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Autumn-cut grass silage as roughage component in dairy cow rations. 2. Rumen degradation, fermentation and kinetics

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Abstract

A 4×4 Latin square experiment was performed to study the effects of dry matter content and/or the extent of fermentation in grass silages on the pattern of rumen fermentation and rumen kinetics. In a separate study two animals were used to measure the rate of degradation using the dacron bag technique. Four rumen cannulated dairy cows were used to measure rumen fermentation pattern, rumen kinetics were measured in three of these animals. The basal diets (70% of total DM) consisted of maize silage, moist ensiled beet pulp, moist ensiled maize gluten feed, moist ensiled brewers' grains and a concentrate mixture. The remainder of the diet (30% of total DM) consisted either of wilted grass silage (WGS), high moisture grass silage with molasses (MGS), high moisture silage with formic acid (FGS) or wilted grass silage with additional water (WW). All diets were fed as totally mixed rations (TMR). The pH of the rumen fluid was lower on the MGS and FGS diets. The concentrations of total VFA, acetic acid, ammonia and branched-chain fatty acids (BCFA) were highest on high moisture diets (MGS and FGS). The rates of clearance and digestion of the OM fractions were or showed tendencies towards being negatively influenced by both high moisture grass silages (MGS, FGS), but remained uneffected by DM content (WGS, WW). Degradability of the grass silages was influenced by fermentation in the silo (lower digestible fractions and higher soluble fractions), as were the rates of degradation (higher). Results of the degradability measured on the basal diet ingredients (maize silage, beet pulp, maize gluten feed, brewers' grains) were in agreement with published literature and showed a strong correlation between OM digestibility *in vitro* and the undigestible fraction.

Keywords: cow, rumen, grass silage, degradability, fermentation, kinetics

Introduction

During the winter period grass silage is the most important roughage component in Dutch dairy diets. These diets are supplemented with by-product based concentrate mixtures to meet energy and protein requirements. The concentrates are fed dry, but occasionally are partly replaced by moist ensiled by-products. In general, the chemi-

cal composition of these moist concentrates is similar to the dried by-products (De Visser & Steg, 1988). However, during the ensiling process easily fermentable components, such as sugars and rapidly degradable starch and protein, are fermented by micro-organisms and are transformed into volatile fatty acids, lactic acid and alcohols (De Visser & Tamminga, 1987; De Visser & Hindle, 1990). Likewise, the energy available in the roughage of dairy diets can easily be converted into fermentation end products with a concomitant reduction of easily fermentable carbohydrates. Highest concentrations of fermentation end products were found in high moisture silage as compared to wilted grass silage (Donaldson & Edwards, 1976; Murphy & Gleeson, 1984; De Visser & Hindle, 1992). Feeding such high moisture diets to dairy cows in early lactation may reduce milk protein output (De Visser & Tamminga, 1987; De Visser & Hindle, 1992). In a fermentation study, Robinson et al. (1986) found lower amounts of microbial protein synthesized in the rumen of dairy cows fed moist instead of dry by-products.

In addition to a feeding trial comparing grass silages either wilted, or moist ensiled with additives, molasses or formic acid, (De Visser & Hindle, 1992), a rumen fermentation and kinetic study was performed. The aim was to investigate the influence of the moisture content and fermentation end products in silages on rumen fermentation pattern and kinetics as well as rumen degradability of various organic matter fractions.

Material and methods

Four dairy cows of the Dutch Friesian Black and White × Holstein breed were used in a 4×4 Latin square design. Three animals were fitted with a large rumen cannula (10 cm internal diameter. Bar-Diamond Inc., Parma, ID, USA); the fourth animal was equipped with a small rumen cannula (5 cm internal diameter. Eriks, Alkmaar, Netherlands). They were housed in a tie stall and offered a totally mixed ration (TMR), using a mixer-forage wagon to minimize selection of dietary intake. The rations fed were similar to those of an accompanying feeding trial (De Visser & Hindle, 1992). The basal diet which supplied 70% of total dry matter intake (DMI) consisted of maize silage (14% DMI), moist ensiled beet pulp (20% DMI), moist ensiled maize gluten feed (15% DMI), moist ensiled brewers' grains (10% DMI) and concentrates (11% DMI). The concentrate-mixture consisted of coconut expeller (40.5%), soya bean hulls (10%), linseed expeller (20%), soya bean meal solv. extr. (14.5%), tallow (3.3%), cane molasses (4%), calcium carbonate (1.7%), magnesium oxide (2%), sodium chloride (2%) and vitamins and minerals (2%). In addition to the basal diet four different grass silages were fed. Treatments consisted of wilted grass silage (WGS), moist grass silage with molasses (MGS; 40 kg per ton fresh grass), moist grass silage with formic acid (FGS; 6 liters per ton fresh grass) and wilted grass silage with added water (WW). This water was added during the preparation of the diet and was equal to the difference in water content between the wilted grass silage and the grass silage with molasses. All grass silages were harvested from the same pastures. Preparation and storage of the silages were as described by De Visser & Hindle (1992). The animals were fed twice daily. Forty percent of total

