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Duration of Regrowth of Ryegrass (*Lolium perenne*) Effects on Grazing Behavior, Intake, Rumen Fill, and Fermentation of Lactating Dairy Cows

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ABSTRACT

The relative importance of duration of sward regrowth and fill and fermentation in the rumen on the control of grazing time and intake rate during the first grazing session of the day was studied. Four lactating dairy cows were allowed to graze ryegrass (*Lolium perenne*) swards, with five different regrowth periods after mowing (6, 9, 16, 22, and 30 d). The cows were allowed to graze until they stopped voluntarily (cessation of grazing activity for at least 15 min). Before and after grazing the rumen contents were evacuated, weighed, sampled, and returned to the animals. Samples of rumen liquid were taken immediately before rumen evacuation and approximately 30, 60, 120, and 240 min after the grazing session was finished. Grazing time and intake rate did not follow a significant trend with period of regrowth. Bite rate did not change significantly with duration of regrowth with cows exhibiting high rates of biting for all the sward conditions. Rumen pools sizes of dry matter, neutral detergent fiber, and volatile fatty acids (VFA), measured after grazing, increased significantly with days of sward regrowth, even though the changes over days were small. Concentration of VFA followed a significant quadratic trend with a maximum concentration observed at approximately 110 min after cessation of grazing. In this study, rumen fill, VFA (either total or major components), ammonia, pH, and osmotic pressure as individual variables were not correlated with grazing time or dry matter intake. (**Key words:** grazing behavior, rumen fill, fermentation)

Abbreviation key: ADL = acid detergent lignin, AG = after grazing, BG = before grazing, GT = grazing time, MBR = mean bite rate, OP = osmotic pressure.

INTRODUCTION

The mechanisms that control DMI in ruminants have received much attention since feed intake is the predominant factor determining animal performance. If DMI is seen as the summation of individual discrete meals (9), understanding what causes an animal to start and stop eating would lead to a better understanding and prediction of daily DMI. Different factors may predominate in ending meals during a 24-h period (12). In dairy cows fed forages, physical limitation has been proposed as the main constraint to obtaining higher DMI (26). However at least two feeding situations where the theory of physical regulation of DMI fails to explain observed DMI exist: dairy cows fed with silage (13) and those fed with high quality fresh forage (38). Under grazing, sward height might appear as the first constraint limiting DMI (e.g., 10, 19). Increasing grazing time (GT) is the main response mechanism exhibited by cows to cope with either changes in their physiological status (3) or with restrictive sward conditions (11, 31). Nevertheless little progress has been made to determine the main factors controlling GT (21). Concentration of fermentative end products (38) have been postulated to control DMI in the grazing ruminant. Reduction in DMI by the infusion of VFA, either into the rumen or the blood stream, has been extensively studied (e.g., 7, 9, 16, 24). In general, a dose response relationship has been observed between the amount of VFA infused and the reduction in DMI (7, 9), which suggests effects of VFA over a wide range of concentrations. Nevertheless, questions remain as to the extent to which the reduction in DMI is due to the quantity of VFA infused or to changes in osmotic pressure (17) or blood insulin concentrations (17, 24).

This study was undertaken to examine the relative importance of duration of regrowth, rumen fill, and fermentation end products in the rumen on the control of GT and DMI during the first grazing bout after a.m. milking.

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MATERIALS AND METHODS

General Procedure

The experiment was carried out from May 13 to June 30, 1996, at the experimental farm "De Ossekampen" of the Wageningen Agricultural University. Four lactating Holstein-Friesian cows previously fitted with a 10-cm rumen cannula (Bar Diamond, Lane, ID) in the dorsal rumen sac were used. At the start of the experiments the cows weighed 533 ± 55 kg and produced 27.5 ± 5.1 kg of milk/d ($\bar{X} \pm \text{SE}$). The cows were milked twice daily at 0600 and 1600 h, and 10 d before the experiments started they were trained to be led and graze while tethered. During the measurement days, cows grazed individually tethered within a circular plot. Between measurement days cows grazed in a contiguous plot of ryegrass. During the whole experiment the cows did not receive any supplementary feed. The experimental plot comprised 2.5 ha of ryegrass (*Lolium perenne*) sown in 1989. In the spring of 1996 the pasture was fertilized with 139 kg of N and 24 kg of P/ha and was not grazed or mown before the start of the experiment.

The treatments comprised six periods of regrowth. At d 0 (May 13) the whole pasture was cut with a mowing machine (cutting height 4 cm), and the cuttings were removed. On d 6, 9, 13, 16, 22, and 30 after cutting (referred to as measurement days) the cows grazed while tethered (4) during their first grazing bout of the day. The general procedure for an experimental day is shown in Figure 1. After morning milking, rumen evacuation of the four cows was conducted between 0900 and 1100 h. At 1100 h the four cows were placed in their respective grazing plots and allowed to graze until they stopped voluntarily. Immediately after grazing, each cow was removed to the barn and the rumen was evacuated again. After replacement of this second

rumen evacuation, the cows were fasted until the next morning when a third rumen evacuation was carried out. Following this third rumen evacuation the cows were allowed to graze freely in a contiguous plot. The sward mass offered per cow and per grazing session on each measurement day was 19.8 ± 4.2 kg DM, measured 2.5 cm above soil surface. Sward mass was obtained by adjusting the length of the rope (the radius of the circle) that restrained the movements of the cows in the plot. The grazing plots were marked and sampled the day before each experimental day. Individual milk production was recorded during four consecutive milkings with the last being the morning of the measurement day.

