

# The effects of added glycerol or unprotected free fatty acids or a combination of the two on silage intake, milk production, rumen fermentation and diet digestibility in cows given grass silage based diets

Hannele Khalili

*Agricultural Research Centre of Finland, North-Savo Research Station, FIN-71750 Maaninka, Finland, current address: Agricultural Research Centre of Finland, Animal Production Research, FIN-31600 Jokioinen, Finland, e-mail: hannele.khalili@mtt.fi*

Tuomo Varvikko, Vesa Toivonen

*Agricultural Research Centre of Finland, Animal Production Research, FIN-31600 Jokioinen, Finland*

Kari Hissa

*Finn Feed Ltd, PO Box 105, FIN-00241 Helsinki, Finland*

Marjatta Suvitie

*Agricultural Research Centre of Finland, North-Savo Research Station, FIN-71750 Maaninka, Finland*

The addition of glycerol or free fatty acids either alone or in combination to concentrate was studied for the effects on feed intake, milk production, rumen fermentation, blood metabolites and diet digestibility in dairy cows given grass silage *ad libitum*. The study was conducted on 12 mid-lactating cows, four of them ruminally cannulated. Barley-based concentrate (control diet, C) was given 7 kg/d as fed. In the other three diets, 36 g/kg of barley was replaced by glycerol (G) or a mixture of free fatty acids (FA) or by a combination of the two, making a total of 72 g/kg (GFA). The experimental design consisted of balanced 4 x 4 Latin squares with a 2 x 2 factorial arrangement of diets: the effects of G, FA and G\*FA interaction. The FA diets significantly decreased silage intake, increased milk yield, decreased milk protein content, increased the concentrations of C<sub>18:0</sub>, C<sub>18:1</sub>, and C<sub>20:1</sub> and decreased those of C<sub>8:16</sub>, and C<sub>18:3</sub> fatty acids in milk fat. The FA diets also increased the concentration of nonesterified fatty acids in plasma, and decreased the digestibility of organic matter and neutral detergent fibre but increased that of fat. Glycerol decreased the molar proportion of acetate and increased the molar proportions of propionate and butyrate in the rumen, but the addition of glycerol did not have any effect on silage intake, milk yield or milk composition. Milk yield was highest when glycerol and free fatty acids were given together, showing a positive interaction.

*Key words:* blood metabolites, digestibility, fatty acids, glycerol, grass silage milk production, rumen fermentation

## Introduction

In Finland dairy cows are typically fed grass silage with barley based supplement. Due to the fermentation type in the rumen however, this diet is not optimal if the aim is to decrease the fat content in milk without simultaneous decreasing the milk protein content (Huhtanen 1988, Jaakkola and Huhtanen 1993). Grass silage with barley produces generally a rumen fermentation pattern relatively high in butyrate and low in propionate, which explains the high milk fat content. In addition, glucose supply may be limited in dairy cows, and glucogenic amino acids may then be used for glucose production. As a result, smaller amounts of amino acids will be available for milk protein synthesis. Therefore, grass silage-based diets should be supplemented with a concentrate mixture producing more propionate and less butyrate in the rumen in order to increase the glucose supply to the host animal.

Energy-yielding components other than carbohydrates or their by-products are alternative sources of ingredients that could be included in the concentrate mixture. We look here at the effects of glycerol and unprotected long-chain free fatty acids as dietary supplements. The use of glycerol and fatty acids separately in concentrate mixtures has commercial interest, for instance if these ingredients affect milk yield and composition. In reported trials glycerol has been fermented under different conditions and therefore the proportions of the end products of rumen fermentation have also varied. Information is lacking on the effects of glycerol supplementation on the performance of dairy cows fed grass silage-based diets. Glycerol in the diet could have the potential to increase the glucose supply if the proportion of propionic acid is mainly increased at the expense of other acids. Glucose carbon can be supplied by propionate or any odd-carbon compounds or by glucogenic amino acids (Bergman and Pell 1985). Carbon sources other than propionate include glycerol from mobilized lipids (Van Soest 1994). The addition of fat to the diet is one way of increasing energy

intake in order to obtain a higher milk yield provided that feed intake and/or digestibility are not much reduced. The level of supplemental fat is important since high levels cause negative effects but even moderate levels have often been reported to reduce milk protein content (Thomas and Chamberlain 1984, Tesfa et al. 1991a). Jenkins (1993) and Doreau and Ferlay (1995) recently showed that unprotected long-chain fatty acids in the diet interfere with the microbial ecosystem. Dietary fat also affects the milk fatty acid composition, e.g. by reducing the content of short- and medium-chain fatty acids and increasing that of long-chain fatty acids (Palmquist and Jenkins 1980, Coppock and Wilks 1991, Tesfa et al. 1991b). Because, however, dietary fat is modified within the rumen, the profile of long-chain fatty acids in milk may not be like that provided in the diet.

Our objective was to study the effects of a low level of free fatty acids because high, and even moderate, levels have negative effects and also because information on the responses of a low level of fatty acids is scarce in grass silage-based diets. We studied the effects of including either glycerol or a mixture of unprotected free fatty acids, mainly C<sub>18:1</sub> and C<sub>18:2</sub>, or a combination of glycerol and fatty acids on feed intake, milk production and composition, rumen fermentation, blood metabolites and diet digestibility in dairy cows fed grass silage and a concentrate mixture.

