

# The effect of number of daily meals for dairy cows on milk yield and composition

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## ABSTRACT

The effect of the number of daily meals on milk yield and composition was studied in a commercial dairy herd. One hundred and eighty Israeli Holstein cows were divided into two groups. One group was given one meal daily and the second group three meals daily. Feeding frequency had no effect on DM intake, milk yield or milk composition. Total VFA and molar proportion of acetate were higher before feeding than 3 h later. The decrease in molar proportion of acetate after feeding was greater in the cows given one meal daily. The propionate molar proportion was higher before feeding and lower 3 h later if cows were given three meals daily. As a result, the acetate to propionate ratio was lower in cows fed three meals daily before feeding and rose after 3 h. Ruminal ammonia-N concentrations, before and after feeding, were lower when cows were fed three meals daily. Plasma urea-N concentrations were higher before feeding, as compared with 3 h later. Plasma urea-N was higher for the one meal treatment, but significantly only 3 h after the morning meal. It is suggested that increasing feeding frequency may improve dietary nitrogen utilization and may shift metabolism towards more gluconeogenesis. Production responses to more frequent feeding are more likely to be revealed in high yielding dairy cows, maintained on high concentrate diets.

KEY WORDS: feeding frequency, rumen, dairy cows

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## INTRODUCTION

Increasing the amount of concentrate in the diet (50% and more of dietary DM) is now a common feeding strategy as a means of increasing the energy and protein density of diets fed to high producing dairy cows. Diets fed in excess of protein requirement may result in high concentrations of urea in blood, milk and urine (Roseler et al., 1993) and might be associated with impaired fertility (Ferguson et al., 1989) and increased environmental pollution (NRC, 1985; Tamminga, 1992). Feeding frequency affects ruminal and blood metabolites (Sutton et al., 1986, 1988); a higher feeding frequency may counteract these effects, enhance diet utilization (Robinson and McNiven, 1994) and decrease ruminal ammonia N ( $\text{NH}_3\text{-N}$ ) and plasma urea-N (PUN) concentrations. In our previous study (Shabi et al., 1998), diets were fed to cows equipped with rumen and abomasum cannulas, four versus two daily meals. In that study, the concentrations of PUN, 2 and 4 h after feeding, were lower when cows were fed four meals than when cows were fed two meals daily. The present study investigates, under commercial conditions, the effect of feeding frequency of total mix ration on performance of high producing dairy cows consuming a high-concentrate diet.

## MATERIAL AND METHODS

### *Cow and treatments*

The experiment was carried out on the herd of the Mevo-Choron kibbutz, on 180 Israeli-Holstein cows (54 primiparous cows,  $120 \pm 30$  DIM) that were divided into two treatments, as follows: 1. Control. The daily ration was given once a day, at 07.00 h, 2. Experimental. The daily ration was given in three meals, at 07.00 h (50% of daily amount), 13.00 h (30% of daily amount) and 22.00 h (20% of daily amount). Cows were assigned to the dietary treatments according to lactation date, parity number, and milk yield during previous lactation. The duration of the experiment was three months.

The diet contained a common blend of ingredients (Table 1), calculated using a least-cost linear program (Gavish, Giv'at Brener, Israel). The diet was formulated to contain (on DM basis) forage 36%, NEL 1.75 Mcal, CP 16.9%, rumen degradable CP (RDCP, % of CP) 69.7%, rumen degradable OM (RDOM, % of OM) 56.1%, NDF 31.5% and NSC 38%. The hourly ratio between RDOM and RDCP, calculated from the *in situ* data, is presented in Figure 1. The cows were group-fed and the ration was offered as a totally mixed ration (TMR) for *ad libitum* intake, and was moved close to the cows a few times daily. The daily consumption of each treatment group was recorded. Refusals from each treatment, which were usually 5%

