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Effects of supplemental dietary fatty acids on milk yield and fatty acid composition in high and medium yielding cows

Martin Riis Weisbjerg*, Lars Wiking, Niels Bastian Kristensen and Peter Lund

Faculty of Agricultural Sciences, University of Aarhus, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark

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The present study tested the hypothesis that supplemental dietary fatty acids (FA) affect the energy corrected milk yield in proportion to the milk production level of dairy cows, and increase both long chain FA proportion of milk FA and milk fat globule diameter. Sixteen Danish Holstein cows were divided into four 4 × 4 Latin squares with two squares of medium yielding cows (32·2 kg energy corrected milk (ECM)/d; 158 days in milk (DIM)) and two squares of high yielding cows (40·0 kg ECM/d; 74 DIM). Experimental length was 12 weeks, with three weeks for each of the four periods. The four treatments were no supplementation (17 g FA/kg dry matter (DM)) and three diets with supplemented FA (29, 40, and 52 g total FA/kg DM, respectively) obtained by substituting barley with Palm Fatty Acid Distillate (PFAD) fat. Diets were offered as total mixed rations with 63% grass/clover silage (DM basis). Dry matter intake decreased with increasing FA supplementation, but net energy intake was not affected. The general linear responses to 10 g/kg DM increase in FA level were 1·1 kg ECM ($P<0·0001$), 0·061 kg milk fat ($P<0·0001$), 0·012 kg milk protein ($P=0·09$) and 0·052 kg lactose ($P=0·0002$) per day, and linear responses in milk composition were 0·39 g fat ($P=0·07$), −0·71 g protein ($P<0·0001$) and 0·05 g lactose ($P=0·3$) per kg milk, and 0·092 μm ($P<0·0001$) in milk fat average globule diameter. Fatty acid supplementation decreased short- and medium-chain FA and $C_{16:0}$ and increased $C_{18:1}$ proportions of total FA in milk. Supplemental dietary FA increased ECM yield but not in proportion to production level as anticipated, and increased average FA chain length and milk fat globule diameter.

Keywords: Milk fat globule diameter, fat, fatty acid, dairy cows, Milk composition.

Milk production responses to increased fat intake are often positive. Østergaard et al. (1981) observed that milk production responses to fat supplementation were greater in early lactation compared with responses later in the lactation (0·92 kg energy corrected milk (ECM) per day in early lactation and 0·56 kg ECM/d in later lactation (week 11–27) per 10 g fatty acid (FA) per kg dry matter (DM). Chilliard (1993) concluded from a literature review that responses generally were lower and contrary to the findings of Østergaard et al. (1981) the greatest responses were observed in late lactation. In a meta analysis by Onetti & Grummer (2004) fat supplementation increased milk production in early lactation, but not in mid lactation due to milk fat depression. Further, Onetti & Grummer (2004) found interactions between forage type and fat supplementation, with positive milk production response to fat supplementation when alfalfa made up at least half of the forage, but no response when corn silage was the main forage. This could be due to less hydrogenation of unsaturated FA in corn silage diets with low physical structure value (Harfoot & Hazlewood, 1988). Hydrogenation of unsaturated FA in the rumen is excessive (Weisbjerg et al. 1992a), and with higher hydrogenation activity negative effects of unsaturated FA on rumen fermentation are reduced. Different fat sources can differ considerably in milk production response, due to different effects on rumen fermentation (Doreau & Chilliard, 1997), caused by differences in FA chain length and saturation, where especially medium chain (C10–C14) and unsaturated FA reduce rumen cellulolytic activity (Weisbjerg & Børsting, 1989). On the other hand, FA are nonfermentable and therefore do not reduce rumen pH.

Also intestinal FA digestibility can vary depending on dietary FA level and composition (Weisbjerg et al. 1992b), and thereby affects energy value.

