The effect of frequency of feeding and supplementation with sucrose on ruminal fermentation of alfalfa silage given ad libitum or restricted to sheep

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[1]*Animal Research Centre and* [2]*Research Program Service, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.* *Contribution nos. 1675*[1] *and R026*[2]*. Received 4 Apr. 1990, accepted 20 Feb. 1991.*

Charmley, E., Veira, D. M., Butler, G., Aroeira, L. and Codagnone, H. C. V. 1991. **The effect of frequency of feeding and supplementation with sucrose on ruminal fermentation of alfalfa silage given ad libitum or restricted to sheep.** Can. J. Anim. Sci. **71**: 725–737. Eight ruminally cannulated wethers were used in a factorial trial to examine the effect of frequency of feeding alfalfa silage and sucrose supplementation on voluntary intake, digestibility, rumen fermentation and rate and extent of digestion in and passage from the rumen. When diets were fed ad libitum, frequency of feeding had no effect on voluntary intake, apparent digestibility or the postfeeding concentrations of rumen ammonia N, volatile fatty acids (VFA) and pH. Similarly, VFA ratios and fluid kinetics in the rumen were unaffected. Supplementation with sucrose reduced ruminal concentrations of ammonia N at the higher level of feeding but failed to influence any other measured parameters. When intake was restricted (18 g DM kg^{-1} body weight), increased feeding frequency reduced the post-feeding ruminal ammonia peak and reduced the post-feeding decline in pH; however, sucrose supplementation had no effect. Kinetics of the liquid phase in the rumen, particulate rate of passage and rate of digestion were not affected by feeding frequency or sucrose supplementation. It was concluded that effects observed at a restricted feeding level may not be apparent when feed is available ad libitum and vice versa.

Key words: Sucrose, feeding frequency, alfalfa, silage, sheep

Charmley, E., Veira, D. M., Butler, G., Aroeira, L. et Codagnone, H. C. V. 1991. **Effet de la fréquence des repas et d'un supplément de sucrose sur la fermentation ruminale d'ensilage de luzerne servi à volonté ou en rationnement à des moutons.** Can. J. Anim. Sci. **71**: 725–737. Huit béliers castrés fistulés du rumen ont servi dans un dispositif factoriel à examiner l'effet de la fréquence des repas à l'ensilage de luzerne d'un supplément de sucrose sur l'ingestion volontaire, la digestibilité, la fermentation ruminale, ainsi que la vitesse et l'importance de la digestion dans le rumen et du passage des nutriments dans le réseau. Lorsque les rations étaient servies à volonté, la fréquence des repas n'avait aucun effet sur l'ingestion volontaire, la digestibilité apparente ou les concentrations postprandiales d'azote ammoniacal, d'acides gras volatils (VFA) et le pH du rumen. De même, les rapports des VFA et la cinétique du liquide ruminal n'étaient pas modifiés. Le supplément de sucrose réduit les concentrations ruminales d'azote ammoniacal à la dose de fourrage la plus élevée, mais n'influe pas sur les autres paramètres mesurés. Lorsque l'ingestion est rationnée (18 g de MS/kg de poids corporel), l'augmentation de la fréquence des repas réduit le pic postprandial d'azote ammoniacal du rumen et la baisse postprandiale du pH, mais le supplément de sucrose n'a aucun effet. La fréquence des repas ou le supplément de sucrose n'influe pas sur la cinétique de la phase liquide dans le rumen,

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la vitesse de passage des matières particulaires ni la vitesse de la digestion. Les effets observés en régime de rationnement peuvent ne pas se manifester en régime d'alimentation libre et vice versa.

Mots clés: Sucrose, fréquence des repas, luzerne, ensilage, moutons

Inefficient ruminal utilisation of silage N has been attributed to rapid and extensive degradation of dietary amino acids and other non-protein N compounds coupled with a shortage of ruminally available energy. Supplementation of silage with a source of readily available energy has been found to reduce peak ruminal ammonia concentration (Chamberlain et al. 1985) and increase flow of microbial protein to the small intestine (Rooke et al. 1987; Huhtanen 1988). In these experiments, animals were offered feed twice a day at a restricted level of intake. Consequently, rumen fermentation had a marked cyclical pattern with concentrations of ammonia and volatile fatty acids rising after feeding and falling to low levels between meals. Under these conditions, microbial utilisation of dietary N is poor and responses to supplemental energy are generally positive (Rooke et al. 1987). However, when feeding is more frequent, improvements in ruminal N utilization to energy supplementation are less evident (Gill and Ulyatt 1977). The objective of this experiment was to compare effects of supplementation with sucrose, a rapidly fermentable energy source, on fermentation in the rumen when alfalfa silage was offered either twice or eight times a day. In addition, the treatments were imposed at a restricted and ad libitum level of intake since it has been shown that restricted feeding, which is the norm for digestion studies, may exacerbate inefficient N utilization in the rumen (Robinson 1989).

MATERIALS AND METHODS

Animals and Diet

Eight Canadian Arcott wether sheep weighing (mean ± SD) 48 ± 4.5 kg and fitted with rumen cannulae were used. The basal diet consisted of first-cut wilted alfalfa silage, containing < 15% timothy (Table 1). Trace mineralized salt licks containing (mg kg^{-1} DM): I, 70 and Co, 40 were available at all times (The Canadian Salt Co., Ltd., Mississauga, ON).