TABLE I

## Composition of experimental diet

Ingredients	% in DM		
Ground maize	8.0	Chemical composition	
Cracked maize	3.1	crude protein	16.9
Barley	9.1	NDF	31.5
Sorghum	2.3	NSC	38.0
Gluten feed	5.9	RDCP <sup>3</sup>	69.7
Whole cotton seed <sup>1</sup>	13.9	RDPM <sup>4</sup>	56.1
Soyabean meal	3.6	NEL, Mcal	1.75
Rapeseed meal	3.2		
Sunflower meal	2.3		
Fish meal	0.7		
Wheat silage	7.2		
Maize silage	19.8		
Pea hay	5.7		
Citrus peels	6.2		
Molasses	6.7		
Urea	0.3		
Ammonium sulfate	0.1		
FA-Ca-salt	0.07		
Oil	0.2		
Vitamins and mineral <sup>2</sup>	1.7		

<sup>1</sup> treated with NaOH

<sup>2</sup> contained (per 20 kg TMR) 16 x 106 IU of vitamin A, 3.2 x 106 IU of vitamin D, 16 x 106 IU of vitamin E, 196 g CaCO<sub>2</sub>, 91 g NaCl, 48 g of Mo, 48 g of Zn, 48 g of Fe, 19.2 g of Cu, 3.4 g of I, 0.32 g of Co, and 0.48 g of Se

<sup>3</sup> RDCP = rumen degradable CP

<sup>4</sup> RDOM = rumen degradable OM

of offered diet, were also recorded every morning. Diets were sampled once a week, and refusals were sampled daily, for DM, CP and NDF analyses.

Rumen fluid and blood from 40 cows of each treatment group were sampled during the third month, before the morning meal was offered and 3 h later. Ruminant fluid samples were taken by ruminal tube. Blood samples for determination of PUN were taken from the coccygeal vessels before feeding and 3 h after feeding.

#### *Milk yield and composition*

Cows were milked at 05.00, 13.00 and 21.00 h. Milk yield was recorded for each milking by the Afimilk System (Afikim, Israel). Milk samples from three consecutive milking events were collected every 14 days and analyzed for fat, protein and lactose by an infrared procedure at the Israel Cattle Association Laboratories.

### *Chemical analyses*

Chemical analyses of feeds were performed on dry, 2-mm milled feed samples. Dry matter was assayed after the sample was dried at 105°C for 12 h, except for silage and citrus peels, which were first dried at 60°C for 48 h. The OM content was determined following ashing at 600°C for 3.5 h. The CP content was analyzed by the Kjeldahl auto analyzer (Tecator, Höganäs, Sweden). The NDF was determined according to the method of Van Soest et al. (1991). Ruminal degradabilities of OM and CP in dietary feeds were determined by the dacron-bag technique *in situ* (Ørskov and McDonald, 1979) and as described by Arieli et al. (1989).

Samples of rumen fluid were divided into subsamples for determination of VFA (10 ml mixed with 750 µl saturated HgCl<sub>2</sub>) and NH<sub>3</sub>-N (5 ml mixed with 5 ml 20% trichloroacetic acid), and were kept frozen (-20°C) until analyzed. Ruminal fluid (0.4 ml + 0.1 ml 25% HPO<sub>3</sub> and 0.1 ml isocaproic acid) was centrifuged at 3000 x g for 15 min and VFA were assessed by GLC (Model 5890, Hewlett Packard, Avondale, PA) on 0.3% Carboxax 20M with 0.1% phosphoric acid (Supelco, Bellefonte, PA). Ammonia-N concentration in ruminal fluid on centrifuged sample (3000 x g for 15 min) was determined by the phenol procedure of Krom (1980).

Plasma urea content was analyzed according to the method described by Tagari (1969).

### *Statistical analyses*

Data were analyzed by ANOVA using the GLM procedure of SAS (1985) to examine the effect of cow, number of lactations, feeding frequency, and their interactions on metabolites, milk yield and milk composition. Treatment means were compared by a contrast t test, following significance for the ANOVA model.