## Material and methods

### Animals, diets, and experimental design

The study was conducted on 12 mid-lactating (115 ± 31 days in milk) Friesian cows, of which four were cannulated in the rumen, in their first lactation period. The cows were individually fed and milked twice a day, at 0700 and 1600. A total of 7 kg of barley-based concentrate (control diet, C) was given twice a day (0800 and 1730)

in two equal meals of 3.5 kg each. The concentrate (C diets) consisted (g/kg) of barley (600), oats (80), sugar beet pulp (150), soya-bean meal (95), molasses (20) and others (55). In other three diets, 36 g/kg of barley was replaced by glycerol (G diets) and a mixture of free fatty acids (FA diets) either alone or in combination (GFA) (36 g/kg of glycerol and 36 g/kg of fatty acids), making a total of 72 g/kg. The fatty acid composition (g/kg) of the mixture of free FA (fractionated vegetable fatty acid blend) was C<sub>16:0</sub> (126), C<sub>18:0</sub> (22), C<sub>18:1</sub> (376), C<sub>18:2</sub> (274), C<sub>18:3</sub> (32) and the rest was a mixture of fatty acids in triglycerides form (170). Wilted grass silage was fed *ad libitum*, and refusals were between 50 to 100 g/kg of the amount offered. Second cut silage was made from a mixture of timothy and meadow fescue, and ensiled with a formic acid based additive (5 l/1000 kg; 690 g/kg formic acid). The experiment was carried out as three balanced 4 x 4 Latin squares, each having 21 day periods comprising 14 days for adjustment and 7 days for data collection.

### Measurements and analytical procedures

Milk yield and silage intake were recorded daily. The results presented are based on the data on intake and milk production during the last 6 days of each experimental period. The silage dry matter (DM) content was determined by oven drying at 105°C for 24 h, and the DM content was corrected for the volatile losses according to Huida et al. (1986). Feed samples were collected on days 15-19 and pooled within the period. Feed samples were analysed for organic matter (OM), nitrogen (N), neutral detergent fibre (NDF) and HCl fat. The silage samples were also analysed for pH, water-soluble carbohydrates, lactic acid, ammonia N and soluble N. Diet digestibility was measured using acid insoluble ash as an internal marker (Van Keulen and Young 1977). During 5 days of each collection period clean (not mixed with urine) faecal grab samples were taken from the four cannulated cows at 0700 and 1900, and pooled within

each cow and frozen. The pooled samples were subsequently thawed and dried at 60°C and stored at room temperature for chemical analyses. Samples from the feeds and faeces were analysed for organic matter by ashing at 600°C for 12 h, nitrogen (Kjeldahl-N), NDF, ADF and lignin (Robertson and van Soest 1981). HCl fat was extracted with diethyl ether (AOAC 1990) after boiling for 1 h in 3 N HCl. Milk samples were taken during four consecutive milkings on days 17 and 18 and analysed for fat, protein and lactose. Milk samples were pooled within diet for analyses. The composition and amount of milk fatty acids were measured after methylation (Anttila and Kankare 1983) using a Hewlett Packard (Böblingen, Germany) HP 5890 gas chromatograph on a 2 mm x 3 m glass column packed with GP 10% SP-2330 on 100/120 Chromosorb WAW (Supelco, Bellefonte USA) and equipped with an HP 7673 automatic injector and flame ionization detector. The column temperature was initially 90°C and then programmed to 220°C at 8°C/min. The split/slitless injector was used in splitless mode at 250°C and the detector also at 250°C. The carrier gas was helium at a flow rate of 40 ml/min. The fatty acid methyl esters were identified and quantitated utilizing the Nu-Check-Prep GLC-60 milk fat standard (Bast of Copenhagen, Denmark) and HP 3365 Chemstation. Blood samples were collected on day 18 before morning feeding and 3 and 6 h later from the coccygeal vein of each cow into 10 ml EDTA tubes and placed in ice. Beta-hydroxybutyrate (BHB) was analysed from acid precipitated blood according to the method described by Työppönen and Kauppinen (1980). Plasma was separated by centrifugation and samples were analysed enzymatically using commercial kits for glucose (Boehringer Mannheim GmbH, Germany) and for non-esterified fatty acids (NEFA) (Wako Chemicals GmbH, Neuss, Germany; Shimizu et al. 1980). Plasma urea was analysed as described by Gutmann and Bergmeyer (1974). Rumen fluid samples were collected from the four cannulated cows on day 19 before morning feeding and 1.5, 3, 4.5, 6, 7.5 and 9 h thereafter. pH was measured immediately. Rumen fluid

samples were first strained through cheese cloth, and then 0.5 ml of saturated mercury(II)chloride and 2 ml of 1 M sodium hydroxide were added to 5 ml of rumen fluid. The samples were kept frozen until analysed. Before analysis a 0.5 ml sample was acidified with 0.25 ml of formic acid (Riedel-de-Haen 33015), diluted to 5 ml with distilled water and centrifuged at 2000 g for 10 min. The VFA content was measured by an HP 5890 gas chromatograph (Hewlett Packard, Avondale, USA) provided with an HP 7673 autosampler, a flame ionization detector and an HP 3365 Chemstation. The capillary column was an HP-FFAP 10 m x 0.53 mm x 1 µm (HP, USA). The sample volume injected was 1 µl. Helium was used as a carrier gas at a flow rate of 9 ml/min. The column temperature was programmed to rise from 60 to 78°C at a rate of 25°C/min, isothermally at a rate of 7.5°C/min to 100°C for 1 min and finally at a rate of 25°C/min to 180°C in the final time of 3 min. The injector was operated in the split mode (split vent flow 45 ml/min). The injector and detector temperatures were 220 and 260°C, respectively. The results were calculated by an external standard method. Ammonia N was analysed as described by McCullough (1967). The number of protozoa of pooled daily (within each cow) rumen samples were counted, after mixing with methyl green-formalin-saline solution, using a haemo-cytometer. The cows were weighed on two consecutive days at the beginning of the experiment and on two days at the end of each period.

