

## Concentrate feeding and ruminal fermentation. I. Influence of the frequency of feeding concentrates on rumen acid composition, feed intake and milk production

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Accepted: 1 May 1981

*Key-words:* feeding frequency, rumen, lactic acid, volatile fatty acids, pH, feed intake, milk production, lactating cow

### Summary

To four cows fitted with rumen cannulae, a quantity of 12 kg of concentrates a day was supplied in two equal portions of 6 kg at 08h00 and 15h30, in one portion of 12 kg at 08h00 or in 4 portions of 3 kg each at 08h00, 11h00, 14h00 and 17h00. Every experimental period lasted 2 weeks. The animals were fed individually. In addition to the concentrates hay was provided at 09h00 and 16h30. The amount of hay fed was adjusted so that per day and per cow the remainder was at least 1 kg. In 1978 the experiments were carried out with concentrates low in starch + sugars (LSS) (23 %) and in 1979 with concentrates high in starch + sugars (HSS) (50 %).

In 1978, when the low-starch concentrates were fed, total dry matter intake, milk production, and milk fat content of each milking were measured daily, the trend in pH and the concentration of L-lactic acid in rumen fluid were estimated on the last day of the preliminary period ( $2 \times 6$  kg) and on the 1st, the 7th and the 14th day of the experimental periods ( $1 \times 12$  kg and  $4 \times 3$  kg). In the experiment in 1979, when the concentrates high in starch and sugars were fed the same measurements were done, but D-lactic acid and volatile fatty acids were also estimated.

Compared to  $2 \times 6$  kg of concentrates the change-over to daily  $1 \times 12$  kg or to  $4 \times 3$  kg of concentrates did not result in significant differences in dry matter intake nor in milk or milk fat production.

Within types of concentrates there was hardly any difference in rumen pH minima between frequencies of concentrate feeding. When the high starch concentrates were fed pH in rumen fluid tended to be somewhat lower than with the low starch concentrates. When feeding  $2 \times 6$  kg of high starch concentrates pH in rumen fluid was for a short time lower than 5.5. Lactic acid concentrations in the rumen fluid reached maximum values within one hour after concentrate

feeding. Maximum L-lactic acid concentrations were 6.5, 5.0 and 2.0 mmol/l respectively for feeding 1 × 12, 2 × 6 and 4 × 3 kg of concentrates low in starch and sugars. For concentrates high in starch and sugars the respective values were 4.5, 2.5 and 1.0 mmol/l. When the HSS concentrates were fed maximum concentrations of D-lactic acid in rumen fluid were 2.5 to 3 times the L-lactic acid concentration. When lactic acid levels were not raised D- and L-lactic acid concentrations were equal. At 08h00 the molar ratio of volatile fatty acids (C<sub>2</sub>:C<sub>3</sub>:C<sub>4</sub>) as a percentage of total VFA in rumen fluid was 62:22:15 and changed gradually to 56:24:20 at 19h00. Differences in VFA between frequencies of concentrate feeding were small. Total concentrations of VFA did not vary between treatments.

### Introduction

Rations with a high content of easily fermentable substances, e.g. rations rich in concentrates, in general stimulate the production of volatile fatty acids in the rumen and cause a strong decrease in the pH of rumen contents. A decrease in pH below 5.5 may cause serious disturbances of the fermentation in the rumen (Counotte & Prins, 1979). This often coincides with an increased concentration of rumen lactic acid, a decreased feed intake and a decreased milk fat content. In order to limit the risk of a disturbance of the rumen function it may be attractive to distribute concentrates in several portions over the day (Kaufmann & Hagemester, 1973). Literature on the effect of feeding frequency (concentrates) is not always easily to interpret for several reasons: (a) incomplete details about the composition of the ration, (b) a limitation of the amount of feed supplied (Bath & Rook, 1963; Jensen & Wolstrup, 1977; Jorgenson et al., 1965; Knox & Ward, 1960; Satter & Baumgardt, 1962); (c) no data about rumen parameters (Burt & Dunton, 1967; Campbell & Merilan, 1961; Johnson et al., 1966; Lindner et al., 1979; Mochrie et al., 1956; Stanley & Morita, 1967; Thomas & Kelly, 1976).