In Danish recommendations (Strudsholm et al. 1999) ECM responses to increased FA supplementation are

*For correspondence; e-mail: martin.weisbjerg@agrsci.dk

Table 1. Composition of concentrates (kg/100 kg)

	No fat concentrate	PFAD concentrate
Barley	37·8	27·9
Dried sugar beet pulp	26·7	26·7
Soybean meal	32·9	—
Soypass†	—	32·9
PFAD‡	—	9·86
Mineral Premix	2·38	2·40
Vitamin Premix	0·23	0·24

† Rumen protected soybean meal (Raiffeisen Hauptgenossenschaft Nord AG, Germany)
‡ Palm Fatty Acid Distillate (palm oil product), Scanfedt A/S, Århus, Denmark

Table 2. Composition of TMR for the four treatments (% of DM)

Treatment†	**FA-17**	**FA-29**	**FA-40**	**FA-52**
Grass/clover silage‡	62·8	62·6	62·5	62·4
No fat concentrate	37·2	24·9	12·5	
PFAD concentrate		12·5	25·0	37·6

‡ FA-17, FA-29, FA-40 and FA-52 refer to 17, 29, 40 and 52 g FA/kg ration DM, respectively
† Botanical composition 82·9% grass and 17·1% clover, % of DM

assumed to be proportional to the milk yield. However, the literature is inconclusive on this question, and in NRC (2001) it was concluded that milk-yield responses to supplemental fat in high producing cows are not well defined. The aim of the present study was to test the hypothesis that milk production response to supplemental dietary fat is proportional to cow's production level and to investigate the effects of supplemental dietary fat on milk production and milk quality variables.

Materials and methods

Cows, design, feeds, and treatments

The experiment compiled with the guidelines of the Danish Ministry of Justice (Act no 726, 1993) with respect to animal experimentation and care of animals under study.

Sixteen Danish Holstein cows were split into two groups of eight cows according to their production level (high vs. medium milk yield) at the start of the experiment, where cows were fed the same standard ration. Both groups of eight cows were further divided into two groups on the basis of parity (2nd and 3rd parity), resulting in four 4×4 Latin squares. Each 4×4 square used four animals. Within square, animals were given one of the four treatments in each of the four periods. The periods lasted three weeks, i.e. a 12-week experiment. Production level was confounded with DIM (DIM; high yielding cows 74 (48–99); medium yielding cows 158 (53–208) at onset of the experiment). The four treatments differed by the dietary FA level: unsupplemented (17 g FA/kg ration DM) and three treatments with partial substitutions of barley with Palm Fatty Acid Distillate (PFAD) fat resulting in 29, 40 and 52 g FA/kg DM, respectively. FA concentration in ration DM is hereafter referred to as FA_{ration} and individual treatments as FA-17, FA-29, FA-40 and FA-52, respectively.

Rations were fed as total mixed rations (TMR) based on grass/clover silage and two concentrates with the same composition except for substitution of barley with PFAD on weight basis, and substitution of soybean meal with Soypass (rumen protected soybean meal) to account for the reduction in metabolizable protein when barley is substituted with PFAD. Composition of concentrates is given in Table 1. Increased FA_{ration} was obtained by substituting no-fat concentrate by increasing amounts of PFAD concentrate in the TMR, as shown in Table 2. Cows had free access to water.

Chemical composition of feeds

Chemical composition of grass/clover silage, concentrates and PFAD fat is given in Table 3, and fat and FA composition of feeds are given in Table 4. Grass silage FA were mainly linolenic, palmitic and linoleic acid. No-fat concentrate FA were mainly linoleic, palmitic, and oleic acid. Concentrate added PFAD contained mainly palmitic, oleic, and linoleic acid, similar to the PFAD fat.

Management, sampling and recordings

Cows were fed equally sized portions at 08·15 and 14·00. The amount was adjusted daily to ensure minimum 5% orts. Feed offered and orts were recorded daily.