## RESULTS AND DISCUSSION

According to data given in Table 2, feeding frequency had no significant effect on DM intake. This finding conforms with other studies in which feeding frequency did not affect DM intake (Yang and Varga, 1989; Klusmeyer et al., 1990). Milk yield and percentage and yield of milk-protein, fat and lactose were higher for multiparous than for primiparous cows, but no effect of feeding frequency could be identified. The lack in milk response to frequency of feedings in our study is in agreement with results published by others (Nocek and Braund, 1985; French and Kennelly, 1990; Klusmeyer et al., 1990). Robinson and McNiven (1994), however, observed an increase in DM intake and milk yield when cows were fed seven vs

TABLE 2

Effect of feeding frequency on DM intake, milk yield and composition of primiparous (P) and multiparous (M) cows

Feeding frequency	x 1		x 3		SEM	Main effect		
	P	M	P	M		LAC	FF	PxFF
Lactation no.								
DM intake, kg/d	22.4	22.4	22.5	22.5				
Milk yield, kg/d	34.0	37.0	34.3	37.6	0.33	0.002	0.820	0.917
Milk protein								
%	2.93	3.07	2.96	3.02	0.01	0.052	0.815	0.990
kg/d	0.98	1.12	0.97	1.10	0.01	0.001	0.937	0.688
Milk fat								
%	3.52	3.73	3.55	3.66	0.03	0.007	0.395	0.146
kg/d	1.18	1.37	1.17	1.34	0.02	0.003	0.620	0.510
Milk lactose								
%	4.60	4.58	4.62	4.57	0.01	0.646	0.414	0.980
kg/d	1.54	1.68	1.52	1.68	0.01	0.001	0.664	0.802

two meals. However, diets high in barley, which is highly fermentable in the rumen, were used in that experiment.

The level of VFA,  $\text{NH}_3\text{-N}$  in rumen fluid and PUN are presented in Table 3. Before the morning meal, the molar proportions of acetate and butyrate were higher and that of propionate was lower when cows were fed one vs three meals daily. As a result, the acetate to propionate ratio was lower before the morning meal was

TABLE 3

Effect of feeding frequency on ruminal fluid pH and on levels of VFA and ammonia-N and on plasma urea-N

Time of sampling	Before meal		3 h after meal		SEM	Main effect		
	x 1	x 3	x 1	x 3		time	FF	time x FF
VFA, % of total:								
acetate (A)	65.2 <sup>a</sup>	63.0 <sup>b</sup>	60.9 <sup>c</sup>	61.0 <sup>bc</sup>	0.40	0.004	0.221	0.167
propionate (P)	21.3 <sup>c</sup>	24.2 <sup>a</sup>	22.6 <sup>b</sup>	21.0 <sup>c</sup>	0.24	0.036	0.155	0.001
butyrate	10.4 <sup>c</sup>	9.9 <sup>c</sup>	13.3 <sup>a</sup>	12.5 <sup>b</sup>	0.11	0.001	0.015	0.336
isovalerate	1.18 <sup>a</sup>	0.99 <sup>b</sup>	1.16 <sup>a</sup>	1.06 <sup>c</sup>	0.01	0.556	0.001	0.070
valerate	1.49 <sup>a</sup>	1.46 <sup>a</sup>	1.55 <sup>a</sup>	1.37 <sup>b</sup>	0.02	0.589	0.017	0.056
A : P	3.11 <sup>a</sup>	2.69 <sup>b</sup>	2.69 <sup>b</sup>	2.92 <sup>a</sup>	0.04	0.353	0.186	0.0001
Total VFA, mM	98.9 <sup>a</sup>	99.7 <sup>a</sup>	84.3 <sup>b</sup>	81.3 <sup>b</sup>	1.30	0.001	0.619	0.536
Ammonia-N, mg/dl	14.9 <sup>b</sup>	10.8 <sup>c</sup>	16.8 <sup>a</sup>	10.8 <sup>c</sup>	0.30	0.049	0.001	0.122
Urea-N, mg/dl	16.8 <sup>a</sup>	16.1 <sup>a</sup>	15.2 <sup>b</sup>	14.1 <sup>c</sup>	0.11	0.001	0.001	0.115

a, b, c – means within rows with different superscripts differ,  $P < 0.05$

offered when cows were fed three meals. A trend towards a higher propionate and lower acetate to propionate ratio was observed when cows were fed four vs two meals daily (Klusmeyer et al., 1990). However, in other studies, no effect of more frequent meals on VFA molar proportion was observed (Sutton et al., 1986; Yang and Varga, 1989). Three h after feeding, the molar proportion of VFA was higher when cows were fed one meal than when cows received three daily meals, suggesting greater consumption in the morning meal when cows were fed once daily. While cows in our experiment were fed with different frequencies, feed remained available to them between feedings. Nevertheless, fermentation was affected by feeding frequency, suggesting that cows consumed the main part of the diet according to the feeding strategy.