Table 1. Experimental design of rumen fermentation and kinetics study.

Period	Cow numbers			
	744[a]	829	843	1563
1	WGS	MGS	FGS	WW
2	MGS	FGS	WW	WGS
3	WW	WGS	MGS	FGS
4	FGS	WW	WGS	MGS

[a] Cow fitted with a small cannula which was not used in the kinetic study.
Treatments: WGS = wilted grass silage; MGS = moist grass silage with molasses; FGS = moist grass silage with formic acid; WW = wilted grass silage with extra water.

DM was offered at 5.00 h and 60 percent at 14.00 h, respectively. Feed intake level was approximately 20 kg DM.

The design of the experiment is given in Table 1. Each experimental period lasted for 6 weeks. Four weeks were used for adaptation to the diet. During the 5th week rumen fermentation was studied, the sixth week was used to determine rumen kinetics. During the last two weeks samples were taken from all diet ingredients and analysed for DM, ash, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), indigestible acid detergent fibre (IADF), sugars, starch and volatiles. The analytical methods used were as described by Robinson et al. (1986, 1987) and De Visser & Hindle (1990).

Rumen fermentation

A period of 48 hours was taken to determine the fermentation pattern with 17 samples of the rumen fluid as described by De Visser et al. (1991). Samples were taken at 5.00, 7.00, 10.00, 14.00, 16.00, 19.00, 22.00, 2.00 and 5.00 hours, respectively. The samples were immediately analysed for pH using a pH-meter (Philips 2000). Subsamples were taken, preserved and stored at –20 °C for the analysis of volatile fatty acids (VFA), lactic acid (LA) and ammonia (NH_3-N) (Robinson et al., 1986). The ratio between the non-glucogenic and glucogenic fatty acids (NGR) was calculated as described by Ørskov et al. (1975).

$$NGR = (HAc+2(IHB+HB)+2MHB+3MHB+HV)/(HP+2MHB+3MHB+HV+HL)$$

Statistical analyses were performed using cow, period and diets as explanatory variables with mean daily values (weighted for time intervals) and daily variations (expressed as standard deviation of daily values), using the statistical package Genstat (Alvey et al., 1982).

Rumen kinetics

Over a period of 36 hours the average daily rumen mass was calculated using manual

evacuation of total rumen contents on three occasions (4.00, 10.00 and 20.00 h). Between evacuations animals had access to food and received at least one meal. The method of evacuation was as described by Robinson et al. (1987).

During the evacuations a sample was taken of the total rumen mass, which was stored at –40 °C, freeze dried and analysed for ash, N, NDF, ADF, ADL and IADF. The total VFA content (g) of the rumen was calculated using two methods. The first method estimated VFA by multiplying total rumen fluid measured by rumen evacuations by the concentration of VFA in the rumen fluid, collected immediately before rumen evacuation (total rumen VFA using fluid: TRVFAF). In the second method the concentration of VFA was estimated after an overnight extraction with water in a subsample (100 g) of evacuated rumen mass (H. Huisert & S.F. Spoelstra, 1987). The concentration analyzed was multiplied by total rumen mass (total rumen VFA using rumen mass: TRVFAM).

Clearance, passage and digestion of OM, N, NDF, ADF, lignin (ADL), digestible acid detergent fibre (DADF), hemicellulose (NDF-ADF) and cellulose (ADF-ADL) were calculated as:

- rate of clearance (k_c) = ((feed intake, kg/d)/(average rumen pool, kg))/24
- rate of passage (k_p) = ((IADF intake, kg/d)/(average rumen IADF pool, kg))/24
- rate of digestion (k_d) = (k_c-k_p).