Table 1. Composition of the silage[z]

Analyte	Silage	
Toluene dry matter (%)	36.3	(0.30)
pH	4.35	(0.010)
Ammonia N (% total N)	10.6	(0.94)
Percent of dry matter		
Organic matter	91.3	(0.94)
Total nitrogen	2.69	(0.032)
Water soluble carbohydrate	1.33	(0.154)
Acid detergent fiber	34.4	(0.59)
Lactic acid	5.53	(0.425)
Acetic acid	4.74	(0.982)
Propionic acid	0.34	(0.130)
Butyric acid	0.42	(0.175)

[z] Values are means (standard deviation of four samples).

Experimental Deisgn

The experiment was designed as a split-plot with subplot treatments in a crossover design. Eight animals and two periods of 32 d duration each were employed. In the first period, four sheep were given alfalfa silage twice daily (low frequency; L), and four were fed eight times daily (high frequency; H). At both frequencies of feeding, alfalfa silage was either fed alone (U) or was supplemented with granular sucrose (S) at 10% of the silage dry matter (DM) intake. Animals assigned to L in period 1 were assigned to H in period 2 and vice versa; however, the same animals received sucrose in both periods. Thus, the effect of supplementation was examined among animals, while the effect of feeding frequency could be examined within animals. Intake, apparent digestibility and rumen parameters were measured at either a restricted feeding level of 18 g DM kg^{-1} body weight (BW) or ad libitum within each period. No statistical comparisons were made between levels of feeding.

Schedule and Measurements

On day 1 of each period, animals were allocated to their respective diets and fed at a rate of 20 g kg^{-1} BW. Feed was given at 08:00 and 16:00 h for the L animals and at 08:00 h and at 3-h intervals throughout the day for the H animals, using automatic feeding machines (Buckley et al. 1985). Sucrose was added to silage at feeding (L) or when

automatic feeders were refilled (H). The amount of feed offered was increased through days 1–5 until daily refusals averaged 10%. On day 6, animals were placed in metabolism cages for the remainder of the period. Ad libitum intake was recorded from days 7 to 17 and during this time, apparent digestibility was determined by collection of total feces. Sucrose was assumed to be completely digested. On day 18, samples of rumen fluid were taken by vacuum extraction just prior to the 08:00 h feeding and at regular intervals over the following 24 h. Samples were taken at least every 2 h, and more frequently from the L animals up to 6 h after each feeding, to monitor changes in rumen pH, and concentrations of ammonia N and volatile fatty acids (VFA) over the 24-h period. The pH was measured immediately. Samples were then strained through cheesecloth and centrifuged (10 000 × g). One portion was frozen for Co analysis and a further portion acidified with 25% metaphosphoric acid for analysis of ammonia N and VFA concentrations. At the time of the first rumen sample, 50 mL of cobalt ethylene diamine tetra-acetic acid (Co EDTA) solution, containing 0.7 g Co, was administered to each animal via the rumen cannula to enable rumen liquid volume and turnover rates to be estimated. Subsequently, level of feeding was reduced to 18 g kg^{-1} BW by day 20 and apparent digestibility was recorded from days 21 to 27. On day 28 rumen sampling was repeated as above. In addition to Co EDTA, 100 g chromium mordanted fiber (Cr mordant) made from alfalfa silage (Uden et al. 1980) containing approximately 2.5% Cr was administered via the rumen cannula at the time of the first rumen sample. Fecal grab samples were obtained 6, 9, 12, 15, 18, 21, 24, 30, 36, 42 and 48 h post-dosing and at 12-h intervals thereafter for 3 d. Change in concentration of fecal Cr with time was used to estimate the particulate rate of passage from the rumen (Aitchison et al. 1986). Fecal Co concentration was also used to compare rate of passage, estimated with Cr or Co. The nylon bag technique was used to determine in situ degradability and rate of disappearance of DM and degradability of N (Aitchison et al. 1986). A portion of silage used in the feeding trial was chopped using a Hobart Food Cutter and approximately 5 g DM was put into eight nylon bags (53-μm pore size) for each sheep. On day 27, four nylon bags were inserted into the rumen of each sheep at 08:00 h and removed after 8, 16, 24 and 48 h of incubation. On day 28, a further three nylon bags were inserted at 08:00 h and removed after 1, 2 or 4 h of incubation. The remaining nylon bag was used to determine the 0-h wash value. Thus, no more than four bags were in the rumen at any one time but measurements of disappearance from nylon bags were obtained for seven discrete sampling times. All bags were thoroughly washed by immersion and rinsing in cold water for 12 min. The residue was freeze-dried and weighed prior to analysis for DM and N. Samples of feed offered were combined for each treatment over days 7–17 and 21–28 of each period; samples of refusals were bulked for each animal over days 11–17. Sheep were weighed at the beginning of each digestibility measurement period (i.e. four times in total).

Silage was analyzed for toluene DM, organic matter (OM), total N, water soluble carbohydrates, organic acids, acid detergent fiber (ADF) and gross energy. Nylon bag contents after 0, 1, 2, 4, 8 and 16 h of incubation were analyzed for N. Fecal grab samples were analyzed for Cr and Co concentrations and bulked feces were analyzed for OM, N and ADF.