Pasture Determinations

The sward mass available before and after grazing was estimated using the double sampling technique (25). Five quadrats (0.5 * 0.5 m) of contrasting sward mass and height were selected per plot. Sward height measured with a plate meter (weight: 350 g, diameter: 0.5 m) and sward mass above 2.5 cm were determined for each square. The square was fitted with six parallel guides to keep the cutting height as uniform as possible. Regression of sward mass against sward height was used to calibrate measurements. About 30 additional measurements of sward height were made at 2 m intervals in six straight parallel lines in each plot. Mean sward mass for the plot was estimated from the mean of these values using the regression equation.

Cut grass samples were collected into plastic bags, weighed fresh, dried at 60°C to constant weight and the dry weight (after acclimatization) registered as air DM. Air DM forage samples were analyzed for DM, ash, N, and NDF content. Dry matter was determined by drying the samples to constant weight in an oven at

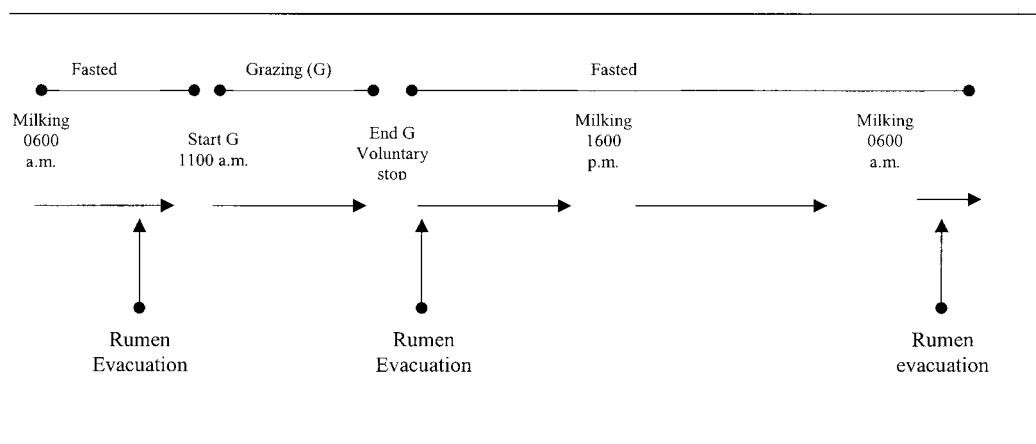


Figure 1. General procedure during an experimental day.

103°C, ash by combustion in a muffle at 550°C, and N by the Kjeldahl method with CuSO_4 as a catalyst. Neutral detergent fiber was measured according to the method of Van Soest et al. (36).

Animal Grazing Behavior

Grazing time was recorded as the time elapsed from the moment the cows were placed on the experimental plot until grazing ceased. Grazing was considered to have finished when a cow met one of two criteria: either it had laid down after an active grazing period or 15 min had passed without any biting activity. In most instances cows met the first criterion. A trained observer continuously recorded grazing activity of the four cows to measure periods of nongrazing activity shorter than 15 min but longer than 1 min. Biting and searching with the head down were considered grazing activities. Other activities (including urination and defecation) were considered as not grazing. Eating time was calculated as GT minus the minutes without biting activity. Bite rate was recorded by the same observer over a period of 1 min every 10 min (18) for each cow. The sound when the cows severed the herbage was easily audible and chosen as the criterion to define and count bites. Mean bite rate (**MBR**) was calculated as the ratio between the total number of bites and GT.

Rumen Contents

The rumen evacuation procedure was conducted as described by Chilibroste et al. (3, 4). To improve the collection of liquid that drained while the evacuation of the solid fraction was taking place, a steel latticework with four legs of 15 cm each was placed within the insulated collection container. The steel latticework and the sides of the container were covered with a double layer of lace curtain. The solid rumen contents were removed by hand and placed in insulated containers that prevented the material from cooling rapidly. Solid material was weighed and mixed by hand in the insulated containers, and two subsamples were taken (approximately 400 g each). Material not removable by hand was collected with a plastic bottle and sieved (pore size, 0.04 mm^2) into a 40-L container. The liquid that drained to the bottom of the insulated container was also sieved through a pore size of 0.04 mm^2 into the liquid container. The liquid fraction was weighed, sampled (approximately 1 L) and returned to the rumen, so that the rumen remained completely empty for only 2 or 3 min. Liquid was added to the solid subsamples in the same proportion (wet basis) as they were in the rumen. The reconstituted samples (solid + liquid) were kept at -20°C until DM content was determined by

freeze drying to constant weight. Freeze dried samples were analyzed for ash, N, NDF, and acid detergent lignin (**ADL**). Ash was determined in a muffle furnace at 550°C , and N by the Kjeldahl method with CuSO_4 as catalyst. Neutral detergent fiber was determined according to Van Soest et al. (36) as modified by Goelma et al. (14) and ADL was determined according to the method of Van Soest (37). Total rumen pool size was calculated as the sum of solid and liquid fractions. Pool size of rumen DM was calculated as the product of the total weight of rumen contents and their DM content.