Because only 3 animals were fitted with a large rumen cannula, the design of the experiment was not completely orthogonal (Table 1). Analysis of variance was performed, using cow, period and diet as dependant variables for rumen kinetic parameters, using the statistical package Genstat (Alvey et al., 1982). The values missing from the fourth animal were estimated as part of the statistical analysis.

Rumen degradability

The degradability of all dietary ingredients was measured using the nylon bag technique. The method used was as described by Van Vuuren et al. (1989). The degradability was measured for OM, N and NDF, using two dairy cows fitted with a large rumen cannula (10 cm internal diameter. Bar-Diamond Inc., Parma, ID, USA). These cows were in the sixth month of lactation and were fed the intermediate diet of the rumen fermentation study (1/3 WGS + 1/3 MGS + 1/3 FGS), offered as TMR. Feeding procedures were similar to those of the rumen fermentation study. The animals received approximately 18 kg DM/day.

The incubation times were 0, 2, 4, 6, 12, 18, 24, 48 and 216 hours. The fractions were divided into a soluble fraction (S), an indigestible fraction (U) and a potentially digestible fraction (D=100-S-U). The rate constant (k_d) of D was estimated by iteration (Robinson et al, 1987).

Results

Apart from the DM content, variations in chemical composition of the diets were limited; only minor differences were observed in ash and cell wall fractions (Table 2). The chemical composition of the basal diet ingredients, maize silage, pressed

Table 2. The chemical composition of total diets (g/kg dry matter).

	Diets			
	WGS	MGS	FGS	WW
Chemical composition total diet				
Dry matter (g/kg)	398	335	331	332
Ash	88	101	98	88
Nitrogen	28	28	28	28
Crude fat	38	38	39	38
Crude fibre	196	183	185	196
Neutral detergent fibre (NDF)	483	459	464	483
Acid detergent fibre (ADF)	228	210	215	228
Acid detergent lignin (ADL)	22	21	20	22
Indigestible acid detergent fibre (IADF)	36	32	31	36
Starch	66	66	66	66
Sugars	22	18	26	22
Vitro OM[a] (%)	77	77	77	77
Chemical composition grass silages				
Dry matter (g/kg)	456	246	233	
Ash	113	156	146	
Nitrogen	30	31	32	
Crude fat	39	42	46	
Crude fibre	268	222	229	
Neutral detergent fibre (NDF)	492	412	429	
Acid detergent fibre (ADF)	286	234	250	
Acid detergent lignin (ADL)	25	20	18	
Indigestible acid detergent fibre (IADF)	54	41	38	
Sugars	26	13	41	
Vitro OM (%)	73	73	73	
Volatile content grass silages				
Acetate	10	18	19	
Butyrate	0	0	2	
Lactate	36	118	26	
Ammonia	3	2	1	
Alcohols	2	7	7	

WGS = Wilted grass silage; MGS = Moist grass silage with molasses; FGS = Moist grass silage with formic acid; WW = Wilted grass silage with extra water.
[a] *Vitro* OM = digestibility measured in vitro (Tilley & Terry, 1963) as modified by IVVO (Van der Meer, 1980)

ensiled beet pulp, ensiled maize gluten feed, ensiled brewer's grains and the concentrate mixture are shown in Table 3. However, the chemical composition in the grass silages varied (Table 2). Wilted grass silage was highest in DM and cell wall fractions compared to both high moisture silages. Grass silages also differed in volatile content. Especially, MGS silage contained large quantities of lactic acid (Table 2). Both high moisture silages (MGS, FGS) showed higher quantities of acetic acid and alcohols (Table 2). The results of the rumen fermentation study are shown in Table 4. Mean pH was lowest for both high moisture silage diets (MGS, FGS), whereas the

Table 3. Chemical composition of basal diet ingredients (g/kg dry matter).