Chemical Analyses

Dry matter of plant material and feces was determined by oven drying at 100°C for 24 h except for silage samples where toluene distillation was used (Dewar and McDonald 1961). Ash concentration was determined by incineration in a muffle furnace at 550°C for 6 h. The concentration for ADF in freeze-dried samples was determined by the method of Goering and Van Soest (1970). Total N was determined on fresh samples (freeze-dried for nylon bag samples) by the Kjeldahl technique and ammonia N was measured by the method of Novazamsky et al. (1974). Determinations of lactic acid (Barker and Summerson 1941) and silage VFA (Erfle et al. 1979) were made on acid extracts of silage. Water soluble carbohydrate (WSC) was measured according to the method of Dubois et al. (1956). Cobalt was measured by atomic absorption in dried feces following extraction (Hart and Polan 1984) and in appropriately diluted samples of rumen fluid. The concentration of Cr in feces was measured by atomic absorption spectrophotometry following nitric/perchloric acid digestion (Charmley and Ivan 1989).

Statistical Analysis

The elimination rate of the fecal markers was established by fitting the model of Dhanoa et al. (1985). The model can be written as in Aitchison et al. (1986).

$$y = A\ [e^{-k_1 t}]\ \exp[-Be^{-(k_2 - k_1)t}], \qquad (1)$$

where y is the fecal concentration (g kg^{-1}) of the marker, t is time in hours and A, k_1, B and k_2 are the parameters of the model. As suggested in Dhanoa et al. (1985), this model can be expressed as a line plus exponential when logarithms are taken as follows:

$$\log y = \log(A) - k_1 t - Be^{-(k_2 - k_1)t}. \quad (2)$$

This was analyzed as a standard model using the Maximum Likelihood Program (MLP; Ross 1987). As discussed in Dhanoa et al. (1985), taking logarithms allows for efficient estimation and stabilizes the variances. There was a lag of between 0 and 12.5 h which consisted of zero values (Cr) or very small values (Co) The lag varied by animal and time period. Fitting all the data to the curve resulted in a poor fit, thus the lag portion of the curve was not included in the fitting of the model; the first non-zero data point after which the data increased steadily was chosen as the first data point.

The Dhanoa model requires a modern computing capability. It was the intention of this work to determine if a simpler model could adequately describe the kinetic parameters. Thus, the elimination phase of the curve (starting at the peak value) was also fit to a line on the logarithmic scale using the model:

$$y = Ae^{-k_1 t}, \quad (3)$$

where y and t are as previously defined and A and k_1 are the parameters of the model. This can be expressed on the logarithmic scale as a line as follows:

$$\log y = \log(A) - k_1 t. \quad (4)$$

Degradation of DM and N in the nylon bags was examined by analysis of variance. Based on this analysis, one observation with a large residual which failed the Tietjen and Moore test for outliers was removed from the data (Tietjen and Moore 1972). Examination of residuals indicated data transformation was not required. The data were then fitted to the following model suggested by Ørskov and McDonald (1979) using the NLIN procedure of the Statistical Analysis System Institute, Inc. (SAS 1985)

$$y = a + b(1 - e^{-ct}), \quad (5)$$

where y is the percentage DM or N disappearance, t is time and a, b and c are parameters of the model.

Variables of intake, digestibility, rumen fermentation, volume and turnover and parameter estimates from models were examined by analysis of variance using the GLM procedure of the SAS Institute Inc. (1985) to determine the effects of frequency of feeding and supplementation. The following model was used:

$$y = \mu + \alpha_i + \gamma_{ij} + \delta_k + \beta_m + \alpha\beta_{im} + \epsilon_{ijk(m)}$$

where μ is the overall mean, α_i is the effect of sucrose supplementation, γ_{ij} is among animal error used to test for sucrose effect, δ_k is the period effect, β_m is the effect of feeding frequency, $\alpha\beta_{im}$ is the interaction of supplementation and feeding frequency and $\epsilon_{ijk(m)}$ is the within animal error used to test the remaining effects.

RESULTS

Voluntary Intake and Digestibility

The alfalfa silage was of intermediate maturity and was well-preserved (Table 1). Feeding frequency and sucrose had no effect ($P > 0.05$) on voluntary intake or apparent digestibility during ad libitum feeding (Table 2). When intake was restricted, sucrose increased apparent OM digestibility of the whole diet ($P < 0.001$) but had no effect on apparent OM digestibility of silage. Sucrose reduced apparent digestibility of N ($P < 0.05$) but not ADF ($P > 0.05$).

Rumen Fermentation

When diets were given ad libitum, feeding frequency had no effect ($P > 0.05$) on rumen ammonia N concentration (Table 3). However, when intake was restricted, the high feeding frequency increased minimum ammonia N concentration ($P < 0.05$) and reduced maximum values ($P < 0.05$) compared with the low frequency. Mean ammonia N concentration over the day was similar at both feeding frequencies. Sucrose supplementation reduced the maximum and mean rumen ammonia concentration ($P < 0.05$) when diets were given ad libitum, but not when they were fed at a restricted level. Increasing the frequency of feeding tended to increase minimum ruminal pH ($P < 0.10$) and reduce maximum pH ($P < 0.05$) but only when feed intake was restricted. Feeding frequency had a marked effect on the diurnal pattern of rumen ammonia concentration (Fig. 1). Increasing meal frequency minimized the fluctuations in ruminal ammonia concentration, but did not eliminate them.

