 | 0.11 | NS | 2.82 | 4.49 | 0.13 | ** | 4.07 | 5.74 | 0.22 | NS |
| <0.071mm | 5.18 | 3.72 | 0.10 | ** | 4.81 | 2.60 | 0.10 | *** | 5.48 | 4.88 | 0.08 | * | 5.46 | 4.20 | 0.16 | NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

When the silages were fed supplemented with 7 kg of concentrates, $NH_3$-N concentrations were lower for all silages, whereas VFA concentrations were higher for all silages as compared to feeding the silages with 1 kg of concentrates. No significant differences in rumen fluid volume, however, were found between silages or between concentrate levels (Bosch *et al.*, submitted[b]).

Degradation rate of the concentrate protein is likely to be lower than degradation rate of the silage protein. Ammonia concentration in the rumen is determined by the ammonia production by microorganisms and the ammount of $NH_3$ available from recycling of urea (saliva and recycling through rumen wall) on one hand and the utilization by microorganisms, rate of passage to the omasum and absorption through rumen wall on the other hand. Higher digestible OM intakes result in higher VFA productions (Haaland *et al.*, 1982; Sutton, 1985). For the high concentrate level, VFA production will be higher, resulting in a better utilization of ammonia by rumen bacteria.

With 1 kg of concentrates, ammonia-N concentration was lowest at all times for G4, due to the lower protein intake. Despite the lower protein intake for G2L as compared to G1L and G3L, the higher ammonia concentration peak and the slower decline after feeding silage plus concentrates resulted in significantly higher ($P<0.05$) average ammonia-N concentrations for G2L (Fig. 2).

According to Satter & Slyter (1974) at least an ammonia-N concentration of 50 mg/l is needed, while Hoover (1986) reports a minimal concentration of 33 mg/l. Hoover (1986) concludes that the ammonia-N concentration for maximum microbial growth rate is not equal to the concentration for an optimal digestion (33 vs 80 mg/l). The lower ammonia concentrations for G4 in Exp. 3, due to the lower protein intake, with minimum values of ammonia-N of less than 50 mg/l may result in a lower microbial growth rate as compared to silage G3. For the high concentrate level, only G2 did not show ammonia-N concentrations below 80 mg/l, indicating that for the other silages during the hours before feeding times the ammonia-N concentrations may have been too low for an optimal degradation.

Except for silage G1 in Exps. 1 and 2, rumination times exceeded 9 hours a day for all silages, regardless of being supplemented with 1 or 7 kg of concentrates. According to Bae *et al.* (1979) and Welch (1982) that is about the maximum time the animals can spent ruminating. Rumination time and cell wall content of the diet are highly ($r$ = 0.94-0.99) correlated (Welch & Smith, 1969a, 1969b). When the animals are ruminating for more than 9 h a day an increase in cell wall content of the diet will not result in an increase in rumination time, but in a decrease in intake. To be able to reduce particle size to a size below the CPS, high cell wall diets require longer RT per kg DM ingested. Apart from silage G3, RT (min/kg DM) increased with an increase in cell wall content of the silage. Although not significantly, RT and ET per kg NDF tended to decrease. According to Balch (1971), the total time spent chewing per kg DM is the most suitable value to use as a roughage index. Apart from G3, total chewing time (CT) per kg DM ingested increased with an increase in cell wall content of the silage ($r$ = 0.93 excluding and $r$ = 0.70 including G3). The "structure" of the feed not only depends on the physical form and the NDF content as such, but also seems to depend on the composition of the NDF fraction. Of G3, the ratio cellulose to hemicellulose of the cell wall fraction was lower as compared to the other silages, resulting in a lower "roughage index", and a higher ad libitum intake.

After particles have left the rumen, hardly any further reduction in particle size takes place (Poppi *et al.*, 1980). The mean size of particles leaving the rumen can thus be measured in faecal samples. The critical particle size (CPS), defined by Kennedy & Poppi (1984) as the sieve aperture retaining the top 5% of faecal particulate dry matter, is the size above which particles have a very low probability of leaving the rumen. They indicated a CPS for cattle of 1.18 mm. The 1.25 mm sieve used in our experiments approaches the CPS adequately, shown by the fraction of faecal dry matter retained on this sieve, varying between 2 and 5%. Therefore, the MFP will be discussed as being the mean size of particles flowing from the rumen to the omasum. The size of particles leaving the rumen decreased when the

proportion of concentrates in the diet increased, due to a bigger pool of particles smaller than 0.071 mm, probably caused by a smaller initial size of the concentrate particles.
There was a positive linear relation between the MFP and the ratio of particles with a size between 1.25 mm and 0.071 mm to particles smaller than 0.071 mm ($R^2$=0.67, $P$<0.001).

Because the major part (>70%) of the rumen content consists of particles smaller than the CPS, passage rate can not only be limited by the size of the particles. Small particles can get trapped in the floating material, and their gas content influences their functional specific gravity (FSG) (Sutherland, 1987).
In experiments 1 and 3, one may assume that the NDF excreted in the faeces is largely originating from silage. Comparing the undegradable NDF fraction with the NDF excreted in the faeces, shows that approximately 48, 57, 61 and 66% of the NDF excreted is undegradable for the silages G1, G2, G3 and G4, respectively (Bosch *et al.*, submitted[a]). The increase in the undegradable fraction of particles with an increase in cell wall content of the silages probably results in a higher FSG and therefore, apart from G3, in an increase in size of particles able to leave the rumen.
Rumination times per kg DM ingested were lowest for silage G3, suggesting a lower resistence to comminution by mastication compared to the other silages. Probably, for silage G3, particles are small enough to leave the rumen before they have a high enough FSG, meaning that there is still considerable microbial activity and thus gas production. These small particles then have to be ruminated again to remove the fermentation gas and the necessary decrease in rumen fill is therefore obtained by passage of, on average, smaller particles.

It seems that the longer CT per kg DM ingested for silages with higher cell wall contents does not compensate enough, resulting in smaller fractions of the small particle pool passing a 0.071 mm sieve, and as a result higher MFP's.

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Chapter V

# Influence of stage of maturity of grass silages on digestion processes in dairy cows. 4. Protein digestion and microbial protein synthesis in the rumen.

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**Abstract.**

In four change-over experiments, wilted grass silages, differing in cell wall content, were fed ad libitum to dairy cows in early and late lactation.

Ruminal degradation rate of the crude protein (CP) fraction of the silages was investigated using nylon bag incubations. No significant relation between the degradation rate ($k_d$, $\%h^{-1}$) and the cell wall content of the silages was found. The soluble ($f_S$, %) and undegradable ($f_R$, %) fractions of the CP both increased with an increase in silage cell wall content. The fraction of dietary protein escaping rumen fermentation ($f_E$, %) increased with cell wall content.

In duodenal protein, the fraction originating from the diet decreased with cell wall content.

**Introduction.**

Feeding grass products to dairy cows usually results in considerable losses of nitrogen (N). These losses can be reduced if the N intake is decreased by lowering the crude protein (CP) content of the grass (Van Vuuren & Meijs, 1987). Ensiling the grass at a more mature stage, results in lower CP contents, but normally also reduces its digestibility (Van Soest, 1982).

The protein entering the small intestine of ruminants consists of microbial protein synthesized in the rumen, dietary protein which escaped rumen fermentation and endogenous protein. The digestion of this protein mixture in the small intestine determines the amount of amino acids coming available for the ruminant.

The degradability of feed protein in the rumen can be estimated using the nylon-bag technique (Mehrez & Ørskov, 1977). This technique gives information on the fraction of feed protein escaping rumen fermentation, though not on the microbial protein synthesis in the rumen.

Different methods to estimate the fraction of duodenal protein

originating from the diet are possible e.g. with markers specific for microbial protein (DAPA, nucleic acids, $^{35}S$, $^{15}N$), or on the basis of the amino acid profiles (AAP) of the different protein sources (Van Bruchem *et al.*, 1985).

In four experiments, in which the effect of chemical composition of grass silages on intake and digestion processes was investigated (Bosch *et al.*, submitted[8]), CP degradation in the rumen was determined using the nylon-bag method. In addition, the fractions of microbial and dietary protein in ruminal and duodenal protein were estimated.

**Materials and Methods.**

In four change-over experiments, grass silages were fed ad libitum to dairy cows, supplemented with 7 (Exps. 2 and 4) or 1 (Exps. 1 and 3) kg of concentrates, in early and late lactation, respectively. The silages (G1 to G5), were harvested at different growth stages, resulting in different chemical compositions. The composition of the silages and the concentrates is given in Table 1.

Table 1. Chemical composition of the five grass silages and the concentrates fed (mean of four experiments).

| | G1 | G2 | G3 | G4 | G5 | concentrates |
|---|---|---|---|---|---|---|
| DM | 59.4 | 54.3 | 60.8 | 38.7 | 55.0 | 88.1 |
| in DM | | | | | | |
| OM | 86.8 | 90.8 | 89.8 | 92.5 | 92.6 | 90.4 |
| CP | 21.3 | 19.6 | 20.9 | 11.9 | 11.2 | 18.2 |
| NDF | 44.6 | 54.7 | 54.8 | 64.1 | 67.3 | 28.6 |
| cellulose | 23.4 | 29.3 | 25.8 | 33.6 | 32.9 | 12.4 |
| hemicellulose | 19.1 | 22.0 | 26.1 | 25.9 | 26.9 | 14.5 |
| lignin | 2.1 | 3.4 | 2.9 | 4.6 | 7.5 | 1.7 |
| $NH_3$[1] | 4 | 6 | 2 | 10 | 5 | |
| harvesting | 7-2 | 5-28 | 8-12 | 6-23 | 7-6 | |
| date | '85 | '85 | '86 | '86 | '87 | |

[1] $NH_3$-N as a % of total N

Table 2. Dry matter (DM) and nitrogen (N) intake, apparent overall digestibility and the mean live weight of the cows in the four experiments.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1 | G2 | SEM | G3 | G4 | SEM | G1 | G2 | SEM | G3 | G5 | SEM |
| silage intake | | | | | | | | | | | | |
| DM | 12.0 | 11.1 | 0.17 NS | 14.1 | 10.7 | 0.10 *** | 10.8 | 10.5 | 0.18 NS | 12.1 | 9.9 | 0.23 * |
| N | 0.42 | 0.35 | 0.01 ** | 0.47 | 0.20 | 0.01 *** | 0.39 | 0.33 | 0.01 * | 0.41 | 0.17 | 0.01 ** |
| total intake | | | | | | | | | | | | |
| DM | 12.8 | 12.0 | 0.17 NS | 15.0 | 11.5 | 0.10 *** | 17.0 | 16.7 | 0.18 NS | 18.2 | 16.0 | 0.23 * |
| N | 0.44 | 0.37 | 0.01 ** | 0.50 | 0.23 | 0.01 *** | 0.57 | 0.52 | 0.01 * | 0.58 | 0.35 | 0.01 ** |
| apparent digestibility | | | | | | | | | | | | |
| DM | 73.0 | 68.2 | 0.22 *** | 68.6 | 62.5 | 0.14 *** | 72.0 | 68.8 | 0.15 *** | 69.0 | 61.5 | 0.26 *** |
| N | 69.7 | 68.4 | 0.21 * | 68.0 | 59.3 | 0.21 *** | 67.5 | 66.4 | 0.19 * | 67.2 | 61.6 | 0.20 ** |
| weight of cows | 546 | 550 | 1.99 NS | 560 | 534 | 2.29 ** | 569 | 576 | 2.19 NS | 579 | 570 | 2.50 NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05

The silages were supplied three times a day; at 7.00, 15.00 and 23.00 h, and the concentrates were fed at 14.45 h (1 kg) or at 6.45 and 14.45 h (two portions of 3.5 kg). The experimental design is described in detail by Bosch *et al.* (submitted[a]). Intake and overall digestibility figures of dry matter (DM) and crude protein (CP) are given in Table 2.

The degradation rate ($k_d$, $\%h^{-1}$), the soluble ($f_S$, %) and the rumen undegradable ($f_R$, %) fractions of the CP of the silages were measured by means of nylon bag incubations, according to the procedure described by Bosch *et al.* (submitted[a]), but without a lagtime in the degradation model. Bags were incubated in the rumen for 0, 3, 5, 8, 16, 24, 48, 72 and 336 h, the first and the last to determine $f_S$ and $f_R$, respectively.

During 72 h, duodenal samples (approximately 0.25 l, after collecting first 1 l which was returned into the fistula after sampling) were taken every two hours, pooled per cow per week, freeze dried, ground through a 1 mm screen and stored till further analysis.

From rumen liquid samples, taken from the ventral rumen sac during the first two days of these periods at 7.00, 9.00, 11.00 and 13.00 h, microbes were isolated by differential centrifugation (550 and 70000 g) with a MSE superspeed 65 centrifuge at 4 °C. The pellet was washed twice with a buffer solution according to the method described by Meyer *et al.* (1967), and then freeze dried, ground and stored till analysis.

Samples of total rumen contents were taken during the last weeks of the experimental periods as described by Bosch *et al.* (submitted[b]).

The silages, concentrates, rumen bacteria and ruminal and duodenal contents were analysed for nitrogen (N) and individual amino acids, including Diaminopimelic acid (DAPA).

Nitrogen was determined according to the Kjeldahl method (micro-Kjeldahl for the rumen bacteria) with $K_2SO_4$ and HgO as catalysts. DAPA and the individual amino acids were determined using a Biotronic LC 6000 automatic amino acid analyser. Samples were hydrolysed under reflux for 22 h with HCl (6 mol $l^{-1}$) at $110^0$C. After formic acid oxidation (Moore, 1963), the sulphur

Table 3. The soluble ($f_S$, %) and undegradable ($f_R$, %) fractions of the crude protein of five silages, and the rate of degradation ($k_d$, %/h) of the crude protein of the silages.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| $f_S$ | 49.7 | 52.4 | | 41.6 | 56.4 | | 49.7 | 52.4 | | 41.6 | 57.3 | |
| $f_R$ | 5.9 | 10.0 | | 9.4 | 18.1 | | 5.9 | 10.0 | | 9.4 | 24.4 | |
| $k_d$ | 4.81 | 4.04 | 0.13 * | 5.19 | 4.30 | 0.13 * | 6.42 | 5.48 | 0.13 * | 4.53 | 3.96 | 0.21 NS |

NS not significant, * $P<0.05$

Table 4. Estimated fractions of silage protein escaping rumen fermentation ($f_E$, %) assuming fractional passage rates ($k_p$, %/h) of 3 and 4.5 %.

| | late lactation | | | | early lactation | | | |
|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | Exp.3 | | Exp.2 | | Exp.4 | |
| | G1L | G2L | G3L | G4L | G1H | G2H | G3H | G5H |
| $f_E$ $k_p$ = 3 %h$^{-1}$ | 19.0 | 23.4 | 24.4 | 26.6 | 15.7 | 18.0 | 25.2 | 30.1 |
| $f_E$ $k_p$ = 4.5 %h$^{-1}$ | 23.0 | 27.0 | 29.0 | 29.1 | 19.1 | 20.9 | 29.8 | 31.8 |

containing amino acids, methionine and cystine, were determined as methionine sulphone and cysteic acid, respectively. In this analysis also DAPA was determined. Tryptophan was not determined.

Data were analysed statistically using the Anova procedure of the spsspc+ statistical package (SPSS Inc., 1988) as described by Bosch *et al.* (submitted[a]).

**Results.**

The degradation characteristics of the CP fraction of the five silages are given in Table 3. The soluble ($f_S$) and the undegradable ($f_R$) fractions both increased with an increase in cell wall content of the silages, except for the $f_S$ of silage G3. Silage G3 had a lower soluble protein fraction as compared to the other silages. The $k_d$ was significantly higher ($P<0.05$) for the low cell wall silages in Exps. 1, 2 and 3. There was a negative, though not significant relation between the $k_d$ and the neutral detergent fibre (NDF) content of the silages ($r=-0.68$).

Using the nylon bag degradation characteristics, and assuming a fractional passage rate from the rumen ($k_p$, $\%h^{-1}$), the fraction of silage protein escaping rumen fermentation ($f_E$) can be calculated according to the equation:

$$f_E = f_R + (100 - f_S - f_R) * (k_p/(k_d + k_p))$$

The thus calculated $f_E$'s are given in Table 4. With an increase in cell wall content, $f_E$ seems to increase. For a higher $k_p$ (4.5 $\%h^{-1}$ vs 3 $\%h^{-1}$), the differences became smaller.