### Statistical methods

Data were analysed by ANOVA for a balanced Latin square design. The model was a mixed one:

$$y_{ijklm} = \mu + A_i + P_j + FA_k + G_l + FA*G_{kl} + f_{ijkl} + T_m + A*T_{im} + P*T_{jm} + FA*T_{km} + G*T_{lm} + FA*G*T_{klm} + e_{ijklm}$$

where  $\mu$  is the overall mean, A and P are the random effects of animal and period, FA, G and FA\*G are the fixed effects of diets,  $f_{ijkl}$  is the random error term for whole-plot (mean square of

the whole-plot), T is fixed effect time, A\*T and P\*T are the random effects of animal\*time and period\*time, G\*T, FA\*T and G\*FA\*T are the fixed effects of diets\*time, and  $e_{ijklm}$  is the random error term for sub-plot (mean square of the sub-plot). The random variables,  $A_i$ ,  $P_j$ ,  $f_{ijkl}$ ,  $A*T_{im}$ ,  $P*T_{jm}$  and  $e_{ijklm}$ , are all assumed to be independent and normally distributed with zero means and variances  $\sigma_A^2$ ,  $\sigma_P^2$ ,  $\sigma_f^2$ ,  $\sigma_{AT}^2$ ,  $\sigma_{PT}^2$  and  $\sigma_e^2$ , respectively. The effects of FA, G and FA\*G on intake, milk production and composition, live weight, mean values of blood metabolites, and mean values of rumen characteristics and digestibility were tested using the whole-plot error mean square as an error variance in the F test. The effects of FA\*time, G\*time and FA\*G\*time on the post-prandial values of blood metabolites and rumen characteristics were tested using the sub-plot error mean square as an error variance in the F test. Statistical significance of treatments was tested 2x2 factorially: the effect of glycerol (G and G\*FA v C and FA); the effect of free fatty acids (FA and G\*FA v C and G) and their respective interaction effect G\*FA (C and G\*FA v G and FA). Blood metabolites and rumen fluid (pH, ammonia, VFA) data were assessed by analysis of variance for repeated measurements. The analysis used the split-plot approach with Greenhouse-Geiser approximate (conservative) significance tests (Littell et al. 1992).

### Results

The chemical composition of experimental feeds is presented in Table 1. The fermentation quality of silage was good. The N contents of all concentrate mixtures were very similar. The fat contents were higher in FA and GFA diets owing additional free fatty acids.

FA diets increased (P<0.001) milk yield despite reduced silage DM intake (P<0.01) (Table 2). However, total DM intake and ME intake were similar in all diets. FA supplementation

Table 1. Chemical composition of experimental feeds.

	Silage <sup>1</sup>	CM <sup>2</sup>	CM+G	CM+FA	CM+GFA
Dry Matter (g kg <sup>-1</sup> )	288	885	888	891	895
In dry matter (g kg <sup>-1</sup> )					
OM	913	930	927	927	928
N	29.0	25.8	25.7	25.6	25.9
Hcl fat	58.2	38.1	36.3	65.3	68.3
NDF	560	236	219	228	215
ADF	309	88.6	77.9	80.1	78.7
Lignin	34.7	16.2	14.5	16.9	14.2
Cellulose	275	72.4	63.4	63.2	64.5
Hemicellulose	250	148	141	148	136

<sup>1</sup> In silage: pH 4.26; in DM (g kg<sup>-1</sup>): water-soluble carbohydrates 48.6; lactic acid 66.3; acetic acid 18.8; propionic acid 0.08; butyric acid 0.47; isovaleric acid 0.028; ethanol 2.6; in total N (g kg<sup>-1</sup>): ammonia N 58.6; soluble N 479. Cellulose digestibility of silage DM 0.752. Cellulose=ADF-lignin, Hemicellulose=NDF-ADF.

<sup>2</sup> CM=concentrate mixture, CM+G=concentrate mixture including glycerol, CM+FA=concentrate mixture including unprotected free fatty acids, CM+GFA=concentrate mixture including both glycerol and unprotected free fatty acids. G=glycerol; FA=free fatty acids; GFA=glycerol and free fatty acids; OM=organic matter; NDF=neutral detergent fibre; ADF=acid detergent fibre

Table 2. Effect of various supplements on feed intake, milk yield, milk composition, yield of milk constituents and live weight.