Table 2. The effects of feeding frequency and sucrose supplementation of voluntary intake and digestibility of the diets in sheep fed alfalfa silage ad libitum or restricted

Sucrose	Unsupplemented (U)		Supplemented (S)				Significance of effects	
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]	L vs. H	U vs. S
Ad libitum feeding								
Dry matter intake								
Total (kg d^{-1})	1.25	1.24	1.23	1.20	0.133	0.162	NS	NS
(g kg^{-1} BW)	29.9	30.3	29.2	31.9	1.24	2.29	NS	NS
Silage (kg d^{-1})	1.25	1.24	1.10	1.03	0.149	0.170	NS	NS
Apparent digestibility (%)								
Organic matter								
Total	65.5	64.2	67.3	66.4	0.97	1.32	NS	NS
Silage	65.5	64.2	63.0	61.9	0.99	1.39	NS	NS
Nitrogen	63.9	64.8	63.8	60.2	1.26	1.90	NS	NS
Acid detergent fiber	53.3	52.1	51.9	51.1	1.71	2.12	NS	NS
Restricted feeding								
Dry matter intake								
(kg d^{-1})	0.91	0.91	0.93	0.97	0.017	0.064	NS	NS
(g kg^{-1} BW)	19.6	19.6	19.5	19.5	0.06	0.07	NS	NS
Apparent digestibility (%)								
Organic matter								
Total	65.6	65.6	69.2	68.2	1.58	1.19	NS	***
Silage	65.6	65.6	65.9	64.7	1.74	1.30	NS	NS
Nitrogen	67.5	67.7	64.5	64.3	1.08	1.16	NS	*
Acid detergent fiber	54.0	53.1	56.3	53.9	2.63	1.96	NS	NS

[z] SED, standard error of the difference appropriate for comparing feeding frequencies within sucrose level; 5 degrees of freedom.

[y] SED, standard error of the difference appropriate for comparing supplementation at the same or different feeding frequencies; 4 degrees of freedom.

NS, *,***, $P>0.05$; $P<0.05$; $P<0.001$, respectively.

Molar proportions of VFA and total ruminal VFA concentrations are given in Table 4. Data from one period at the restricted level of feeding were discarded owing to laboratory analytical problems. At the ad libitum level of intake, increasing the frequency of feeding increased the molar proportion of acetate in the rumen 2 h post-feeding ($P<0.01$) whereas sucrose supplementation tended to reduce it. A similar pattern was apparent at the restricted level.

Rumen Volume, Fluid and Solid Turnover and Digestion

Results in Table 5 show no effect ($P<0.05$) of feeding frequency or sucrose supplementation on estimated rumen liquid volume or any indices of fluid turnover within the rumen. Rumen liquid volume was between 5.5 and 6.5 L and liquid flow, estimated from Co dilution in the rumen, was between 0.59 and 0.65 L h^{-1} at the ad libitum intake level. Corresponding values were approximately 20% lower at the restricted feeding level.

Rates of passage of liquid (Co) and particulate matter (Cr) from the rumen, estimated from fecal concentrations of markers, were only measured at the restricted intake level (Table 6). Estimates obtained using the model of Dhanoa et al. (1985) were similar ($R>0.90$) to those obtained by simply taking the descending slope of the logarithmically transformed data. Examples from two animals for both Co and Cr are given in Fig. 2. Between the markers, Co tended to give higher elimination rates than Cr. Although there were no significant effects of diet or feeding frequency, sucrose tended to reduce ruminal particulate rate of passage.

Table 3. Effects of feeding frequency and sucrose supplementation on ruminal concentrations of ammonia N and pH in sheep fed alfalfa silage ad libitum or restricted

Sucrose	Unsupplemented (U)		Supplemented (S)				Significance of effects	
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]	L vs. H	U vs. S
Ad libitum feeding								
Ammonia N (mg dL^{-1})								
Minimum	2.03	3.87	0.71	1.26	1.008	1.138	NS	NS
Maximum	23.0	22.6	17.2	12.6	2.17	3.37	NS	*
Mean	10.8	10.2	6.76	6.39	0.946	1.653	NS	*
pH								
Minimum	6.47	6.55	6.39	6.42	0.084	0.110	NS	NS
Maximum	6.94	6.96	6.85	6.84	0.043	0.065	NS	NS
Mean	6.71	6.74	6.61	6.65	0.051	0.072	NS	NS
Restricted feeding								
Ammonia N (mg dL^{-1})								
Minimum	1.87	6.58	1.66	6.49	1.811	1.806	*	NS
Maximum	23.6	17.8	21.3	16.0	2.93	3.31	*	NS
Mean	12.6	11.5	12.2	11.2	1.60	1.97	NS	NS
pH								
Minimum	6.29	6.41	6.23	6.35	0.079	0.095	NS	NS
Maximum	7.05	6.76	6.94	6.76	0.117	0.111	*	NS
Mean	6.65	6.61	6.54	6.56	0.086	0.090	NS	NS

[z] Standard error of the difference appropriate for comparing feeding frequencies within sucrose level.
[y] Standard error of the difference appropriate for comparing supplementation at the same or different feeding frequencies.
*, $P>0.05$; NS, $P<0.05$, respectively.

Digestion parameters of DM determined using nylon bags indicate that none of the treatments influenced either the extent or rate of DM digestion in the rumen (Table 7). Approximately 76% of silage DM was potentially degradable. The effective degradability of N in the rumen was approximately 80% and was unaffected ($P>0.05$) by the treatments.