Ammonia-N concentrations in ruminal fluid, before and 3 h after the ration was offered, were lower when cows were fed three meals as compared with one meal (Table 3). Lower  $\text{NH}_3$ -N concentration 2 and 4 h after feeding when cows were fed more frequent meals was in line with data shown by Yang and Varga (1989). Plasma urea-N concentrations were higher before the morning meal was offered, as compared with 3 h later. The PUN concentration was higher when cows were fed one meal, but it was significantly higher only 3 h after the morning meal was offered. Plasma urea-N concentrations can be indicative of ammonia loss from the rumen and represent the efficiency of processes related to protein metabolism. Therefore, lower PUN concentrations might indicate a higher efficiency of N utilization in the cow. Furthermore, a decrease of PUN concentrations might reduce milk non-protein N (Roseler et al., 1993).

The relation between ruminal VFA and ruminal  $\text{NH}_3$ -N concentration is presented in Table 4. A high proportion of ruminal acetate before the morning meal correlated with a reduced  $\text{NH}_3$ -N concentration:  $\text{NH}_3$ -N (mg/dl) =  $34.74 (\pm 10.4) - 0.34 (\pm 0.16) \times \text{acetate (molar proportion)}$ ,  $n = 58$ ,  $R = 0.27$ ,  $P < 0.05$ . The linkage between ruminal acetate and ammonia may be due to incorporation of ammonia by fibre-digesting microbes (Nocck and Russell, 1988). When cows were fed one daily meal, increases in ruminal butyrate and ammonia concentrations were observed. The increase in the proportion of ruminal butyrate was found to be affected by the presence of ruminal protozoa (Grummer et al., 1983; Whitelaw et al., 1984). The presence of protozoa in the rumen might explain the increased concentration of ruminal ammonia, suggesting a lower number of ruminal protozoa when cows were fed three vs one meal daily. Three h after the morning meal, increased molar proportion of ruminal butyrate and ruminal ammonia concentrations were found in both feeding frequencies. It seems that the number and/or activity of ruminal protozoa were increased, and recycling of bacteria was increased after feeding. These data concur with those published by Martin et al. (1994) in which the number of Holotrichs was found to be higher two hours after feeding than five or 23 h later. When cows were fed three meals, an increase in propionate molar

TABLE 4

Effect of feeding frequency on the relation between ruminal VFA and ammonia-N

Feeding frequency	Before meal:
x 1	Ammonia N (mg/dl) = acetate (%) x -0.83 (+ 0.3) + 68.94 (± 19.9) R=0.46, P<0.05, n=30 Ammonia N (mg/dl) = butyrate (%) x 2.18 (± 0.6) - 8.18 (± 6.0) R=0.58, P<0.01, n=30 Ammonia N (mg/dl) = total VFA (mM) x 0.16 (± 0.1) -1.46 (± 7.4) R=0.38, P<0.05, n 30
x 3	Ammonia N (mg/dl) = acetate (%) x -0.56 (± 0.1) + 45.81 (± 8.2) R=0.64, P<0.01, n=28 Ammonia N (mg/dl) = propionate (%) x 0.51 (± 0.1) -1.57 (± 3.5) R=0.57, P<0.01, n=28 Ammonia N (mg/dl) = total VFA (mM) x 0.15 (± 0.1) - 4.01 (± 3.7) R=0.62, P<0.01, n=28
	3 h after meal:
x 1	Ammonia N (mg/dl) = butyrate (%) x 1.23 (+ 0.4) + 0.75 (± 5.6) R=0.45, P<0.01, n=36
x 3	Ammonia N (mg/dl) = butyrate (%) x 0.71 (± 0.3) + 1.88 (± 3.6) R= 0.40, P<0.05, n=34