	Treatment				SEM	Significance of effect		
	C	G	FA	GFA		G	FA	GxFA
Feed intake (kg DM day <sup>-1</sup> )								
Silage	10.18	10.06	9.94	9.74	0.108	NS	**	NS
Supplement	6.09	6.01	6.11	6.15	0.058			
Total intake	16.27	16.06	16.05	15.89	0.115	NS	NS	NS
Silage+suppl. g/W <sup>0.75</sup>	148	146	147	146	1.1	NS	NS	NS
ME intake (MJ)	187	185	189	188	1.3	NS	NS	NS
ECM <sup>1</sup> yield (kg day <sup>-1</sup> )	22.52	22.08	23.07	23.19	0.206	NS	***	NS
Milk yield (kg day <sup>-1</sup> )	22.98	22.21	23.47	23.69	0.258	NS	***	P=0.07
Milk composition (g kg <sup>-1</sup> )								
fat	39.1	39.2	40.1	39.8	0.47	NS	P=0.09	NS
protein	31.8	32.1	31.1	31.2	0.17	NS	***	NS
lactose	49.7	49.4	49.6	49.9	0.12	NS	P=0.09	*
Yield of milk constituents (g day <sup>-1</sup> )								
fat	891	873	934	937	11.0	NS	***	NS
protein	726	714	727	740	8.0	NS	P=0.10	NS
lactose	1148	1124	1170	1195	12.5	NS	***	P=0.06
Live weight (kg)								
mean	527	527	522	523	1.6	NS	**	NS
change (day <sup>-1</sup> )	+0.424	+0.408	+0.325	+0.143	0.1238	NS	NS	NS

Statistical significance: NS, P>0.10 not significant; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

<sup>1</sup> ECM=energy corrected milk (Sjaunja et al. 1990)

C=concentrate mixture (CM); G=CM+glycerol; FA=CM+fatty acids; GFA=CM+glycerol+fatty acids

Table 3. Effect of various supplements on milk fatty acid composition (g kg<sup>-1</sup> fat).

	Treatment				SEM	Significance of effect		
	C	G	FA	GFA		G	FA	GxFA
C <sub>4</sub>	55.3	56.5	56.5	59.3	1.95	NS	NS	NS
C <sub>6</sub>	29.0	29.8	28.0	28.5	0.93	NS	NS	NS
C <sub>8</sub>	16.0	16.0	14.0	14.5	0.50	NS	**	NS
C <sub>10</sub>	36.0	36.3	30.0	30.8	1.04	NS	***	NS
C <sub>12</sub>	41.3	43.0	35.5	35.5	0.89	NS	***	NS
C <sub>14:0</sub>	128.5	133.5	118.3	121.3	2.12	NS	***	NS
C <sub>14:1</sub>	15.5	16.3	13.8	14.0	0.29	NS	***	NS
Total C <sub>4</sub> -C <sub>14</sub>	321.5	331.3	296.0	303.8	6.59	NS	**	NS
C <sub>16:0</sub>	293.5	278.8	268.8	264.3	10.28	NS	P=0.10	NS
C <sub>16:1</sub>	26.0	27.8	24.8	26.0	0.61	*	*	NS
Total C <sub>16</sub>	319.5	306.5	293.5	290.3	9.80	NS	P=0.07	NS
C <sub>18:0</sub>	111.0	109.0	128.5	129.8	2.54	NS	***	NS
C <sub>18:1</sub>	218.0	222.0	252.3	246.8	4.70	NS	***	NS
C <sub>18:2</sub>	20.5	22.0	21.3	22.0	1.33	NS	NS	NS
C <sub>18:3</sub>	7.5	6.8	6.5	6.3	0.20	*	**	NS
C <sub>20:1</sub>	3.3	2.8	4.0	3.5	0.25	P=0.09	*	NS
Total C <sub>18</sub> -C <sub>20</sub>	360.3	362.5	412.5	408.3	6.61	NS	***	NS

Statistical significance: NS, P>0.10 not significant; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

C=concentrate mixture (CM); G=CM+glycerol; FA=CM+fatty acids; GFA=CM+glycerol+fatty acids

tended (P=0.09) to increase milk fat content and increased (P<0.001) milk fat yield. Milk protein content decreased (P<0.001) but, due to the greater milk yield, protein yield tended (P=0.10) to increase in response to FA supplementation. Mean live weight was lower (P<0.01) with FA diets. Inclusion of glycerol alone did not significantly affect silage intake, milk production, milk composition or live weight. However, glycerol tended to decrease milk yield without supplemental fat (-0.77 v +0.22 kg/d; P=0.07) and to increase milk yield with supplemental fat. Lactose yield also tended to decrease without supplemental fat (-24 v +25 g/d; P=0.06) and to increase with supplemental fat.

FA diets increased the proportions of C<sub>18:0</sub>, C<sub>18:1</sub> (P<0.001) and C<sub>20:1</sub> (P<0.05) long-chain fatty acids and simultaneously decreased the total proportions of short-chain fatty acids (C<sub>4</sub>-C<sub>14</sub>) (P<0.01) and the proportion of the total medium-chain fatty acids C<sub>16</sub> (P=0.07) in milk fat

(Table 3). Due to the increased fat yield (Table 2) and proportions (Table 3) of C<sub>18:0</sub> and C<sub>18:1</sub>, secretions of C<sub>18:0</sub> and C<sub>18:1</sub> increased significantly (P<0.001) (C diet 99.1 and 194.9 v FA diet 120.2 and 235.7 for C<sub>18:0</sub> and C<sub>18:1</sub>, respectively,) but secretions of total C<sub>4</sub>-C<sub>14</sub> and total C<sub>16</sub> remained unaltered (results not shown in Table 3). Glycerol supplementation increased (P<0.05) the proportion of C<sub>16:1</sub> and decreased that of C<sub>18:3</sub> (Table 3). No interaction affecting for the proportion or production of milk fatty acids was noticed between glycerol and free fatty acids.