This experiment was carried out to compare the feeding of a given amount of concentrates in 4, 2 and 1 daily portions. The experiment was done with lactating cows on a high feeding level and with concentrate mixtures of different composition.

### Material and methods

Four lactating dairy cows (Holstein × Friesian, liveweight about 600 kg) fitted with rumen cannulae (Ø 5 cm) were housed in a tied stall and individually fed. In 1978 concentrates with a low content of starch + sugars (ca. 23 %, LSS), in 1979 concentrates with a high content of starch + sugars (ca. 50 %, HSS) were fed. In both years the protein content of the concentrates was almost equal. The percentual composition of the concentrates is given in Table 1 and the proximate analysis of the hay in Table 2. In the preliminary period hay was provided ad libitum and the concentrates were offered twice a day, in equal portions of 6 kg at

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Table 1. Ingredient composition of the concentrates (g/100 g).

1978	1979
10 coconut, expeller	19 soya bean meal, solv. extr.
9 rice bran, solv. extr.	70 maize
6 linseed, expeller	8 molasses, cane
21 maize gluten feed 21-24 %	3 minerals + vitamins
3 soya bean meal, solv. extr.	
3 grass meal	crude protein 15
18 beet pulp, dried	starch + sugars 44 + 6
13.5 citrus pulp, dried	VEM* 980/kg
1.5 fat	
8 molasses, cane	
4 wheat middling	
3 minerals + vitamins	
crude protein 15	
starch + sugars 9 + 14	
VEM* 940/kg	

\* 1000 VEM = 6900 kJ (1650 kcal) nett energy.

Table 2 The chemical composition of the hay.

	1978	1979
Dry matter (g/kg)	840	850
Crude protein* (g/kg)	141	134
Crude fiber* (g/kg)	291	298
Ash* (g/kg)	94	110
DCP <sup>1</sup> (g/kg)	83	80
VEM* <sup>2</sup> (g/kg)	764	746

\* In dry matter.

<sup>1</sup> DCP = digestible crude protein.

<sup>2</sup> 1000 VEM = 6900 kJ (1650 kcal) nett energy.

08h00 h and 15h30. In the subsequent experimental period two animals received 12 kg of concentrates once a day at 08h00, the other two cows in 4 equal portions at 08h00, 11h00, 14h00 and 17h00. After 14 days treatments were reversed. Throughout the experimental periods hay was given twice a day, at 09h00 and 16h00; the refusals were collected and weighed in the morning just before the first feeding. The amount of hay was adjusted, so that refusals were at least 1 kg per day. When 12 kg of concentrates were fed at once, in several cases the cows refused part of it. In such cases refusals after one hour were weighed and again provided at 15h30.

On the last day of the preliminary period and on the 1st, 7th and 14th day of each experimental period rumen fluid was sampled from the ventral rumen sac. The pH of these samples was measured immediately with an Electrofact pH meter. Sub-samples were preserved and frozen for analysis of lactic acid and vola-

tile fatty acids (VFA). For lactic acid analysis 1 ml of rumen fluid was added to 1 ml of 5 % ZnSO solution. After thawing the lactic acid was measured enzymatically (Bergmeyer, 1970). In 1978 L-lactate, and in 1979 L- and D-lactate and VFA were measured. VFA was measured by gas chromatography (Di Carcia & Samperi, 1974). From the last two weeks of the preliminary period the daily feed intake, milk production and milk fat content were estimated. At the time of the start of the experiment the cows were producing milk for 108 days (1978) and 30 days (1979) on average. From each milking the fat content was estimated (Gerber method). Milking times were at 07h00 and 16h30.

