Reprod Nutr Dev (1994) 34, 9-23

Original article

Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet

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(Received 2 October 1993; accepted 18 October 1993)

Summary — The effects of grass maturity on the mean retention time of liquid and particles, digestibility and duodenal nutrient flows were measured by feeding second cuts of perennial ryegrass to 3 fistulated lactating dairy cows over 2 successive periods (late June and mid-July, 28 and 49 d regrowth, respectively). Fresh forage was given *ad libitum* with 12% (dry matter (DM) basis) concentrate. The increase in maturity lowered grass nitrogen content, organic matter (OM) digestibility and non-ammoniacal nitrogen flow into the duodenum, but did not affect OM intake (13.2 kg/d). Neither the fractional outflow rate of liquid in the rumen (FOR; 17.3%/h) nor the concentrate total mean retention time (TMRT; 32.9 h) were affected. With maturity forage TMRT tended to increase (43.0–48.8 h), certainly because of the longer comminution time of coarse particles. These results were compared with data obtained 2 months earlier with the same cows fed on a maize silage diet (25% DM basis of the same concentrate) *ad libitum* (17.4 kg OM/d). Despite a lower total OM intake, FOR was much higher with the grass than with the maize silage diet (12.1%/h) and this may explain the lower retention time in the rumen of the small particles when grass diets were given (19.0 *vs* 24.3 h for maize silage diet).

dairy cow / passage rate / digestion / fresh grass / maize silage

Résumé — Temps de séjour moyen dans le tube digestif et digestion du ray-grass anglais par des vaches laitières : influence de l'âge des repousses et comparaison avec de l'ensilage de maïs. *L'effet de l'âge des repousses du ray-grass anglais sur le temps de séjour des liquides et des particules, la digestibilité et les flux de nutriments à l'entrée du duodénum ont été étudiés chez 3 vaches laitières au cours de 2 périodes successives (fin juin et début juillet, 28 et 49 j de repousse). Le régime distribué* ad libitum *a été complémenté avec 12% (sur la base de la MS) de concentré. Le vieillissement de l'herbe a entraîné une diminution de la teneur en azote non ammoniacal à l'entrée du duodénum, mais les quantités ingérées n'ont pas été affectées (13,2 kg MO/j). Le taux de renouvellement des liquides dans le rumen (Kl ; 17,3%/h) et le temps de séjour moyen total (TSMT) du*

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concentré (32,9 h) n'ont pas été modifiés. Le TSMT du fourrage a eu tendance à augmenter (43,0 à 48,8 h), sûrement en raison d'un temps de comminution des particules plus long. Ces résultats ont été comparés à des données obtenues 2 mois auparavant sur les mêmes animaux, alors alimentés ad libitum *(17,4 kg MO/j) avec un régime à base d'ensilage de maïs contenant 25% du même concentré. Bien que les quantités ingérées aient été inférieures, le KI a été bien plus élevé avec le régime herbe qu'avec le régime ensilage de maïs (12,5%/h), ce qui peut expliquer un temps de rétention des petites particules dans le rumen beaucoup plus faible avec le régime herbe (19,0 contre 24,3 h pour l'ensilage de maïs).*

vache laitière / vitesse de passage / digestion / ray-grass anglais / ensilage de maïs

INTRODUCTION

Food intake and the extent of digestion of forage are related to the retention time of feed residues in the digestive tract (Bull *et al,* 1979; Mertens and Ely, 1982). Factors influencing the rate of passage of undigested residues have been extensively studied in sheep, whilst more specific information in dairy cows has been obtained over the last 10 yr (Hartnell and Satter, 1979; Snyder *et al,* 1984; Shaver *et al,* 1986, 1988; Mambrini *et al,* 1988; Peyraud *et al,* 1989). However, data regarding fresh forage are still lacking, although it is a major feedstuff given to dairy cows for half of the year in western Europe. Indeed, the digestive transit of fresh forage has only been examined in steers grazing low fertilized native rangeland (Krysl *et al,* 1987) or in tropical pastures (Pond *et al,* 1987). These studies concluded that there was an increase in the retention time of particles with advancing plant maturity. However, these data need confirmation for highly digestible fresh forage given to dairy cows. Compared generally to conserved forages, fresh grass has high water content, high digestibility and low level of lignified cell walls. These characteristics may lead to particular features in the rumen dynamics of liquid and particles. However, there appears to be no direct comparison between fresh and conserved diets in the literature.

The aims of this study were to measure the effect of plant maturity (28 and 49 d regrowth) of fresh perennial ryegrass on the rate of passage of liquid and forage and concentrate particles, and on the duodenal flows in 3 dairy cows and to compare them with those of a conserved diet based on maize silage and the same concentrate. A preliminary report of part of this work has previously been published (Mambrini and Peyraud, 1992).