proportion and a decrease in ruminal ammonia concentration were noted before feeding. The negative correlation between ruminal propionate and ammonia concentration might be caused by a decrease in ruminal acetate and butyrate in that feeding frequency. A reduced acetate to propionate ratio in ruminal fluid as a result of increasing feeding frequency were shown in our previous work (Shabi et al., 1997). In that work, increased feeding frequency was associated with a higher proportion of by-pass non-structural carbohydrates and protein in the abomasal digesta. It seems that increasing the feeding frequency may improve dietary nitrogen utilization and carbohydrate metabolism, with a shift toward more gluconeogenesis. Improvement of feed utilization may sometimes be a result of a reduction in DM intake without affecting milk yield. In the current experiment, the cows were group-fed, and the experimental group included primiparous and multiparous cows that were not in the same stage of lactation. Accordingly, the average DMI of each group (Table 2) might not reflect the actual experimental effect on DMI of the individual cow. Production response to more frequent feeding is more likely to be expressed in cows undergoing large perturbations in metabolite supply. Such a situation may occur in high-yielding dairy cows, maintained on high concentrate diets.

TABLE 5

Summary of results of studies on the effect of feeding frequency (FF) on dry matter intake, milk yield and rumen metabolites

	n	Milk Grains in diet,		FF	FF of:	Effects of feeding frequency on:					
		kg/d	g/kg			DMI	milk	milk-fat	milk-protein	NH <sub>3</sub> -N	VFA
Nocck and Braund (1985)	4	28	600	1,2,4,8	TMR	NS	NS	NS	NS		
	30			1 vs 4		NS	NS	NS	NS		
Robinson and Sniffen (1985)	3	26	260	1 vs 4	TMR	NS	NS	NS	NS		
Sutton et al. (1988)	16	21	700/900	2 vs 6	C		NS	¶			
Yang and Varga (1989)	3	21	450	1,2,4	C	NS	NS	*	*		
French and Kennelly (1990)	4	30	600	2 vs 12	C		NS	#	NS		
	6	37		2 vs 12	C, TMR		NS	NS	NS		
Klusmeyer et al. (1990)	20	30	450	2 vs 4	TMR	NS	NS	NS	NS		
	4		450							NS	Ý
Macleod et al. (1994)	20	28	650	2 vs 3	C	NS	NS	NS	NS	NS	NS
	32	29	600	2 vs 6		NS	NS	NS	NS		
Robinson and McNiven (1994)	24	36	490	2 vs 7	Barely	§	§				
Robinson and McQueen (1994)	24	36	550	2 vs 5	Protein	NS	NS	NS	NS	NS	□
Shabi et al. (1997)	4	26	470	2 vs 4	TMR	†	NS	†	†	NS	†
Current experiment	180	36	650	1 vs 3	TMR	NS	NS	NS	NS	‡	‡

TMR = total mix ration, C = concentrate. NS = not significantly affected by feeding frequency P > 0.1

¶ milk-fat increased when cows were fed concentrate six vs two times daily

\* milk-fat and -protein yield increased when cows were fed concentrate more than once daily. Mean daily concentration of ammonia was NS

# milk-fat increased when cows were fed concentrate 12 vs two times daily

Ý total VFA was increased when cows were fed more frequent meals

§ cows fed raw barley seven vs two times daily, increased DM intake and milk yield

□ total VFA and proportion of propionate decreased when cows were fed more frequent supplemental protein

† milk-fat and -protein percentage and yield increased, proportion of propionate increased and proportion of acetate decreased when cows fed 4 vs 2 meals

‡ before feeding, proportion of propionate increased, acetate decreased, ammonia concentration decreased when cows fed four vs two. After feeding ammonia concentration decreased and plasma urea concentration decreased when cows were fed four vs two meals



Gibson (1984) summarized the effect of the frequency of feeding on milk production of cattle. In his review, significant responses of milk production to increased frequency of feeding occurred in comparisons of one or two with three or more meals per day. However, that review dealt with low-production cows. Data shown in Table 5 review published results of effect of feeding frequency on milk production and rumen fermentation from 1984 until recently. Milk yield, milk composition, and ruminal ammonia were not affected by variable experimental feeding strategy in most cases. Increasing the feeding frequency improved fibre digestion and increased milk-fat (Sutton et al., 1988; Yang and Varga, 1989; French and Kennely, 1990; Shabi et al., 1998). Milk-fat increased when concentrates were fed more frequently. Rumen fermentation was also affected by increased feeding frequency in half of the reported cases. The greatest response to an increased number of meals in milk yield, milk composition, and metabolites was observed in cows when they were fed time-limited meals (Shabi et al., 1997).