## Results

### *Dry matter intake*

The dry matter intake is given in Table 3. As the 12 kg of concentrates were consumed fully, the differences in total dry matter intake were caused by differences in hay intake. From Table 3 it can be deduced that on average 9.5 kg hay dry matter was consumed daily, varying between cows from 6.8 kg to 12.2 kg. Within cows differences in dry matter intake between treatments were small. The dry matter intake of cow N 240 in 1978 on the treatment 1 × 12 kg decreased during a period when the temperature inside the barn rose to about 25 °C. After the warm period, which lasted for 5 days, the dry matter intake returned to its original level.

Changing the frequency of concentrate feeding from 2 × 6 kg or 4 × 3 kg to 1 × 12 kg did not bring about any clear change in dry matter intake, when LSS concentrates were fed. On HSS concentrates, after changing to 1 × 12 kg, dry matter intake decreased in all cows on the first day. This decrease was temporary and was apparently due to the sudden change to feeding 1 × 12 kg of HSS concentrates, not to the feeding frequency itself. Therefore for HSS concentrates the first day is not taken into account (Table 3). After changing to 4 × 3 kg of concentrates there was no difference in dry matter intake.

Table 3. The mean daily total dry matter intake (kg) at three concentrate feeding frequencies.

Year	Cow	2 × 6 kg	1 × 12 kg	4 × 3 kg
1978	L 234	17.4	17.0	17.2
	S 218	20.2	20.1	20.1
	N 240	22.4	20.1	22.1
	Hetty	19.4	19.4	19.4
	Mean	19.9	19.2	19.7
1979	N 240	20.8	21.3	21.5
	S 218	20.0	20.3	20.3
	I 332	20.1	18.9	18.9
	N 248	17.7	17.1	17.8
	Mean	19.7	19.6	19.6

When the LSS concentrates (1978) were fed once a day, about 9 to 10 kg were consumed within one hour, the remainder was provided at 15h30. HSS concentrates (1979) were consumed almost completely within the stated term of one

*Lactic acid in rumen fluid*

As can be seen in Fig. 1, the concentration of L-lactic acid raised sharply after feeding and normally reached the highest value within one hour, followed by a rapid decrease. Maximum concentration of L-lactic acid was strongly influenced by the amount of concentrates consumed per feeding, both with the concentrates low and high in starch and sugars. In each feeding regimen the highest concentration of L-lactic acid in rumen fluid after feeding HSS concentrates was lower than with LSS concentrates. After changing the feeding regimen there seemed to be no difference in level of lactic acid peak value between the 1st, the 7th and the 14th day on both types of concentrates.

In 1979 when feeding the HSS concentrates the concentration of D-lactic acid in rumen fluid, sampled at times when peak values were noted, was 2.5 to 3 times as high as L-lactic acid concentration. When concentrations of lactic acid were not raised the levels of D- and L-lactic acid in rumen fluid were equal.

*Volatile fatty acids in rumen fluid*

Analysis of volatile fatty acids were only carried out in 1979 when HSS concentrates were fed. Immediately before the first feeding (08h00) the molar propor-