The fraction of protein entering the duodenum originating from the diet, was also estimated, based on the DAPA/N ratio in the microbes isolated from the rumen liquid relatively to the DAPA/N ratio in the duodenal samples, and based on the amino acid profile (AAP) of dietary, microbial and duodenal proteins. The AAP of the protein consumed (silage + concentrates), and the AAP's of ruminal, microbial and duodenal proteins are given in Table 5. Dietary and microbial proteins were mixed by a

computerized iterative procedure in such proportions that the computed AAP matched best the actual AAP of duodenal protein, on the basis of minimizing the objective function (Van Bruchem *et al.*, 1985) :

$$\sum_{AA=1}^{17} (1-AA_{computed}/AA_{actual})^2$$

The results of both methods are given in Table 6. In addition, the fraction of protein in the rumen, consisting of dietary protein was calculated according to the same methods. No significant ($P>0.05$) differences in the fractions of protein in the duodenum originating from the diet were found, except for Exp. 3, in which the AAP method gave significantly ($P<0.001$) lower figures for silage G4 than for silage G3. Though not significant, within experiments both methods gave a lower fraction of dietary protein in the duodenum for the silage with the highest cell wall content. Compared to the other results, the figures obtained in Exp. 2 using the DAPA-method were too low. Except for Exp. 2, the DAPA-method gave higher values than the AAP-method. The fraction of protein in the rumen consisting of dietary protein, estimated according to both methods is also given in Table 6. The DAPA-method resulted in most cases in estimated fractions consisting of microbial protein of over 100%, and therefore in negative fractions of dietary protein. In experiments 2, 3 and 4, the AAP-method gave lower microbial protein fractions for the low cell wall silages than for the high cell wall silages. In Exp. 1, however, the opposite was found. A higher fraction of protein in the rumen was estimated to originate from the diet for the low cell wall silage (G1L).

**Discussion.**

During incubation in nylon bags in the rumen the contents of the bags becomes initially contaminated with microbial N, which, according to Nocek & Grant (1987), is largely responsible for the lag time (t') occasionally observed. Microbial contamination is

Table 5A. Amino acid profiles (AAP, mol per 100 mol amino acid) of dietary protein (F), microbial protein (M), ruminal protein (R) and duodenal protein (D) in experiments 1 and 2 and the DAPA-N contents of microbial, ruminal and duodenal proteins (μg/g N).

| | Experiment 1 | | | | | | | | Experiment 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G1L | | | | G2L | | | | G1H | | | | G2H | | | |
| | F | M | R | D | F | M | R | D | F | M | R | D | F | M | R | D |
| Cys | 1.02 | 0.79 | 0.67 | 0.99 | 0.76 | 0.83 | 0.68 | 1.54 | 0.82 | 0.67 | 0.89 | 0.89 | 0.96 | 0.67 | 0.98 | 0.93 |
| Asp | 9.55 | 11.48 | 10.24 | 10.12 | 11.83 | 11.47 | 11.32 | 10.17 | 9.39 | 11.21 | 10.32 | 9.87 | 10.41 | 11.29 | 10.39 | 10.06 |
| Met | 1.45 | 2.10 | 2.12 | 1.69 | 1.40 | 2.07 | 2.10 | 1.80 | 1.54 | 2.08 | 2.26 | 1.51 | 1.48 | 2.04 | 2.37 | 1.50 |
| Tre | 5.35 | 6.34 | 5.71 | 5.83 | 5.35 | 6.41 | 5.77 | 5.82 | 4.97 | 6.14 | 5.69 | 5.59 | 4.71 | 6.17 | 5.69 | 5.70 |
| Ser | 6.13 | 5.74 | 5.97 | 6.08 | 6.41 | 5.78 | 6.02 | 6.14 | 6.08 | 5.73 | 6.00 | 6.20 | 6.02 | 5.80 | 6.06 | 6.07 |
| Glu | 10.23 | 11.11 | 10.77 | 10.71 | 10.36 | 10.97 | 10.80 | 10.80 | 11.71 | 11.09 | 11.23 | 11.20 | 11.95 | 11.10 | 11.44 | 11.32 |
| Pro | 8.57 | 3.75 | 5.11 | 4.66 | 7.83 | 3.94 | 5.04 | 4.68 | 8.76 | 3.05 | 5.12 | 4.58 | 9.12 | 3.04 | 5.03 | 4.85 |
| Gly | 9.51 | 9.46 | 9.84 | 11.13 | 9.11 | 9.43 | 9.72 | 10.73 | 8.99 | 9.34 | 9.49 | 11.68 | 8.58 | 8.99 | 9.50 | 10.92 |
| Ala | 10.30 | 10.80 | 10.02 | 9.60 | 10.37 | 10.57 | 9.98 | 9.42 | 9.38 | 10.39 | 9.30 | 9.11 | 9.05 | 10.23 | 9.41 | 9.26 |
| Val | 7.51 | 6.98 | 7.16 | 6.95 | 7.31 | 6.95 | 7.05 | 6.86 | 7.04 | 7.08 | 7.27 | 6.81 | 6.97 | 7.13 | 7.29 | 6.86 |
| Ile | 4.90 | 5.38 | 5.74 | 5.37 | 4.92 | 5.47 | 5.63 | 5.40 | 4.88 | 5.70 | 5.76 | 5.27 | 4.76 | 5.75 | 5.71 | 5.36 |
| Leu | 8.22 | 6.99 | 8.42 | 8.00 | 7.99 | 7.04 | 8.15 | 8.01 | 8.11 | 7.31 | 8.40 | 8.02 | 7.71 | 7.33 | 8.09 | 7.96 |
| Tyr | 2.54 | 3.60 | 2.90 | 3.18 | 2.17 | 3.61 | 2.74 | 3.08 | 2.39 | 3.34 | 2.96 | 3.01 | 2.12 | 3.47 | 2.79 | 2.98 |
| Phe | 4.46 | 3.85 | 4.59 | 4.37 | 4.12 | 3.94 | 4.37 | 4.28 | 4.14 | 3.87 | 4.46 | 4.15 | 3.84 | 3.91 | 4.26 | 4.10 |
| Lys | 4.80 | 6.88 | 5.26 | 5.96 | 5.12 | 6.83 | 5.23 | 5.98 | 4.23 | 6.89 | 4.73 | 5.59 | 4.17 | 6.95 | 4.93 | 5.73 |
| His | 1.73 | 1.41 | 1.91 | 1.85 | 1.69 | 1.40 | 1.90 | 1.79 | 3.38 | 2.51 | 2.57 | 2.81 | 4.34 | 2.54 | 2.57 | 2.72 |
| Arg | 3.73 | 3.34 | 3.57 | 3.51 | 3.26 | 3.29 | 3.50 | 3.48 | 4.19 | 3.60 | 3.55 | 3.71 | 3.81 | 3.59 | 3.49 | 3.68 |
| DAPA-N | | 29.1 | 20.8 | 12.8 | | 31.2 | 22.5 | 14.3 | | 32.1 | 30.0 | 20.3 | | 31.2 | 33.3 | 20.9 |

Table 5B. Amino acid profiles (AAP, mol per 100 mol amino acid) of dietary protein (F), microbial protein (M), ruminal protein (R) and duodenal protein (D) in experiments 3 and 4 and the DAPA-N contents of microbial, ruminal and duodenal proteins (μg/g N).

| | Experiment 3 | | | | | | | | Experiment 4 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G3L | | | | G4L | | | | G3H | | | | G5H | | | |
| | F | M | R | D | F | M | R | D | F | M | R | D | F | M | R | D |
| Cys | 0.56 | 0.69 | 0.89 | 0.91 | 0.86 | 0.75 | 1.21 | 0.98 | 0.68 | 0.65 | 0.72 | 0.95 | 0.60 | 0.70 | 0.82 | 0.93 |
| Asp | 9.49 | 11.60 | 10.24 | 9.80 | 8.22 | 11.36 | 10.58 | 10.03 | 9.20 | 11.31 | 10.10 | 9.71 | 8.55 | 11.12 | 10.24 | 10.28 |
| Met | 1.41 | 2.05 | 2.01 | 1.63 | 1.23 | 2.00 | 2.05 | 1.60 | 1.46 | 1.93 | 1.97 | 1.71 | 1.38 | 1.80 | 1.97 | 1.67 |
| Tre | 5.27 | 5.80 | 5.65 | 5.70 | 4.45 | 6.24 | 5.61 | 5.68 | 5.15 | 6.16 | 5.69 | 5.73 | 4.66 | 6.08 | 5.61 | 5.73 |
| Ser | 6.04 | 5.43 | 5.85 | 6.06 | 5.32 | 5.96 | 5.67 | 6.08 | 6.05 | 5.74 | 5.94 | 6.09 | 5.75 | 6.17 | 5.88 | 5.97 |
| Glu | 9.64 | 10.63 | 10.62 | 10.23 | 9.93 | 10.90 | 11.19 | 10.51 | 9.64 | 10.99 | 10.52 | 10.04 | 9.66 | 11.79 | 11.40 | 10.51 |
| Pro | 7.20 | 2.73 | 5.39 | 4.22 | 10.33 | 2.74 | 4.89 | 3.58 | 7.84 | 2.76 | 5.72 | 4.29 | 9.67 | 2.97 | 4.69 | 3.79 |
| Gly | 9.37 | 9.49 | 9.73 | 10.87 | 8.61 | 9.43 | 9.74 | 12.35 | 9.28 | 9.32 | 9.41 | 10.49 | 8.74 | 9.28 | 9.52 | 11.59 |
| Ala | 9.98 | 10.61 | 9.64 | 9.01 | 11.79 | 10.72 | 10.15 | 9.10 | 10.16 | 10.40 | 9.73 | 8.94 | 11.55 | 10.27 | 10.16 | 9.11 |
| Val | 7.42 | 7.71 | 7.29 | 6.95 | 7.51 | 7.11 | 7.44 | 6.87 | 7.32 | 7.61 | 7.32 | 6.98 | 7.47 | 7.01 | 7.37 | 6.84 |
| Ile | 4.41 | 5.49 | 5.66 | 5.46 | 4.21 | 5.41 | 5.70 | 5.24 | 4.37 | 5.47 | 5.65 | 5.54 | 4.28 | 5.22 | 5.88 | 5.32 |
| Leu | 7.87 | 7.25 | 8.46 | 8.36 | 6.95 | 7.12 | 7.60 | 7.59 | 7.63 | 7.09 | 8.39 | 8.43 | 7.14 | 7.03 | 7.89 | 7.61 |
| Tyr | 2.19 | 3.32 | 2.85 | 3.13 | 1.53 | 3.18 | 2.64 | 2.73 | 2.08 | 3.26 | 2.94 | 3.08 | 1.65 | 3.10 | 2.66 | 2.72 |
| Phe | 3.99 | 3.73 | 4.61 | 4.47 | 3.47 | 3.73 | 4.16 | 3.97 | 3.92 | 3.60 | 4.52 | 4.43 | 3.55 | 3.55 | 4.08 | 3.88 |
| Lys | 4.65 | 6.98 | 4.81 | 5.81 | 4.07 | 6.78 | 5.25 | 6.31 | 4.63 | 7.31 | 4.79 | 5.84 | 4.24 | 6.87 | 5.10 | 6.36 |
| His | 6.41 | 2.89 | 2.59 | 3.50 | 8.80 | 3.00 | 2.70 | 3.61 | 6.74 | 2.83 | 3.12 | 3.68 | 8.29 | 3.30 | 3.61 | 3.96 |
| Arg | 4.10 | 3.60 | 3.71 | 3.89 | 2.72 | 3.54 | 3.42 | 3.77 | 3.85 | 3.57 | 3.49 | 4.06 | 2.82 | 3.75 | 3.12 | 3.73 |
| DAPA-N | | 33.0 | 29.7 | 17.4 | | 33.1 | 39.2 | 21.2 | | 40.5 | 39.3 | 22.2 | | 40.8 | 59.2 | 23.8 |

Table 6. Fractions of duodenal protein originating from the diet, based on the AAP- (D-$F_{AAP}$, %) and the DAPA (D-$F_{DAPA}$, %) method, and the fraction of ruminal protein consisting of dietary protein, based on the AAP- (R-$F_{AAP}$, %) and the DAPA (R-$F_{DAPA}$, %) method.

| | late lactation | | | | | | early lactation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | | | Exp.3 | | | Exp.2 | | | Exp.4 | | |
| | G1L | G2L | SEM | G3L | G4L | SEM | G1H | G2H | SEM | G3H | G5H | SEM |
| D-$F_{AAP}$ | 35 | 32 | 0.45 NS | 34 | 13 | 0.64 *** | 38 | 34 | 0.86 NS | 42 | 32 | [1]) |
| D-$F_{DAPA}$ | 51 | 48 | 3.20 NS | 47 | 42 | 1.25 NS | 31 | 29 | 1.24 NS | 48 | 40 | [1]) |
| R-$F_{AAP}$ | 34 | 48 | 1.64 * | 21 | 10 | 0.99 ** | 42 | 34 | 0.45 ** | 38 | 33 | 0.79 NS |
| R-$F_{DAPA}$ | 27 | 27 | 1.91 NS | 10 | -9 | 2.69 * | 7 | -9 | 1.82 * | 2 | -50 | 2.90 * |

[1]) 2 cows with a duodenal cannula, no SEM could be calculated
NS not significant, *** P<0.001, ** P<0.01, * P<0.05

proportionally higher for feeds high in cell walls and lower for higher protein feeds (Varvikko & Lindberg, 1985; Varvikko, 1986). After correcting for this microbial protein, the t' decreases and the degradation pattern of the protein changes. For different feeds, N-disappearance from the nylon bags as well as DM-disappearance was in all cases lower if not corrected for microbial contamination. Because of microbial contamination, a lag time was particularly observed for the high roughage diets in these experiments. It may be assumed that in the course of degradation, not only the feed protein but also microbial protein dissappears from the nylon bag, resulting in a too fast decline of protein in the bags. Thus, if the $k_d$-CP is calculated using a model without a lag-time, the $k_d$ will be less biassed due to microbial contamination.

Disappearance rate from the nylon bags of DM and cell wall components was negatively related to the cell wall content of the silages (Bosch *et al.*, submitted[a]). A decrease in $k_d$ for the cell wall fraction makes the protein, which is in the cell content or associated with the cell walls, less accessible for the bacteria. As a result, the $k_d$-CP is, though not significantly, decreasing with cell wall content of the silages.

The $f_s$-CP seems to increase with cell wall content, except for silage G3, which had an even lower $f_s$-CP than silage G1. Tamminga *et al.* (1990) showed, that the $f_s$-CP of grass silages was best described by a regression equation with DM content of the silage and number of days elapsed since May 1st at harvesting. Both factors had a negative effect on $f_s$-CP. For the silages used in our experiments, this regression equation predicted the lowest $f_s$-CP for silage G3, which agrees with our observations.

It is often assumed that the $f_s$ is rapidly and fully degraded in the rumen. The $f_s$ is likely to have a high degradation rate, but also a higher outflow rate from the rumen. Therefore it does not seem correct to assume a 100% degradation of $f_s$ in the rumen. This underestimation is considered to compensate the slight overestimation which may result from microbial contamination. It is, however, not possible to give a precise estimate of the fraction of $f_s$ that is escaping rumen fermentation.

According to Bosch *et al.* (submitted[b]), the $k_p$ increases with an increase in cell wall content of the silage. As shown in Table 4, the differences in $f_E$ between silages will be bigger when passage rate is higher for the high cell wall diets than for the low cell wall diets. The higher $f_E$ for the high cell wall diets mainly consists of rumen undegradable protein. The rumen undegradable protein fraction is also undegradable in the small intestines (Tamminga *et al.*, 1990), and therefore not available for the animal. The fraction of dietary protein escaping rumen fermentation and digestible in the small intestine is thus higher for the low cell wall diets.