Rumen Fluid

Samples of rumen fluid (approximately 250 ml) were collected with a 85-cm plastic tube (2.5 cm diameter), closed at the bottom, with about 270 holes (1.5 mm diameter) drilled in the lower 27 cm. This tube was inserted into the rumen through the cannula and positioned in such a way that the bottom of the tube reached the liquid phase in the ventral rumen sac. Flexible tubing (0.5 cm diameter) was placed into this plastic tube and the rumen liquid siphoned into a plastic bottle. The collected rumen fluid was sieved through a sieve with apertures of 0.04 mm^2 , and samples were taken for pH, osmotic pressure (**OP**), ammonia, and VFA determinations. The pH was measured immediately after collection (pH electrode type 62, Testo 252, Testo GmbH & Co, Germany). A subsample (5 ml) was mixed with 5 ml of TCA and frozen at -20°C until analyzed for ammonia. The subsamples were thawed at room temperature, centrifuged for 10 min at $2564 \times g$ (Sigma 2-15, Laborzentrifugen GmbH, Germany), and the ammonia concentration in the supernatant determined (31). Another subsample (10 ml) of the rumen liquor sample was acidified with 0.5 ml of 85% phosphoric acid and kept frozen at -20°C pending analysis for VFA. For VFA analysis the samples were thawed at room temperature and centrifuged for 10 min at $13,600 \times g$ (IEC Centra-M, International Equipment Company, USA). Subsequently $500 \mu\text{l}$ of the supernatant liquid was mixed with $200 \mu\text{l}$ water and $300 \mu\text{l}$ of an internal standard (iso-caproic acid). One milliliter of this mixture was injected into a GLC (Packard Becker model 419, packed column filled with Chromosorb 101, carrier gas N_2 saturated with formic acid, temperature 190°C). Concentrations of acetic, propionic, iso-butyric, butyric, iso-valeric, and valeric acid were determined. The total concentration of VFA in the rumen liquor was calculated as the sum of the individual VFA. Another subsample of the rumen liquid (5 ml) was kept frozen at -20°C until analysis for OP. After thawing, the samples were centrifuged for 10 min at $2564 \times g$ and diluted twice

with distilled water and OP was determined, by freezing point depression (Halfmikro-Osmometer; Knauer & Co GmbH, Germany).

Dry Matter Intake

The DMI was estimated from the changes in the DM rumen pool as follows:

$$\text{DMI} = (\text{RPAG} - \text{RPBG}) + \text{RPBG} (1 - e^{-k_{cl} \times \text{GT}}) + \text{CNGI}$$

where

- RPAG = DM rumen pool after grazing (kg),
- RPBG = DM rumen pool before grazing (kg),
- k_{cl} = clearance rate (h^{-1}) of RPBG during the grazing session,
- CNGI = clearance of rumen DM ingested during the grazing session (kg), and
- GT = grazing time (h).

To calculate CNGI: a uniform pattern of ingestion through the grazing session and a mean residence time of the particles ingested of 0.5 GT were assumed. The calculations were conducted as follows:

$$\text{CNGI} = [(\text{RPAG} - \text{RPBG}) + \text{RPBG} (1 - e^{-k_{cl} \times \text{GT}})] \times (1 - e^{-k_{cl} \times 0.5 \text{GT}})$$

Rumen DM clearance rate (k_{cl}) was estimated over the starvation period following the grazing session (Figure 1) assuming first-order kinetics (29) with one pool being cleared at a constant fractional rate.

Statistical Analysis

Analysis of a compound symmetric variance structure was tested using the MIXED procedure with cow as a repeated subject (33). Because the compound symmetric structure was not significant, a general linear model was applied. Linear and quadratic effects of days of regrowth on sward chemical composition, grazing behavior, and DMI were estimated by the GLM procedure of SAS (33). Cows were treated as blocks and heterogeneity of slopes tested according to the model:

$$Y_{ij} = \mu + \text{cow}_i + \text{day}_j + \text{day}_j^2 + (\text{cow} * \text{day})_{ij} + (\text{cow} * \text{day}^2)_{ij} + e_{ij}$$

Where:

Y_{ij} = dependent variables,

- μ = general mean,
- cow = cow effect (class variable),
- day = linear effect of day of regrowth (continuous variable),
- day₂ = quadratic effect of day (continuous variable),
- (cow*day) = interaction terms, and
- and
- (cow*day²) = interaction terms, and
- e = error term (0, s²).

A similar model without the quadratic terms (day², cow*day²) was applied to estimate the effects of treatments on rumen pool sizes.