FA diets increased (P<0.001) the mean value of the three sampling times of plasma NEFA concentration (Table 4). There was an interaction between glycerol and free fatty acids in the mean value of the plasma urea concentration, resulting in a decrease (P<0.05) in urea concentration when FA was included in the diet with glycerol. Postprandial values of BHB and urea were highest and those of glucose and NEFA

Table 4. Effect of various supplements on the concentrations (mmol l<sup>-1</sup>) of some blood and plasma metabolites.<sup>1</sup>

	Treatment				SEM	Significance of effect		
	C	G	FA	GFA		G	FA	GxFA
BHB <sup>2</sup> (mmol l <sup>-1</sup> )	0.58	0.58	0.58	0.58	0.017	NS	NS	NS
Glucose (mmol l <sup>-1</sup> )	3.35	3.28	3.33	3.36	0.041	NS	NS	NS
Urea (mmol l <sup>-1</sup> )	4.87	4.88	5.14	4.76	0.083	*	NS	*
NEFA <sup>3</sup> (mmol l <sup>-1</sup> )	0.096	0.096	0.133	0.119	0.0052	NS	***	NS

Statistical significance: NS, P>0.10 not significant; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

<sup>1</sup> Means of three sampling times. <sup>2</sup> BHB=Beta-hydroxybutyrate. <sup>3</sup> NEFA=nonesterified fatty acids.

C=concentrate mixture (CM); G=CM+glycerol; FA=CM+fatty acids; GFA=CM+glycerol+fatty acids

Table 5. Effect of various supplements on digestibilities of dry matter (DM), organic matter (OM), (N), fat and neutral detergent fibre (NDF) (four ruminally cannulated cows).

	Treatment				SEM	Significance of effect		
	C	G	FA	GFA		G	FA	GxFA
DM	0.759	0.752	0.744	0.747	0.0029	NS	**	NS
OM	0.776	0.768	0.760	0.762	0.0032	NS	**	NS
N	0.726	0.723	0.713	0.718	0.0045	NS	P=0.09	NS
FAT	0.625	0.618	0.648	0.669	0.0041	NS	***	**
NDF	0.734	0.719	0.715	0.705	0.0070	NS	*	NS

Statistical significance: NS, P>0.10 not significant; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

C=concentrate mixture (CM); G=CM+glycerol; FA=CM+fatty acids; GFA=CM+glycerol+fatty acids

lowest 3 h after morning feeding compared with the values before feeding and 6 h after feeding with each diet (results not shown here).

FA diets decreased (P<0.01) digestibilities of DM, OM and (P<0.05) NDF and tended to decrease the digestibility of N (P=0.09) but increase (P<0.001) that of ether extract fat (Table 5). Glycerol inclusion had no significant effect on digestibility. An interaction (P<0.01) affecting fat digestibility was observed between glycerol and FA.

FA diets did not significantly affect the average (seven sampling times) rumen fermentation parameters or numbers of protozoa (Table 6). In contrast, G diets altered the rumen fermentation

type, the molar proportion of acetate decreasing (P<0.001) and the proportions of propionate, butyrate and valerate increasing (P<0.05 at least). Glycerol decreased (P<0.05) the number of Holotrich protozoa. There were no effects of an interaction between glycerol and fatty acids on rumen fermentation parameters or protozoal numbers.

Postprandial changes in molar proportions of acetate, propionate and butyrate are shown in Figure 1a-c. G diets caused interactions with time for acetate and butyrate, decreasing (G\*h P<0.01) the postprandial value of acetate and increasing (G\*h P<0.001) that of butyrate.

Table 6. Effect of various supplements on rumen fermentation characteristics<sup>1</sup> (four ruminally cannulated cows, mean values of seven sampling times).

	Treatment				SEM	Significance of effect		
	C	G	FA	GFA		G	FA	GxFA
pH	6.27	6.29	6.28	6.37	0.058	NS	NS	NS
NH <sub>4</sub> -N (mmol l <sup>-1</sup> )	10.45	9.02	9.48	9.35	0.638	NS	NS	NS
Total VFA (mmol l <sup>-1</sup> )	120.6	122.4	120.6	120.4	1.74	NS	NS	NS
Molar proportion of VFA's (mmol mol <sup>-1</sup> )								
Acetate	656	637	655	637	2.9	***	NS	NS
Propionate	170	176	172	178	2.3	*	NS	NS
Isobutyrate	9.5	9.4	8.9	9.8	0.28	NS	NS	NS
Butyrate	133	144	134	142	1.3	*	NS	NS
Isovalerate	12.5	12.5	11.9	13.1	0.40	NS	NS	NS
valerate	13.9	15.4	13.6	14.8	0.40	**	NS	NS
Caproate	6.3	6.5	6.6	6.5	0.25	NS	NS	NS
Protozoa (x10 <sup>4</sup> ml <sup>-1</sup> )								
Total	19.1	19.2	18.3	17.5	1.00	NS	NS	NS
Holotricha	1.4	1.0	1.8	1.2	0.20	*	NS	NS
Entodiniomorph	17.7	18.2	16.5	16.3	1.17	NS	NS	NS

Statistical significance: NS, P>0.10 not significant; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

<sup>1</sup> Means of seven sampling times

C=concentrate mixture (CM); G=CM+glycerol; FA=CM+fatty acids; GFA=CM+glycerol+fatty acids

## Discussion

### Effects of free fatty acids

Inclusion of unprotected supplementary fats has had both beneficial and adverse effects on dairy cow feeding and production, depending on factors such as the amount and type of fat given. The mixture of unprotected free fatty acids used in the present trial consisted mainly of C<sub>18:1</sub> (376 g/kg) and C<sub>18:2</sub> (274 g/kg) fatty acids. The effects of supplementary fats with very different fatty acid compositions are not therefore referred to in this paper.