Cows were milked at 04·50 and 16·00. Milk yield and concentration of fat, protein, lactose, and citrate were recorded the three last days of each period. Milk yield was recorded and milk samples obtained using Tru Tester (Tru Test Distributors Ltd., Auckland, New Zealand). Milk samples for analysis of milk fat globule (MFG) diameter, milk free fatty acids (FFA), and milk FA were obtained from the morning milking on the last day of each period.

Cows were weighed and scored for body condition at 13·00 at onset of the experiment and on the second last day in each period. Body condition score (BCS) was recorded using a scale from 1 (thin) to 5 (fat).

Feed samples (silage and concentrates) were collected weekly and composited in samples covering the first half and the second half of the experiment, respectively.

Milk analysis

Milk samples were analysed for fat, protein, lactose (monohydrate), and citrate using a MilkoScan 4000 instrument (Foss Electric A/S, Hillerød, Danmark).

Table 3. Chemical composition of feedstuffs

	Grass/clover silage	No fat concentrate	PFAD concentrate	Fat (PFAD)
DM %	46·4	89·0	89·4	99·8
% in DM				
Ash	8·79	8·55	6·72	
Crude fat	2·70	2·28	12·48	100
Crude protein	14·8	22·8	21·4	
NDF	41·4	20·7	20·7	
Sugar	12·8	6·6	6·4	
Starch		17·9	17·1	
In vitro digestibility of OM (%)				
Rumen fluid method	74·6			
Enzymatic method		94·7	96·7	

Tabel 4. Fat and fatty acid composition of ingredients in TMR and of PFAD

	Grass/clover silage	No fat concentrate	PFAD concentrate	Fat (PFAD)
Fatty acids (% in DM)	1·63	1·91	11·08	95·0
Fatty acids (% in crude fat)	60·5	83·8	88·8	95·0
Fatty acid composition (weight %)				
$C_{12:0}$	0·14	0·05	0·25	0·35
$C_{14:0}$	0·42	0·23	0·91	1·10
$C_{16:0}$	17·6	21·6	38·5	43·2
$C_{16:1}$	0·16	0·19	0·17	0·15
$C_{18:0}$	1·94	2·87	4·39	4·71
$C_{18:1}$	3·41	14·3	37·0	38·6
$C_{18:2}$	16·1	51·4	16·3	10·4
$C_{18:3\omega3}$	55·8	6·45	1·14	0·30
$C_{20:0}$	0·65	0·27	0·37	0·39
$C_{20:1}$	0·12	0·60	0·24	0·16
$C_{20:2}$	0·31	0·16	0·04	0·00
$C_{22:0}$	1·20	0·57	0·16	0·08
$C_{24:0}$	1·02	0·45	0·13	0·04
Other FA†	1·15	0·93	0·36	0·47

† Fatty acids below 0·25% of total FA ($C_{8:0}$, $C_{10:0}$, $C_{13:0}$, $C_{14:1}$, $C_{15:0}$, $C_{17:1}$, $C_{20:3\omega6}$, $C_{20:4}$, $C_{20:3\omega3}$, $C_{20:5}$, $C_{22:1\omega11}$, $C_{22:1\omega9}$, $C_{22:5\omega6}$, $C_{22:5\omega3}$, $C_{22:6\omega3}$, $C_{24:1}$)

Particle size distribution of MFG was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) as previously described by Wiking et al. (2003). The volume-weighted diameter, $d_{(4,3)}$ (μm), was calculated by the integrated software.

Milk FFA were analysed using the B.D.I. method (International Dairy Federation, 1991). The milk was stored for 24 h at 4 °C before determination of FFA. FFA were analysed on composite samples within treatment and within period (n=16).

Milk FA composition was determined by gas chromatography (GC) after methylation of the lipids. The GC used a FFAP-column (terephtalic acid modified polyethylene glycol 25m × 200 μm × 0·30 μm) with helium as carrier and a flame ionization detector as described in detail by Wiking et al. (2003). One sample was lost and data on milk FA are based on 63 observations.