The DAPA-N/N ratio in total rumen contents was in most cases higher than the DAPA-N/N ratio in bacteria isolated from the rumen fluid. According to McAllan and Smith (1983), the DAPA-N content of mixed rumen bacteria is rather constant. In our experiments however, DAPA-N content in bacteria isolated from the rumen fluid, varies from approximately 16 μmol/g to 23 μmol/g. In duodenal samples, the DAPA-N/N ratio was lower than in the isolated rumen bacteria, resulting in positive fractions of the protein originating from the feed. A larger fraction of duodenal microbial protein, in comparison with total rumen microbial protein, seems to originate from bacteria normally present in rumen fluid. A smaller fraction then comes from bacteria attached to the feed particles, which seem to have a higher DAPA-N/N ratio. Hence, using DAPA as a marker for microbial protein does not seem to give very reliable results.

The amino acid composition of bacterial cell wall is close to the amino acid composition of bacterial cell content, with the exception of DAPA, which is only in the cell wall fraction (Hoogenraad & Hird, 1970). Probably the bacteria in the rumen fluid have a lower cell wall fraction than the bacteria attached to the feed particles.

The amino acid composition of the undegraded feed protein can differ considerably from the amino acid composition of the original feed protein (Varvikko, 1986; Susmel *et al.*, 1989). The AAP of mixed rumen bacteria, however, is very constant and is not really affected by the dietary amino acid profile (Storm &

Ørskov, 1983). Differences between the dietary AAP and the AAP of undegraded feed protein probably interfere with the AAP-method to estimate the fraction of duodenal protein consisting of dietary protein.

According to van Soest (1982), the amount of amino acid nitrogen absorbed in the intestines exceeds the dietary intake of nitrogen below about 12.5% CP in the diet. The amount of N entering the duodenum on cell wall rich diets (G4) can be considerably higher than the N-intake, whereas on protein rich diets, great losses of N in the rumen occur. A high fraction of dietary protein escaping rumen fermentation on diets low in protein, still results in a low absolute dietary-N flow to the duodenum. When the N-flow to the duodenum exceeds N-intake, the fraction of duodenal protein originating from the diet will be lower than $f_E$.

In general, despite the higher $f_E$-values for the high cell wall silages, the fraction of duodenal protein originating from the diet is bound to decrease with cell wall content. An increase in $f_E$ with cell wall content, still results in a low dietary protein content in the duodenum, which consists for a higher proportion of indigestible protein.

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Chapter VI

# Ruminal passage rate as affected by Cr-NDF particle size.

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**Abstract.**

Fractional rumen passage rates of three particle sizes CrNDF (<0.3 mm, 0.6-1.0 mm and 15-25 mm) and of CoEDTA were estimated in an experiment with three lactating and three non-lactating rumen fistulated cows, fed grass silage ad lib and 7 and 1 kg of concentrates, respectively. Total feed intake was higher for the lactating cows, but silage intake did not differ significantly.

Fractional passage rates of CrNDF and CoEDTA were derived from the descending parts of the faecal excretion curves and from CoEDTA measured directly in the rumen fluid, following a pulse dose into the rumen. The faecal excretion curves of the 15-25 mm CrNDF particles showed no decline until the last sampling time at 86 h after dosage, so fractional passage rates of these particles could not be determined. Fractional passage rates did not differ significantly between lactating and non-lactating cows and were on average 2.0, 4.1, 8.6 and 14.9 $\%h^{-1}$ for CrNDF (0.6-1.0 mm), CrNDF (<0.3 mm), CoEDTA-faeces and CoEDTA-rumen, respectively.

The results of this experiment indicate that particle size of the CrNDF marker has a great influence on the determined passage rates.

**Introduction.**

Nutrient supply in ruminants is for a considerable part determined by reticulorumen contents and clearance rate of the ingested feed.

Reticulorumen contents depend among others on the animal's physiological demands for energy and the chemical and physical characteristics of the feed. Values for cattle range from 10 to 20 % of body weight (Owens & Goetsch, 1988; Van Soest, 1982). Reticulorumen contents can be subdivided in a liquid and a particulate phase. The liquid phase partly contains particles and nutrients which behave like liquid and is partly

bound to particles. The particulate phase consists of particles which are able, and of particles which are as yet not able to leave the reticulorumen (Owens & Goetsch, 1986). The limits between the different particle pools depend on particle size, particle shape and functional specific gravity (Kennedy & Murphy, 1988). When particle size is considered, particles smaller than 0.15 mm are expected to behave like liquid (Faichney, 1986). Particles passing a sieve with 1.18 mm pore size are believed to have a relatively high probability to leave the reticulorumen; on the other hand particles retained on this sieve have a low probability to leave and hence need further physical size reduction before they can leave (Poppi *et al.*, 1980; Kennedy & Poppi, 1984).

Clearance rate of the ingested feed from the reticulorumen is the result of microbial degradation and of passage to the omasum. The extent of microbial degradation within the reticulorumen depends, in addition to the chemical and physical feed characteristics, on the retention time of the feed in this compartment (Van Soest, 1982). Passage rate is inversely related to rumen retention time and depends amongst others on feed intake, chemical composition of the feed, particle size, functional specific gravity and site of sampling. Passage rate increases with a higher feed intake level and decreases with high concentrate levels in the diet (Colucci *et al.*, 1984; Owens & Goetsch, 1986). Particle size influences passage rate by the increased probability of smaller particles to leave the reticulorumen (Kennedy & Poppi, 1984). A higher functional specific gravity results in an increased probability to escape from the reticulorumen (Sutherland, 1987). Welch (1986) observed maximal passage rates of plastic particles with a specific gravity of 1.17 to 1.42. The site of sampling results in higher estimated passage rates by ruminal sampling compared with faecal sampling (Snijder *et al.*, 1984; Bosch & Bruining, submitted).

Passage rates of the particulate ($k_p$) and the liquid ($k_l$) phases can be estimated with the external markers CrNDF and CoEDTA, respectively. The objective of this investigation was

to determine the influence of CrNDF particle size on the estimated passage rate of particles measured by faecal sampling. The second objective was to check whether the smallest particles flow with the liquid phase through the digestive tract.

**Material and methods.**

**Animals.** In this experiment, 3 lactating and 3 non-lactating 4 year old FH * HF cows were used. At the onset of the experiment the 3 cows were lactating for 1, 2 and 2 months, respectively. Two of the non-lactating cows were pregnant, both 6 months. The cows were provided with a large cannula in the dorsal rumen sac. Average live weight of the lactating and non-lactating cows was 606 ± 50 kg and 702 ± 79 kg, respectively.

**Diets.** The cows were fed ad libitum with chopped prewilted grass silage 3 times daily at 7.00 h, 15.00 h and 23.00 h. The lactating cows were supplied 2 times daily 3.5 kg mixed concentrates at 7.00 and 15.00 h, the non-lactating cows were fed 1 kg mixed concentrates at 15.00 h (Table 1). Drinking water was freely available through an automatic drinking device. Dry matter (DM) content of the silage and concentrates was determined by drying at 103 °C, N content according to Kjeldahl and cell wall constituents according to Goering & Van Soest (1970).

Table 1. Chemical composition of the experimental feeds (%).

| | grass silage | concentrates |
|---|---|---|
| Dry Matter (DM) | 53.6 | 87.4 |
| in the DM: | | |
| Organic Matter (OM) | 90.4 | 91.0 |
| Crude Protein (CP) | 18.3 | 18.6 |
| Neutral Detergent Fibre (NDF) | 47.4 | 32.5 |
| Acid Detergent Fibre (ADF) | 26.7 | 17.7 |
| Lignin (ADL) | 2.2 | 2.5 |

**Markers.** As liquid and particulate phase markers were used CoEDTA and CrNDF, respectively (prepared according to Uden *et al.*, 1980). Three CrNDF particle sizes were prepared: <0.3 mm (Extra Fine), 0.6-1.0 mm (Fine) and 15-25 mm (Coarse) containing 5.44, 4.47 and 3.96 % Cr in air dry matter, respectively.

Table 2. Scheme of administration of the three CrNDF particle sizes[1] to the six cows during the three experimental weeks.

| Cow | Stage of lactation | Week 1 | Week 2 | Week 3 |
|---|---|---|---|---|
| 1 | non-lactating | EF | F | C |
| 2 | " " | F | C | EF |
| 3 | " " | C | EF | F |
| 4 | lactating 1 month | C | EF | F |
| 5 | " 2 months | F | C | EF |
| 6 | " 2 months | EF | F | C |

[1] EF = particle size < 0.3 mm (Extra Fine)
F = " " 0.6-1.0 mm (Fine)
C = " " 15-25 mm (Coarse)

**Experiments.** In a preliminary period of 3 weeks the cows could adapt to diet, feeding times and stable. Then 3 weeks experimental period followed in which feed organic matter intake was determined per day. A Latin square design was used to allocate the 3 CrNDF particle sizes over the weeks, to the lactating and non-lactating cows (Table 2). At the onset of each experimental week, on Monday at 6.00 a.m., pulse doses of 30 g CoEDTA and 100 g CrNDF of a distinct particle size were given through the rumen cannula. Subsequently rumen liquid samples were taken by sucking up a sample with a perforated tube through the cannula every 2 h, from Monday morning 9.00 h till Tuesday morning 7.00 h. Faeces were collected over 2-h collecting periods, from Monday 10.00 h till Tuesday 24.00 h. On Wednesday and Thursday, faeces were collected over 4 h collecting periods from 6.00 h till 22.00 h. Faecal collections were weighed and samples were dried and ground to

pass a 1 mm sieve for analysis. Co in rumen centrifugated liquid and Co and Cr in dried and wet destructed faeces were determined by atomic absorption spectrophotometry (Varian, SpectrAA-300) at wavelengths of 251.0 and 357.9 nm for Co and Cr, respectively.

**Calculations.** Passage rate constants of Co in rumen liquid and of Co and Cr in faeces were derived from the equation:

$$C_t = C_0 * e^{-kt}$$

in which: $C_t$ = marker concentration (ruminal or faecal) at time t (ppm)

$C_0$ = marker concentration at time 0 (ppm), calculated intercept from descending part of the curve

k = fractional rate constant ($\%h^{-1}$)

t = hours after pulse dose of CoEDTA and CrNDF.

**Statistics.** An analysis of variance was performed to test the effects of lactation stage, animal within lactation stage, experimental week and the interaction of lactation stage and experimental week on ruminal and faecal CoEDTA passage rates. The Fine and Extra Fine faecal CrNDF passage rates were also analysed by analysis of variance taking into account the same effects, but instead of the interaction between lactation stage and experimental week particle size was included. With two-sided Student t values significances were tested between lactating and non-lactating cows for feed intake and CrNDF passage rates. Comparisons between ruminal and faecal CoEDTA passage rates and between faecal CoEDTA and Extra Fine CrNDF passage rates were made by using a paired t-test.

**Results and discussion.**

The lactating cows had a significantly ($P<0.001$) higher average feed intake than the non-lactating cows (Table 3). The

higher feed intake of the lactating cows was primarely caused by the higher intake of concentrates, since average grass silage intake did not differ significantly. Also expressed as average intake per kg body weight, grass silage intake did not differ significantly between the lactating and non-lactating cows.

Table 3. Average total and grass silage DM and OM intake (kg/day) of the lactating (L) and non-lactating (NL) cows and average DM intake per bodyweight (g/kg BW; average ± standard deviation).

| | Total intake | | | Grass silage intake | | |
|---|---|---|---|---|---|---|
| | DM | OM | DM/BW | DM | OM | DM/BW |
| L | 18.0±0.8 | 16.4±0.7 | 29.9±2.7 | 11.9±0.8 | 10.8±0.7 | 19.8±2.1 |
| NL | 13.6±1.6 | 12.3±1.5 | 19.3±1.2 | 12.7±1.6 | 11.5±1.5 | 18.0±1.2 |

Daily water consumption of the lactating and non-lactating cows was 76 ± 9 and 47 ± 11 kg per day, respectively. The lactating cows had an average fat corrected milk production of 29.6 ± 1.2 kg per day.

Fractional passage rates of Fine and Extra Fine CrNDF particles and of CoEDTA, derived from the descending parts of the faecal excretion curves ($k_{p\text{-}Cr}$ and $k_{l\text{-}Co}$-faeces, respectively), and of CoEDTA measured directly in the rumen ($k_{l\text{-}Co}$-rumen), are given in Table 4. The faecal excretion curves of the Coarse CrNDF particles in some cases showed a decline in Cr concentration during the last few sampling times, but in most cases no decline even until 86 h after administration of the Cr could be observed. Fractional passage rates of the Coarse CrNDF particles administered in weeks 1 and 2 were calculated using the part of the curve where a decline in Cr concentration was found, including a sample taken at 168 h after administration. The latter sample was taken just before the next administration of the markers on Monday at 6.00 h. When administration of Extra Fine CrNDF particles was preceded

by Coarse CrNDF particles, the Cr concentrations were corrected for the residues originating from the Coarse CrNDF particles. Average and standard deviation of the Coarse particles passage rates used for this correction was 1.5 ± 0.2 % per hour. Probably the time needed for physical reduction delays the decline of the faecal excretion curve of the Coarse particles.

Table 4. Fractional passage rates (average ± standard deviation, $\%h^{-1}$) of Medium and Small CrNDF particles ($k_{p-Cr}$) and of CoEDTA ($k_{l-Co}$), derived from the faecal and ruminal marker decline.

| | lactating cows | non-lactating cows |
|---|---|---|
| $k_{p-Cr}$ (0.6-1.0 mm) | 2.1 ± 0.5[a] | 2.0 ± 0.2[a] |
| $k_{p-Cr}$ (<0.3 mm) | 4.0 ± 1.0[b] | 4.2 ± 0.4[b] |
| $k_{l-Co}$-faeces | 8.6 ± 0.9[c] | 8.6 ± 0.6[c] |
| $k_{l-Co}$-rumen | 14.7 ± 2.1[d] | 15.1 ± 1.2[d] |

[a,b,c,d]Data with different superscripts differ significantly ($P<0.001$).

There were no significant differences between lactating and non-lactating cows in passage rates of the particulate and liquid phase. Particulate and liquid phase passage rates usually increase with a higher feed intake (Colucci *et al.*, 1984; Owens & Goetsch, 1986; Tamminga *et al.*, 1989). As the lactating cows in this experiment had a higher total feed intake, a faster passage rate for the lactating cows was expected. However, the lactating cows can be expected to have a higher reticulorumen content than the non-lactating cows, as was found in other experiments (Bosch *et al.* (submitted), Bosch & Bruining (submitted)). Hence, with a higher flow of the particulate phase, fractional particle passage rates don't necessarily have to increase.

The higher water consumption of the lactating cows did not

result in faster particulate or liquid passage rates. This agrees with results of Harrison *et al.* (1975). They found in sheep no significant effect of water infusions into the rumen on the proportion of rumen volume leaving the rumen per unit of time. In this experiment, rumen liquid volume was calculated as the amount of Co administered in the rumen divided by the Co concentration at time zero established by regression analysis. Although fractional liquid passage rates were the same, because the lactating cows had a greater rumen liquid volume than the non-lactating cows (86 ± 11 versus 75 ± 17 kg) the flow of liquid was higher for the lactating cows.

Analysis of variance of the CrNDF and CoEDTA passage rates showed significant between animal within stage of lactation effects ($P<0.05$ for CrNDF and $P<0.01$ for ruminal and faecal CoEDTA passage rates). Differences between cows within lactation stage were considerably greater than average differences between lactating and non-lactating cows. For the lactating cows there was no relation between milk production and CrNDF or CoEDTA passage rates.

The $k_{l\text{-}Co}$-rumen was significantly higher ($P<0.001$) than the $k_{l\text{-}Co}$-faeces. This agrees with other observations (Bosch & Bruining (submitted)). The postruminal delay is possibly due to mixing and flow resistance in omasum, abomasum and gut.

Passage rate of the Extra Fine CrNDF particles was significantly lower ($P<0.001$) than passage rate of the liquid phase as measured in the faeces. This means that CrNDF particles <0.3 mm flow more slowly than the liquid through the gastrointestinal tract. This is in line with findings of Faichney (1986), who observed that particles passing the finest sieve (0.15 mm) and expected to behave as solutes, had a larger retention time than the fluid. The longer retention time of the Extra Fine CrNDF particles is presumably caused by sieving effects of the reticuloruminal mass (Sutherland, 1987). It is not likely that the longer retention time was caused by more flow resistance of the Extra Fine particles compared to the liquid phase in the digestive tract after the rumen, because other observations (Bosch & Bruining

(submitted)) showed a greater proportional decline between ruminal and faecal passage rates for $k_{l\text{-}Co}$ than for $k_{p\text{-}Cr}$.