Concentration of fermentation end products. Linear and quadratic effect of sampling time on pH, VFA, ammonia, and OP were tested with the GLM procedure of SAS (33) as follows:

$$Y_{ijk} = \mu + \text{cow}_i + \text{day}_j + (\text{cow} \times \text{day})_{ij} + \text{TL}_k + \text{TQ}_k + (\text{TL} \times \text{day})_{jk} + (\text{TQ} \times \text{day})_{jk} + e_{ijk}$$

Where:

- Y_{ijk} = dependent variables,
- μ = general mean,
- cow = cow effect (class variable), $i = 1$ to 4,
- day = day effect (class variable), $j = 1$ to 5,
- cow \times day = interaction term,
- TL = linear effect of time of sampling, $k = 1$ to 5,
- TQ = quadratic effect of time of sampling, $k = 1$ to 5,
- (TL \times day) = interaction terms, and
- (TQ \times day) e_{ijk} = error term (0, s²).

To test day and cow effect the interaction (cow \times day) was used as the error term. All means reported are least square means unless otherwise indicated.

RESULTS

Weather conditions during the experiment are presented in Table 1. The information was collected from a meteorological station (Maandoverzicht Weergegevens Station Wageningen "De Haarweg") located approximately 300 m from the experimental plot. Except for experimental d 16 and 22, that exhibited higher temperatures and lower relative humidity, weather conditions were relatively stable. However due to rain each day (mean: 2.36 h/d) from experimental d 9 to 13 and symp-

Table 1. Mean weather conditions during the experimental days.¹

Day of regrowth	Date	Air Temp. (°C)	Relative humidity (%)	Wind (m/s)	Rain fall (mm)
6	05-20-96	11.0	59.0	2.5	0.0
9	05-23-96	12.7	83.0	5.9	3.3
13	05-27-96	11.0	86.0	5.1	4.0
16	05-30-96	20.4	59.0	3.0	0.0
22	06-05-96	21.6	51.0	3.4	0.0
30	06-13-96	13.5	61.0	2.5	0.0

¹Data source: Maandoverzicht Weergegevens Station Wageningen "De Haarweg."

toms of illness shown by one cow on d 13, data from d 13 were omitted from the analyses.

The sward characteristics before grazing are given in Table 2. Sward N content exhibited a significant deviation from linearity. No important changes were evident for grass N content during the first 2 wk of regrowth but a significant decline occurred thereafter. A similar trend, but in opposite direction, was observed for sward NDF content. Sward DM content decreased during the first 16 d, and thereafter increased significantly.

Grazing behavior and DMI are summarized in Table 3. Neither DMI nor intake rate increased with the age of regrowth. A large increase was observed for GT from d 6 to 9, then a decrease from d 9 to 16, and then a relatively constant value up to d 30. Nevertheless, neither the linear nor the quadratic term was significant. Bite mass tended to increase linearly ($P < 0.08$) with period of regrowth, although neither MBR nor adjusted bite rate showed any such effect. Milk production before each measurement day exhibited random variations during the experiment (Table 3).

Statistical significance of the effect of cow, days of regrowth, and their interaction on rumen pool sizes before (BG) and after (AG) grazing are shown in Table 4. Least square means, slopes, and standard error of the regression analysis are shown in Tables 5 and 6.

Rumen pool sizes BG (with exception of ammonia) exhibited significant linear effects with days of regrowth (Tables 4 and 5). Because of the linear trend observed, the rumen pool sizes BG was included as a covariant in the regression model for rumen pool sizes AG. The interaction effect between cow and days of regrowth was not significant for any of the measured variables (except NDF) either BG or AG. Total rumen pool size AG increased ($P < 0.05$) with age of regrowth. Neutral detergent fiber and ADF rumen pools increased ($P < 0.05$) with days of regrowth but ADL rumen pool did not. Rumen pool of total and major VFA also increased with days of regrowth. Rumen ammonia pools were not affected by days of regrowth.

In Table 7 and Figure 2, the time course of the fermentative end products, pH, and OP are shown. There was a significant cow effect for all measured variables (except OP) reflecting high individual variability. The inclusion of a quadratic term was highly significant ($P < 0.01$) for all the fermentative end-product concentrations and OP, indicating the presence of a maximum or minimum within the 4-h period of sampling. Except for rumen ammonia concentration, there was no significant interactive effect of day and sampling time, either linear or quadratic, on the concentration of fermentation products.

Table 2. Plot areas, sward masses, and chemical composition of herbage before grazing.

	Days of regrowth						Linear slope	Q ¹ Slope	SEM
	6	9	13	16	22	30			
Plot area, m ²	299	165	123	87	71	57			
Sward mass, kg of DM/ha	907	1022	1466	1797	2524	4007	129***	NS ²	248
Sward height, cm	6.2	6.4	9.6	13.1	19.3	28.0	0.38**	0.02**	1.10
DM content, g/kg of fresh grass	201.5	192.5	166.0	150.7	189.0	199.7	-0.86**	0.024**	2.24
OM, g/kg of DM	904.8	902.6	892.7	893.1	906.8	909.4	NS	NS	7.09
N, g/kg of DM	40.4	42.6	43.3	43.5	34.8	29.0	0.85	-0.039**	3.54
NDF, g/kg of DM	445.7	424.9	423.0	444.4	466.9	531.9	-5.9**	0.272	16.82

¹Q = Quadratic.

²NS = Nonsignificant.

** $P < 0.05$.

*** $P < 0.01$.

Table 3. Effect of the days of regrowth on short-term DMI, intake rate, grazing behavior, and rumen pool sizes and kinetics.