Feed Analysis

Concentrate DM was determined after drying in a forced air oven for 20 h at 103 °C, and silage DM after drying at 80 °C. Samples for chemical analyses were ground through a 1·0 mm screen. Ash was determined by combustion at 525 °C for 6 h. Total N content was determined according to the Dumas principle (Hansen, 1989). Ash free neutral detergent fibre (NDF) was analysed using a Fibertec according to Mertens (2002). Starch was determined as described by Åman & Hesselman (1984). Crude fat was determined using a Soxhlet-apparatus with petroleum ether extraction after 3 M-HCl hydrolysis (Stoldt, 1952). Feed FA were analysed using gas chromatography on the extracted crude fat (Knarreborg et al. 2004), except for PFAD which was extracted using Bligh & Dyer extraction according to Knarreborg et al. (2004). In vitro

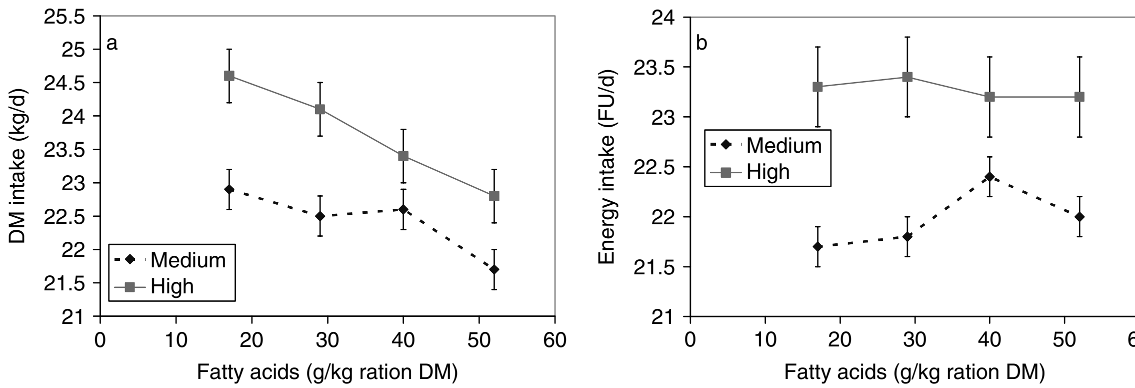

Fig. 1. Effect of FA intake on DM (a) and net energy (FU) (b) intake for the high and medium yielding cows. Significant linear effect for DM intake (P=0·001). Linear effect for FU, quadratic effects, and interactions between linear and quadratic FA_{ration} and production level for both dry matter and FU intake were non significant.

digestibility of forage organic matter (OM) was determined using the two step rumen fluid method described by Tilley & Terry (1963). In vitro enzyme digestibility of concentrate OM was determined according to Weisbjerg & Hvelplund (1993). Sugar was analysed as reducing sugars according to Jacobsen (1981).

Calculations

Feed intake calculations were based on registrations from the seven last days in each period, and milk production on registrations the last three days in each period. Net energy intake based on chemical composition and in vitro OM digestibility is reported as feed unit cattle (FU), where one FU equals 7·89 MJ net energy (Weisbjerg & Hvelplund, 1993).

Energy corrected milk was calculated as: ECM (kg)=0·01M+12·2F+7·7P+5·0339L, where M is milk yield in kg and F, P, and L are fat, protein, and lactose (monohydrate) in kg, respectively (Sjaunja et al. 1990).

Data were analysed using PROC GLM for analysis of variance and using PROC MIXED for random regression (SAS, 2001). Test for general linear effect and correlation between individual cow level (intercept) and response to FA supplementation (regression coefficient) were analysed by likelihood ratio tests using random regression in PROC MIXED with a model with period and cow as class variables and FA_{ration} as regression variable. Calculations were performed with a model assuming no correlation (type=VC) and a model assuming correlation (type=UN). Difference in -2 log likelihood between the two models was assumed χ^2 distributed with one degree of freedom (=difference in estimated parameters in the two models), and ranges for P-values were obtained from statistical tables.

Error bars in figures are based on standard error of LSMEANS from a GLM model by production level and with FA_{ration}, block, and period as class variables.