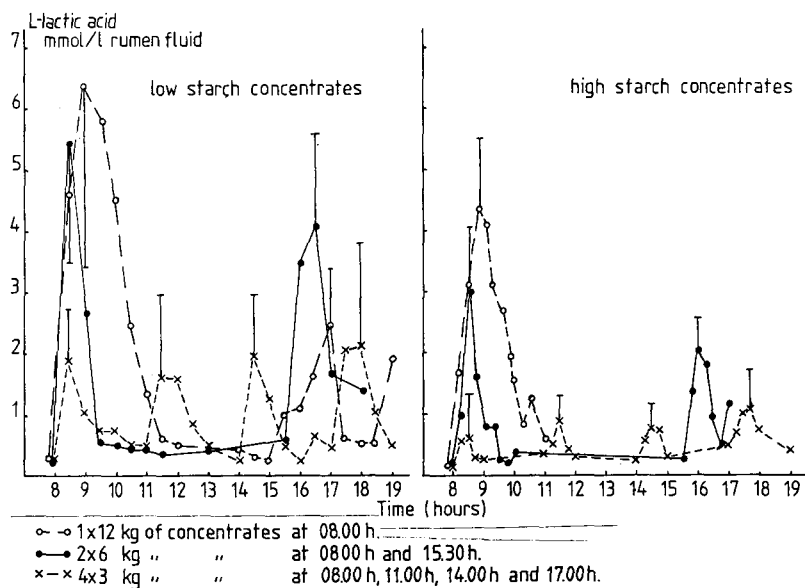


Fig. 1. L-lactic acid concentration in rumen fluid after feeding 12 kg of concentrates low or high in starch and sugars with different feeding frequencies. Vertical bars gives S(x) at peak values.

tion of acetic acid ( $C_2$ ) was about 62 % while the proportions of propionic acid ( $C_3$ ) and butyric acid ( $C_4$ ) were respectively 22 % and 15 %. At that time differences between feeding regimens were small. During the day the relationship between VFAs changed and greatest differences were stated at 19h00. The relationship  $C_2:C_3:C_4$  at that time was about 56:24:20, with some greater differences between feeding regimens than at 08h00. The relationship between VFAs after changing the feeding regimen differed not clearly from the 7th or from the 14th day. Mean concentrations of total VFA at  $t=08h00$  were 93, 98 and 107 mmol/l for respectively  $1 \times 12$ ,  $2 \times 6$  and  $4 \times 3$  kg of concentrates. These differences were not significant. At  $t=19h00$ , when differences in VFA concentration were greatest, the mean total VFA concentration was 118, 136 and 138 mmol/l respectively for  $1 \times 12$ ,  $2 \times 6$  and  $4 \times 3$  kg of concentrates. The value of 118 mmol/l observed with  $1 \times 12$  kg of concentrates differed significantly from the other values at the same time ( $P \leq 0.05$ ).

*pH in rumen fluid*

From Fig. 2 it appeared that shortly before feeding at 08h00 the pH values of rumen fluid sampled in the different periods were almost equal. After 08h00 the decrease in pH appeared to depend upon the amount of concentrates consumed at that time. With  $2 \times 6$  kg of LSS concentrates, the lowest pH of 5.6 was reached soon after the second feeding at 16h30. With  $1 \times 12$  kg of LSS concentrates, the lowest pH of 5.8 was reached at about 10h00, with  $4 \times 3$  kg of LSS concentrates the lowest pH (5.8) was reached at 18h00. At 18h00 the pH in rumen fluid was similar in the three feeding regimens. From a number of measurements carried

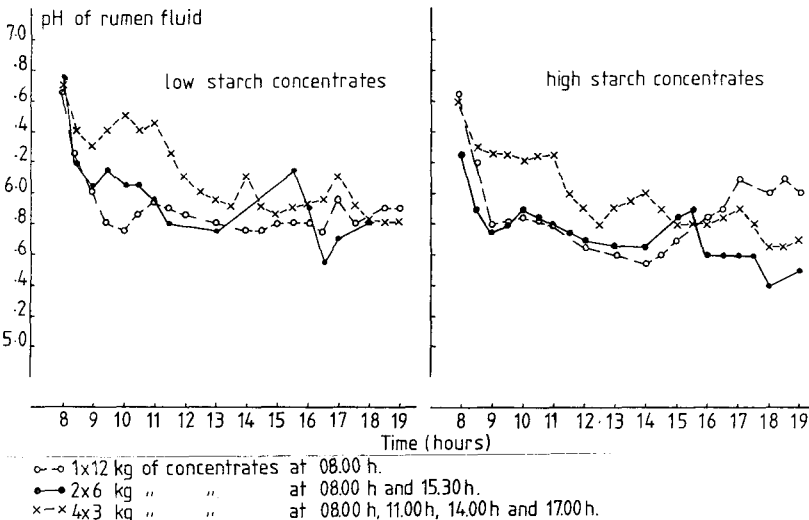


Fig. 2. pH in rumen fluid after feeding 12 kg of concentrates low or high in starch and sugars with different feeding frequencies.