	Maize silage	Beet pulp	Maize gluten feed	Brewers grains	Concentrate mixture
Dry matter (g/kg)	269	213	401	229	898
Ash	57	86	61	53	139
Nitrogen	13	16	34	44	37
Crude fat	25	–	22	100	83
Crude fibre	227	196	103	161	127
Neutral detergent fibre (NDF)	453	530	435	614	360
Starch	259	–	168	22	17
Sugars	–	13	10	–	88
vitro OM (%)	71	87	88	56	80

Beet pulp = moist ensiled pressed beet pulp: Maize gluten feed = moist ensiled maize gluten feed; Brewers' grains = moist ensiled brewers' grains.

Table 4. Rumen characteristics (pH, osmolality and concentrations of volatile fatty acids, lactic acid, ammonia and branched chain fatty acids (mMol/l).

Treatments	WGS	MGS	FGS	WW	SED
DM intake (kg/ day)	20.7	20.5	20.8	20.6	0.80
pH, mean	6.10^{a}	5.98^{b}	6.03^{ab}	6.12^{a}	0.03
pH, range	0.35	0.34	0.33	0.33	0.03
Osmolality, mean	0.321^{a}	0.333^{b}	0.328^{b}	0.320^{a}	0.001
Osmolality, range	0.03	0.03	0.03	0.02	0.002
Total VFA, mean	121^{a}	129^{b}	130^{b}	120^{a}	3.03
Total VFA, range	15.5	16.5	17.9	14.3	1.88
NGR[1], mean	4.64	4.44	4.50	4.63	0.16
NGR, range	0.63	0.54	0.58	0.62	0.05
Lactate, mean	2.74	3.19	2.29	1.79	0.63
Lactate, range	4.45	4.44	3.95	2.81	1.48
Ammonia, mean	9.27^{ab}	10.41^{b}	9.86^{ab}	8.99^{a}	0.37
Ammonia, range	7.26^{a}	7.58^{a}	7.25^{a}	6.50^{b}	0.25
Acetate, mean	79^{b}	82^{b}	84^{b}	76^{a}	1.80
Acetate, range	7.87	8.14	9.70	7.75	1.27
Propionate, mean	23	26	25	23	1.06
Propionate, range	5.00	5.16	5.30	4.63	0.29
Butyrate, mean	16	18	17	17	0.51
Butyrate, range	2.01	2.44	2.97	2.05	0.34
BCFA[2], mean	3.16^{a}	3.83^{b}	3.97^{b}	3.33^{a}	0.23
BCFA, range	0.44	0.46	0.43	0.41	0.03

Figures with a different superscript are significantly different ($p<0.05$).
SED = Standard error of difference; mean = mean daily values (weighted for time intervals); range = calculated as standard deviation of daily values.
[1] NGR = Non-glucogenic Glucogenic Ratio (Ørskov, 1975).
[2] BCFA = branched chain fatty acids (iso butyrate, 2- and 3-methyl butyrate and valerate).