Increasing the feeding frequency maintained a higher ratio of RDOM/RDCP from 16.00 h until the morning feeding (Figure 1). Higher RDOM/RDCP ratios during those hours improved N utilization but did not enhance milk synthesis. The diet in the present experiment contained a variety of feedstuffs. Such a variety could maintain the supply RDOM and RDCP during the day. It seems that a difference of 0.2 units of the RDOM/RDCP ratio, obtained by more frequent feeding, had small impact of diet utilization for milk synthesis. This suggests that the RDOM/RDCP ratio can be used to predict the benefit from an increase of feeding frequency.

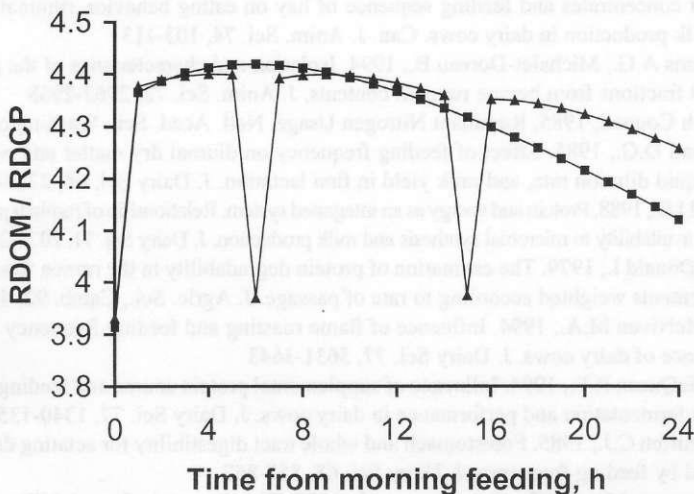


Figure 1. Hourly ratio between ruminally degradable OM (RDOM) and ruminally degradable CP (RDCP) when cows were fed once (■) or three times daily (▲)

## CONCLUSIONS

Increased feeding frequency may change the ruminal fermentation to more gluconeogenic one, and this may improve utilization of carbohydrates in the diet. More frequent meals might be a useful tool to reduce ruminal ammonia and plasma urea concentration and may reduce excretion of N to the environment when high-protein diets are fed to dairy cows.

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## STRESZCZENIE

### Wpływ liczby odpasów na wydajność i skład mleka krów

Doświadczenie przeprowadzono w przemysłowej fermie bydła na 180 krowach rasy holsteńskiej (Israel Holstein), podzielonych na 2 grupy. W grupie pierwszej paszę podawano raz dziennie, w drugiej – trzy razy dziennie. Suma LKT i molarny udział kwasu octowego były wyższe przed karmieniem niż w 3 godz. po podaniu paszy. Obniżenie udziału kwasu octowego po odpasie było większe u krów otrzymujących paszę raz niż trzy razy dziennie. Udział kwasu propionowego był większy przed karmieniem i niższy w 3 godz. po odpasie u krów, którym paszę podawano 3 razy dziennie, w następstwie czego stosunek kwasu octowego do propionowego u krów żywionych 3 razy dziennie był niższy przed karmieniem i wzrastał po 3 godzinach. Stężenie  $\text{NH}_3\text{N}$  w żwaczu, przed i po karmieniu, było niższe u krów otrzymujących paszę 3 razy dziennie. Zawartość N-mocznikowego w osoczu krwi była wyższa przed odpasem niż 3 godz. później i była wyższa u krów żywionych jednorazowo, ale istotną różnicę stwierdzono tylko w 3 godz. po rannym karmieniu. Można sądzić, że zwiększenie częstotliwości podawania paszy może poprawiać wykorzystanie azotu paszy i może wpływać na zmianę kierunku przemian, zwiększając glukonogenezę.

Zmiany w wydajności w zależności od częstotliwości podawania paszy mogą się ujawnić u krów wysokowydajnych, żywionych dawkami z dużym udziałem paszy treściwej.