MATERIAL AND METHODS

Digestive characteristics of fresh grass diet

Management of sward, animals and feed

A plot of perennial ryegrass (*Lolium perenne*, L cv Vigor) was fertilized with 60 kg nitrogen per ha after the first cut (20 May, 1988) and was harvested at 2 different stages of leafy regrowth: 23–34 d (young) and 44–55 d (more mature). Three holstein cows (658 ± 70 kg) with a ruminal and T-piece duodenal cannula between 40 and 43 weeks of lactation (13 ± 3 kg/d of milk) were housed in metabolic crates and milked twice daily. They were fed *ad libitum* (10% refusals) during 2 successive periods of 2 weeks on ryegrass and received a constant supply of 1.7 kg dry matter (DM) of concentrate per day. The diet finally (in % of the total DM) consisted of 85% fresh grass, 12% concentrate and 3% mineral

mix. The diet supplementation was performed in order to compare the transit of the same concentrate when associated with grass or with maize silage (see below). Additional mineral and vitamins were provided by means of 0.3 kg of a feed containing 30% minerals. The chemical composition of the feeds are shown in table I. The animals had free access to water and to a mineral block. The grass was harvested daily at 09.00 h and held at 4°C until required. The herbage was cut to 5 cm above ground level and picked up without being chopped by means of a forage wagon equipped with a motorscythe. The grass was given in 3 equal meals at 07.30, 14.30 and 21.30 h, and the concentrate was distributed in 2 meals (07.30 and 21.30 h). Feeds refusals and water intake were recorded once daily at 07.30 h during each experimental period. Representative samples of the grass offered and refused were taken daily to determine DM content. The samples for each week were pooled for the determination of the organic matter (OM), nitrogen (N) and neutral detergent fiber (NDF) contents. Samples of concentrate and mineral feed were taken once a week for DM, OM, N and NDF analysis as appropriate.

Organization of experimental period

Since the nutritional characteristics of fresh forage change quickly, an experimental schedule was developed to measure the rate of passage and duodenal nutrient flows simultaneously over an 11-day experimental period. Polyethylene glycol 4000 (PEG) was pulse-dosed into the rumen for the determination of liquid dynamics. Ytterbium chloride (YbCl) and PEG were then perfused simultaneously into the rumen for duodenal flow determination, and forage and concentrate labelled respectively with europium (Eu) and dysprosium (Dy) were given as a test meal to follow their passage through the digestive tract.

Fractional outflow rate of liquid in the rumen (FOR) was measured on days 1 and 2. A dose of PEG (25 g/kg DM ingested diluted in 1 l water) was introduced into the rumen on day 1 at 08.00 h. Rumen fluid was sampled at 10.00, 10.30 and 11.00 h on days 1 and 2, strained through 6 layers of cheesecloth and stored at 4°C until analysis. Duodenal nutrient flows were obtained from continuous intra-ruminal perfusions of PEG (250 g/d/cow) and YbCl (2 g Yb/d/cow). Perfusions performed using 2 multichannel peristaltic pumps were begun on day 2 at 14.00 h, after a priming dose, and maintained until day 11. Eighteen spot samples of duodenal contents (350 ml) were taken between days 8 and 10 in order to distribute them evenly over a mean day (every 80 min). A 20-ml aliquot was stored at 4°C for PEG analysis and the remaining samples were pooled and frozen at –20°C. DM, OM, N and ammonia were determined on fresh samples; Yb, neutral detergent fiber and acid detergent lignin were analyzed after freeze-drying. Rumen fluid was sampled at the same time as duodenal content; pH was measured immediately, the sample was strained and the filtrates were pooled, frozen, and analyzed for volatile fatty acids (VFA) and ammonia.

For the measurement of particle passage, feedstuffs were labelled with rare earth metals

Table I. Chemical composition of fresh perennial ryegrass harvested at 2 stages of regrowth 23–24 d (YG) and 44–55 d (MG) and of concentrate [a].

	YG	*MG*	*Concentrate* [b]	*Mineral mix*
DM content (g/100 fresh weight)	18.1	19.8	90.2	91.4
Organic matter (g/100 g DM)	89.1	91.6	91.7	65.7
Crude protein (g/100 g DM)	17.4	9.5	13.1	6.0
Neutral detergent fiber (g/100 g DM)	51.6	52.3	28.1	13.2

[a] The diets contained (g/100 g DM): 85 fresh grass, 12 concentrate and 3 mineral mix; [b] concentrate composition (g/100 g DM): 25 barley, 25 wheat bran, 30 dry beet pulp, 10 dehydrated lucerne, 5 sugar beet molasses, 1 fat, 2 Na_2CO_3, 1 $CaHPO_4$, 1 NaCl.