DISCUSSION

It was not the purpose of this study to compare ad libitum vs. restricted feeding per se, but to evaluate feeding frequency and sucrose supplementation within each feeding level. However, it is clear that responses to feeding frequency and energy supplementation differed according to level of feeding. These findings indicate that results from trials conducted at restricted feeding levels cannot necessarily be applied to production situations in which animals are normally fed at or near ad libitum intake.

Ad Libitum Feeding Level

When the diets were offered ad libitum, the effects of feeding frequency on all measured parameters were small. This was not surprising, since it was observed that feed was continuously available to sheep on both feeding frequencies. In common with other research, when the diets were fed either as a mixed ration or were composed largely of forage, there was no effect of feeding frequency on voluntary intake (Stanley and Morita 1967; Sutton et al. 1985).

Ruminal fermentation of silage-based diets shortly after silage ingestion is characterised by a paucity of readily fermentable carbohydrate in the presence of an excess of degradable dietary protein (Thomas and Thomas 1985). This results in excessive variation in ruminal ammonia concentration throughout the day and reduced passage of nonammonia N to the intestine. These factors have been implicated in reduced voluntary intake of silages by Beever and Gill (1987) and Egan (1977).

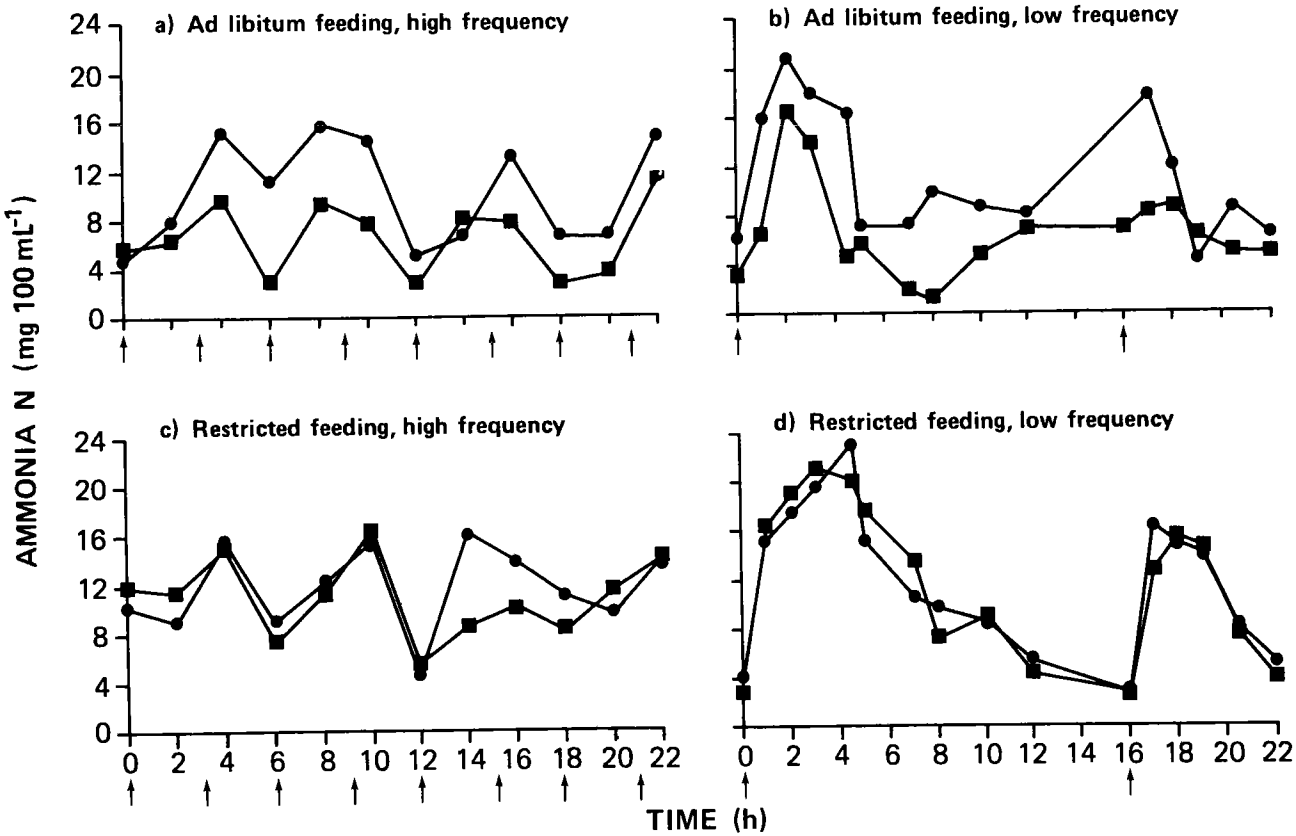

Fig. 1. Changes in rumen ammonia N concentration (mg 100 mL^{-1}) over 22 h in sheep fed silage without (●) or with sucrose supplementation (■) either ad libitum, at (a) high and (b) low frequency or at restricted feeding at (c) high of (d) low frequency.