Passage rate of CrNDF particles <0.3 mm was significantly ($P<0.001$) higher than passage rate of CrNDF particles 0.6-1.0 mm. According to Kennedy & Poppi (1984), particles which are able to pass a sieve with a pore size of 1.18 mm, have a high probability of leaving the rumen of cattle, while bigger particles have a lower probability. So the Fine and Extra Fine CrNDF particles both have a high probability to pass. However, size of reticuloruminal particles alone does not regulate rate of passage (Martz & Belyea, 1986). In the present experiment, the difference in passage rate is presumably caused by a variation in interaction with the reticuloruminal filterbed matrix. The Fine particles might stay longer in this matrix. However, according to Welch (1982), specific gravity has a bigger influence on passage rate of small particles than the sieving effects of the reticuloruminal mass. In general, passage rate increases with increasing specific gravity (Sutherland, 1987). This might be of influence in this experiment because the Extra Fine CrNDF particles contained relatively more Cr than the Fine CrNDF particles (5.44 and 4.47 % Cr, respectively). Also Ehle (1984) found that rumen turnover rate was positively related to chromium concentration and density of particles.

In the air dry CrNDF material without gas spaces, a specific gravity of the Fine and Extra Fine particles can be calculated (1.35-1.45 and 1.36-1.46, respectively) with the by Lange (1966) mentioned range of 1.3-1.4 for the specific gravity of cellulose, which is also likely for the hemicelluloses (Sutherland, 1987) and used here for the specific gravity of NDF. In the reticulorumen the Extra Fine particles can be expected to contain less water than the Fine particles, because the relative enclosed spaces which can be occupied by liquid, are less for the Extra Fine particles. So, the specific gravity of the Fine particles is presumably more decreased by the liquid than of the Extra Fine particles.

These results indicate that, in experiments in which passage

rates are determined using CrNDF as a marker, the particle size of the marker has a great influence on the calculated passage rates. Perhaps CrNDF is only representative for the fraction of rumen particles with the same size and density as the marker. Hence care should be taken that particle distribution of the marker should be comparable to that of the particles leaving the reticulorumen.

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Chapter VII

# Passage rate and total clearance rate from the rumen of cows fed grass silages differing in cell wall content.

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**Abstract.**

Four dry, rumen fistulated cows were fed ad libitum grass silages (silages G6 and G7) harvested at different growth stages, resulting in different crude protein (CP) and neutral detergent fibre (NDF) contents (G6, 21.0 % CP, 44.2% NDF and G7, 15.2 % CP, 51.5 % NDF).

Voluntary intake and rumen contents, total as well as kg organic matter (OM) were higher for silage G7. Ruminal passage rate, calculated from the logarithmic decline in Cr-NDF rumen pool, was higher for silage G7 (3.95%/h and 4.46%/h for silages G6 and G7, respectively). When ruminal passage rates were calculated by dividing the intake of indigestible organic matter (iOM) by the mean rumen pool of this fraction, the same differences between silages were found, although the level was much lower (2.58%/h and 3.00%/h for silages G6 and G7, respectively).

The results from this experiment suggest that disappearance rate from the rumen of particles with a size between 1.25mm and 0.071mm is the rate limiting step in reduction in rumen fill.

**Introduction.**

Nutrient supply and as a result production in dairy cows is influenced by the capacity of the rumen to clear the ingested feed, either by microbial degradation or by passage to the lower gut. Rate of microbial degradation in the rumen has been investigated widely and was shown to be very variable and to depend on feed component, type of feedstuff, particle size and pH in the rumen. Much less is known on passage of undegraded feed particles to the lower gut and its variation. Aspects which are considered important in this respect are chewing during eating, rumination, (rate of) particle size reduction, particle size distribution and extent of degradation and functional specific gravity (Sutherland, 1987). The probability of feed particles to escape from the rumen is inversely related to particle size (Poppi *et al.*, 1980), but passage rate from the rumen of the finest

particles is still lower than of the fluid (Faichney, 1986; Bruining & Bosch, submitted). Particles with a size above the critical particle size (CPS), defined by Kennedy & Poppi (1984) as the sieve aperture retaining the top 5% of faecal particulate dry matter, have a low probability of leaving the rumen. Not only (rate of) degradation and (rate of) passage are important for the total rumen capacity, so is rumen fill. Degradation and passage are usually expressed as fractional rate constants. For the total capacity the pool sizes from which such fractional rate constants are derived may be equally important.

Rate of passage of particles from the rumen is often determined using Cr-Neutral Detergent Fibre (Cr-NDF) as a marker. Robinson *et al.* (1987) and Bosch *et al.* (1988, submitted[a]) suggest that the rate of passage ($k_p$, $\%h^{-1}$), as calculated from the Cr-NDF faecal excretion curve, is likely to overestimate ruminal passage as a fraction of total rumen contents. It is not clear for which part of rumen contents Cr-NDF can be regarded as representative and if differences between diets can be measured using Cr-NDF.

An experiment with dry cows, fed grass silages differing in quality because of different growth stages, was conducted. Objectives of the experiment were: (1) How rumen content is influenced by silage quality, (2) How kinetics of rumen particles are influenced by silage quality, (3) To establish if particle size reduction can act as a limiting factor for total rumen capacity and (4) Whether Cr-NDF is a reliable marker for measuring passage rate from the rumen.

**Materials and methods.**

Two grass silages, from the same field, were harvested at different growth stages, resulting in different cell wall and crude protein (CP, N*6.25) contents. The chemical composition of the two silages is given in Table 1. The silages were fed ad libitum to four rumen fistulated non-lactating cows in a change-over design. The cows were offered feed twice a day, at 7.00 and 19.00 h. Left overs were removed at 9.00 and 7.00 h.

Table 1. Chemical composition (%) of the two grass silages and the soluble and indigestible OM fractions ($f_S$ and $f_R$, respectively).

| | Silage G6 | Silage G7 |
|---|---|---|
| dry matter (DM) | 56 | 62 |
| in DM: | | |
| organic matter (OM) | 88.7 | 90.9 |
| crude protein (CP) | 21.0 | 15.2 |
| neutral detergent fibre (NDF) | 44.2 | 51.5 |
| acid detergent fibre (ADF) | 25.0 | 28.0 |
| lignin (ADL) | 2.2 | 2.8 |
| indigestible ADF (iADF) | 4.9 | 6.5 |
| $f_S$ | 28.0 | 22.2 |
| $f_R$ | 12.4 | 15.0 |
| harvesting date | 1-8-'88 | 13-6-'88 |

Feed intake was recorded per day, and was corrected for the ash content in the refusals.

The experiment consisted of two experimental periods of two weeks, preceded by adaptation periods of three weeks. The first week of the experimental periods on Monday at 6.00 h 100 g of Cr-NDF (0.2-1 mm, ±5% Cr) and 30 g of Co Ethylene Diamino Tetra Acetate (Co-EDTA, ±14.8% Co) were injected into the rumen as markers for passage rates of the particulate ($k_p$, $\%h^{-1}$) and liquid ($k_l$, $\%h^{-1}$) phases from the rumen, respectively. Both markers were prepared as described by Uden *et al.* (1980). Samples of rumen fluid were taken on Monday every hour from 9.00 till 20.00 h and analysed for Co. Faeces were collected on Tuesday, Wednesday and Thursday from 8.00 till 18.00 h and were sampled every two hours. Faecal samples were dried at 70°C, ground at 1 mm and analysed for dry matter (DM), Cr and Co. In the second week of the experimental periods on Monday at 6.00 h 300 g of Cr-NDF was injected in the rumen. In this week rumen evacuations were done for five days according to the scheme in Table 2; four days twice a day with a five hour interval, and the fifth day once early in the morning without feeding the animals the night before.

Table 2. Scheme of rumen evacuations for the four cows.

| time | Monday | Tuesday | Wednesday | Thursday |
|---|---|---|---|---|
| 9.30 | 1 | 3 | 4 | 2 |
| 10.30 | 2 | 4 | 1 | 3 |
| 11.30 | 3 | 1 | 2 | 4 |
| 12.30 | 4 | 2 | 3 | 1 |
| 14.30 | 1 | 3 | 4 | 2 |
| 15.30 | 2 | 4 | 1 | 3 |
| 16.30 | 3 | 1 | 2 | 4 |
| 17.30 | 4 | 2 | 3 | 1 |

On Friday morning between 7.00 and 8.00 h rumen evacuations were done for all cows in order 2, 3, 4 and 1.

Rumen evacuations were done according to the procedure described by Robinson *et al.* (1987). Rumen contents were weighed and sampled. A 1% sample was dried at 70$^0$C, ground and analysed for DM, ash and Cr, and two 2% samples were stored at -20 $^0$C until wet sieve analysis.

Particle size distributions of rumen samples of the 9.30, 12.30, 17.30 h and the Friday morning evacuations, were determined by wet sieving of about 50 g of wet rumen contents in a 'Fritsch Analysette 3' for 30 minutes, using the method described by Bosch *et al.* (submitted[b]). Three sieve pore sizes were used; 5, 1.25 and 0.071 mm.

The dry matter fractions retained on the sieves were determined, and the fraction passing the 0.071 mm sieve was calculated. Rumen contents were divided in two pools, a large particle pool (dry matter retained on the 5 and the 1.25 mm sieve, LP), and a small particle pool (dry matter passing the 1.25 mm sieve, SP). Of the same evacuations, the acid detergent fibre (ADF) and the indigestible ADF (iADF) contents of the LP, of the particles retained on the 0.071 mm sieve and of the total rumen contents were determined. Rumen pools were corrected for the Cr-NDF, assuming that all Cr-NDF was in the iADF fraction in particles with a size between 1.25 mm and 0.071 mm.

Samples of about 70 g of total rumen contents of the 9.30h,

12.30h, 17.30 h, and of the Friday morning evacuations were weighed into nylon bags (Nybolt, Switzerland) of 9 * 18 cm with a pore size of 41 micron (8 bags per rumen sample), and washed in the washing machine, twice with cold water. The OM loss from the bags was determined as the soluble fraction ($f_S$, %). From the same samples, and from the LP from the same evacuation times, 8 nylon bags per sample and per fraction, were incubated in the rumen for 336 h, and washed afterwards in the washing machine twice with cold water to determine the undegradable OM fraction ($f_R$, %). According to the same method $f_S$ and $f_R$ of samples of the two silages cut at about 1 cm were determined. The undegradable fractions of OM were used to calculate intake and rumen pools of undegradable OM (iOM).

Dry matter was determined by drying to constant weight at 103$^0$C and ash in an oven at 550$^0$C. Nitrogen (N) was determined by the Kjeldahl method with $K_2SO_4$ and HgO as catalysts. ADF was measured according to the method described by Goering & van Soest (1970), and iADF according to the method described by Penning & Johnson (1983). Co-concentration in the rumen fluid was determined using the standard addition method, with an atomic absorption spectrophotometer (Varian, SpectrAA-300), at a wavelenght of 251.0 nm. Marker concentrations in dried faeces (Cr and Co), and dried rumen contents (Cr) were determined by atomic absorption using the concentration method after wet destruction at wavelenghts of 357.9 (Cr) and 251.0 nm (Co).

Poppi *et al.* (1981) developed a model to describe kinetics of large and small ruminal particles. At steady state, the amount of material entering the rumen in a certain time is equal to the amount cleared during that time. When intake as well as the average rumen pool are known, the amount of material disappearing from the rumen by passage plus digestion as a fraction of total rumen pool can be calculated. Based on a comparable model (Figure 1) various rate constants were calculated.

Assuming passage following first order kinetics, the outflow rate of rumen fluid ($k_{l\text{-}Co}$-rumen, $\%h^{-1}$) was calculated from the logarithmic decline in Co-concentration in the rumen fluid. The descending parts of the faecal excretion curves of Cr and Co were

used, under the same assumptions, to calculate the particle passage rate ($k_{p\text{-}Cr}$-faeces, $\%h^{-1}$) and the fluid outflow rate ($k_{l\text{-}Co}$-faeces, $\%h^{-1}$), respectively. The total clearance rate of OM from the rumen ($k_{c\text{-}OM}$, $\%h^{-1}$) as well as the $k_{p\text{-}Cr}$-rumen were estimated directly in the rumen from the logarithmic decline in the different pool sizes.

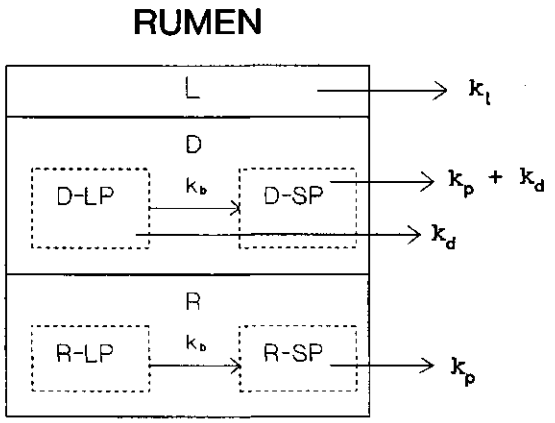


Figure 1. Rumen kinetics. (L, liquid; D, digestible, non-soluble material; R, rumen undegradable material; LP, long particle pool; SP, small particle pool; $k_l$, outflow rate of rumen fluid; $k_b$, rate of physical breakdown of long particles; $k_p$, rate of passage of small particles; $k_d$, rate of ruminal degradation of the degradable, non-soluble material).

Passage rates of the indigestible OM ($k_{p\text{-}iOM}$, $\%h^{-1}$) and of the iADF ($k_{p\text{-}iADF}$, $\%h^{-1}$) from the rumen were calculated by dividing the intake of iOM or iADF by the mean rumen pool of these fractions. A logarithmic decline in pool sizes, using the 0.5, 3.5 and 8.5 h after feeding evacuations, was calculated for the iOM and the iADF fractions. The mean rumen pool of these fractions was calculated as:

$$\text{mean rumen pool} = 1/10 * \int_0^{10} V_0 * e^{-kct} dt$$

in which $V_0$ = calculated rumen volume at t=0
$k_c$ = calculated clearance rate from logarithmic decline in pool sizes

The passage rate of small particles was calculated from the decline in iADF in SP ($k_{p\text{-}iADF\text{-}SP}$, $\%h^{-1}$), corrected for the iADF entering the SP from LP through particle size reduction.

Rate of size reduction of LP ($k_{b\text{-}OMLP}$, $\%h^{-1}$) was calculated from the decline in pool size of iOM in the LP. The decline in total OM in the LP (from 0.5 till 8.5 h after feeding) was used to calculate clearance rate of OMLP ($k_{c\text{-}OMLP}$, $\%h^{-1}$). The rate of digestion of OM in the LP ($k_{d\text{-}OMLP}$, $\%h^{-1}$) was subsequently calculated as the difference between $k_{b\text{-}OMLP}$ and $k_{c\text{-}OMLP}$.
The clearance rate of digestible, non-soluble OM ($k_{c\text{-}OM\text{-}D,NS}$, $\%h^{-1}$) was calculated from the decline in the pool size of this fraction with time after feeding. The rate of digestion of this fraction ($k_{d\text{-}OM\text{-}D,NS}$, $\%h^{-1}$), was calculated as the difference between $k_{c\text{-}OM\text{-}D,NS}$ and the $k_{p\text{-}iOM}$.

Data were analysed statistically using the anova procedure of the SPSSPC+ statistical package (SPSS Inc., 1988). If applicable, comparisons were made by using a paired T-test.

**Results.**

Intake was higher ($P<0.01$) for G7, the silage with the highest cell wall content, than for G6 (10.1 and 9.1 kg OM per day, respectively).
For silage G6, $f_{S\text{-}OM}$ was higher (28.0 *versus* 22.2%) and the $f_{R\text{-}OM}$ was lower (12.4 *versus* 15.0%) than for silage G7.

Rumen contents, total as well as kg OM, were at all times higher for silage G7 (Table 3). However, the differences in total rumen contents were only significant ($P<0.05$) after more than 6 h after feeding. Significant differences in OM content in the rumen were found at a few times after feeding, divided over the day.