using a competitive binding technique adapted from Ellis and Beever (1984) and Poncet (personal communication). The cell-wall constituents of feedstuffs were extracted by means of a commercial detergent (Ergamatic AC, Société Chimiotechnique, Lyon, France) at 80°C for 1 h. The residues were soaked in a water bath containing the rare earth element (20 g/kg DM) and citric acid as a competitive ligand, the pH was brought to 2.2–2.5 using 2 N HCl. After an immersion of 24 h, the labelled feedstuffs were carefully rinsed. The grass was dried by centrifugation and the concentrate was dried at 60°C for 48 h. Labelled fresh grass contained 11.8 g Eu/kg DM and concentrate, 9.0 g Dy/kg DM. Labelled feedstuffs (600 and 500 g DM for grass and concentrate, respectively) were offered on day 3, 30 min before the morning meal. After 45 min, any uneaten grass was chopped (5 cm long), soaked in artificial saliva at 40°C and introduced into the rumen *via* the cannula. The total faecal output was collected 22 times during the 178 h post-dosing with increasing intervals (from 2–12 h). Faecal samples (500 g) were dried, ground through a 0.8 mm screen and analyzed for Eu and Dy. During the last 5 d (days 6 to 11), the faecal samples collected were also used to determine the digestibility of the diet and the recovery rate of markers used for duodenal flow determination.

Calculations

FOR was estimated as the slope of the regression of the natural logarithm of PEG concentration *vs* time post-dosing. Ruminal liquid volume was obtained after dividing the quantity of PEG dosed by the calculated concentration at zero time. Rumen liquid outflow was calculated as the product of FOR and the volume. Duodenal digesta flow was calculated by dividing daily quantities of Yb and PEG recovered in faeces by their respective equilibrium concentrations in duodenal digesta. The mean value of the 2 estimates was retained. Total mean retention time in the digestive tract (TMRT) of forage or concentrate particles was calculated using the equation:

$$\text{TMRT} = \frac{\sum m_i t_i}{\sum m_i}$$

where t_i is the time elapsed between dosing the mid-point of each interval i during which faeces were collected, and m_i the quantity of marker excreted in the ith interval. Faecal marker excretion curves were analyzed in order to estimate retention time in the rumen according to Uden (1984). The calculations were as follows. The natural logarithm of marker concentration was plotted *vs* time. The curve was divided into a linear descending part, a curve linear ascending part and a delay (TT). TT was determined by the time elapsed between dosing and the mid-point of interval where marker appeared for the first time. T1 was calculated by the reverse of the slope of the descending part of the curve. T2, corresponding to the ascending part, was obtained by subtracting T1 + TT from TMRT. The physiological implications of these 3 parameters were determined by Mambrini (1990). Briefly, 4 fistulated cows were fed a hay-based diet and received a test meal consisting of coarse hay labelled with thullium and ground hay (mean particle size 0.64 mm) labelled with Yb. Duodenal digesta and faeces were collected and TMRT, T1, T2 and TT were calculated as previously described. The time T1 was associated with the retention time of small particles in the rumen (*ie* having a high probability of exit) because it did not differ according to the hay particle size and to the sampling site. The time T2 was associated with: i) the time of the comminution of coarse particles, because the difference between long and ground hay TMRT was totally recovered in T2, regardless of the sampling site; and ii) the time spent in a small distal mixing compartment (*ie* caecum), as the sum T1 + T2 in faeces was highly correlated with but 3 h longer than TMRT calculated from duodenal digesta. The time TT calculated on faecal kinetics and not observed on duodenal kinetics, was assumed to be the time necessary for the labelled feedstuffs to mix with the rumen content and to transit through the tubular segments.

Chemical analysis

Dry matter was determined by drying at 80°C over a period of 48 h and OM content by ashing for 5 h at 550°C. Nitrogen was obtained by the Kjeldahl method. Neutral detergent fiber was analyzed according to Van Soest (1963) on Fibertec (Tecator) as described by Giger and Pochet (1987). For ammonia determination, the method of Berthelot was adapted to an autoanalyzer. Volatile fatty acid composition was analyzed using gas-liquid chromatography on a

column 1.5 m long x 2 mm id packed with nitrogen as the vector gas, according to Jouany (1982). Concentrations of PEG were analyzed using Hyden's turbidimetric method (1955) modified by Malawer and Powell (1967). The rare earth metals were determined by atomic absorption spectrophotometry, after the samples had been ashed (550°C, 6 h) and digested in a solution containing 2% nitric acid and 2 g/l potassium chloride. Previous studies had indicated no interaction between the determination of the 3 rare earth metals.