Table 4. Effects of feeding frequency and sucrose supplementation on molar proportions and concentration of volatile fatty acids in sheep fed alfalfa silage ad libitum or restricted

Sucrose	Unsupplemented (U)		Supplemented (S)				Significance of effects	
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]	L vs. H	U vs. S
Ad libitum feeding								
Acetate (Molar %)								
Pre-feeding	73.9	71.6	71.9	68.4	1.67	2.13	NS	NS
2 h post-feeding	67.0	73.2	62.5	69.0	1.91	2.37	**	NS
Propionate (Molar %)								
Pre-feeding	17.7	18.1	17.1	20.4	1.89	1.78	NS	NS
2 h post-feeding	20.7	17.7	22.4	20.7	1.78	1.93	NS	NS
Total VFA (mmol L^{-1})								
Pre-feeding	61.2	60.6	61.6	83.3	9.51	10.30	NS	NS
2 h post-feeding	95.2	74.6	93.6	87.5	12.36	12.50	NS	NS
Restricted feeding								
Acetate (Molar %)								
Pre-feeding	78.2	74.7	75.9	71.3	3.51[x]		NS	NS
2 h post-feeding	68.0	71.2	64.7	69.0	3.14		NS	NS
Propionate (Molar %)								
Pre-feeding	17.2	16.7	16.6	19.5	1.96		NS	NS
2 h post-feeding	21.7	19.2	22.8	20.9	1.65		NS	NS
Total VFA (mmol L^{-1})								
Pre-feeding	41.7	67.1	51.8	92.7	15.39		*	NS
2 h post-feeding	73.9	73.2	79.4	93.3	12.30		NS	NS

[z] Standard error of the difference is appropriate for comparing frequencies within sucrose level; 5 degrees of freedom.
[y] Standard error of the difference appropriate for comparing supplementation at the same or different feeding frequencies; 4 degrees of freedom.
[x] Data from period 1 only ($n=2$) with 4 error degrees of freedom is appropriate for comparing any of the four means.
NS, *,**, $P>0.05$; $P<0.05$; $P<0.01$, respectively.

Increased feeding frequency should lessen variation in ruminal ammonia N concentration and improve microbial protein yield (Robinson 1989). Consequently, one would expect concomitant increases in voluntary intake to occur with increased feeding frequency. However, data from the ad libitum feeding level indicate otherwise, since there was no effect of feeding frequency on voluntary intake. Under conditions where silage availability is not limiting, it would seem that feeding frequency is unimportant. This possibility was alluded to in a recent review by Robinson (1989).

Despite availability of feed to both groups for approximately 23 h per day, rhythmic variations in ruminal ammonia concentration were observed. When sheep were fed twice daily, peaks were observed after each feeding, confirming visual observations that eating was stimulated by the appearance of fresh feed. Similar responses have been reported with meal size decreasing as time from feeding increases (Robinson 1989). When sheep were fed eight times daily, ammonia N concentration fluctuated less than with four cycles per day. These cycles were unrelated to feeding times and may reflect the animals' need for rest and rumination. A similar observation was made by Satter and Baumgardt (1962).

Feeding frequency had no effect on digesta turnover rates or rumen liquid volume due to the similarity in voluntary intake and availability of feed. This observation agrees with the findings of Robinson and Sniffen (1985).

Sucrose supplementation at the ad libitum level of feeding had no effect on total voluntary intake but tended to reduce the intake of silage. England and Gill (1985) found the same effect in young cattle given grass silage supplemented with sucrose at 5–15% of the DM. A similar observation has also been reported by Huhtanen and Robertson (1988).

Table 5. Effects of feeding frequency and sucrose supplementation on rumen liquid volume and turnover in sheep fed alfalfa silage ad libitum or restricted

Sucrose	Unsupplemented (U)		Supplemented (S)			
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]
Ad libitum feeding						
Rumen volume (L)	5.58	6.48	5.76	6.40	0.845	1.082
Dilution rate						
(h^{-1})	0.113	0.100	0.102	0.098	0.0107	0.0142
(L h^{-1})	0.635	0.655	0.589	0.631	0.0842	0.1095
Turnover time (h)	9.27	10.4	9.98	10.8	0.942	1.380
Restricted feeding						
Rumen volume (L)	4.92	4.64	4.73	4.87	0.471	0.489
Dilution rate						
(h^{-1})	0.093	0.092	0.091	0.097	0.0070	0.0108
(L h^{-1})	0.446	0.425	0.423	0.475	0.0398	0.0342
Turnover time (h)	11.4	11.1	12.0	10.2	1.28	1.66

[z]This error is appropriate for comparing frequencies within sucrose level.
[y]This error is appropriate for comparing supplementation at the same or different feeding frequencies.
All contrasts were not significant ($P>0.05$).

Table 6. Effects of feeding frequency and sucrose supplementation on k_1, fractional rate of passage (h^{-1}) from the rumen, as estimated by two fecal markers according to two models

Sucrose	Unsupplemented (U)		Supplemented (S)			
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]
Dhanoa model[x]						
Chromium mordant	0.057	0.053	0.050	0.047	0.0060	0.0087
Cobalt EDTA	0.061	0.060	0.056	0.059	0.0074	0.0080
Elimination only model[w]						
Chromium mordant	0.052	0.049	0.041	0.044	0.0041	0.0090
Cobalt EDTA	0.060	0.056	0.058	0.056	0.0058	0.0070

[z]This error is appropriate for comparing frequencies within sucrose level.
[y]This error is appropriate for comparing supplementation at the same or different feeding frequencies.
[x]Model fitted log y = log $(A) - k_1 t - Be^{-(k_2 - k_1)t}$ (Dhanoa et al. 1985).
[w]Model fitted to the elimination portion of the curve log y = log $(A) - k_1 t$.
All contrasts were not significant ($P>0.05$).