out between 19h00 and 23h00 there appeared a faster increase in pH when  $1 \times 12$  kg of concentrates were fed than with  $4 \times 3$  kg of concentrates. In 1979 the same trend as in 1978 was found, except that in general pH in rumen fluid was somewhat lower. Besides, in 1979 with  $1 \times 12$  kg a faster increase in pH appeared in the afternoon than in the other feeding regimens within this experiment as well as with the same feeding regimen in 1978. With  $2 \times 6$  kg of HSS concentrates pH was for a short time lower than 5.5.

Despite the very fast consumption of  $1 \times 12$  kg of HSS concentrates in 1979, the decrease in pH was less than in 1978 with LSS concentrates.

In 1978, no difference in pH level with each feeding regimen between 1st, 7th and 14th day was seen. The mean of these 3 days is shown in Fig. 2. When the HSS concentrates were fed (1979), after changing to  $1 \times 12$  kg, the pH throughout the first day was about 0.2 pH unit lower than that at the 7th and 14th day. The mean pH of the 7th and 14th day is given in Fig. 2. No difference in pH was observed between the 7th and the 14th day.

#### *Milk and milk fat production*

Milk fat content differed considerably between evening and morning milk. Within cows great day-to-day differences in milk fat content of every milking time were found. Data about milk and milk fat production are summarized in Table 4. Between the experimental periods no real differences in production could be observed.

Between 1978 and 1979 differences in milk production were mainly due to differences in the stage of lactation. Additional influences because of the different composition of the concentrates were not found.

Table 4. The mean daily production of milk (in kg) and of milk fat (in g) at three concentrate feeding frequencies.

Year		$2 \times 6$ kg		$1 \times 12$ kg		$4 \times 3$ kg	
		milk	milk fat	milk	milk fat	milk	milk fat
1978	L 234	21.1	718	20.3	701	19.8	694
	S 218	24.1	824	22.8	785	23.4	815
	N 240	27.6	1023	26.1	951	25.1	950
	Hetty	17.8	547	16.9	642	17.0	622
	Mean	22.7	778	21.5	770	21.3	770
	4% F.C.M.	19.4		19.2		19.2	
1979	N 240	31.0	1174	33.9	1208	34.6	1054
	S 218	33.8	1080	30.1	938	32.7	1047
	I 332	28.5	1058	26.7	939	27.3	879
	N 248	26.3	982	25.5	798	27.1	914
	Mean	29.9	1074	29.1	971	30.4	974
	4% F.C.M. <sup>1</sup>	26.8		24.3		24.3	

<sup>1</sup> F.C.M. = fat-corrected milk.

## Discussion

### *Dry matter intake*

In our experiments feeding dairy cows 12 kg of concentrates in 1, 2 or 4 daily portions did not affect dry matter intake. This does not support the results of Campbell & Merilan (1961), Lindner et al. (1979) and Palmquist et al. (1964). The reason for this difference can be that in our experiments the roughage was available during the whole day and our cows consumed a high amount of roughage. In the experiments of Campbell & Merilan (1961), roughage was available for only 3 hours after feeding, in the experiments of Lindner et al. (1979) only 3.5 hours and in the experiments of Palmquist et al. (1964) only 2 hours. The authors mentioned offered concentrates and roughage together when feeding two times a day, but not during higher frequencies of feeding. Moreover, the ration was available to the animals for a shorter period when feeding twice a day than with higher feeding frequencies. Although we do not have exact measurements about behaviour of feed intake, we observed a different pattern of roughage intake with different frequencies of concentrate feeding. In other experiments with various frequencies of feeding differences in dry matter intake were not mentioned (Johnson et al., 1966; Jorgenson et al., 1965; Rohr & Daenicke, 1973; Stanley & Morita, 1967).