Statistical analysis

The results were analyzed with an analysis of variance with grass maturity (1 degree of freedom) and individual effects (2 degrees of freedom) as main factors, using the general linear models procedure (GLM; SAS, 1987). To compare the retention times of forage and concentrate particles, the data were pooled and analyzed by means of an analysis of variance taking into account the effects of the mature of the feedstuff (1 degree of freedom), the nature of the diet and the individual effects. Significance was determined at $P < 0.10$ unless otherwise indicated.

Comparison with maize silage diet

The maize silage diet was given to the same animals 9 weeks before the fresh grass diet. Cows (673 ± 76 kg) were then in mid-lactation (32–35 weeks *post-partum*) and produced 32 ± 2 kg of milk. The diet, given in 2 equal meals (08.00 and 17.00 h), consisted of (% of the total DM) 65% maize silage, 25% of the same concentrate as used in experiment 1, 8% soybean meal, 0.5% urea and 1.5% mineral vitamin mix. The composition of the maize silage was 37.2% DM and, on a DM basis, 95.3% OM and 8.0% crude protein. The diet was given *ad libitum* (10% refusals) and feed refusals were recorded once daily. Cows had free access to water and to a mineral block. Digestion and rate of passage were measured as previously described. However, only 3 markers were used and the experimental period lasted for 23 d. The duodenal nutrient flows (Yb and PEG) were measured after the determinations of liquid dynamics in the rumen and mean retention times of maize silage and concentrate labelled with Yb and Eu respectively. Maize silage was labelled after the grains had been discarded.

The statistical analysis was performed with an analysis of variance. Main effects were partitioned into the nature of the diet (young, more mature grass and maize silage, 2 degrees of freedom) and individual effect (2 degrees of freedom). The GLM procedure was used (SAS, 1987). When the effect was significant the means were compared by the Newman and Keuls multiple range test at the 0.05 significance level.

RESULTS

Characteristics of fresh grass diets

With advancing maturity, crude protein content (N*6.25) of grass decreased markedly but the other chemical parameters were not changed (table I). The increase in maturity did not affect OM intake but OM digestibility was reduced ($P < 0.03$) and the proportion of digestible OM intake (DOMI) apparently digested in the rumen was also slightly reduced ($P < 0.07$; table II), whilst this proportion remained unchanged for NDF (0.94). NDF digestibility was reduced as a consequence of delayed harvesting (0.794 and 0.695 for young and more mature grass, respectively; $P < 0.01$). With the older regrowth, N intake was strongly decreased ($P < 0.002$; table II) due to the low N content of grass. Total apparent digestibility of N was also reduced although the quantity of N excreted in faeces decreased ($P < 0.03$) from 8.3 for the young grass to 6.9 g/kg OM intake for the more mature grass. The duodenal flow of non-ammoniacal N (NAN) was always higher than the total N intake. This difference was even more striking for the more mature grass. Nonetheless, the greater maturity reduced total NAN flow by 17% ($P < 0.05$) and tended to lower NAN supply

Table II. Digestion of organic matter and nitrogen in 3 dairy cows fed a diet based either on fresh perennial ryegrass harvested at 2 stages of regrowth (trial 1) 23–34 d (YG) and 44–55 d (MG) or on maize silage (MS; trial 2).

	YG	*MG*	*SEM* [a]	*MS*	*SEM* [b]
Organic matter					
Intake (kg/d/)	13.1	13.2	0.44	17.4	0.12
Entering the duodeum (kg/d)	6.7	7.0	0.11	10.1	0.74
Apparent digestibility	0.803	0.750	0.007	0.725	0.012
Ruminal digestion (kg/kg DOMI [c])	0.612	0.626	0.033	0.581	0.077
Nitrogen (N)					
Intake (g/d)	374	240	5	426	10
Entering the duodenum as NAN[d] (g/d)	412	340	12	416	19
(g/kg DOMI [a])	39.3	34.1	1.6	32.7	2.2
(g/g N intake)	115	135	5.7	89	7.2
Apparent digestibility	0.710	0.623	0.001	0.676	0.008

[a] Standard error of mean of the analysis of variance testing the effect of advancing maturity of the fresh grass diet (trial 1); [b] standard error of mean of the analysis of variance testing the difference between the 3 diets (trial 2); [c] DOMI, digestible organic matter intake; [d] NAN, non-ammoniacal nitrogen.

as a fraction of DOMI although the difference failed to be significant (table II). Ruminal pH was slightly increased with the more mature grass ($P < 0.10$; table III). The total amount of VFA was not significantly influenced by the diet, greater maturity caused a decrease in the proportion of isoacids, propionate ($P < 0.05$; table III). The rumen ammonia level was also reduced ($P < 0.01$).