The distribution of rumen particle sizes, as well as the ratios iADF:ADF at different times after feeding are given in Table 4. The amount (kg DM) of long particles (>1.25 mm), as well as the amount of small particles (passing the 1.25 mm sieve), was at all times higher for G7 than for G6. However, within the small

particle pool, the amount of DM passing the 0.071 mm sieve was higher for G6. The amount of rumen DM washable from the nylon bags (<0.041 mm) was not significantly different between silages. The iADF:ADF ratio in rumen digesta was considerably higher than in the silages and increased for all rumen pools with time after feeding. With a decrease in particle size, the iADF:ADF ratio increased.

Table 3. Rumen contents (kg and kg OM) at different times after feeding, for the two grass silages.

| Time after feeding | kg | | | kg OM | | |
|---|---|---|---|---|---|---|
| | G6 | G7 | SEM | G6 | G7 | SEM |
| 0.5 | 95.7 | 97.9 | 1.29 NS | 8.3 | 9.5 | 0.04 ** |
| 1.5 | 90.2 | 96.9 | 2.89 NS | 7.8 | 9.2 | 0.37 NS |
| 2.5 | 89.8 | 94.0 | 1.63 NS | 7.1 | 8.9 | 0.09 ** |
| 3.5 | 88.9 | 94.6 | 0.74 NS | 7.3 | 8.2 | 0.19 NS |
| 5.5 | 82.4 | 93.2 | 1.73 NS | 6.5 | 8.1 | 0.19 NS |
| 6.5 | 80.2 | 93.9 | 1.25 * | 6.1 | 7.5 | 0.32 NS |
| 7.5 | 80.1 | 88.7 | 0.54 * | 5.9 | 7.2 | 0.08 * |
| 8.5 | 76.5 | 85.4 | 0.79 * | 5.8 | 6.7 | 0.01 *** |
| 22.5 | 60.2 | 65.9 | 0.48 * | 3.4 | 4.1 | 0.14 NS |

NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Fractional passage rates from the rumen of liquid and particulate phases, derived from the descending parts of the faecal excretion curves, as well as measured directly in the rumen are given in Table 5. There were no differences between silages in passage rate of the liquid phase. The $k_{l\text{-}Co}$-rumen was significantly higher ($P<0.001$) than the $k_{l\text{-}Co}$-faeces. The passage rate of the Cr-NDF was higher ($P<0.05$) for the high cell wall silage when measured directly in the rumen, but was not significantly different between silages when calculated from the faecal excretion curves. The $k_p$, like the $k_l$, was higher when measured directly in the rumen, than when calculated from the faecal excretion curves ($P<0.001$). Passage rates of iOM ($k_{p\text{-}iOM}$,

Table 4. Distribution of rumen particles (kg DM of the different particle sizes), and the iADF:ADF ratio in different rumen pools for the two silages at 0.5, 3.5, 8.5 and 22.5 h after feeding.

| Time after feeding | 0.5 | | | 3.5 | | | 8.5 | | | 22.5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G6 | G7 | SEM | G6 | G7 | SEM | G6 | G7 | SEM | G6 | G7 | SEM |
| kg DM in different rumen pools | | | | | | | | | | | | |
| >5mm | 1.68 | 1.27 | 0.09 NS | 1.30 | 0.76 | 0.04 * | 0.65 | 0.44 | 0.02 * | 0.16 | 0.15 | 0.01 NS |
| >1.25mm, <5mm | 1.44 | 2.31 | 0.15 NS | 1.11 | 1.80 | 0.09 NS | 0.73 | 1.08 | 0.01 * | 0.30 | 0.45 | 0.03 NS |
| >0.071mm, <1.25mm | 2.59 | 3.57 | 0.08 * | 2.40 | 3.49 | 0.10 * | 2.49 | 3.49 | 0.10 * | 1.75 | 2.28 | 0.07 NS |
| >0.041mm, <0.071mm | 0.96 | 0.56 | 0.09 NS | 1.01 | 0.67 | 0.04 NS | 0.64 | 0.21 | 0.12 NS | 0.67 | 0.67 | 0.04 NS |
| <0.041mm | 2.94 | 2.98 | 0.04 NS | 2.67 | 2.56 | 0.05 NS | 2.29 | 2.30 | 0.07 NS | 1.14 | 1.29 | 0.03 NS |
| Ratio iADF:ADF | | | | | | | | | | | | |
| total rumen DM | 0.29 | 0.31 | 0.01 NS | 0.31 | 0.33 | 0.00 * | 0.37 | 0.37 | 0.01 NS | 0.47 | 0.46 | 0.01 NS |
| >1.25mm | 0.21 | 0.27 | 0.01 NS | 0.24 | 0.27 | 0.01 * | 0.30 | 0.31 | 0.01 NS | 0.44 | 0.43 | 0.01 NS |
| >0.071mm, <1.25mm | 0.31 | 0.33 | 0.01 NS | 0.33 | 0.33 | 0.01 NS | 0.35 | 0.36 | 0.01 NS | 0.46 | 0.45 | 0.01 NS |
| <0.071* | 0.70 | 0.76 | 0.05 NS | 0.61 | 0.66 | 0.08 NS | 0.73 | 0.94 | 0.15 NS | 0.59 | 0.61 | 0.04 NS |

NS not significant, *** P<0.001, ** P<0.01, * P<0.05
*calculated

$P$=0.02) and of iADF ($k_{p\text{-}iADF}$, $P$=0.06) were higher for G7 than for G6. Within silages, both were lower than $k_{p\text{-}Cr}$-rumen ($P$<0.001), and $k_{p\text{-}iOM}$ was lower than $k_{p\text{-}iADF}$ ($P$<0.01). However, there was a significant correlation between $k_{p\text{-}Cr}$-rumen and $k_{p\text{-}iOM}$ ($r$ = 0.98, $P$<0.001). On average, the level of $k_{p\text{-}Cr}$-rumen was not significantly different from $k_{p\text{-}iADF\text{-}SP}$, but only a very low correlation between these two fractional passage rates was found ($r$=0.20).

Table 5. Fractional passage rates from the rumen of liquid and particulate phases, measured in the faeces ($k_{l\text{-}Co}$-faeces and $k_{p\text{-}Cr}$-faeces (%$h^{-1}$), respectively), and measured directly in the rumen ($k_{l\text{-}Co}$-rumen and $k_{p\text{-}Cr}$-rumen (%$h^{-1}$), respectively), the passage rate of indigestible organic matter ($k_{p\text{-}iOM}$, %$h^{-1}$), of indigestible acid detergent fibre ($k_{p\text{-}iADF}$, %$h^{-1}$), the fractional passage rate of small particles ($k_{p\text{-}iADF\text{-}SP}$, %$h^{-1}$), the fractional clearance rate of OM from the rumen ($k_{c\text{-}OM}$, %$h^{-1}$), the rate of physical breakdown, total clearance rate and digestion rate of the om in the long particle pool ($k_{b\text{-}OMLP}$, $k_{c\text{-}OMLP}$ and $k_{d\text{-}OMLP}$, (%$h^{-1}$) respectively) and the rates of total clearance and digestion of the digestible, non-soluble OM ($k_{c\text{-}OM\text{-}D,NS}$ and $k_{d\text{-}OM\text{-}D,NS}$ (%$h^{-1}$), respectively).

| | Silage G6 | Silage G7 | SEM | |
|---|---|---|---|---|
| $k_{l\text{-}Co}$-faeces | 6.10 | 6.30 | 0.13 | NS |
| $k_{p\text{-}Cr}$-faeces | 3.03 | 3.30 | 0.11 | NS |
| $k_{l\text{-}Co}$-rumen | 11.20 | 12.32 | 0.65 | NS |
| $k_{p\text{-}Cr}$-rumen | 3.95 | 4.46 | 0.02 | * |
| $k_{p\text{-}iOM}$ | 2.56 | 2.98 | 0.03 | * |
| $k_{p\text{-}iADF}$ | 2.82 | 3.18 | 0.05 | NS |
| $k_{p\text{-}iADF\text{-}SP}$ | 3.03 | 4.88 | 0.13 | * |
| $k_{c\text{-}OM}$ | 4.42 | 4.22 | 0.11 | NS |
| $k_{b\text{-}OMLP}$ | 5.49 | 7.51 | 0.40 | NS |
| $k_{c\text{-}OMLP}$ | 10.33 | 10.67 | 0.56 | NS |
| $k_{d\text{-}OMLP}$ | 4.84 | 3.16 | 0.23 | NS |
| $k_{c\text{-}OM\text{-}D,NS}$ | 9.76 | 8.90 | 0.20 | NS |
| $k_{d\text{-}OM\text{-}D,NS}$ | 7.32 | 6.03 | 0.18 | NS |

NS, not significant; *, $P$<0.05

Total clearance rate of OM from the rumen did not differ between silages, but was for silage G7 even lower than the $k_p$ calculated from the decline in rumen Cr-pool (Table 5). Though not significant ($P$=0.13), the rate of breakdown of large particles ($k_{b\text{-}OMLP}$, $\%h^{-1}$), calculated from the decline of the iOM pool in the LP, was highest for G7. The $k_{c\text{-}OMLP}$ was not different for both silages. Although differences were not significant, the higher $k_{b\text{-}OMLP}$ in combination with the same $k_{c\text{-}OMLP}$ resulted in a somewhat lower ($P$=0.07) degradation rate of OM in the LP pool ($k_{d\text{-}OMLP}$, $\%h^{-1}$) of silage G7.

Total clearance rate of digestible, non-soluble OM, the $k_{c\text{-}OM\text{-}D,NS}$, was, though not significant ($P$=0.16), higher for G6, resulting in a somewhat higher degradation rate of this fraction for G6 ($k_{d\text{-}OM\text{-}D,NS}$, $\%h^{-1}$, Table 5).

**Discussion.**

Cell wall content and lignin content of the cell walls increase when the plant matures (Van Soest, 1982), resulting in a decrease in digestibility (Reid *et al.*, 1988). The silages in this experiment were grown at the same field and were harvested at different growth stages. Lignin content of silage G7 was highest, and thus this silage was expected to have the lowest digestibility.

The capacity of the rumen can be limiting roughage intake in high producing dairy cows (Mertens, 1987). Because the cows in this experiment were not in lactation and nutrient requirements of the animals was relatively low, feed intake was not limited by the capacity of the rumen, but probably chemostatically by digestion products. Therefore intake of silage G7 could be higher.

Rumen content is determined by feed intake on one hand and clearance rate from the rumen on the other. The higher intake of silage G7, in combination with the same total clearance rate ($k_{c\text{-}OM}$), resulted in a constant higher rumen content for G7.

The rumen DM pool can be divided into two pools: the large

particle pool (LP, in this experiment defined as the material retained on a 1.25 mm sieve) and the small particle pool (SP, here defined as the material passing a 1.25 mm sieve). Both pools exist of a degradable fraction (dOM) and an undegradable fraction (iOM) (Figure 1). The dOM in the LP pool can be physically broken down to small particles (with a fractional rate constant $k_b$, $\%h^{-1}$), or can be fermentatively degraded by rumen microbes ($k_{d\text{-}OMLP}$, $\%h^{-1}$). The iOM in the LP pool can move over to the SP by particle size reduction only, mainly through rumination (Ulyatt *et al.*, 1986). The dOM in the SP can pass to the omasum undegraded or can be fermented by rumen microbes. The iOM in the SP can pass to the omasum only. According to Poppi *et al.* (1981), clearance rate of small particles from the rumen is the most important factor influencing DM retention time in the rumen.

In most experiments reported in literature, $k_p$ is calculated from the faecal excretion curve of Cr-NDF. Bosch *et al.* (1988, submitted[8]) reported a 25-30% lower $k_{1\text{-}Co}$ when derived from the faeces than when measured directly in the rumen. The same observation was made in this experiment, except that the differences here were much larger with 45 to 50% lower values when measured in the faeces. For the particulate marker, a similar delay as for the soluble marker is found when measured in the faeces as compared to measured directly in the rumen. However, in this case the difference was is only 25%. This indicates that, after the rumen, there is also another mixing compartment for the particulate phase. When the decline in rumen Cr-NDF pool is measured, this results in an even greater overestimation of the $k_p$ than when measured in the faeces. Cr-NDF can be considered to be representative only for particles able to leave the rumen (passing a 1.25 mm sieve). Compared to the clearance of undegradable material from the small particle pool, which has to be corrected for undegradable material entering the SP pool from the LP pool, here calculated as the $k_{p\text{-}iADF\text{-}SP}$, the $k_{p\text{-}Cr}$-rumen was not significantly different from the fractional passage rate of undegradable material in small particles. The high correlation between $k_{p\text{-}Cr}$-rumen and $k_{p\text{-}iOM}$ ($r$=0.98, $P$<0.001), suggests that differences between silages in rates of passage

from the rumen of total iOM can be demonstrated using Cr-NDF as a marker.

As with iOM, Cr-NDF particles are not degradable in the rumen (Robinson *et al.*, 1987), so no fermentation gases are trapped in these particles. Gas reduces the functional specific gravity (FSG) of particles, and so delays their passage rate (Sutherland, 1987). Tamminga *et al.* (1989) demonstrated in dairy cows that undegradable material passes from the rumen at a much faster rate than the potentially degradable material. The faster rate of passage of smaller particles, even within the SP (Bruining & Bosch, submitted), and the higher iADF:ADF ratio for smaller particles in this experiment, agree with this.

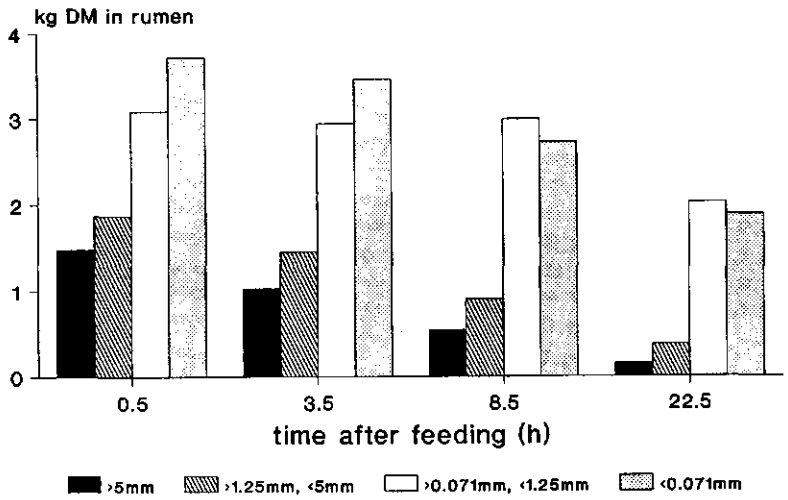


Figure 2. Average rumen contents (G6 + G7) of the different particle pools for 0.5, 3.5, 8.5 and 22.5h after feeding.

Although the $k_{p\text{-}iOM}$ values thus probably overestimate the passage rate of total rumen OM, the figures calculated in our simplified rumen model, suggest that the retention of DM in the rumen is influenced most by the retention time of small particles, which agrees with the findings of Poppi *et al.* (1981). In Figure 2, the

average rumen contents (G6 + G7) of the different particle pools at different times after feeding are given. This figure shows a decline for all pools with time after feeding, except for the pool containing particles with a size between 1.25mm and 0.071mm. The latter fraction seems to be rather constant for at least 8.5h after feeding, indicating that during this time the amount of material cleared from this pool is close to the amount entering this pool. This suggests that clearance rate from this pool is the rate limiting step in reduction in rumen fill, and that particle size reduction as such is not the only limiting factor for rumen capacity. A combination of size reduction and extent of digestion, the latter influencing the FSG of the particles, seems more likely.

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Chapter VIII

# General discussion

The mechanisms controlling feed intake, especially roughage intake, in dairy cows are still not fully understood. Since the introduction of a milk quota system in 1984 in the Netherlands, on an increasing number of farms there's a surplus of grass and grass silage. Therefore, increasing the roughage proportion in dairy diets seems interesting. This is, however, only possible if our knowledge concerning the regulation of roughage intake is improved.

It is generally accepted that many factors, such as digestibility and volume of the feed, demand of the animal for nutrients, rumen volume and clearance rate of feed from the rumen, and for good quality forages probably also amount and type of fermentation end products (Van Soest, 1982), are influencing ad libitum intake of forages in ruminants.