#### *Feed intake, milk production and composition*

Replacement of barley by 36 g/kg of a mixture of free fatty acids decreased silage DM intake in the cows given FA diets. Generally, high concentrations of supplementary fat have decreased

feed DM intake but the results of many trials with supplementary fat have been less consistent (Coppock and Wilks 1991). FA supplementation increased milk yield in the trial discussed here. Such an increase has often been observed if the effect on feed intake has been slight or non-negative, resulting in increased energy intake. The estimated ME intake did not increase significantly with FA diets and there were no differences in ME balances (intake-requirement). As shown by Coppock and Wilks (1991), FA diets tended to increase milk fat content and decrease milk protein content. A decrease in milk protein content has often, but not invariably, been reported and the reasons for such a decrease are not clear. It is not easy to explain the decrease in milk protein here either, because there were no changes in the concentrations of plasma glucose or rumen ammonia. Furthermore, the amino acid supply from the small intestine was estimated to be higher than that recommended (maintenance and milk production, ignoring live weight change)



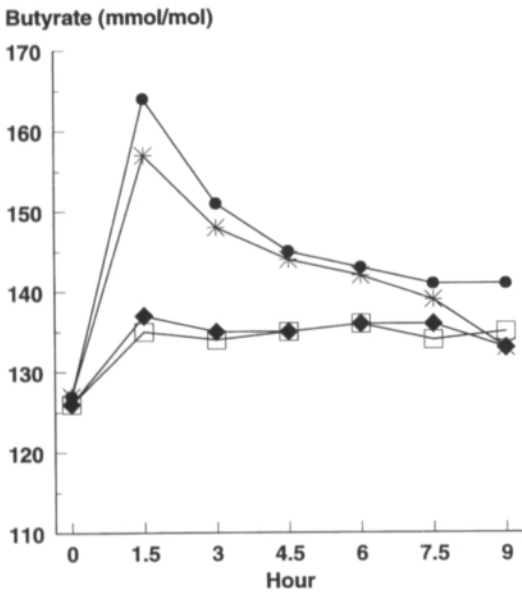
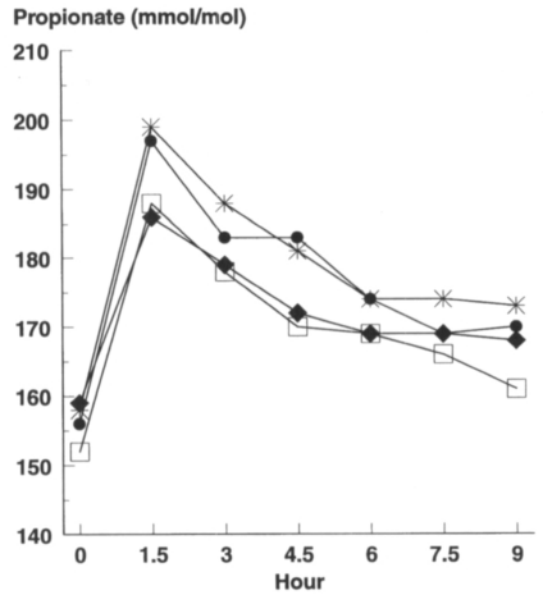
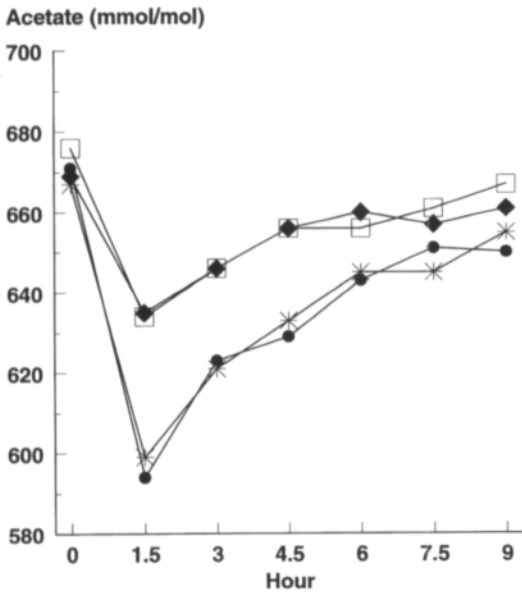


Fig. 1 (a-c). The effects of various supplements on molar proportions of acetate, propionate and butyrate. -□- control diet, -●- glycerol, -◆- a mixture of free fatty acids and -\*- combination of glycerol and free fatty acids.

(Madsen et al. 1995) in each diet. The main cause of the decrease in milk protein content here was the dilution effect due to increased milk yield. Wu and Huber (1994) attribute the decrease in milk protein concentration associated with supplemental fat partly to increased milk yield.