	Days of regrowth					SEM	Linear ³	Q ⁴
	6	9	16	22	30			
Milk production, L/d	26.2	23.7	27.3	29.0	22.5	3.95	NS ⁵	NS
DMI, kg/first meal	2.8	3.3	2.6	3.1	3.5	0.81	NS	NS
Intake rate, kg/h	2.1	1.2	2.1	2.0	2.2	0.74	NS	NS
Grazing behavior								
Grazing time, min	89.5	166.7	92.0	94.5	98.7	44.6	NS	NS
Eating time, min	82.7	160.7	82.5	90.0	97.2	43.8	NS	NS
MBR, ¹ bites/min	56	51	50	55	50	6.2	NS	NS
ABR, ² bites/min	58	52	53	57	51	5.5	NS	NS
Bite mass, g/bite	0.62	0.40	0.66	0.61	0.72	0.18	NS	NS

¹MBR = Mean bite rate.²ABR = Adjusted bite rate.³Linear = Linear effect.⁴Quadratic = Quadratic effect.⁵NS = Nonsignificant.

DISCUSSION

Experimental Protocol

We have worked with tethered grazing animals as experimental units in previous grazing experiments (4). After a short training period the animals become adapted to the procedure and exhibited normal behavior. Nevertheless conclusions from this work have to be treated carefully because of some limitations of the experimental protocol. We succeeded in achieving the

target herbage allowance per cow during each grazing session, but sward height and chemical composition were confounded in this experiment. Although we did not expect carry-over effects from one experimental day to another (6) the protocol did not allow us to estimate or eliminate them.

Sward Measurements

The N content of the herbage increased moderately from d 1 to 16 and then declined rapidly (Table 2). Van

Table 4. Statistical significance of the main effects tested (cow, day of regrowth and their interaction) on rumen pool sizes before and after grazing and after fasting.

Variable	Evacuation before grazing			Evacuation after grazing			Evacuation after fasting		
	Cow	Day	Cow × day	Cow	Day	Cow × day	Cow	Day	Cow × day
Total rumen pool, kg	**	***	NS ³	NS	**	NS	**	***	NS
DM content, %	NS	**	NS	NS	NS	NS	***	NS	NS
Particulate matter pool sizes									
OM rumen pool, kg	NS	***	NS	NS	**	NS	**	***	NS
N rumen pool, kg	NS	**	NS	NS	NS	NS	NS	***	NS
NDF rumen pool, kg	NS	***	NS	NS	**	**	**	***	**
ADF rumen pool, kg	NS	***	NS	NS	**	NS	**	***	NS
ADL ¹ rumen pool, kg	NS	***	NS	NS	NS	NS	**	**	NS
Liquid phase pool sizes									
Acetic, mol	NS	***	NS	NS	**	NS	NS	***	NS
Propionic, mol	**	***	NS	NS	**	NS	NS	NS	NS
Butyric, mol	***	***	NS	NS	**	NS	NS	***	NS
Branched, ² mol	NS	***	NS	NS	NS	NS	NS	***	NS
Total VFA, mol	**	***	NS	NS	**	NS	NS	***	NS
Ammonia, g	***	NS	NS	NS	NS	NS	NS	***	NS

¹ADL = Acid detergent lignin.²Branched = Iso-butyric + isovaleric.³Nonsignificant ($P > 0.05$).** $P < 0.05$.*** $P < 0.01$.

Table 5. Total, OM, N, NDF, ADF, and acid detergent lignin (ADL) rumen pools size and DM content of rumen content before grazing (BG), after grazing (AG), and after fasting (AF).

Rumen pool		Days of regrowth					Slope ¹	SEM
		6	9	16	22	30		
Total, kg	BG	49.2	51.4	64.7	67.5	73.8	1.06	5.02
	AG	54.4	63.5	69.1	73.6	78.6	0.71	4.60
	AF	23.5	32.9	31.8	38.4	48.3	0.89	5.21
DM, %	BG	10.9	10.3	12.2	11.3	12.0	0.057	1.00
	AG	11.7	10.8	12.6	12.1	12.9	NS ²	0.68
	AF	5.5	6.8	8.1	6.5	7.6	NS	1.24
OM, kg	BG	4.76	4.65	7.13	6.86	8.00	0.143	0.93
	AG	5.60	6.09	7.86	7.91	9.13	0.088	0.61
	AF	1.08	1.88	2.25	2.12	3.21	0.072	0.50
N, kg	BG	0.23	0.20	0.34	0.31	0.31	0.0043	0.06
	AG	0.29	0.30	0.40	0.37	0.36	NS	0.04
	AF	0.03	0.06	0.07	0.07	0.09	0.0018	0.017
NDF, kg	BG	2.05	2.29	3.34	3.31	4.16	0.085	0.38
	AG	2.22	2.67	3.44	3.49	4.69	0.059	0.30
	AF	0.68	1.22	1.44	1.28	2.11	0.047	0.37
ADF, kg	BG	1.22	1.36	2.02	1.91	2.38	0.047	0.24
	AG	1.37	1.67	2.10	2.10	2.69	0.028	0.19
	AF	0.46	0.83	0.98	0.79	1.26	0.025	0.27
ADL, kg	BG	0.25	0.25	0.40	0.40	0.47	0.0098	0.05
	AG	0.28	0.29	0.42	0.42	0.49	NS	0.024
	AF	0.13	0.23	0.30	0.23	0.30	0.005	0.083

¹Slope = Slope of linear effect of days of regrowth.²Nonsignificant.