When energy requirements are high (early lactation), the ad libitum intake of forages can be limited by the capacity of the rumen. Capacity of the reticulorumen may be limited by rumen fill (Mertens, 1987), or by rates of clearance, determining the throughput capacity of the rumen (Weston, 1982). Rumen fill (kg dry matter, DM) can be limited by rumen volume or by a maximum packing density (DM%). According to Grovum (1987), there is a large excess capacity to transport bulk in the intestines, but rumen volume and throughput capacity of the rumen can limit roughage intake.

1. *Rumen fill*

The rumen pool (kg DM) is determined by input (intake) and output (passage + digestion) (Fig. 1). The rumen pool can be determined by rumen evacuation of fistulated animals. Rumen pools vary between animals, feeds, level of feed intake and feeding scheme. In Table 1, a literature overview of maximum rumen contents (g DM/kg Body Weight) and rumen clearance, calculated as average rumen fill times clearance rate ($k_c$, $\%h^{-1}$ (DM Intake/average rumen DM content)) of different animals fed different diets is given. Maximum rumen DM contents (g DM/kg BW) were calculated as determined rumen contents corrected for the estimated amounts that had disappeared between the end of the meal (2 hours after

feeding time) and the time of rumen evacuation using the following equation:

$$\text{maximum rumen DM content} = \text{determined rumen content} * \frac{1}{e^{-((\text{DMI}/\text{det. rumen content})/24)* (\text{h after feeding}-2)}}$$

Rumen capacity is partly determined by rumen fill which depends on rumen volume on the one hand and on packing density on the other. Maximum fill appears to vary between 14 and 28 g DM/kg BW. Clearance rate in the results in Table 1 varies between 5.2 for a brome grass fed in early lactation and 10.6 $\%h^{-1}$ for fresh lucerne fed to dry cows. The relation between maximum fill and clearance rate was poor ($r$ = -0.22, Table 2); hence the two phenomena appear to operate independent from one another. As a result, total capacity (clearance) varied from less than 300 to over 1000 g/hour.

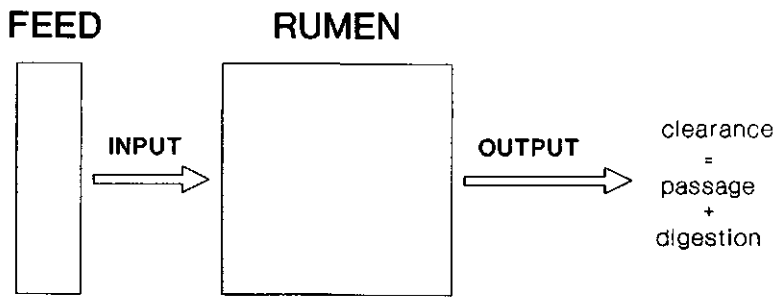


Figure 1. Rumen pool as determined by input of the feed and output (=clearance) through passage and digestion.

Table 1. Literature data on rumen contents of dairy cows, determined by rumen evacuations (BW, body weight (kg); FCM/MBW, g fat corrected milk per kg $BW^{0.75}$; C%, concentrate percentage in diet; NDFR, neutral detergent fibre content of roughage proportion of diet (g/kg DM); DMI, kg dry matter intake; n, number of animals in experiment; fill/BW, calculated maximum rumen fill (g/kg BW); $k_c$, rumen clearance rate (%/h); clear., mean rumen clearance (g/h); roughage, roughage fed; Ref, reference.

| BW | FCM/ MBW | C% | NDFR | DMI | n | fill/ BW | $k_c$ | clear. | roughage | Ref. * |
|---|---|---|---|---|---|---|---|---|---|---|
| 648 | 239 | 39 | 402 | 24.3 | 3 | 23.3 | 7.65 | 1013 | alfalfa hay | 1 |
| 689 | 151 | 40 | 421 | 20.2 | 3 | 20.2 | 6.81 | 842 | (prebloom) | 1 |
| 744 | 0 | 40 | 417 | 14.5 | 3 | 14.7 | 6.17 | 604 | ,, | 1 |
| 685 | 192 | 39 | 407 | 19.8 | 3 | 19.6 | 6.92 | 825 | ,, | 1 |
| 690 | 194 | 40 | 416 | 20.0 | 3 | 19.7 | 6.92 | 833 | ,, | 1 |
| 676 | 187 | 39 | 418 | 19.2 | 3 | 19.0 | 7.04 | 800 | ,, | 1 |
| 606 | 233 | 55 | 494 | 16.9 | 4 | 26.4 | 5.01 | 704 | alfalfa hay | 2 |
| 628 | 195 | 43 | 494 | 19.8 | 4 | 25.5 | 5.99 | 825 | (second cut) | 2 |
| 656 | 125 | 33 | 494 | 17.6 | 4 | 23.9 | 5.36 | 733 | ,, | 2 |
| 700 | 0 | 17 | 494 | 10.8 | 4 | 13.7 | 5.38 | 450 | ,, | 2 |
| 555 | 204 | 67 | 520 | 20.9 | 4 | 29.0 | 6.36 | 873 | grass hay | 3 |
| 569 | 227 | 36 | 446 | 17.0 | 6 | 20.8 | 7.14 | 708 | grass silage | 4 |
| 579 | 217 | 34 | 548 | 18.2 | 4 | 23.9 | 6.44 | 758 | (wilted) | 4 |
| 576 | 211 | 38 | 547 | 16.7 | 6 | 23.4 | 6.00 | 696 | ,, | 4 |
| 570 | 177 | 39 | 641 | 16.0 | 4 | 24.6 | 5.47 | 667 | ,, | 4 |
| 546 | 114 | 7 | 446 | 12.8 | 6 | 18.4 | 6.21 | 533 | ,, | 4 |
| 560 | 167 | 6 | 548 | 15.0 | 6 | 22.2 | 5.83 | 625 | ,, | 4 |
| 550 | 96 | 7 | 547 | 12.0 | 6 | 18.8 | 5.56 | 500 | ,, | 4 |
| 534 | 109 | 8 | 673 | 11.5 | 6 | 18.6 | 5.57 | 497 | ,, | 4 |
| 583 | 295 | 40 | 422 | 23.3 | 5 | 24.2 | 7.86 | 971 | prebloom alfalfa | 5 |
| 584 | 253 | 43 | 543 | 20.2 | 5 | 24.8 | 6.52 | 842 | midbloom alfalfa | 5 |
| 583 | 253 | 41 | 562 | 20.0 | 5 | 24.7 | 6.47 | 833 | full bloom alfalfa | 5 |
| 582 | 232 | 43 | 718 | 17.9 | 5 | 28.1 | 4.99 | 746 | bromegrass | 5 |
| 588 | 272 | 40 | 446 | 23.7 | 5 | 26.7 | 7.13 | 988 | corn silage | 5 |

Table 1. Continued

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 704 | 0 | 0 | 442 | 10.3 | 4 | 14.1 | 5.33 | 429 | grass silage | 6 |
| 704 | 0 | 0 | 515 | 11.1 | 4 | 15.7 | 5.13 | 463 | (wilted) | 6 |
| 550 | 0 | 0 | 601 | 9.6 | 6 | 17.1 | 5.21 | 400 | midbloom alfalfa | 7 |
| 550 | 0 | 0 | 580 | 10.5 | 6 | 17.8 | 5.54 | 438 | (second cut) | 7 |
| 550 | 0 | 0 | 614 | 9.2 | 6 | 15.8 | 5.46 | 383 | ,, | 7 |
| 420 | 0 | 0 | 368 | 6.9 | 3 | 13.8 | 8.14 | 288 | red clover | 8 |
| 420 | 0 | 0 | 475 | 8.6 | 3 | 17.1 | 8.19 | 358 | fresh lucerne | 8 |
| 420 | 0 | 0 | 592 | 8.5 | 3 | 17.1 | 8.05 | 354 | lucerne hay | 8 |
| 460 | 0 | 0 | 569 | 9.2 | 5 | 17.0 | 6.22 | 383 | ryegrass | 9 |
| 460 | 0 | 0 | 394 | 9.1 | 5 | 14.8 | 7.27 | 379 | alfalfa | 9 |
| 200 | 0 | 0 | 688 | 6.0 | 6 | 28.1 | 5.50 | 250 | straw | 10[1] |
| 200 | 0 | 10 | 688 | 6.2 | 6 | 28.7 | 5.59 | 258 | straw + barley | 10[1] |
| 200 | 0 | 10 | 688 | 6.5 | 6 | 32.3 | 5.13 | 271 | straw + cottonseed | 10[1] |

* 1: Shaver et al., 1986
2: Hartnell & Satter, 1979
3: Robinson et al., 1987
4: Bosch et al., this thesis
5: Shaver et al., 1988
6: Bosch & Bruining, this thesis
7: Belyea et al., 1989
8: Waghorn, 1986
9: Waghorn et al., 1989
10: Spragg et al., 1986

[1]) not included in the multiple regression analysis

Table 2. Correlation matrix for the factors involved in the regression analysis (DMI, dry matter intake, kg; MAXBW, maximum rumen fill, g/kg body weight; $k_c$, rumen clearance rate, %/h; clearance, rumen clearance, g/h; FCM/MBW, g fatt corrected milk per kg body weight$^{0.75}$; NDFR, neutral detergent fibre content of the roughage, g/kg DM; C%, concentrate proportion in the diet; BW, body weight of the animals, kg).

| | DMI | MAXBW | $k_c$ | clearance | FCM/MBW | NDFR | C% | BW |
|---|---|---|---|---|---|---|---|---|
| DMI | -- | | | | | | | |
| MAXBW | 0.78 ** | -- | | | | | | |
| $k_c$ | 0.16 | -0.07 | -- | | | | | |
| clearance | 0.99 ** | 0.78 ** | 0.16 | -- | | | | |
| FCM/MBW | 0.91 ** | 0.87 ** | 0.13 | 0.91 ** | -- | | | |
| NDFR | -0.23 | 0.24 | -0.57 ** | -0.22 | -0.05 | -- | | |
| C% | 0.87 ** | 0.78 ** | 0.03 | 0.87 ** | 0.82 ** | -0.14 | -- | |
| BW | 0.48 * | 0.07 | -0.37 | 0.48 * | 0.24 | -0.28 | 0.45 * | -- |

**, $p < 0.001$; *, $p < 0.01$

In the experiments described in chapters II to V, maximum rumen contents varied from approximately 19 g DM/kg BW in late lactation (ad lib grass silage, 1 kg of concentrates) to approximately 23 g DM/kg BW in early lactation (ad lib grass silage, 7 kg of concentrates). Dry matter percentage in the rumen increased when the proportion of concentrates in the diet increased. No effect of roughage composition on the DM% in the rumen was found. Rumen liquid volume (kg/kg BW) in these experiments varied between 0.14 and 0.16 kg/kg BW and was not different between diets, cows or stages of lactation. Hence, not only rumen volume is an important factor for the holding capacity of the rumen, packing density even seems to be of more importance.

For dry cows (Chapter VII), maximum rumen DM content was influenced by silage quality and was 14 g DM/kg BW for the grass silage containing 44% NDF and 16 g DM/kg BW for the grass silage containing 52% NDF.

According to Baile & Forbes (1974), the capacity of the rumen is increased during early lactation. When energy deficit is high, there is a strong signal to stimulate intake in spite of the level of fill in the reticulum and cranial sac (Grovum, 1987). In those parts of the forestomachs the tension receptors are located, and probably fill in these organs determines whether or not satiety is signalled. The threshold value might be higher during lactation. Gill *et al.* (1988), pointed out the fact that the receptors for rumen distension respond to stretch or mechanical stimulation. The weight of the digesta in the rumen may therefore not be the most appropriate index of distension. However, measuring the exact volume of the rumen, including the gas, has not yet been done. As mentioned earlier, in our experiments with lactating cows, only a higher proportion of concentrates increased rumen fill, indicating that the density of the feed would be of more influence than the volume of the rumen.

Regression analysis, using the data presented in Table 1 (of which the correlation matrix is given in Table 2), based on milk production (g FCM/kg Metabolic Body Weight, MBW), the proportion

of concentrates in the diet (C%), the NDF content of the roughage part of the diet (g/kg DM) and the body weight of the cows (BW, kg), showed that the first two parameters each explained a significant part of the variation in maximum rumen content (g DM/kg BW). Multiple regression analysis showed that in combination, these parameters could explain 89% of the total variation in maximum rumen content of mature cows (Table 3). For growing animals, maximum rumen content per kg body weight seems higher, as the results of Spragg *et al.* (1986) in heifers weighing 200 kg show (Table 1). Therefore these data were not included in the regression analysis.

Table 3. Stepwise multiple regression analysis on factors responsible for the variation in maximal rumen dry matter content per kg body weight (g DM/kg BW). FCM, g FCM/kg $BW^{0.75}$; NDFR, NDF content of the roughage (g/kg DM); C%, concentrate proportion in the diet; BW, body weight (kg).

| Constant | FCM | NDFR | C% | BW | $R^2$ |
|---|---|---|---|---|---|
| 15.74 | 0.037 *** | | | | 0.76 |
| 14.28 | | 0.012 NS | | | 0.06 |
| 16.26 | | | 0.17 *** | | 0.61 |
| 18.17 | | | | 0.004 NS | 0.01 |
| 8.48 | 0.037 *** | 0.014 *** | | | 0.83 |
| 15.54 | 0.029 *** | | 0.05 NS | | 0.77 |
| 19.89 | 0.038 *** | | | -0.007 NS | 0.77 |
| 7.35 | 0.026 *** | 0.016 *** | 0.07 * | | 0.86 |
| 13.63 | 0.024 *** | 0.014 *** | 0.10 ** | -0.010 * | 0.89 |

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$, NS, not significant

Also in the experiments reported in this thesis, rumen content seemed to be more closely related to the amount of concentrates fed than to silage quality (Chapter III). The concentrate particles have a higher density and thus a smaller volume. Within concentrate level, except for silage G1, which was the only silage with an apparent DM digestibility of more than 70%, rumen

contents were not different, despite the lower intakes of the high cell wall silages. For dry cows, however, intake as well as rumen contents increased with cell wall content of the silages (Chapter VII), probably because under these conditions rumen capacity was not the factor limiting intake, but the energy requirement of the animals was.

2. *Rumen clearance capacity*

The average rumen fill (g DM/kg BW) of the data given in Table 1, was estimated according to the following equation:

$$\text{average rumen fill} = 1/T * \int_0^T V_0 e^{-kct} dt$$

in which T = time between the end of the first meal (t=0) and the start of the next meal, calculated as (24/number of feedings per day)-2 hour
$V_0$ = maximum rumen fill, rumen contents at t=0
$k_c$ = fractional rate fo DM clearance

At steady state situations, the material entering the rumen per unit of time is equal to the amount disappearing from the rumen during that time. A steady state was assumed and rumen clearance ($gh^{-1}$) for the data given in Table 1, was calculated as DM intake/24 h. The results of this calculation are also given in Table 1. Regression analysis based on g FCM/kg MBW, concentrate proportion in the diet (C%), the NDF content of the roughage (NDFR, g/kg DM) and body weight of the animal (BW, kg), showed that three of these parameters (FCM/kg MBW, C% and BW) explained a significant part of the variation in rumen clearance ($gh^{-1}$) (Table 4). FCM/kg MBW alone even explained 83% of this variation. Multiple regression analysis showed that in combination these four parameters explained 93% of the variation in rumen clearance or throughput ($gh^{-1}$).