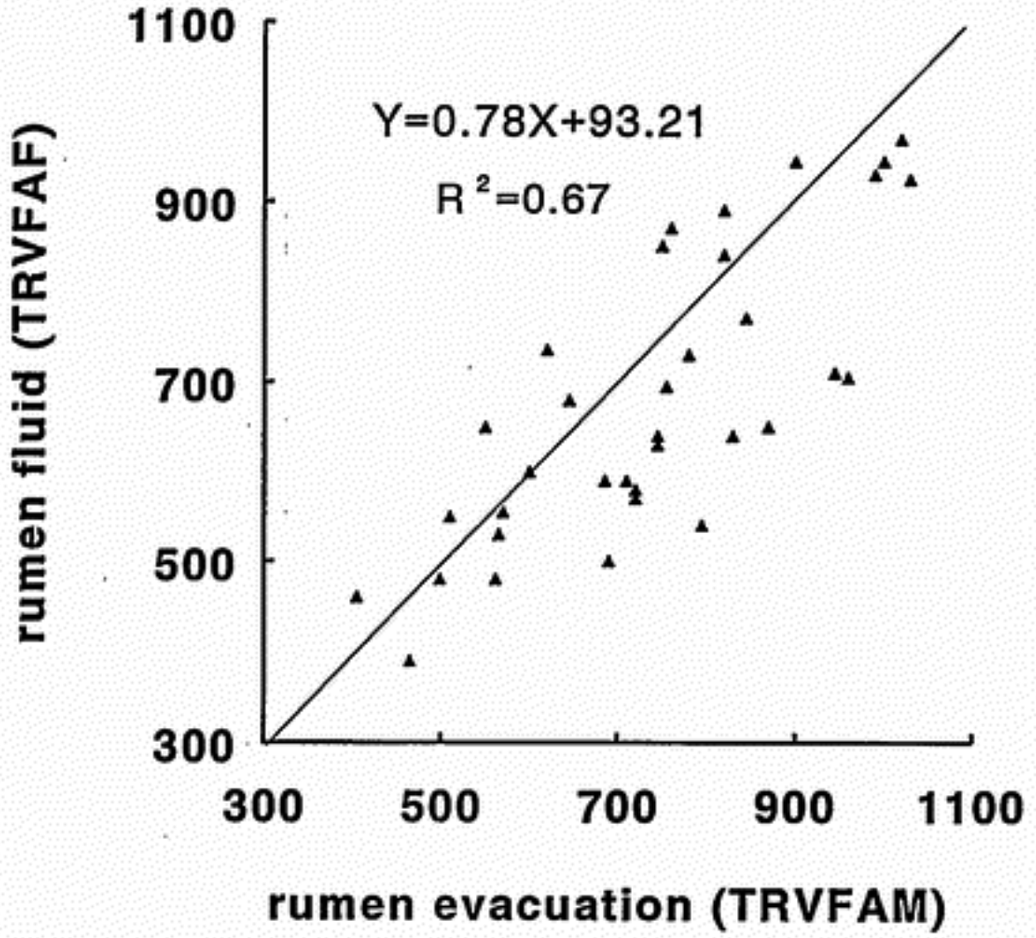

Fig. 1. Calculation of rumen VFA from rumen fluid or evacuation data.

mean concentration of total VFA was highest for these diets. The NGR tended to be lower for MGS and FGS reflecting changes in the major VFA's (acetic and propionic acid). The concentration of NH_3-N, branched-chain fatty acids and valerate (BCFA) were higher for high moisture diets (MGS, FGS). Lactic acid tended to be higher in cows fed MGS. Except for the NH_3-N concentrations, no significant effect on ranges in fermentation parameters were observed. The total amount of VFA present in the rumen was significantly higher for TRVFAM, than for TRVFAF (Fig. 1).

Rumen pool sizes of different OM components did not differ between diets, except for ADL and IADF (Table 5). ADL and IADF followed the same pattern and were lowest for both moist diets (MGS and FGS). The clearance rate of OM, NDF, ADF, DADF, hemicellulose and cellulose was lower or tended to be lower for the groups fed high moisture silage (MGS, FGS, Table 6). The rate of clearance was similar for both ADL and IADF fractions. Substractions, using the average rate of clearance of the IADF and ADL fractions as the rate of passage of particles, resulted in tendencies towards reduced rates of digestion of the OM, NDF, ADF, DADF, hemicellulose and cellulose fractions on high moisture diets (MGS, FGS; Table 6).

The S, D and U fractions of the various dietary components observed *in situ* are shown in Table 7. The basal-diet components showed large differences in the rate of degradation for the various OM fractions. Pressed beet pulp was lowest in S and U

Table 5. Total dry matter intake (TDM) and average rumen pool sizes of dry matter (DM), organic matter (OM), nitrogen (N) and cell wall constituents.

Treatments	WGS	MGS	FGS	WW	SED
TDM intake (kg)	24.4	23.7	23.5	23.8	1.16
Body weight (kg)	610	595	588	612	
DM pool/kg bodyweight (g)	18.7	18.0	18.5	18.5	
Total rumen contents					
Non-dry matter (kg)	74.5	75.4	73.5	73.0	2.65
Dry matter (kg)	11.2	11.1	10.9	11.0	0.51
Total ingesta (kg)	85.9	86.5	84.4	84.0	3.16
Percentage DM	13.1	12.8	12.9	13.1	0.17
Rumen pool sizes					
OM (kg)	10.1	10.0	9.7	10.0	0.46
N (g)	394	372	383	398	10.32
NDF (kg)	5.9	5.6	5.4	5.7	0.32
ADF (kg)	3.2	3.1	2.8	3.2	0.21
ADL (kg)	0.56^a	0.52^b	0.47^c	0.53^b	0.005
IADF (kg)	0.96^a	0.89^b	0.73^c	0.93^a	0.05
DADF (kg)	2.3	2.3	2.1	2.3	0.21
Hemicellulose (kg)	2.7	2.3	2.5	2.7	0.21
Cellulose (kg)	2.6	2.6	2.5	2.7	0.27