Vuuren et al. (40) observed the same trend measured over a longer period (week 1 to 8). In our experiment, the stubble height could have influenced the chemical analyses during the first weeks of regrowth, since the experimental plot was mown at 4 cm and the individual experimental samples were cut at 2.5 cm. The significant decline in sward N content after 16 d of regrowth may have been due to an increase in the proportion of stem,

or a decrease in N content in leaf and stem fractions, or both (32). As with N, sward NDF content changed little during the first 2 wk of regrowth, then increased rapidly, agreeing with the pattern observed by Van Vuuren et al. (40). A slight decrease in NDF content during the first week of regrowth may be a reflection of an increase in the leaf to stem ratio, compensating for the effect of maturation on the fiber content and quality.

Table 6. VFA and ammonia rumen pool sizes before (BG) and after (AG) the grazing session.

Rumen pools		Days of regrowth					Slope ¹	SEM
		6	9	16	22	30		
Acetic, Mol	BG	2.64	1.92	2.61	2.88	3.76	0.057	0.572
	AG	2.70	2.75	2.91	3.28	4.20	0.042	0.502
Propionic, Mol	BG	0.87	0.81	1.11	1.11	1.19	0.015	0.174
	AG	0.88	1.02	1.12	1.17	1.35	0.019	0.172
Butyric, Mol	BG	0.59	0.51	0.61	0.60	0.78	0.0086	0.102
	AG	0.53	0.65	0.63	0.67	0.98	0.0133	0.146
Branched, ² Mol	BG	0.092	0.065	0.123	0.124	0.156	0.003	0.024
	AG	0.102	0.101	0.115	0.126	0.145	NS ³	0.023
Total VFA, Mol	BG	4.27	3.43	4.60	4.85	5.99	0.085	0.794
	AG	4.25	4.54	4.90	5.40	6.93	0.091	0.781
Ammonia, g	BG	5.14	5.43	9.96	6.65	7.72	NS	2.347
	AG	9.89	11.5	9.87	8.70	12.1	NS	3.343

¹Slope = Slope of linear effect of days of regrowth.²Branched = Iso-butyric + isovaleric.³Not significant.

Table 7. Statistical significance of different effects on VFA, ammonia, pH, and osmotic pressure (OP) in rumen liquor after grazing.

Variable	Cow	Day	TL ¹	TQ ²	Day × TL	Day × TQ	Intercept	Slope L ³	Slope Q ⁴	SEM	R ²
Fermentation products											
Acetic, mmol/L	***	NS ⁶	NS	***	NS	NS	51.6	0.079	-0.00041	6.90	0.75
Propionic, mmol/L	NS	NS	***	***	NS	NS	17.5	0.055	-0.00023	2.75	0.72
Butyric, mmol/L	NS	NS	***	***	NS	NS	10.7	0.037	-0.00015	1.76	0.82
Branched, ⁵ mmol/L	NS	NS	***	***	NS	NS	1.69	0.011	-0.00003	0.34	0.81
Total VFA, mmol/L	**	NS	**	***	NS	NS	83.3	0.194	-0.00088	11.9	0.72
Ammonia, g/L	NS	NS	***	***	***	***	160.3	0.772	-0.00348	28.6	0.86
Rumen environment											
pH	***	NS	NS	NS	NS	NS	6.5	-0.0004	0.000004	0.19	0.81
OP, mosmol ml ⁻¹	NS	NS	NS	**	NS	NS	307	0.0307	-0.00069	20.8	0.64

¹TL = Linear effect of time of sampling.

²TQ = Quadratic effect of time of sampling.

³Slope L = Slope of linear effect of sampling time.

⁴Slope Q = Slope of quadratic effect of sampling time.

⁵Branched = Iso-butyric + isovaleric.

⁶Not significant.

Dry Matter Intake

Neither DMI nor intake rate exhibited significant changes with age of regrowth. As DMI under grazing is the product of GT and intake rate (19) results will be discussed in this terms.

Grazing time. In accordance with the results of previous research (10, 19), we expected a maximum meal duration on the shortest sward, with a decline in GT as sward height increased and then a plateau after a certain threshold had been reached. On measurement d 9 the cows grazed longer than on d 16, 22, and 30, agreeing with previous research, where cows have attempted to compensate for reductions in bite mass and intake rate on short swards by increasing GT. However on d 6, the cows did not increase GT. The observed decline in GT on d 6 may be caused by the presence of a physical limit to grass prehension. In short grasses the height of pseudostems has been considered a potential barrier for grazing (8) and may have prevented subsequent grazing after the first grazing horizon had been removed. A reduction in GT has been reported under strip grazing (23), under controlled grazing conditions with hand-constructed swards (35), and with continuous grazing (31), when cows have been exposed to short swards. After d 16, GT showed only small variations (Table 3) and the observed values of GT in this period were within the range of reported values for the first grazing bout (5, 31).