Linear and quadratic effects and interactions between FA_{ration} and production level were tested by orthogonal contrasts in a model including FA_{ration}, production level, block, and period analysed with PROC GLM (data given in Figures).

Results and discussion

Feed intake

Feed intake responses to increased FA_{ration} are shown in Fig. 1. DM intake decreased (P=0·001) from 24·6 to 22·8 and from 22·9 to 21·7 kg/d for high and medium yielding cows, respectively, as FA_{ration} increased from 17 to 52 g/kg DM (Fig. 1a). However, as ration energy concentration increased with increased FA_{ration}, net energy intake was not affected by treatments (Fig. 1b). The general linear effect on DM intake with increasing FA_{ration} was −0·42 kg/d per 10 g FA/kg DM, and the response to increased FA_{ration} was not affected by cows actual feed intake level (Table 5). Total FA intake increased from 0·412 to 1·154 kg/d with increasing dietary FA level.

The negative response in DM intake with increasing FA_{ration} was greater than reported in a literature review by Chilliard (1993) who found DM intake to decrease by 0·20 kg when crude fat in feed DM was increased with 10 g/kg. The response to increased FA compared with increased crude fat should be 5–15% higher, as FA proportions of supplemental fat normally vary from 0·85–0·95. However, this can only explain part of the greater decrease in DM intake observed in the present study. In the experiments reviewed by Chilliard (1993) increased fat intake reduced DM intake less in very early lactation compared with later in lactation. In a meta analysis Onetti & Grummer (2004) concluded that cows in mid lactation reduced feed intake more than cows in early lactation when the level of dietary fat increased. In the present experiment high yielding cows (early lactation) numerically

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Table 5. Linear effects of increased dietary FA concentration on milk yield and composition, and test for correlation between individual cow parameter level (intercept) and response on FA concentration (regression coefficient) analysed by random regression

	General linear effect of FA		Correlation between intercept and regression coefficient†		
	β‡	*P* value	Correlation	χ^2	*P* value
DM intake (kg)	−0·42	<0·0001	Neg.	1	Ns§
Milk (kg)	1·1	<0·0001	Neg.	5·6	0·005<P<0·01
ECM (kg)	1·1	<0·0001	Neg.	5·2	0·005<P<0·01
Fat (kg)	0·061	<0·0001	Neg.	0·4	Ns
Protein (kg)	0·012	0·09	Neg.	12·0	P<0·001
Lactose (kg)	0·052	0·0002	Neg.	6·1	0·01<P<0·025
Fat (%)	0·039	0·07	Pos.	6·5	0·01<P<0·025
Protein (%)	−0·071	<0·0001	Neg.	0·2	Ns
Lactose (%)	−0·005	0·3	Neg.	0·4	Ns
Protein/fat ratio	−0·024	<0·0001	Pos.	2	Ns
Citrate (%)	0·0064	<0·0001	Pos.	0	Ns
Fat globule diameter (μm)	0·092	<0·0001	Neg.	0·1	Ns
FA composition (% of total milk FA)					
$C_{4:0}$–$C_{10:0}$	−0·59	<0·0001	Pos.	0·1	Ns
$C_{12:0}$–$C_{14:0}$	−2·2	<0·0001	Neg.	1·8	Ns
$C_{16:0}$	−0·52	0·0009	Neg.	19·2	P<0·001
$C_{18:1}$	1·8	<0·0001	Neg.	1·5	Ns

† Likelihood ratio test
‡ Increase when FA is increased with 10 g/kg DM (1% of DM)
§ Non significant

decreased DM intake slightly more than medium yielding cows (later lactation). However, the effect was far from significant (Figure 1, Table 5).