Digestibility of the whole diet was unaffected by the addition of sucrose. This effect has been observed elsewhere (England and Gill 1985) and was attributed to the off-setting effect of reduced fiber digestion in the rumen, as a consequence of sucrose lowering ruminal pH (Rooke et al. 1987). In the present study, digestibility of ADF was reduced by about one percentage unit by the addition of sucrose at 10% of intake. However, the effect of sucrose on ruminal pH was small and was unlikely to have influenced ruminal cellulolytic activity.

Sucrose supplementation reduced rumen ammonia N concentration which resulted in protracted time periods (5–6 h) when rumen ammonia N concentration was below 5 mg 100 mL^{-1}, a value cited as being necessary to support microbial cellulolytic activity (Satter and Slyter 1974). This may indicate why sucrose supplementation tended to reduce ADF digestibility, in the absence of a pH depressing effect. The reduced ammonia N concentration, following addition of sucrose, may have been indicative of increased microbial protein synthesis in response to

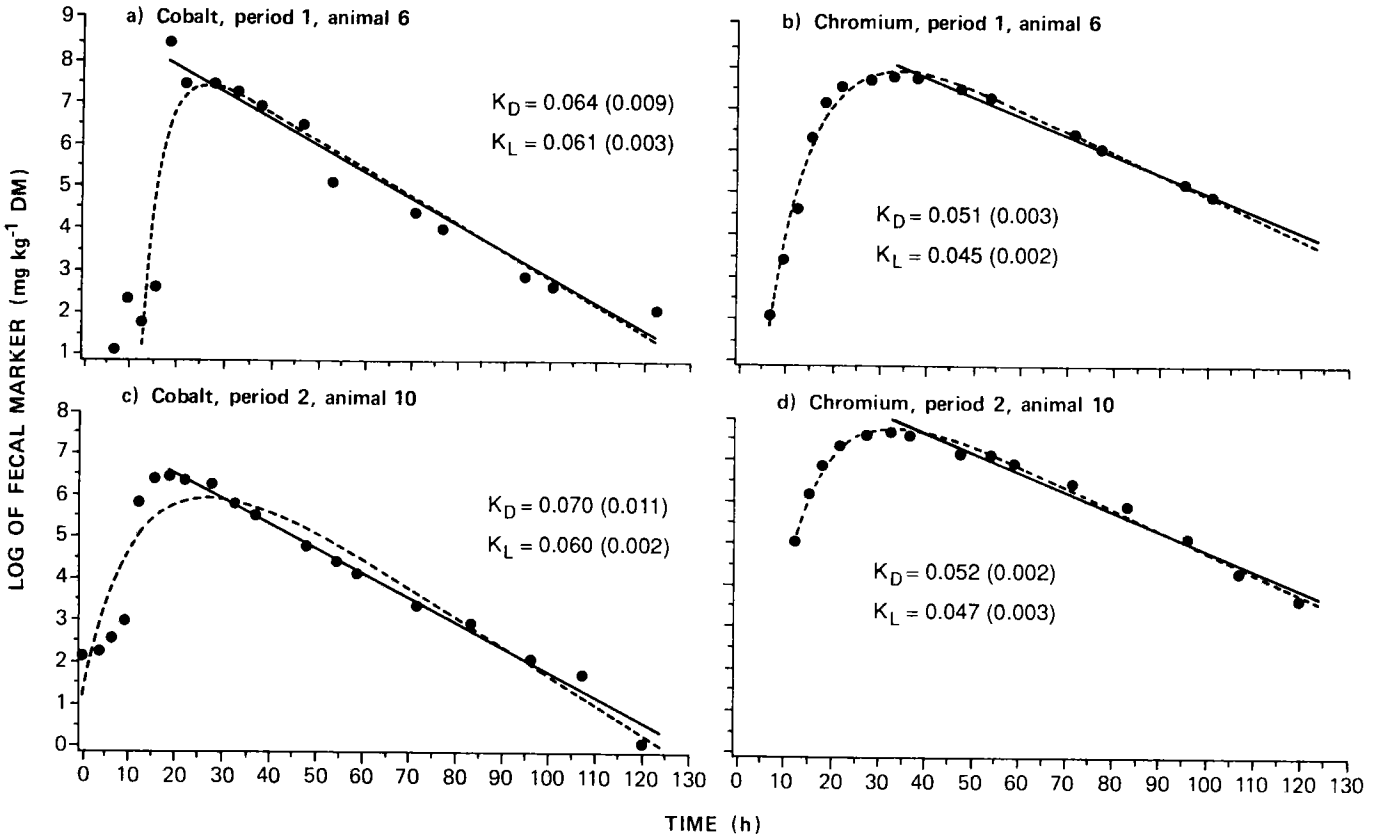

Fig. 2. Four examples of changes in fecal marker concentration (●) and the fitted lines according to the Dhanoa model (---) and the elimination model (−). The estimated fractional rate of passage from the rumen is indicated as k_D for the Dhanoa Model and k_L for the elimination model with Standard Errors in parentheses.