*Milk fatty acids*

It is well documented that dietary fats affect milk FA composition (Palmquist and Jenkins 1980). In the present trial, free FA in FA diets modified milk FA composition by increasing long-chain

FA derived from the diet. However, because the dietary supply of free fatty acids (main components in the FA mixture were  $C_{18:1}$  and  $C_{18:2}$ ) is modified within the rumen, the long-chain FA profile of milk changed, increasing the proportions of  $C_{18:0}$  and  $C_{18:1}$ . Unsaturated free fatty acids are rapidly hydrogenated by rumen microbes into more saturated fatty acids at rates depending on factors such as the nature and amount of lipids fed (Jenkins 1993). According to Doreau and Ferlay (1994), the higher the linoleic acid concentration in the diet the greater is the extent of hydrogenation of linoleic acid. Judging by the lack of an increase in the linoleic acid in milk despite the addition of linoleic acid, this is most probably what occurred here. The hydrogenation of linoleic acid ( $C_{18:2\ n-6}$ ) is not complete, but provides stearic acid and different isomers of  $C_{18:1}$  (Tamminga and Doreau 1991). This hydrogenation was also indicated here by the increase in  $C_{18:0}$  and  $C_{18:1}$  with FA diets. A supply of dietary fatty acids in FA diets could have reduced *de novo* synthesis of  $C_{18:0}$  and  $C_{16:0}$  fatty acids by rumen bacteria (Jenkins 1993) probably because of enhanced uptake of exogenous lipids by bacteria (Poncet et al. 1995). Furthermore, a decrease in the content of  $C_{16}$  in milk with FA diets was affected by the supply of  $C_{18}$  fatty acids and their subsequent uptake by the mammary gland, which inhibits *de novo* synthesis of short-chain fatty acids (Palmquist and Jenkins 1980).

FA diets increased the intake of free fatty acids by about 170 g/d, which increased the production of  $C_{18}$  fatty acids by 62.9 g/d in milk and simultaneously slightly decreased *de novo* synthesis of short-chain fatty acids in the mammary cell. Doreau and Ferlay (1994) concluded that it is currently difficult to predict the amount of absorbable FA, since the factors controlling the synthesis and disappearance of FA in the rumen are not well known.

#### Blood metabolites

FA diets did not affect the blood BHB concentration, a finding supporting the conclusion that BHB concentrations do not usually markedly

increase during fat supplementation (Grummer and Carroll 1991). As found in fat studies (Dhiman et al. 1995, Choi and Palmquist 1996), FA diets did not have any effect on the glucose concentration in plasma. According to Grummer and Carroll (1991), it seems likely that fat supplementation spares glucose from oxidation, but that the metabolic consequences are unknown. They concluded that fat supplementation does not consistently increase blood glucose. The plasma NEFA concentrations of lactating cows fed supplemental fat almost invariably increase (Grummer and Carroll 1991). In the present trial too, FA diets increased the NEFA concentration, which did not indicate increased lipolysis since the cows were in mid-lactation and their energy balance was positive, as shown by the positive live weight change and energy balance. An increased plasma NEFA concentration was probably related to an increased dietary supply of additional free fatty acids rather than to net changes in the balance between lipolysis and lipogenesis.

#### Digestibility

FA inclusion has been shown to reduce digestibilities of OM and fibre (Palmquist and Jenkins 1980, Tesfa 1992, Jenkins 1993), as was also observed in the present trial with FA diets. However, free fatty acids reduced the digestibilities of OM and NDF in FA diets only slightly, partly due to the high proportion of  $C_{18:1}$  in the mixture of FA used and to the total amount of FA given. Note that the mixture of free FA included polyunsaturated FA. According to Chalupa et al. (1984), lipids rich in short-chain, medium-chain or polyunsaturated fatty acids have a greater negative effect on carbohydrate, especially fibre, digestion than have saturated or mono-unsaturated long-chain fatty acids. Here, a slight depression in NDF digestibility might be one reason for the observed decrease in feed intake. Although several mechanisms may be operative in the rumen to explain the reduction in fibre digestion when fatty acids are given, Palmquist and Jenkins (1980) concluded that most data suggest an inhibitory effect on microbial activi-

ty. When fibre digestibility has been depressed by lipids, the fermentation type, too, has often been modified through an increased proportion of propionate in the rumen. This is probable due to a decrease in the activity of cellulolytic bacteria. According to a recent review by Doreau and Ferlay (1995), lipid supplements decrease the protozoal counts in the rumen but this effect is very variable. They also noted that the negative effect on protozoa seems to be more marked with free than with esterified fatty acids and that the strongest decrease in protozoal number has been associated with a significant drop in the ammonia concentration in the rumen. Including a low level of free FA in a silage-based diet did not affect the rumen ammonia concentration or the protozoal number in FA diets. In the present trial, FA supplementation did not affect the rumen fermentation pattern, which is consistent with no change in protozoal numbers. In contrast, Tesfa (1992) reported a decrease in protozoal number, an increase in the proportion of propionate and a decrease in that of butyrate in the rumen in response to a daily supplement of 0.5 kg rapeseed oil.

### Effects of glycerol

In the present trial, inclusion of glycerol did not affect feed intake or milk yield and had only a slight effect on milk fatty acid composition and plasma urea concentration. Few papers have been published on the effects of glycerol as an energy yielding substrate in dairy cow feeding. Remond et al. (1991) reported no effects on milk yield, milk composition or food intake when glycerol was given in amounts to those in the present trial.