Cows weight and body condition

The cows gained weight during the experiment; average live weight (LW) was 620±15, 634±16, 641±18, 654±18 and 659±20 kg, and BCS was 2·7±0·2, 2·7±0·2, 2·9±0·2, 2·9±0·2 and 3·0±0·2 at experimental onset and at the end of period 1, 2, 3 and 4, respectively. Medium yielding cows increased LW with 53±9 kg and BSC with 0·5±0·1, whereas high yielding cows LW only increased with 25±6 kg and BSC with 0·1±0·1 during the experiment.

Milk yield

Milk (*P*=0·002) and ECM (*P*=0·004) yield responded positively to increased FA_{ration} (Fig. 2a,b). It was expected that ECM response on FA_{ration} was almost proportional to actual milk production, but the opposite was found. The response in ECM to the increase in dietary FA from 17 to 52 g FA/kg feed DM was 16% for the medium yielding cows, and only 8% for the high yielding cows. In absolute amounts, the response to increased FA_{ration} was almost similar for the medium and the high yielding cows, although numerically slightly higher for the medium yielding cows, as response seemed to be moderate on the higher FA_{ration} levels for the high yielding cows. However, interaction between FA_{ration} and production level was not significant. The response in ECM analysed on individual cow basis was negatively correlated to cows' production level. The general linear response was 1·1 kg ECM per 10 g/kg DM increase in FA_{ration} (Table 5). The milk production response was high compared to literature values, especially for the medium yielding cows. Onetti & Grummer (2004) found milk production to increase with 1·40 kg/d in early lactation, and decrease with −0·11 kg/d in mid lactation when ether extract was increased with an average of 3% of DM, and Østergaard et al. (1981) found fat corrected milk production to increase with 0·92 kg/d in early and 0·56 kg/d in later lactation when FA was increased with 10 g/kg feed DM. However, Chilliard (1993) in his review, found higher responses in later lactation compared with early, more in agreement with the present experiment.

Energy evaluation systems might also be responsible for varying conclusions in different studies. Different classical energy evaluation systems differ considerably in the ratio between fat and starch energy value. Also milk production responses to increased supplemental fat related to energy supply will therefore differ according to the energy evaluation system and thereby affect conclusions. The considerable positive effects of increased FA_{ration} on milk production, despite constant net energy intake (Fig. 1b), indicate that the energy factor used in the Danish FU system (Weisbjerg & Hvelplund, 1993; Hvelplund et al. 2007) severely underestimates net energy value of supplemental fat compared to starch, when the supplemental fat is rich in FA as in the present experiment.