### *Lactic acid in rumen fluid*

To obtain a correct picture of lactic acid content in rumen fluid, sampling every 15 to 30 minutes proved to be desirable. The D(-) and L(+) lactic acid ratio varied strongly in our experiments. With low lactic acid concentrations D/L ratio was about 1, while at times of peak concentrations of lactic acid this ratio was about 2.8. This D/L ratio agrees with results of Mackie et al. (1978). No change was observed in this ratio in the first peak compared with the subsequent peak of each day. An effect of the pH on this ratio as found by Giesecke & Stangassinger (1976) was not observed here.

The lactic acid content of rumen fluid not only depends upon rate of lactic acid production but also on its rate of disappearance. The rate of lactic acid disappearance is mainly determined by absorption and less by the capacity of fermentation and passage rate to the lower gut (Counotte, 1981).

Absorption of lactate increases with decreasing pH of rumen fluid, while fermentation capacity decreases at pH values less than about 6.0 (Brüggeman & Giesecke, 1968; Counotte, 1981). With pH values lower than 6.0, the risk of accumulation of lactic acid increases. In our experiments there appeared to be a fast disappearance of lactic acid even at low pH.

Sugar content (mono- and disaccharides) of the concentrates low in starch was higher than that in concentrates high in starch. The observation that the lactic acid content in rumen fluid peaked higher when LSS concentrates were fed than when HSS concentrates were offered, indicates that lactic acid production is more stimulated by mono- and disaccharides than by starch.



*VFA in rumen fluid*

Kaufmann et al. & Hagemeister (1973) in researching the effect of feeding frequency in cows suggested that the  $C_2/C_3$  ratio in rumen fluid influences the milk fat content. From data of our experiments it can be deduced that with the concentrates high in starch, the  $C_2/C_3$  ratio decreased from about 3 at 08h00 to 2.4 at 19h00, while during a great part of the day this ratio fluctuated around 2.6. Using the relation between  $C_2/C_3$  and milk fat content by Kaufmann & Hagemeister (1973) we should have found a milk fat content of about 3 %, but found a considerably higher content. Based on the  $C_2/C_3$  ratio the highest milk fat content could be expected when feeding  $4 \times 3$  kg. Instead, we found the lowest milk fat content in that experimental period. Possibly the high roughage intake in our experiments were responsible for these differences.

*pH of rumen fluid*

The minima in pH levels recorded by McCullough & Smart (1968) and Rohr & Daenicke (1973) in their experiments remained higher when feeding concentrates more frequently than with less frequent feeding. However, after they had stopped sampling of rumen fluid, they offered some additional food only to the cows fed more frequently. Although levels of feeding in our experiments were much higher (dry matter intake was ca. 3 % of body weight) our results support those results in which frequencies of feeding (concentrates) do not result in differences in lowest pH level (Bath & Rook, 1963; Jensen & Wolstrup, 1977; Jorgenson et al., 1965; Satter & Baumgardt, 1962).

Disturbances in rumen function because of too low a pH are not to be expected as long as the pH remains higher than 5.5. Optimum pH for microbial growth for most micro-organisms is 6.0; for some of them the pH optimum is about 6.5 (Kistner et al., 1979; Russel et al., 1979). Probably the pH of rumen fluid during the experimental periods in which  $1 \times 12$  kg or  $2 \times 6$  kg of concentrates were fed, was below this optimum for a longer period than with  $4 \times 3$  kg. This may have inhibited microbial growth. In addition, at a low pH of the environment micro-organisms need more energy to maintain the integrity of the bacterial cell. Therefore at a low pH in the rumen, microbial energy utilization could be less efficient.

*Production of milk and milk fat*

The higher milk production in the experimental period with  $2 \times 6$  kg of concentrates can easily be explained by the somewhat earlier stage of lactation. Great differences in milk fat content coincided with milking interval. Perhaps even more striking are the great day-to-day differences in milk fat content of each cow in every evening or morning milking. In retrospect, if milk was sampled two days per week the mean milk fat content would have been totally different.

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