Table 4. Stepwise multiple regression analysis on factors responsible for the variation in maximum rumen dry matter clearance (=throughput, g/h) (equal to maximum dry matter intake per h). FCM, g FCM/kg $BW^{0.75}$; NDFR, NDF content of the roughage (g/kg DM); C%, concentrate proportion in the diet; BW, body weight (kg).

| Constant | FCM | NDFR | C% | BW | $R^2$ |
|---|---|---|---|---|---|
| 405.5 | 1.83 *** | | | | 0.83 |
| 913.6 | | -0.54 NS | | | 0.05 |
| 418.0 | | | 9.02 *** | | 0.76 |
| -55.9 | | | | 1.19 ** | 0.23 |
| 630.9 | 1.82 *** | -0.44 * | | | 0.86 |
| 388.8 | 1.19 *** | | 4.01 ** | | 0.88 |
| 20.6 | 1.70 *** | | | 0.69 *** | 0.90 |
| 92.9 | 1.37 *** | | 2.24 * | 0.54 ** | 0.92 |
| 265.9 | 1.39 *** | -0.26 * | 2.11 * | 0.47 ** | 0.93 |

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; NS, not significant

3. *Clearance rate*

Fractional rumen clearance (clearance as a fraction of average rumen fill, $k_c$) was also calculated (Table 1). For $k_c$, the same regression analyses as for maximum rumen fill and rumen clearance were performed. As shown in Table 5, level of milk production and concentrate proportion in the diet had no effect on $k_c$. Cell wall content of the roughage and body weight of the animal both could explain a significant part of the variation in clearance rate. So, besides rumen volume, which is likely to be related to body weight of the animal, especially roughage characteristics seem to determine $k_c$. In combination, these two factors (BW and NDFR) explained 63% of the variation, which was raised to 68% after addition to the model of the factors level of milk production and concentrate proportion in the diet.

Dry matter is cleared from the rumen by microbial degradation or by passage to the lower gut. The feed as well as the rumen content consists of a soluble fraction (S), a non-soluble but potentially degradable fraction (D) and a rumen undegradable fraction (R) (Fig 2). Generally it is assumed that the soluble

fraction is completely and rapidly degraded in the rumen. The non-soluble but degradable fraction can be cleared from the rumen by microbial degradation or by passage to the lower gut. The rumen undegradable fraction, however, can leave the rumen by passage only.

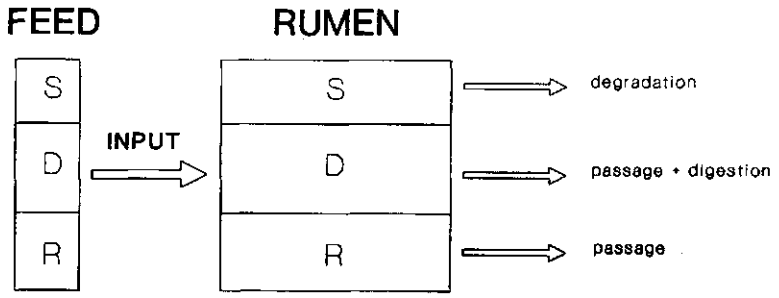


Figure 2. Rumen clearance. The soluble fraction (S) is degraded in the rumen, the degradable, non-soluble fraction (D) is cleared by passage and digestion and the rumen undegradable fraction (R) is cleared by passage only.

Table 5. Stepwise multiple regression analysis on factors responsible for the variation in rate of clearance of dry matter from the rumen ($k_c$, %/h). (FCM, g fatt corrected milk/kg $BW^{0.75}$; NDFR, NDF content of the roughage (g/kg DM); C%, concentrate proportion in the diet; BW, body weight (kg)).

| Constant | FCM | NDFR | C% | BW | $R^2$ |
|---|---|---|---|---|---|
| 6.19 | -0.0015 NS | | | | 0.02 |
| 9.54 | | -0.006 ** | | | 0.33 |
| 6.29 | | | -0.016 NS | | 0.01 |
| 8.75 | | | | -0.004 * | 0.14 |
| 14.17 | | -0.008 *** | | -0.006 *** | 0.63 |
| 14.40 | -0.0018 NS | -0.008 *** | 0.003 NS | -0.007 *** | 0.68 |

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; NS, not significant

3.1 *Rate of degradation*

The degradation rate (%/h) of D can be measured using the nylon bag technique (Mehrez & Ørskov, 1977). When plants mature, cell wall content increases, lignin content of the cell walls also increases and crude protein (CP) content decreases. In temperate grasses, generally both digestibility and voluntary intake decrease with stage of maturity (Van Soest, 1982). The soluble fraction ($f_S$-DM, %) of grass silages, which is assumed to have a very high degradation rate, decreases with an increase in cell wall content. The $f_S$-CP is more related to the DM content of the silages and the date of harvesting than to cell wall content (Tamminga *et al.*, 1990), and therefore did not show a consistent relation with cell wall content (Chapter V). The undegradable fraction ($f_R$, %) of the different chemical components increases with stage of maturity, and is according to Mertens & Ely (1979) the parameter with the most negative effect on (maximum) digestible organnic matter intake. Lignin, which is not degraded by rumen microbes, acts as a barrier for cell wall degradation. As the lignin content in the cell walls increases, the cell wall fraction bound to the lignin also increases, which explains the increase in indigestible hemicellulose. Lignin has linkages mainly with hemicellulose (Van Soest, 1982), so the, even faster increase in the indigestible cellulose fraction can not be explained by this. Probably the fraction of cellulose which is surrounded by the hemicellulose-lignin complex increases as the plant matures.

More CP is incorporated in the cell wall fraction (Krishnamoorthy *et al.*, 1982; Van Soest, 1982) and the fraction of non protein nitrogen (NPN) decreases with maturity (Van Soest, 1982) explaining the rise in $f_R$-CP.

Rates of degradation of the non-soluble, degradable fraction, measured using the nylon bag method (Chapter II), declined for all components with increasing cell wall content of the silages. This means that degradation rate as a fraction of total rumen contents declined even faster, due to the decline in S and the increase in R.

3.2 *Rate of passage*

The rumen fluid, containing the soluble fraction, flows to the omasum with a fractional rate constant $k_l$ ($\%h^{-1}$). Of the particulate phase, not all particles can leave the rumen. Particles have to be smaller than the critical particle size (CPS) to have a high probability of leaving the rumen (Kennedy & Poppi, 1984). The rumen DM pool can therefore be divided into two pools: a large particle pool (LP), particles with a low probability of leaving, in these experiments defined as the material retained on a 1.25mm sieve, and a small particle pool (SP), containing particles with a high probability of leaving, considering their size (Poppi *et al.*, 1981), here defined as particles passing a 1.25mm sieve. The LP as well as the SP pool both consist of a potentially degradable and a rumen undegradable dry matter fraction (dDM and iDM, respectively) (Fig 3).

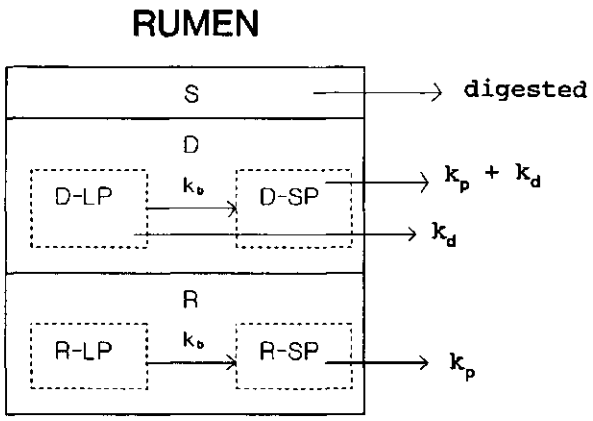


Figure 3. Rumen kinetics. (S, soluble fraction; D, digestible, non-soluble fraction; R, rumen undegradable fraction; LP, long particle pool; SP, small particle pool; $k_b$, rate of physical breakdown of long particles; $k_p$, rate of passage of small particles; $k_d$, rate of ruminal degradation of D).

The material not degradable in the rumen has to leave by passage, whereas the potentially degradable fraction can disappear from the rumen by either microbial fermentation or by passage to the lower gut. Particles from the LP pool can enter the SP pool after being reduced in size, mainly through rumination (Ulyatt *et al.*, 1986).

The fraction of rumen DM in the SP pool was slightly decreased ($r$ = -0.57) by an increase in cell wall content of the silages (Table 6). The distribution of particles within this pool changed with cell wall content, resulting in a smaller fraction passing the 0.071mm sieve with increasing cell wall content (Chapter IV). Rumination time per kg DM ingested increased with cell wall content, but not enough to achieve the same distribution in particle sizes in the rumen. The probability of passage from the rumen is inversily related to particle size (Poppi *et al.*, 1980), resulting in a negative correlation between the mean faecal particles size (MFP) and the rumen pool passing a 0.071mm sieve (Chapter IV, Table 6).

Rate of passage increases with cell wall content because more undegradable material has to leave the rumen. This does not seem to agree with the fact that smaller particles have a better chance of leaving by passage and that for silages low in cell walls the mean rumen particle size is lower. Hence, not only the size of particles, but also their functional specific gravity (FSG) determines their chance of passage (Sutherland, 1987). Fermentation gas lowers the FSG, but can be removed from the particles by rumination and rumen contractions. Within concentrate level, high correlations between Cr-NDF passage rate and rumination time per kg silage DM ingested were found ($r$=0.97 and 0.96 for the low and high concentrate level, respectively (Table 6)). When particles have a higher fraction of undegradable material, the FSG will be higher, raising their chance for passage (Sutherland, 1987), although their size is likely to remain larger. The higher fraction of undegradable material, the slower rate of degradation in the rumen and the longer RT/kg silage DM for the high cell wall silages can explain the higher rates of passage from the rumen.

Table 6. An overview of parameters measured in experiments 1 to 4.

| | 1 kg of concentrates | | | | 7 kg of concentrates | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | G3 | G2 | G4 | G1 | G3 | G2 | G5 |
| max. rumen fill (g DM/kg BW) | 18.4 | 22.2 | 18.8 | 18.6 | 20.8 | 23.9 | 23.4 | 24.6 |
| Rumen DM <1.25mm (%) | 81 | 76 | 74 | 74 | 81 | 78 | 78 | 79 |
| Rumen DM <0.071mm (%) | 58 | 43 | 40 | 29 | 53 | 45 | 41 | 33 |
| Rumination time per kg silage DM | 42.4 | 36.9 | 50.5 | 52.5 | 47.5 | 45.7 | 54.0 | 57.3 |
| $k_c$ (%/h) | 6.6 | 6.2 | 5.9 | 6.1 | 7.7 | 6.9 | 6.4 | 5.8 |
| $k_p$-Cr | 4.1 | 3.7 | 5.2 | 5.0 | 3.6 | 3.6 | 4.2 | 4.2 |
| $k_d$-NDF | 5.9 | 4.6 | 3.8 | 3.9 | 6.4 | 3.8 | 4.0 | 2.7 |

More than 70% of rumen DM is in the SP pool (Chapters IV, VII), and this fraction increases with time after feeding (Chapter VII). Rate of particle size reduction through rumination can therefore not be regarded as the rate limiting factor in reduction in rumen fill.

For lactating dairy cows fed rations high in cell walls, the capacity of the rumen to contain DM seems to be limiting intake. Rumen DM content does not increase, while intake decreases when the cell wall content of the silage further increases. When rumen DM content has come to a point where a further increase is not possible, rate of clearance of feed from the rumen determines feed intake. Because of a higher fraction of undegradable material for the high cell wall silages, rate of passage of (undegraded) small feed particles to the lower gut seems to be of more importance than rate of degradation of the potentially degradable fraction.

The influence of the NDF content of grass silages on the parameters determined in the experiments described in this thesis, are summarized in Table 7.

4. *Markers*

Generally, rate of particle passage is determined using particulate markers. In literature, a lot of different markers have been described. Commonly used external markers (added to the diet or given separately) are among others Chromic oxide ($Cr_2O_3$) and Chromium mordanted Neutral Detergent Fibre (Cr-NDF). As an internal marker (occurs naturally in the diet) often lignin is used. In the experiments reported here, Cr-NDF with a particle size of 0.2-1 mm was used, and in some of the experiments also Tritium (T) labeled hay (particle size 10-20 mm). A single pulse dose of external markers results in a marker excretion curve in the faeces. The fractional rate of passage from the rumen ($k_p$, $\%h^{-1}$) of the marker is defined as the slope of the logarithmic decline of marker concentration in the faeces (Grovum & Williams, 1973). A marker has to be representative for the fraction that

Table 7. Influence of an increase in the NDF content of grass silages on the here determined parameters (0, no influence on parameter; +, the parameter increases; -, the parameter decreases with an increase in NDF content of grass silages).

| Parameter | | Chapter |
|---|---|---|
| Silage composition | | |
| CP content | - | II |
| lignin content | + | II |
| iADF/ADF | + | VII |
| DM degradation characteristics | | |
| $f_R$ | + | II, VII |
| $f_S$ | - | II, VII |
| $k_d$ | - | II |
| Rumination parameters | | |
| RT/kg DM | + | IV |
| RT/kg NDF | - | IV |
| Passage rate | | |
| $k_p$ | + | III |
| Rumen | | |
| NDF content | + | III |
| size reduction of LP | + | VII |
| fraction of DM in SP | 0 | IV, VII |
| mean size SP | + | IV, VII |
| iADF/ADF total rumen DM | 0 | VII |
| iADF/ADF in SP | 0 | VII |
| Faeces | | |
| mean particle size | + | IV |
| indigestible fraction | + | IV |
| Digestibility | - | II |
| Intake lactating cows | - | II* |
| Intake dry cows | + | VII* |
| Milk production | - | II |

* depends on nutrient demand and intake capacity of the animal

has to be measured. Assuming the marker is representative for the particulate fraction in the rumen, rumen particulate DM pool times $k_p$ should give total passage of DM from the rumen to the lower gut. Calculations of total DM escaping rumen fermentation according to this equation (Chapter III), show that the so determined $k_p$ can not be the fraction of rumen particles escaping per hour. As shown in Figure 3, particles have to be reduced to a size below the CPS to have a high probability of leaving the rumen by passage. Based on particle size, the Cr-NDF can only be respresentative for particles smaller than the CPS, meaning that when this marker is used, also the distribution of particle sizes in the rumen has to be measured. However, as mentioned earlier, not just the size of particles, but also their FSG determines their change for passage. Because the Cr-NDF is not fermented in the rumen and thus no fermentation gas is produced within the particles, their FSG does probably not change. The density as well as the size of Cr-NDF particles results in a faster outflow rate of these particles than of the feed particles. In this respect, T labeled hay should be a better marker than Cr-NDF. The T labeled hay particles have to be reduced in size through rumination and have a changing FSG due to microbial fermentation. However, rate of particle size reduction ($k_b$, Fig. 3) is influenced by the physical structure of the particles. The latter differs between feeds, and the ideal marker would need the same $k_b$ as the feed particles.

One of the criteria markers have to meet is indigestibility (Kotb & Luckey, 1972), which is not accounted for with the T labeled hay. T not only disappears from the rumen by passage, but also by degradation. Van Bruchem *et al.* (1990), after simulation of faecal excretion curves assuming degradation rates of 4 and 6 %/h and an undegradable fraction of 15% of the T labeled hay, concluded that the fraction of the T hay used to calculate $k_p$ was almost completely indigestible. Compared to the undegradable fractions of the grass silages used in the experiments described in this thesis (Chapter II), however, a $f_R$ of 15% seems too high. This would mean that the degradable fraction of the T labeled hay was higher at the part of the curve used to calculate $k_p$ than

assumed by Van Bruchem *et al.* (1990), probably resulting in an overestimation of $k_p$.

Despite the differences between Cr-NDF and the T labeled hay, both markers gave a considerable overestimation of the rate of passage of particles from the rumen and even the ratio $k_p$-Cr/$k_p$-T varied between diets and concentrate levels.

Therefore, in the experiment described in Chapter VII, $k_p$ was also calculated from the decline in rumen indigestible material with time after feeding ($k_{p\text{-}iOM}$, $\%h^{-1}$). Besides this, fractional rate of passage of Cr-NDF from the rumen was calculated from the decline in rumen Cr pool as well as measured in the faeces. It was shown that the clearance rate of Cr even gave a bigger overestimation of $k_p$ than would be expected from the faecal excretion curves.

Comparing the different values of $k_p$ with the total clearance rate from the rumen ($k_c$), it can be concluded that using the undegradable fraction of the feed as an internal marker and calculating the $k_p$ from the decline in rumen pool of this fraction presumably gives the best estimate of ruminal passage rate.

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# Summary

Because of the introduction of a milk quota system in 1984 and the subsequent decrease of the number of dairy cows with some 25%, an increasing number of farms in the Netherlands has a surplus of grass and grass silage, which makes it interesting to increase the roughage proportion in the diet. However, roughage intake by dairy cows in early lactation is limited and the mechanisms controlling roughage intake are still insufficiently understood. Factors presumably influencing roughage intake are the volume of the feed, the rate of physical size reduction, degradation rate in the rumen, passage rate of undigested particles from the rumen and the removal of fermentation end products.