Intake rate. Intake rate is the product of bite mass and bite rate (22). Estimated bite masses were the smallest on experimental d 9 and the largest on d 30. The low sward height on experimental d 9 undoubtedly restricted bite mass (10, 19, 21) because of a restriction in bite depth (22) and could not be compensated by either bite rate or by density of the grazed horizon (42).

However bite mass tended to be heavier on experimental d 6 than on d 9, even though the sward height and mass were less than on d 6. Rook et al. (31) and Soca (1996, unpublished) working with very short swards (approximately 4 cm), found heavier bite masses when the GT of the cows was reduced either intentionally or as a consequence of supplementation with concentrates. Average bite rate was not affected by the day of regrowth (Table 3). However, cows started the grazing bouts with a high bite rate independent of the initial sward conditions, in line with our previous results (3). These initial, high rates were probably the result of the period of fasting (3, 15) and, possibly, induced by a feeling of hunger caused by the rumen-emptying procedure prior to grazing.

Rumen Pools

Total and OM rumen pool sizes AG (Table 5) were far below the observed rumen pools sizes in other studies (2, 4, 30). Only on d 30 did the observed DM rumen pool size approach that expected of a full rumen (2, 4). Bosch et al. (2) summarized values of total rumen pool from 18 to 25 g/kg of BW for cows eating silage (lower values) and hay (higher values), which contrasts with our observations (10 to 14.7 g/kg of BW). Lower OM rumen pool size AG during the first two measurement days might have been related to the low DMI imposed by the sward height of the available pasture. Changes in sward chemical composition may have also affected the observed rumen pools AG (Table 2). The analysis of fermentation characteristics of grass samples by the gas production technique (5) showed a linear decline both in gas production and in the extent of fermentation during the regrowth period.

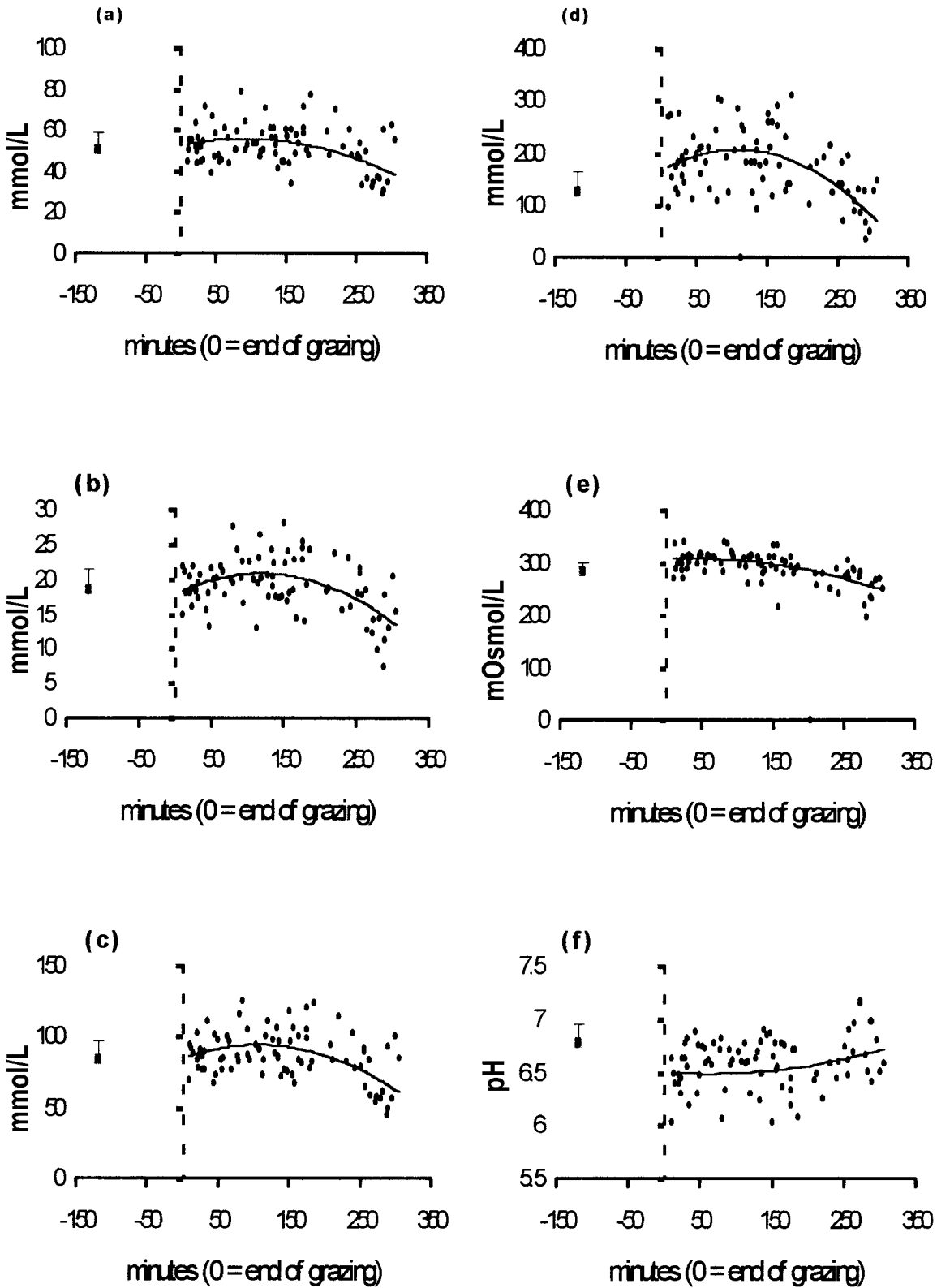


Figure 2. Fermentative end-product concentration (a, acetic; b, propionic; c, total VFA; d, ammonia) and rumen environmental variables (e, osmotic pressure; f, pH) before (filled square) and after grazing (dots, observed values; lines, predicted values from model Table 7).