Figures with a different superscript differ significantly ($p<0.05$). SED = Standard error of difference.

fractions of the OM, N and NDF. Brewers' grains and maize silage showed the highest U fractions for OM and NDF, while the S fraction of N was highest in maize gluten feed. As a result the rate of degradation differed between basal diet ingredients. Degradation of the OM, N and NDF fractions was slower in WGS silage compared to MGS and FGS silage.

Discussion

WGS silage showed the highest NDF values in comparison to the high moisture silages (MGS, FGS). This finding agreed with results from earlier laboratory studies performed by Van Vuuren et al. (1989). They concluded that the drying process itself positively influenced NDF levels; probably as a result of a Maillard reaction. The high moisture silages (MGS, FGS) were higher in acetic acid and alcohols than the WGS silage. MGS silage displayed the highest lactic acid content, which agreed with previous findings (Donaldsen & Edwards, 1976; Murphy & Gleeson, 1984; De Visser & Hindle, 1992), as a result of differences in type and rate of fermentation during the ensiling process.

The differences in the soluble fractions between N and OM of the grass silages suggested differences in NH_3-N between silages, which was confirmed by the higher amount of NH_3-N found in WGS silage and the lowest value in FGS silage (Tables 2 and 7).

The results of the *in situ* study with the grass silages agreed with results presented

Table 6. Turnover of organic matter (OM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), digestible acid detergent fibre (DADF), hemicellulose and cellulose calculated from rumen evacuation data (rates %/hour).

Treatments	WGS	MGS	FGS	WW	SED
ADL rates					
$k_{PASSAGE}$	4.1	3.9	3.8	4.2	0.10
IADF rates					
$k_{PASSAGE}$	4.0	3.7	3.8	4.0	0.43
OM rates					
$k_{CLEARANCE}$	9.3	8.8	8.4	9.2	0.45
$k_{PASSAGE}$	4.0	3.7	3.8	4.0	0.43
$k_{DIGESTION}$	5.3[a]	5.1[a]	4.6[b]	5.2[a]	0.17
N rates					
$k_{CLEARANCE}$	7.5	7.3	6.7	7.9	0.44
$k_{DIGESTION}$	5.3[a]	3.6[b]	2.9[b]	5.2[a]	0.22
NDF rates					
$k_{CLEARANCE}$	8.1	7.4	7.2	8.2	0.56
$k_{DIGESTION}$	4.1	3.7	3.6	4.2	0.30
ADF rates					
$k_{CLEARANCE}$	7.5	6.4	6.5	7.3	0.65
$k_{DIGESTION}$	3.5	2.7	2.7	3.3	0.45
DADF rates					
$k_{CLEARANCE}$	8.3	7.1	7.2	8.2	0.55
$k_{DIGESTION}$	4.3	3.4	3.4	4.2	0.29
Hemicellulose rates					
$k_{CLEARANCE}$	8.6	8.2	8.1	8.6	0.34
$k_{DIGESTION}$	4.6	3.5	3.3	4.6	0.55
Cellulose rates					
$k_{CLEARANCE}$	7.9	6.8	7.1	8.1	0.70
$k_{DIGESTION}$	3.9	3.1	3.3	4.1	0.58

Figures with a different superscript are significantly different ($p<0.05$). WGS = diet with wilted grass silage; MGS = diet with moist grass silage with molasses; FGS = diet with moist grass silage with formic acid; WW = diet with wilted grass silage and extra water.

by Bosch (1991). The results obtained with WGS silage compared favourably with the relationships that Bosch (1991) found between S and U fractions and the NDF content of grass silages. However, our findings for MGS and FGS silage did not compare as well as the others, due to the lower NDF contents measured in both of these high moisture silages.

The OM of the maize silage displayed a higher S fraction and lower U fraction compared to earlier findings published by De Visser et al. (1991), although the chemical composition of the maize silage appeared to be similar. Differences are probably attributable to the stage of maturity of the maize silage (De Visser, unpublished). However, the degradation rate of OM fell within the range published. The OM fraction in pressed beet pulp had a lower S fraction compared to earlier results published

Table 7. Degradability characteristics for diet components using nylon bag incubations in dairy cows.