In literature, the results of various experiments are reported in which one or a few of these factors, though not all together in an integrated system, were studied. Therefore, in the experiments described in this thesis, the effect of stage of maturity of grass silages on intake, digestibility, rumen fermentation pattern, rumination activity, passage rate from the rumen, degradation rate of the potentially degradable fractions and composition of the rumen contents was studied in an integrated approach.

Four experiments with lactating dairy cows fed grass silages harvested at different growth stages ad libitum and a fixed amount of concentrates, 1 or 7 kg depending on stage of latation, were performed.

When grasses matured, the chemical composition changed, the cell wall content (Neutral Detergent Fibre) of the silages and the lignin content of the cell wall fraction increased while crude protein (CP) content decreased. Together with this change in composition, the degradation characteristics changed. The soluble fraction ($f_S$,%), which is supposed to be fully and rapidly degraded in the rumen decreased, the rumen undegradable fraction (residue after 336 h nylon bag incubation, $f_R$, %) increased whereas the rate of degradation of the degradable, non-soluble fraction ($k_d$, $\%h^{-1}$) decreased. This resulted in a decrease in digestibility of the silages (Chapter II). Of the CP

fraction of the silages, $f_S$ as well as $f_R$ increased with an increase in cell wall content of the silages. The soluble fraction of the CP was more closely related to the DM content than to the NDF content of the silages. The $f_S$-CP decreased with an increase in DM content of the silage. Rate of degradation of the CP decreased, as for the other components, with an increase in cell wall content. Thus, the fraction of dietary protein escaping rumen fermentation increased with an increase in cell wall content, mainly because of the higher undegradable fraction. The latter fraction, however, is also not digestible in the intestines. Thus, the fraction of dietary protein escaping rumen fermentation and digestible in the intestines was presumably higher for the low cell wall silages (Chapter V).

Milk production (g Fat Corrected Milk/kg Metabolic Body Weight) and the amount of concentrates consumed (g/kg BW) together explained 64% of the variation in silage dry matter (DM) intake. Silage DM intake decreased with an increase in concentrate intake. A significant contribution to the explanation of variation in intake was given by the N/OM ratio in the silage and the $f_R$. The addition of these factors increased the percentage of the variation in silage DM intake explained to 68% (Chapter II).

The pH in the rumen declined with an increase in concentrate intake, whereas VFA concentrations increased. Diurnal variations in ammonia concentrations were higer for the high concentrate level, but the average ammonia concentrations were lower for the high concentrate level (Chapter IV).

Chewing and rumination time were recorded for six 24 h periods per cow. When rumination time (RT) exceeded 9 h a day, an increase in cell wall content of the silages did not result in a further increase in RT, but in a decrease in intake. RT per kg silage DM ingested increased with an increase in NDF content of the silages, whereas RT per kg silage NDF tended to decrease. Average size of particles in the rumen increased with maturity of the silages, resulting in an increased faecal particle size (Chapter IV).

Total rumen contents (kg) and rumen DM contents (kg) increased when the proportion of concentrates in the diet increased, but

no significant relation with cell wall content of the silages was found. Passage rate of the fluid from the rumen was not affected by diet composition. Rate of passage of undigested particles from the rumen, calculated from the logarithmic decline in faecal marker excretion, increased with an increase in NDF content of the silages and with an increase in intake level (Chapter III). In these experiments, it was concluded that the marker used to measure particle passage rate, Cr mordanted NDF, was not representative for total rumen contents. Cr-NDF particles with a size of 0.2-1mm do not have to be reduced in size, but it was calculated that not even they could be representative for particles with a size able to leave the rumen. Therefore an experiment was conducted in which three different particle sizes of Cr-NDF (<0.3 mm, 0.6-1.0 mm and 15-25 mm) were used to determine particle passage rate. In this experiment, 3 lactating and 3 non-lactating dairy cows were fed grass silage ad libitum, with 7 or 1 kg of concentrates, respectively. No significant differences between lactating and non-lactating cows were found, but rate of passage decreased with an increase in Cr-NDF particle size. Passage rate of the smallest particles (<0.3 mm) was considerably lower than passage rate of the fluid phase. The size of the particulate marker has a great influence on the calculated passage rates (Chapter IV).

Kinetics of rumen particles are considered to be important in intake regulation, but the rate limiting step in reduction in rumen fill was still not identified. In an experiment with four dry rumen fistulated cows, rumen contents and rates of disappearance of different ruminal fractions were determined (Chapter VII). The cows were fed ad libitum grass silages, differing in cell wall content. Ruminal passage rates were calculated from the logarithmic decline in rumen Cr pool, as well as by dividing the intake of undegradable organic matter (iOM) or indigestible acid detergent fibre (iADF) by the mean rumen pool of these fractions. The thus calculated passage rates using the pools of iOM or iADF were much lower than using the Cr-NDF (particle size 0.2-1 mm), but the differences between silages

were comparable.
Particle size distributions of rumen contents, collected at different times after feeding were determined. The iADF content of particles passing a 1.25 mm, but retained on a 0.071 mm sieve was determined. From the results of this experiment it was concluded that clearance rate of particles with a size between 1.25 mm and 0.071 mm is the rate limiting step in reduction in rumen fill, which in turn seems to depend on the degree of digestion.

In the General Discussion (Chapter VIII), the results from these experiments were combined with literature data on rumen fill and clearance rate from the rumen. Multiple regression analysis showed that milk yield and concentrate proportion of the diet explained 77% of the variation in rumen fill. Roughage characteristics did not have a significant influence on rumen DM fill. Rate of clearance, however, significantly decreased with an increase in roughage NDF content. Rumen fill and rate of clearance were not significantly correlated.
From this study it may be concluded that important factors such as intake, digestibility and milk production all three were negatively influenced by an increase in silage cell wall content. When intake is not limited by the capacity of the rumen, as for the dry cows in the experiment described in chapter VII, intake increases with an increase in silage cell wall content.

# Samenvatting

Vanwege de invoering van de superheffing in 1984 en de, als gevolg daarvan, afname van het aantal melkkoeien met ongeveer 25%, is in Nederland op een toenemend aantal bedrijven een overschot aan gras en grassilages ontstaan. Om deze reden is het interessant om het ruwvoer aandeel in de rantsoenen te verhogen. In het begin van de lactatie is de ruwvoeropname door melkkoeien echter beperkt, en de mechanismen waardoor de ruwvoeropname bepaald wordt zijn nog niet geheel bekend. Factoren die mogelijk een invloed op de ruwvoeropname hebben zijn het volume van het voer, de snelheid van voerdeeltjes-verkleining, de microbiële afbraaksnelheid in de pens, de passage snelheid van onverteerde voerdeeltjes uit de pens en de snelheid waarmee de eindprodukten van de fermentatie worden afgevoerd.

In de literatuur worden vele proeven gerapporteerd waarin één of enkele van deze factoren onderzocht werden, doch niet allen tezamen in een geïntegreerd systeem. Daarom zijn in de proeven beschreven in dit proefschrift, de effecten van het groeistadium van grassilages op opname, vertering, pensfermentatie patroon, herkauwaktiviteit, passage snelheid uit de pens, afbraaksnelheid van de potentieel verteerbare fractie in de pens en samenstelling van de pensinhoud onderzocht in een geïntegreerd systeem.

Vier proeven met lacterende koeien, ad libitum gevoerd met grassilages geoogst in verschillende groeisatdia, en een gefixeerde hoeveelheid krachtvoer, 1 of 7 kg, afhankelijk van lactatiestadium, werden uitgevoerd.

Bij het ouder worden van het gras veranderde de chemische samenstelling. Het celwandgehalte (Neutral Detergent Fibre) en het lignine gehalte in de celwandfractiename toe, en het ruw eiwitgehalte (CP) nam af. Met deze verandering in samenstelling veranderden ook de afbraak krakteristieken. De oplosbare fractie ($f_s$, %), waarvan aangenomen wordt dat ze volledig en snel in de pens wordt afgebroken, daalde. De in de pens onverteerbare fractie (residu na 336 uur incubatie in de pens in nylon zakjes, $f_R$, %) nam toe en de afbraaksnelheid van de verteerbare, niet oplosbare fractie ($k_d$, $\%h^{-1}$) nam af. Dit resulteerd in een daling van de verteerbaarheid van de silages (Hoofdstuk II). Van de ruw

eiwitfractie van de silages, de $f_s$ zowel als de $f_R$, namen toe met een toename van het celwand-gehalte van de silages. De oplosbare fractie van het ruw eiwit was meer gerelateerd aan het droge stof (DM) gehalte dan aan het NDF gehalte van de silages. De $f_s$-CP nam af met een toename van het DM gehalte van de silages. Afbraaksnelheid van ruw eiwit nam, evenals voor de andere componenten, af met een toename in het celwandgehalte. De fractie van het voereiwit die aan fermentatieve afbraak in de pens ontsnapte, nam toe met een toename van het celwandgehalte, vooral vanwege de grotere onverteerbare fractie. De in de pens onverteerbare fractie is echter ook in de dunne darm niet verteerbaar. Het aandeel van het voereiwit dat aan fermentatie ontsnapt en verteerbaar is in de darm was dus hoger voor de silages met de lagere celwandgehalten (Hoofdstuk V).
Melkproduktie (g meetmelk/kg metabool gewicht) en de hoeveelheid opgenomen krachtvoer (g/kg lichaamsgewicht) samen verklaarden 64% van de variatie in DM opname uit silage. De droge stof opname uit silage nam af met een toename in de krachtvoeropname. De hoeveelheid stikstof in de organische stof in de silages en de $f_R$, droegen beide significant bij aan de verklaring van de variatie in DM opname uit silage. Een toevoeging van deze factoren verhoogde het percentage van de variatie in DM opname uit silage dat door het model verklaard werd naar 68% (Hoofdstruk II).

De pH in de pens daalde met een toename in krachtvoeropname, terwijl de concentratie aan vluchtige vetzuren in de pens toenam. De dagelijkse variaties in de ammoniak concentraties in de pens waren hoger, maar de gemiddelde concentraties waren lager voor het hoge krachtvoer niveau dan voor het lage krachtvoer niveau (Hoofdstuk IV).

Kauw- en herkauwtijden werden geregistreerd gedurende 6 x 24 uur per koe. Wanneer er langer dan 9 uur per dag herkauwd werd, resulteerde een toename in het celwandgehalte van de silages niet in een verdere stijging van de herkauwtijden, maar in een daling van de opname. Herkauwtijden per kg opgenomen DM uit silage name toe met een toename in het celwandgehalte. Per kg opgenomen celwanden werd er echter een dalende tendens in herkauwtijden waargenomen.

De gemiddelde deeltjesgrootte in de pens nam toe met het ouder worden van het gras, wat resulteerde in een toename van de deeltjesgrootte in de mest (Hoofdstuk IV).

De totale pensinhoud (kg) en de DM inhoud (kg) namen toe wanneer het krachtvoeraandeel in het rantsoen toenam, maar er werd geen significante relatie met het celwandgehalte van de silages gevonden. Passagesnelheid van de vloeistof uit de pens werd niet beinvloedt door de samenstelling van het rantsoen. Passagesnelheid van onverteerde voerdeeltjes, berekend uit de logaritmische afname van de mestconcentratie van de indicator, steeg met een toename in celwandgehalte van de silages en met een toename in opnameniveau (Hoofdstuk III).

In deze proeven werd geconcludeerd dat de indicator, gebruikt om de passage van de deeltjes te meten (Cr-NDF), niet representatief was voor de gehele pensinhoud. Cr-NDF deeltjes, met een grootte van 0.2-1mm behoeven niet verkleind te worden, maar uit berekeningen bleek dat zij zelfs niet representatief konden zijn voor deeltjes die gezien hun grootte de pens konden verlaten. Daarom werd een proef uitgevoerd waarin Cr-NDF met drie verschillende deeltjesgroottes (<0.3 mm, 0.6-1.0 mm and 15-25 mm) gebruikt werd om de snelheid van deeltjespassage te bepalen. In deze proef werden drie lacterende en drie droge koeien ad libitum gevoerd met grassilage, gesupplementeerd met respectievelijk 7 en 1 kg krachtvoer. Er werd geen verschil gevonden in passage snelheid van deeltjes tussen lacterende en droge koeien. De snelheid van passage nam af met een toename van de deeltjesgrootte van het Cr-NDF. De passage van de kleinste deeltjes (<0.3 mm) was echter nog aanzienlijk langzamer dan van de vloeistof. De deeltjesgrootte van de indicator heeft een grote invloed op de berekende passage snelheid (Hoofdstuk VI).

Het is algemeen aanvaard dat de kinetiek van de deeltjes in de pens een grote invloed heeft in de opnameregulatie. Wat de beperkende factor is in de afname van de pensinhoud is echter nog niet bekend. In een proef met vier droge koeien, werden de pensinhouden en de verdwijningssnelheden van de verschillende fracties uit de pens bepaald (Hoofdstuk VII). De dieren werden

ad libitum gevoerd met grassilages met verschillende celwand-gehalten. De passagesnelheid uit de pens werd berekend uit de logaritmische afname in Cr hoeveelheid in de pens. Door de opname aan onverteerbare organische stof (iOM) of onverteerbare celwandbestanddelen (iADF) te delen door de gemiddelde penspool van deze fracties, werden de passage-snelheden eveneens berekend. De op deze laatste manier berekende passagesnelheden waren aanzienlijk lager dan de uit de Cr (deeltjesgrootte 0.2-1 mm) afname berekende waarden, hoewel de verschillen tussen de silages vergelijkbaar waren.
Van pensinhouden verzameld op verschillende tijden na voeren werd de deeltjesgrootte verdeling bepaald. Het iADF gehalte van deeltjes >0.071mm, en <1.25mm, werd bepaald. Uit de resultaten van deze proef werd geconcludeerd dat verdwijnings-snelheid van deeltjes met een grootte tussen 0.071 en 1.25 mm de beperkende factor is in de afname van de pensinhoud. De afname van de pensinhoud hangt eveneens af van de mate van verteerd zijn van de deeltjes.

In de General Discussion (Hoofdstuk VIII) worden de gegevens uit deze proeven gecombineerd met gegevens uit de literatuur omtrent pensinhoud en verdwijningssnelheid uit de pens. Multipele regressie analyse toonde aan dat melk produktie en krachtvoer aandeel in het rantsoen, 77% van de variatie in pensinhoud konden verklaren. kenmerken van het ruwvoer hadden geen significante invloed op pensinhoud. Verdwijningssnelheid echter, nam significant af met een toename van het celwandgehalte van het ruwvoer. Pensinhoud en verdwijningssnelheid waren niet significant gecorreleerd.
Uit deze studie kan geconcludeerd worden dat belangrijke factoren, zoals opname, vertering en melkproduktie alle drie negatief beïnloed worden bij een toename in het celwandgehalte van grassilage. Indien de opname niet beperkt wordt door de capaciteit van de pens, zoals voor de droge koeien in de proef beschreven in Hoofdstuk VII, neemt de opname toe met een toename van het celwandgehalte van grassilages.

**Curriculum vitae**

Maria Wilhelmina Bosch werd op 1 februari 1960 geboren te Hulst. Zij behaalde in 1978 het Atheneum-B diploma aan het Jansenius Lyceum te Hulst. In september van datzelfde jaar begon zij met haar studie Zöotechniek aan de toenmalige Landbouw-hogeschool te Wagenningen. In juli 1985 behaalde zij het doctoraalexamen, met Veevoeding en Dierfysiologie als hoofdvakken en Agrarische Bedrijfseconomie als bijvak. Op 1 september 1985 werd zij aangesteld bij de vakgroep Fysiologie van Mens en Dier als universitair docent in tijdelijke dienst, en werkte zij o.a. aan het onderzoek wat resulteerde in dit proefschrift. Van 1 oktober 1989 tot 15 februari 1991 was zij werkzaam als veevoedkundig onderzoeker bij TNO afdeling ILOB. Sinds 15 februari 1991 is zij werkzaam als universitair docent bij de vakgroep Veevoeding van de Landbouwuniversiteit.