The ability to increase the proportion of propionate in the rumen at the expense of other volatile fatty acids by using glycerol was investigated in the present trial with grass silage-based diets. The effects of glycerol on rumen fermentation reported elsewhere have varied due to differences in diets; and the effects have not been studied using grass silage-based diets. Wright (1969) observed increases in acetic and propi-

onic acids but Remond et al. (1993) reported that glycerol increased butyrate more than it did propionate at the expense of acetate, as was noticed here, too. Glycerol did not affect either blood glucose or BHB concentrations, but Remond et al. (1991) observed that glycerol increased the plasma 3-hydroxybutyrate concentration. In the present trial the increase in butyrate was not due to the pH effect since the mean value of rumen pH was not affected with G diets. It is not easy to explain why butyrate increased more than did propionate with glycerol supplementation. It is possible, however that propionate formation depressed when fermentation proceeds rapidly in the rumen. Our results with grass silage-based diets confirmed the observations of Remond et al. (1993) that an increase in butyrate is not evidence of the glucogenic ability of glycerol in dairy cows given maize silage. According to Broster et al. (1970), both glucose and sucrose depressed the proportion of acetate and increased the proportions of propionate and butyrate when a basal diet of hay and concentrates was supplemented with glucose or sucrose in dairy cow feeding. They reported that glucose diets increased butyrate more than sucrose. Similarly, when cattle were fed purified diets, glucose diets increased the proportion of butyrate more than that of propionate (Ørskov and Oltjen 1967). Glucose and sucrose supplements tended to increase the proportion of propionate and butyrate. However, these changes were not statistically significant in goats given a silage diet (Chamberlain et al. 1985).

### Conclusions

The low level of fatty acids used only slightly decreased silage intake and diet OM digestibility. Fatty acids did not change the rumen fermentation type or the ammonia concentration or protozoal numbers in the rumen. On the other hand, fatty acids increased the milk yield and simultaneously decreased the milk protein content but

not the protein yield. Furthermore, milk composition changed and the proportions of C<sub>18:0</sub> and C<sub>18:1</sub> fatty acids in milk fat increased.

Our findings suggest that the addition of glycerol to the diet does not have any positive ef-

fects when cows are fed grass silage-based diets. However, when glycerol and fatty acids are given in combination there tended to be a positive effect on milk production.

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## SELOSTUS

**Väkirehuun lisätyn glyserolin tai vapaiden rasvahappojen tai näiden yhdistelmän vaikutus maidontuotantoon ja pötsifermentaatioon ruokittaessa lypsylehmiä säilörehuun perustuvalla ruokinnalla**

Hannele Khalili, Tuomo Varvikko, Vesa Toivonen, Kari Hissa ja Marjatta Suvitie  
*Maatalouden tutkimuskeskus ja Suomen Rehu Oy*

Tutkimuksessa selvitettiin lypsylehmille annettavaan väkirehuun lisätyn glyserolin tai suojaamattomien vapaiden rasvahappojen tai näiden yhdistelmän vaikutuksia rehun syöntiin, maidontuotantoon, maidon koostumukseen, pötsifermentaatioon, veriarvoihin ja ruokinnan sulavuuteen. Koejärjestelyssä mukana olleita 12 friisiläislehmää ruokittiin säilörehuun perustuvalla ruokinnalla. Koeruokintoja oli neljä. Kontrolliruokinnassa lehmille annettiin säilörehua ja perusväkirehua. Säilörehua lehmät saivat vapaasti ja perusväkirehua 7 kiloa päivässä. Kilogrammassa perusväkirehua oli 600 g ohraa, 80 g kauraa, 150 g leikettä, 95 g soijarouhetta, 20 g melassia ja muita 55 g. Kahdessa koeväkirehussa 36 g/kg ohraa oli korvattu joko glyserolilla tai rasvahapposeoksella. Yhdessä koeväkirehussa 72 g/kg ohraa oli korvattu glyserolin (36 g/kg) ja rasvahappojen (36 g/kg) yhdistelmällä. Rasvahappolisä koostui palmitiini-  $C_{16:0}$  (126 g/kg), steariini-  $C_{18:0}$  (22 g/kg), öljy-  $C_{18:1}$  (376 g/kg), linoli-  $C_{18:2}$  (274 g/kg), linoleenihaposta  $C_{18:3}$  (32 g/kg) ja vapaita rasvahappoja triglyseridimuodossa (170 g/kg).

Rasvahapot heikensivät ruokinnan sulavuutta ja säilörehun syöntiä. Rasvahapot eivät kuitenkaan muuttaneet pötsifermentaatiotyyppiä, pötsin ammoniakkipitoisuutta eikä alkueläinten lukumäärää pötsissä. Maitotuotos lisääntyi ja samalla maidon valkuaispitoisuus pieneni hieman, mutta valkuaistuotos ei vähentynyt. Maidon rasvahappokoostumus parani siten, että steariini- ja öljyhapon suhteelliset osuudet lisääntyivät.

Glyserolilisä muutti pötsifermentaatiotyyppiä vähentäen etikkahapon suhteellista osuutta ja lisäten propioni- ja voihiapon osuuksia. Muutokset fermentaatiossa olivat kuitenkin niin pieniä, etteivät beta-hydroksivoihapon ja glukoosin pitoisuudet veressä, maidontuotanto eikä maidon koostumus muuttaneet. Tämän kokeen tulosten perusteella glyserolilisäyksestä ei ollut hyötyä ruokittaessa lypsylehmiä säilörehuun perustuvalla ruokinnalla. Kuitenkin on todettava, että maidontuotanto oli suurinta annettaessa glyserolia ja rasvahappoja yhdessä.