Greater maturity of the grass did not alter FOR, caused a slight but non-significant increase of the rumen volume and increased the rumen liquid outflow ($P < 0.05$; table IV). When eating the more mature grass, the cows drunk less ($P < 0.002$), and due to the higher DM content of this grass, the total water intake was lower ($P < 0.002$). Concentrate TMRT was unaffected by advancing maturity. Grass

Table III. Rumen parameters of dairy cows fed a diet based on fresh ryegrass harvested at 2 stages of regrowth, 23–34 d (YG) and 44–55 d (MG).

	YG	*MG*	*SEM* [a]
pH	6.41	6.55	0.06
NH_3 (mg/l)	64.9	25.1	3.12
VFA [b] (mM/l)	97.2	91.5	3.70
C2 (molar %)	65.0	66.3	0.58
C3 (molar %)	20.9	19.1	0.52
C4 (molar %)	11.3	12.5	0.17
Isoacids [c] (molar %)	1.7	1.2	0.17
Other minor acid (molar %)	1.1	0.9	0.12

[a] Standard error of mean of the analysis of variance testing the effect of advancing maturity of the fresh grass diet; [b] volatile fatty acids; [c] isobutyric and isovaleric acids.

Table IV. Water intake and rumen liquid dynamics in dairy cows fed a diet based either on fresh perennial ryegrass harvested at 2 stages of regrowth (trial 1) 23–34 d (YG) and 44–55 d (MG) or on maize silage (MS; trial 2).

	YG	*MG*	*SEM* [a]	*MS*	*SEM* [b]
Water drunk (l/d)	32	15	1.1	59	0.5
Total water intake [c] (l/h)	97	67	4.3	83	2.5
Liquid fractional outflow rate (%/h)	16.9	17.6	0.3	12.1	0.2
Rumen liquid volume (l)	61	70	2.7	99	3.4
Rumen liquid outflow (l/d)	247	296	9	288	12

[a] Standard error of mean of the analysis of variance testing the effect of advancing maturity of the fresh grass diet (trial 1); [b] standard error of mean of the analysis of variance testing the difference between the 3 diets (trial 2); [c] water drunk + water contained in feedstuffs.

Table V. Total and partial mean retention times (h) of forage and concentrate in dairy cows fed a diet based either on fresh perennial ryegrass harvested at 2 stages of regrowth (trial 1) 23–34 d (YG) and 44–55 d (MG) or on maize silage (MS; trial 2).

	YG	*MG*	*SEM* [a]	*MS*	*SEM* [b]
Forage particles					
Total mean retention time	43.0	48.8	2.1	49.7	1.5
T1 [c]	19.0	19.0	1.5	24.3	1.8
T2 [c]	11.6	19.8	1.6	12.6	2.5
TT [c]	12.4	10.0	0.7	12.8	0.8
Concentrate particles					
Total mean retention time	32.6	33.1	0.3	36.5	0.3
T1 [c]	17.6	16.1	0.7	20.2	0.9
T2 [c]	6.5	9.1	0.7	3.5	0.8
TT [c]	8.5	7.9	0.4	12.8	0.8

[a] Standard error of mean of the analysis of variance testing the effect of advancing maturity of the fresh grass diet (trial 1); [b] standard error of mean of the analysis of variance testing the difference between the 3 diets (trial 2); [c] from the analysis of the faecal marker excretion curves according to Uden (1984).

TMRT tended to increase (5.8 h on the mean; table V) but there were large between-cow variations. Forage TMRT increased up to 8.9 h for the 2 cows with the shortest TMRT (mean of 38 h for the young grass) but was not affected for the cow with the longest TMRT (52.6 h for the young grass). However, T2 was always longer with more mature grass, either for forage ($P < 0.07$) or for concentrate ($P < 0.10$) particles whilst T1 and TT were not affected (table V). Forage TMRT (45.9 h)

was strikingly longer than concentrate TMRT (32.9 h; $P < 0.01$), as a consequence of the longer T2 ($P < 0.05$) of forage particles (15.7 *vs* 7.8 h for concentrate), T1 and TT being similar for all feedstuffs.