Component	Fraction (%)			k_d
	S	U	D	
Organic matter				
Wilted grass silage	27	13	60	4.85
Grass silage with molasses	30	11	59	5.64
Grass silage with formic acid	31	10	59	5.65
Maize silage	45	17	38	2.73
Beet pulp (moist ensiled)	10	4	85	6.55
Maize gluten feed (moist ensiled)	37	3	60	4.36
Brewers' grains (moist ensiled)	16	18	66	3.68
Concentrates	37	6	57	9.71
Nitrogen				
Wilted grass silage	54	10	36	6.25
Grass silage with molasses	52	10	38	7.95
Grass silage with formic acid	48	8	44	7.85
Maize silage	67	22	11	1.30
Beet pulp (moist ensiled)	15	77	8	6.10
Maize gluten feed (moist ensiled)	73	3	24	5.85
Brewers' grains (moist ensiled)	27	7	66	2.95
Concentrates	30	3	67	9.15
Neutral detergent fibre				
Wilted grass silage	0	18	82	4.28
Grass silage with molasses	0	14	86	4.85
Grass silage with formic acid	0	13	87	4.91
Maize silage	0	29	71	2.24
Beet pulp (moist ensiled)	0	6	94	5.95
Maize gluten feed (moist ensiled)	0	5	95	3.69
Brewers' grains (moist ensiled)	0	24	76	4.76
Concentrates	0	13	87	9.30

S = soluble fraction; U = undigestible fraction; D = potential digestible fraction; k_d = rate of degradation of the digestible fraction.

by De Visser et al. (1991). The U fraction, however, was low and agreed with De Visser et al. (1991) and corresponded well with the digestibility *in vitro* (Table 3). This lower S fraction was compensated for by a rate of OM degradation that was higher than that observed earlier by De Visser et al. (1991). Results agreed favourably with data for dried beet pulp (Tamminga et al., 1991; De Visser et al., 1991). The degradation characteristics of moist maize gluten feed agreed with previous results (Firkins et al., 1984; Steg, unpublished; Klop & De Visser, unpublished). The results displayed the highest U fraction in moist brewers' grains, which was in agreement with previous findings (Tamminga et al., 1991; Steg, unpublished). The relationship between the U fraction and the digestibility *in vitro* of the OM (Table 3) seems to be poor, when compared to the results obtained with maize silage. Probably OM digestibility measured *in vitro* was underestimated due to the high fat content of the brewers' grains (Table 3). Differences in U fraction of the OM of the dietary ingre-

dients would appear to be strongly related to the U fraction of the NDF of these feedstuffs, whereas the S fraction appeared to be more strongly related to nitrogen and the non-structural carbohydrates (sugars, starch; Tables 2 and 7).

In situ results with the grass silages showed a more rapid degradation of the N as compared to energy sources (OM minus N; NDF; Table 7). The increased loss of N, due to amino acid fermentation was supported by increased concentrations of NH_3-N and BCFA measured in the rumen fluid on both high moisture diets (MGS and FGS), indicating reduced microbial protein synthesis, due to the discrepancy between the availability of N and energy. This was also confirmed by the pool size of N, which tended to be larger on WGS and WW (Table 4). Our results confirm previous findings when: feeding diets differing in degradation rate between N and carbohydrates (barley vs. maize; beetpulp vs. maize bran; De Visser et al., 1992); feeding diets differing in the amount of energy available for microbial protein synthesis, due to higher indigestible fractions in combination with lower rates of degradation for the NDF fraction (beet pulp vs. maize silage; De Visser et al., 1991) and with diets in which energy sources are less efficiently utilized by rumen micro-organisms (fermentation end products vs. carbohydrates) due to fermentation of carbohydrates in the silo (Robinson et al., 1987). These indications for reduced synthesis of microbial protein on high moisture silage diets (MGS, FGS) were confirmed by the results of the accompanying feeding trial (De Visser & Hindle, 1992), showing reduced milk protein output on both high moisture diets. Chamberlain et al. (1985), Chamberlain, Thomas & Quig (1986) and Rooke, Lee & Armstrong (1987) showed reduced amounts of microbial protein entering the small intestine, when feeding diets with an inbalance in ruminal N and available energy, which agreed with our results.