Rumen NDF pools AG on measurements d 6 and 9 were comparable with NDF pools observed in grazing dairy cows after an overnight fast (4) but were lower than NDF rumen pools after 1 h of grazing. Van Vuuren et al. (39) observed low NDF rumen pools (2.27 kg) in dairy cow fed highly fertilized, fresh ryegrass, probably associated with a low DM content of the grass. Low NDF rumen pools have been also observed in dairy cows fed fresh ryegrass and supplemented with 7 kg of a high starch concentrate (38). From d 16 onwards NDF rumen pools AG were greater than on d 6 and 9 (Table 5), but still within the lower range of NDF rumen pools reported elsewhere (2, 4, 30). The highest NDF rumen pool (measurement d 30), was below the threshold of 1.1 to 1.2% of BW described by Mertens (26) as the average NDF holding capacity of dairy cows. This suggests that NDF rumen pool as an individual entity was not the main signal received by the cows to stop the grazing session.

The VFA rumen pool sizes (total and major VFA) increased linearly with days of regrowth (Tables 4 and 6). In general a dose response relationship has been observed between the amount of VFA infused and reduced DMI (7, 9) which suggests that slight increases in VFA from basal level should reduce DMI. In this experiment, VFA rumen pools AG on d 6 and 9 were similar to the rumen pools observed after 1.75 h of grazing following an overnight fast (4). At the other extreme, VFA rumen pool sizes AG measured on d 30 were similar to the maximum rumen pool sizes (approximately 7 mols) observed immediately after the first morning grazing session (4). Nevertheless, the observed VFA rumen pool sizes AG in this experiment were below the range where they would be expected to cause DMI depression (1, 9).

The change in VFA concentration AG followed a quadratic ($P < 0.01$) trend with time (Table 7; Figure 2). If the animals were able to sense rumen VFA concentration or VFA rumen pool sizes (9, 16) they should interrupt the grazing session before this maximum level had been reached (24). Maximum VFA concentrations in rumen liquid (either total or major components) were observed at approximately 110 min after grazing. The maximum VFA concentration observed was lower than values reported for grazing cattle (28, 41). The lack of a significant interaction between experimental day and time of sampling (either linear or quadratic) indicates a degree of stability between experimental days in the observed pattern.

Rumen pool size of ammonia was not affected by age of regrowth (Table 6). Rumen pool of ammonia AG exhibited a greater correlation ($r = 0.65$, $P < 0.01$) with DMI than VFA rumen pools ($r = 0.34$, $P > 0.15$). Studying the fermentation pattern during the first grazing

session in the morning of lactating dairy cows, Chilibroste (1996, unpublished) found that ammonia concentration in the rumen liquor increased linearly with GT and that VFA concentration exhibited a plateau during approximately 1 h of grazing and then sharply increased. Irrespective of the mechanism leading to these differences, these results suggest that ammonia is perhaps more important than VFA concentration in regulating meal size under grazing. Short-term imbalances in the supply of ammonia and VFA, as our results suggested, should be considered in further research (20). Rumen liquid ammonia concentration AG did not follow a clear trend (Table 7; Figure 2) with day of regrowth. The branched chain VFA (isobutyric and isovaleric) did not change with the day of regrowth, although the chemical composition of the grass, particularly N content, changed significantly with grass maturity (Table 2). This trend might reflect changes in N utilization (5), a better synchrony of carbohydrates and N availability in the rumen (39), or simply that rumen microbes that require branched chain VFA can only take up branched VFA when concentrations are above this level.

Grovum (17) has shown dose-response reductions in DMI with the addition of NaCl in the rumen of sheep. As in our experiment, high variability between animals for OP values has been observed (17). The time course AG of the rumen liquid OP exhibited a significant ($P < 0.05$) quadratic term (Table 7; Figure 2) with the maximum value observed around 22 min after the end of the grazing session. In this experiment OP values AG were within a normal range (9) and far below 450 to 550 mosmol/L, the range at which DMI either ceased (27) or was severely depressed (17) in sheep.

CONCLUSIONS

Rumen fill (as represented by total, OM, or NDF rumen pool sizes), and ruminal VFA, ammonia, pH, and OP, when taken in isolation, are unlikely to be the primary factors controlling the length of the first grazing bout. For all regrowth periods evaluated, the cows stopped grazing before a maximal ruminal capacity was reached. During the first measurements days behavioral restrictions imposed by the sward characteristics appeared to be important in terminating the grazing session. After 16 d of regrowth, sward condition per se did not play an important role controlling GT.

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