Comparison with maize silage diet

Compared with fresh grass diets (table II), OM and N intakes were much higher ($P < 0.05$) with the maize silage diet, OM digestibility was lower ($P < 0.05$) but the proportion of DOMI apparently digested in the rumen was not affected ($P > 0.10$; table II). Total apparent N digestibility ranked the diets in the following order: mature grass < maize silage < young grass ($P < 0.05$). The NAN flow relative to DOMI tended to be higher with fresh grass diets, unless the difference was not significant ($P > 0.10$). Cows drunk more water with the maize silage diet ($P < 0.05$) but considering the dry matter of feedstuffs, total water intake was not different between young grass and maize silage ($P > 0.10$; table IV). Compared with fresh grass diets, FOR was much lower ($P < 0.05$) and rumen volume higher ($P < 0.05$) with the maize silage diet (table IV). There was no direct effect ($P > 0.10$) of the nature of the diet on rumen liquid outflow (table IV). Maize silage TMRT tended to be greater than young grass TMRT but was not different from that measured with the more mature grass ($P < 0.10$; table V). This was explained by a relatively shorter T1 for grass diets ($P < 0.10$), balanced only for the more mature grass diet by a relatively longer T2. The total mean retention time of the concentrate associated with maize silage was lower than forage TMRT ($P < 0.05$), as already observed for the grass diets. Concentrate TMRT was longer ($P < 0.05$) when associated with maize silage than with fresh grass. This difference was explained by longer T1 ($P < 0.05$). The opposite was true for T2, which was lower ($P < 0.05$) with the maize silage diet (table V). There were large individual variations of TMRT (variation coefficient of 13 and 8% for forage and concentrate, respectively). Individual differences outlined for grass diets were also found with the maize silage. The cows showing the highest TMRT when fed on grass, also had the highest TMRT when fed on maize silage (54.9 *vs* 48.3 and 45.8 for the other 2 cows). These differences were explained by higher T1, T2 and TT ($P < 0.05$). Between-cow differences were also found in concentrate TMRT.

DISCUSSION

Knowing the high individual variability of digestive transit (Hartnell and Satter, 1979; Ørskov *et al,* 1989), the experimental schedule was chosen in order to feed the 3 diets consecutively to the same cows. However, due to the time elapsed between the periods of measurement, it must be borne in mind that treatment effects may be confounded by time and stage of lactation. This confounding is unimportant in the first experiment (2 successive 14-d periods) but might not be completely excluded for the comparison between maize silage and grass diets (9-week interval). Nonetheless, passage of liquid and particles in the digestive tract of dairy cows is strongly affected only during the dry period and the first month of lactation (Doreau and Rémond, 1982; Doreau *et al,* 1990; Mambrini, 1990). Otherwise a reversal design experiment would have required several long periods for adaptation to the diet.

Without a reference value, validation of duodenal flow estimates is difficult. One approach could be to compare lignin flow in duodenum and lignin intake or output in faeces. Faecal acid detergent lignin was

determined on grass diets. The estimated duodenal lignin flow was higher (+15%) than, but highly correlated (r = 0.90) with, faecal acid detergent lignin output. This suggests that the method used in the pesent experiment is reliable to compare diets although the actual flows might be slightly overestimated. Actual values of NAN flows were 8% higher than the estimates obtained by a linear regression established by Peyraud (1994) on 40 data reported in the literature on fresh grasses given to cattle and dairy cows (NAN flow = 0.18 N ingested + 28.6 DOMI, giving 368 and 326 g/d for the young and the more mature grass, respectively).

Different models have been proposed to study particle dynamics within the gastrointestinal tract. Besides the proposed method, models described by Grovum and Williams (1973, GW model), Ellis *et al* (1979) with a time-dependent passage rate following a gamma 3 distribution (E3 model) and Dhanoa *et al* (1985; DH model) were also examined but they were not retained. Firstly, in some cases, the nonlinear iterative procedure did not converge (NLIN procedure, Marquardt algorithm, SAS, 1987). On 2 occasions faecal marker excretion patterns could not fit the DH model. For the same reason, the GW model had to be solved by the linear method initially proposed by Grovum and Williams (1973). Secondly, TMRT (algebraic method) may be considered as a reference value because it is based on the quantitative recovery of markers and is calculated without any assumption about marker excretion. In contrast, all models assume that faecal excretion is a continuous process. Fitting models provided mean estimates of total MRT that did not differ from TMRT but increased the variability of the estimates as shown by the residual standard deviation of the relationships between those estimates and TMRT (table VI and Mambrini *et al,* 1988). This may reflect curve fitting inadequacy and surely limits the use of such models. Lastly, the physiological allocation of the different compartments of the models remains unclear. Times spent in the first compartment of the DH model, assumed to be the rumen retention times, provided mean values that were close to, and highly correlated (r = 0.95) with, T1. However this model has dramatically overestimated the time of first appearance of the marker in faeces (table VI). Similarly, physiological components of the GW model are obscure for forages because the time of first appearance of marker in the faeces was also overestimated. In contrast, the E3 model provided a delay and a time spent in the second compartment that were close to TT and T2, suggesting that this model fits the first steps of the faecal kinetics adequately. However, the time spent in the first compartment was lower than T1 (table VI). So, we inferred that no confidence could be placed in the estimations of rumen retention time due to their variations with the different models. We preferred to use the method for which we had determined the physiological implications of the parameters.