Table 7. Effects of feeding frequency and sucrose supplementation on digestion of dry matter (DM) and nitrogen (N) in the rumen of sheep fed alfalfa silage at a restricted level

Sucrose	Unsupplemented (U)		Supplemented (S)			
Feeding frequency	Low (L)	High (H)	Low (L)	High (H)	SED[z]	SED[y]
Digestion of DM in the rumen[x]						
Rapidly degraded (*a*)(%)	29.2	28.9	29.4	26.2	1.80	1.65
Slowly degraded (*b*) (%)	46.8	47.8	47.1	49.5	1.00	0.99
Fractional degradation rate (*c*) (h^{-1})	0.064	0.065	0.070	0.075	0.0128	0.0130
Potential degradability ($a+b$) (%)	76.0	76.7	76.5	75.7	1.76	1.76
Effective degradability[w] (%)	54.2	55.0	56.2	56.7	1.54	1.71
Digestion of N in the rumen						
Potential degradability ($a+b$) (%)	91.7	89.6	90.2	88.6	1.46	1.57
Effective degradability (%)	82.4	80.4	81.6	81.5	1.09	1.45

[z] This error is appropriate for comparing frequencies within sucrose level.
[y] This error is appropriate for comparing supplementation at the same or different feeding frequencies.
[x] Model fitted $a+b(1-e^{-ct})$ (Ørskov and McDonald 1979).
[w] Effective degradability = $a+(bc/(c+k))$ (Ørskov and McDonald 1979), k is the estimated rate of passage of the chromium marker from the fitted Dhanoa model (see Table 6).
All contracts were not significant ($P>0.05$).

additional energy availability in the rumen (Rooke et al. 1987).

Sucrose supplementation also had no influence on the kinetics of the fluid phase and rate of digestion within the rumen. Huhtanen (1987) reached the same conclusions when dairy cows were fed supplemental energy in the form of sugar beet pulp and molasses. Huhtanen (1988) also observed that supplementation with a soluble sugar source failed to alter in sacco rate of DM digestion.

Restricted Feeding Level

We intended to restrict intake to 18 g DM kg^{-1} BW; however, observed intakes were higher than this. Increased feeding frequency stabilized diurnal concentrations of rumen metabolites, compared with the twice daily feeding regime. The diurnal variations in ammonia N concentration, pH and total VFA concentration were lessened and there was an indication that the post-feeding reduction in the molar proportion of acetate was reduced. These effects suggest that conditions in the rumen may have been more conducive to sustained microbial activity when restricted feed was offered frequently. This is in contrast to the situation when feed was offered ad libitum, where no effect of feeding frequency was observed on ruminal ammonia N concentration or pH. Thus, diurnal patterns in ruminal metabolite concentration can be reduced, either by feeding more frequently or by offering feed ad libitum. Both approaches effectively increase the time throughout the day that feed is available. Despite the effects of feeding frequency on rumen metabolite concentration, there was no effect on whole tract digestibility, rate of passage from and rate of digestion in the rumen.

When feed intake was restricted, sucrose supplementation increased apparent digestibility of OM and N, compared with no effect at the ad libitum feeding level. During restricted feeding, sucrose intake was approximately 66% of that at the ad libitum level (0.2% BW). Under these conditions of limited feed intake, additional ruminally available energy, such as sucrose, may enhance cellulolytic activity. This was contrary to the effect observed during ad libitum feeding. Similarly, England and Gill (1985) found that sucrose, included at 5% of the diet (approximately 0.1% BW), increased fiber digestibility but had the opposite effect when included at 10 and 15% of the diet (0.2–0.3% BW).

Sucrose supplementation failed to influence peak, mean or minimum ruminal ammonia N concentrations and pH during restricted feeding,

contrasting with the observation at the ad libitum feeding level. This difference may relate to the fairly low inclusion rate of sucrose in the diet (10%). In other experiments where feed was restricted and sucrose supplementation influenced rumen ammonia concentration, sucrose has constituted between 20 and 25% of the diet (Chamberlain et al. 1985; Rooke et al. 1987).

Rate of Passage Methods

Particulate outflow rate was estimated from the descending curve for marker excretion in the feces and corresponded to k in Eqs. 1 and 2. Rates were approximately half those obtained for fluid outflow rates from the rumen, which agrees with other reports (Robinson and Sniffen 1985; Huhtanen 1987). Rate of passage, according to fecal Cr concentration decline, was consistently lower than rate of passage values obtained using cobalt EDTA. This was expected since Co is a fluid marker, and its increased rate of passage from the rumen would have some bearing on the elimination rate in the feces. In addition, the mordanting procedure renders the chromium-treated fiber unavailable for microbial digestion which would further slow its rate of passage.

The estimates of rate of passage obtained in this experiment using the model of Dhanoa et al. (1985) were greater than those obtained by Dhanoa et al. (1985) using the same techniques and the same animal species. However, diets used in that study were mostly dried forages rather than silages. Similarly, Aitchison et al. (1986) obtained lower rates of passage in sheep fed hay. Estimates based on the exponential model were consistently lower than those based on the model of Dhanoa et al. (1985). The two approaches can be compared visually in Fig. 2. The data chosen represent the extremes in curve shape. It was concluded that in the absence of appropriate statistical software, the simpler exponential model can be used to represent the biological rate of passage of particulate and fluid phases from the rumen and to compare treatments within the same experiment.

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