Reducing the DM content of a diet by adding water (WW) had no significant influence on rumen fermentation and rumen kinetic parameters (Tables 4, 5, 6), when compared to WGS. However, high moisture silages in combination with increased fermentation in the silo (MGS and FGS) negatively influenced rumen fermentation and rumen kinetics.

During wilting the association of nitrogen/NDF complexes are probably responsible for the lower rate of degradation of N and NDF in WGS silage, as compared to MGS and FGS silage, which agrees with results observed by Van Vuuren et al. (1989).

On the one hand, the lower rumen pH and the higher concentrations of total VFA and lactic acid originated from higher concentrations of acetic acid (FGS) and lactic acid (MGS) fed with moist grass silages in combination with large amounts of moist ensiled by-products (Table 2). On the other hand the higher rates in degradation for MGS and FGS silage (Table 6) found in the *in situ* experiment (Table 7) will have initiated a further decrease in cellulolytic activity (Russell & Sniffen, 1984), a lower ruminal pH and a shift towards propionic acid production. Confirming the lower NGR values, measured on high moisture diets (MGS, FGS, Table 4).

The decrease in cellulolytic activity, is believed to be responsible for the reduced rates of clearance and digestion of the cell wall constituents on MGS and FGS diets (NDF, ADF, DADF, hemicellulose and cellulose; Table 6). These negative effects on rate of clearance of OM in cows fed high moisture diets was confirmed by the lower

DM intake found in the accompanying feeding trial (De Visser & Hindle, 1992).

Total rumen content as related to bodyweight (BW) did not differ between diets and was approximately 18.5 g/kg BW. Our results were within the range found and reviewed by Bosch (1991). However, Bosch (1991) found lower values in silage cut in a more mature stage and found higher values in silages similar in quality to those studied here. The higher proportion of concentrates fed in our experiment compared to Bosch (1991) may have been responsible for the difference found, because the rate of degradation and rate of passage of concentrates are higher than those of roughage components.

The rate of passage of particles, using $k_{PASSAGE}$ of IADF as a marker, was similar for all diets (Table 6) and agreed with earlier results published by Tamminga et al. (1989). The diets fed here and by Tamminga et al. (1989) were relatively high in IADF, due to the use of brewers' grains, which contains large quantities of IADF. Brewers' grains are considered to constitute to the small particle fraction of the rumen pool and may leave the rumen relatively sooner than IADF which originates from large particles such as maize silage or grass silages.

This hypothesis agrees with the findings of Poppi et al. (1981), who indicated a negative influence from smaller particles on the retention time in the rumen. Bosch (1991), Van Vuuren et al. (1992) and De Visser et al. (1992) showed lower rates of passage measured using IADF as a marker, but those diets consisted to a larger extent of grass silage (Bosch, 1991) or fresh grass (Van Vuuren, unpublished data) or included concentrates with a relatively low IADF content (De Visser et al., 1992), resulting in minor effects on the retention time of IADF in the rumen.

Total clearance of the OM fraction (Table 6) in the rumen was higher than those found by Bosch (1991). However, in our experiment a relatively larger proportion of the total diets fed consisted of by-products and a concentrate mixture, which were mainly part of the small particle pool in the rumen, negatively influencing the retention time in the rumen (Poppi et al., (1981). The higher rate of degradation of the concentrate mixtures and by-product ingredients relative to the roughage fed by Bosch (1991) was another influencing factor.

Total VFA present in the rumen was underestimated, when using the common rumen fluid method (TRVFAF) instead of the total rumen mass method (TRVFAM; Fig. 1). Differences between both methods are related to the concentration gradient which exists between fluid and OM being fermented by micro-organisms. Differences between both methods are of importance in relation to absorption coefficients of various VFA's (Murphy, Baldwin and Koong, 1982).

Grass silages, varying in DM and fermentation end products, differ in degradation characteristics, which negatively influence rumen kinetics. Attention should be given to the chemical composition and degradation characteristics of supplemented concentrates consisting largely of by-products, in an attempt to avoid or minimize negative effects on DM intake, microbial protein synthesis and milk protein output.

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