Characterization of grass diets

The decrease of OM digestibility with plant maturity is well known (INRA, 1989). Nonetheless, the values remained high for the more mature grass. This result is explained by the decrease of NDF digestibility. Actually, the level of indigestible cell-wall material (total NDF content minus digestible NDF content) increased from 112 for the young to 162 g/kg OM for the more mature grass. Because this study suggests that the decrease in digestibility is not due to a lower rumen mean retention time, it may be related either to characteristics of plant cell walls or to lower cellulolytic activity in the rumen. Nylon bag incubations

Table VI. Comparison between modelling methods for estimating total and partial mean retention times (h) in the gastrointestinal tract and the proposed method ($n = 18$).

	Proposed method	*GW* [a]	*E3* [b]	*DH* [c]
Total mean retention time				
Forages	47.1	45.6	47.6	48.4
Concentrates	33.9	35.0	32.7	38.2
rsd [d]	–	2.9	1.8	2.5
Mean retention time in the first compartment				
Forages	20.8	20.3	17.1 *	19.7
Concentrates	17.8	18.9	14.6*	18.3
rsd [d]	–	2.3	3.0	2.2
Mean retention time in the second compartment				
Forages	15.3	10.4*	17.7	6.3*
Concentrates	6.4	5.6	6.7	3.5*
rsd [d]	–	2.1	2.9	0.8
Time of first appearance of marker				
Forages	10.6	14.8*	12.2*	22.3
Concentrates	9.4	10.1	10.9	15.0*
rsd [d]	–	2.2	1.6	4.6

[a] Model proposed by Grovum and Williams (1973); [b] model proposed by Ellis *et al* (1979) with a time-dependent passage following a gamma 3 distribution; [c] model proposed by Dhanoa *et al* (1985); [d] residual standard error of the regression between the parameter estimated after fitting the model and the parameter of the proposed method (Uden, 1984); * significantly different from the parameter calculated with the proposed method (tested with a paired *t*-test, $P < 0.05$).

were not carried out in the present study but Legoffe (1991) showed that the rate of degradation of DM decreases as the plant matures. Similar results were obtained from a first and a second cut of fresh ryegrass in our laboratory (JL Peyraud, unpublished results). The lower fiber digestibility may be also related to ruminal characteristics and, in particular, the low levels of ammonia could have limited cellulolytic activity (Hoover, 1986). Indeed, Kennedy *et al* (1992) found that digestion rate of forage cell wall incubated *in situ* was depressed below the rumen ammonia level of 25 to 50 mg N/l.

The advanced maturity had no major effect on the site of digestion of DOMI. Apparent rumen digestion accounted for 0.62 of DOMI, which is slightly less than data recorded for fresh ryegrass diets fed to growing cattle (Beever *et al*, 1985). This may be either related to the slight overestimate of duodenal flow previously mentioned or to species differences. The decrease of NAN flow with grass maturity is in accordance with previously reported results (Beever *et al*, 1978, 1985, 1986; Vérité *et al*, 1984). It is probably due to lower microbial nitrogen production. In particular, the ammonia concentration was close to the minimal value required for maximal microbial synthesis (22 mg/l according to Slyter *et al*, 1979). Due to the decrease of apparent digestibility of NAN, the small in-

testinal content of apparently digested NAN sharply decreased when plant has matured (*ie* 292 and 212 g/kg OM, for the young and the more mature fresh grass, respectively). Thus it appears that plant maturity affects more strikingly nitrogen than energy value.

Rate of passage of grass diets

The advanced maturity did not affect FOR, in agreement with the results of Funk *et al* (1987). Grass TMRT was increased to small extent and this agrees with data previously obtained either with hay (Robles *et al,* 1981; Hunt *et al,* 1988) or with fresh grass (Krysl *et al,* 1987; Pond *et al,* 1987). By contrast with the present study, advanced maturity in those studies always led to a marked reduction of intake and a sharp fall in digestibility. Aitchinson *et al* (1986) limited grass intake per sheep to 18 g DM/kg live weight and did not observe any influence of the stage of grass regrowth on the retention time. The retention time of the small particles in the rumen did not change with grass maturity probably because FOR was not affected, the small particles being supposed to flow out of the rumen with the liquid phase. Thus, the slight increase in TMRT is fully explained by a longer T2. As the level of intake remained unchanged, it should be assumed that particulate retention time in the abomasum and caecum-proximal colon is not modified (Coombe and Kay, 1965; Grovum and Hecker, 1973). Thus the factor which is most likely to induce the longer TMRT observed with more mature grass may be an increase in the time required for the comminution of the coarse particles. This hypothesis is consistent with the higher content of non-digestible cell-wall material in more mature grass which would have required longer mastication than the young grass comminution being achieved primarily by mastication (Ulyatt *et al,* 1986). This longer mastication would have stimulated salivary flow and the increase of the net inflow of water into the rumen (rumen outflow minus intake) with advancing maturity (230 l *vs* 150 l/d) might also support this.

Concentrate TMRT is 13 h shorter than forage TMRT. This faster passage has been previously observed in dairy cows eating diets based on conserved forages (Prange *et al,* 1979; Colucci *et al,* 1982; Snyder *et al,* 1984; Shaver *et al,* 1986; Peyraud *et al,* 1989) and also, in this experiment, when maize silage diet was given. This difference is largely accounted for by a lower T2 and so may be mainly due to differences in comminution time, since the passage in caecum-proximal colon is not different in forage and concentrate particles (Peyraud and Mambrini, 1992).

Comparison of grass with maize silage diets

Water dynamics in the rumen are greatly influenced by the nature of the forage. FOR was 43% higher and volume 35% lower with grass than with maize silage diet. The high FOR values observed with the grass diets are similar to the results obtained for ryegrass, cocksfoot and white clover diets by JL Peyraud (average of 15%/h, unpublished results) with dairy cows eating 9–11 kg DM/d. Carruthers *et al* (1988) measured liquid dynamics in grazing dairy cows in mid-lactation and also obtained high values for FOR in spite of a low feed intake (6.3 kg/d). FOR with the maize silage diet was comparable with the values generally observed when cows were fed conserved forages at the same level of intake (Snyder *et al,* 1984; Peyraud *et al,* 1989). The lower rumen volume observed with the grass diets was partly

explained by the lower OM intake, but may also be a particular feature of fresh forage diets. Actually, at similar levels of intake, the fibrous mass in the rumen, and therefore the water content, might be lower due to the higher digestibility of fresh grass. Rumen liquid outflow showed only small variations between the 3 diets. This could be related to the fact that the frequency of reticular contractions generally show only minor changes (Baumont and Deswysen, 1991). Therefore, the higher FOR observed with grass diets could be the consequence of the lower rumen volume. The increase of NAN flow relative to DOMI with the grass compared to the maize silage diet may indicate a higher efficiency of microbial synthesis in association with the more rapid FOR. Indeed, it is well known that the efficiency of microbial synthesis is related to FOR (Harrison *et al,* 1975).

TMRT of grass particles was shorter than for maize silage particles only in the case of the young highly digestible grass (–6.7 h). Obviously, this difference could have been influenced by the lower intake observed with the grass diets, since TMRT is known to increase when intake decreases (Hartnell and Satter, 1979; Snyder *et al,* 1984; Shaver *et al,* 1986). In our laboratory, we have previously observed that reducing the level of intake (17–15 kg OM/d) of cows fed the same diet as in experiment 2, increased forage TMRT (47–53 h) whilst TMRT was longer during the dry period (64 h; 12 kg OM/d) than in mid-lactation (53 h; 17.2 kg OM/d; Mambrini, 1990). Indeed, the passage of grass and maize silage particles was quite different. The retention time of small particles in the rumen was lower for the 2 grass diets probably as a consequence of the higher FOR. This is consistent with the suggestions of Faichney (1986) assuming that water is the major vehicle for the removal of small particles. By contrast, T2 was not shorter for the particles of the young grass but was even higher for the more mature grass. This difference may originate in a longer comminution time for the coarse particles of more mature grass distributed without being chopped. However, it may also be partly ascribed to a longer transit in the caecum-proximal colon with the grass diets due to the lower level of intake, as previously observed in sheep (Coombe and Kay, 1965; Grovum and Hecker, 1973).

Concentrate TMRT was always lower than, but highly correlated with, forage TMRT (r = 0.84; n = 9). Thus when the same concentrate was used with the 2 grasses and the maize silage, shorter transit times were associated with the fresh forage. This effect is explained by the lowest retention of the small particles in the rumen with the grass diet as a consequence of the water dynamics already mentioned for forage particles. Widyobroto and Peyraud (1993) showed that concentrate TMRT was not affected by the level of concentrate (20–35%, DM basis). Thus the transit of the concentrate, and consequently its nutritive value, seems to be dependent on the nature of diet.

CONCLUSION

Increasing the maturity of fresh grass influenced liquid and particle dynamics. The index of comminution time of coarse particles was increased with more mature grass compared to the highly digestible grass, yet the retention time of small particles in the rumen remained unaffected. Compared to conserved forages, fresh grass showed a rapid FOR which was probably responsible for the lower rumen retention time of small particles. The retention time of concentrate particles seemed to depend on the nature of the associated forage, more so than content of concentrate in the whole diet.

ACKNOWLEDGMENTS

The authors are grateful to R Vérité for making helpful criticisms and C Chabanet for her advice in statistics and her availability.

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