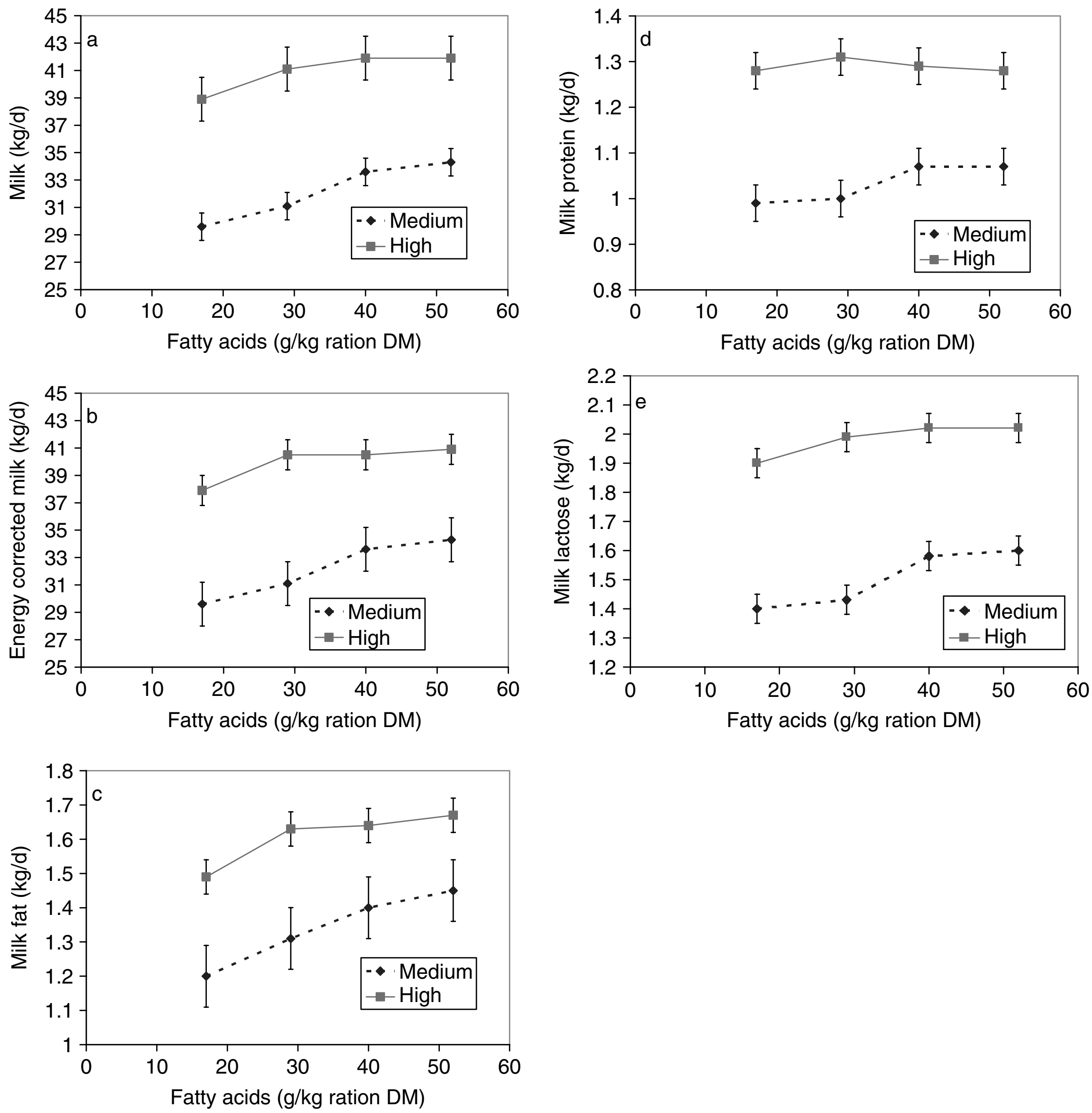

Fig. 2. Effect of FA intake on milk (a), energy corrected milk (ECM) (b) and solid yield (c, d, e) for the high and medium yielding cows.
Significant linear effects except for protein yield (P for a 0·002, b 0·004, c 0·003, d 0·3, e 0·0004). Quadratic effects, and interactions between linear and quadratic FA_{ration} and production level were non significant for all shown yield measures.

Increased FA_{ration} increased fat and lactose, but not protein yield in kg/d (Fig. 2c,d,e). The responses to increased FA_{ration} in daily yield of fat, protein and lactose were negatively correlated to the cows' actual yield of fat, protein, and lactose, respectively, but not significantly for fat (Table 5). General linear responses (kg/d) per 10 g increase in FA_{ration} were 0·061 ($P<0·0001$), 0·012 ($P=0·09$) and 0·052 ($P=0·0002$) for fat, protein, and lactose, respectively (Table 5).

Milk composition

Increased FA_{ration} increased fat, and decreased protein ($P=0·0002$) and lactose concentration in milk. However, only the effect on protein was significant (Fig. 3a,b,c). For milk fat concentration, response was positively correlated to cows' actual milk fat concentration (Table 5). General linear responses (percent units) per 10 g increase in FA_{ration} were 0·039 ($P=0·07$), −0·071 ($P<0·0001$) and −0·005 ($P=0·3$) for fat, protein, and lactose concentration, respectively (Table 5). However, the response was significant only for protein. The negative effect of dietary fat supplementation on milk protein concentration is normally regarded as a dilution of a constant protein production with an increased milk production in kg, which this experiment also indicates as total protein production in kg was positively, although insignificant, affected by increased FA_{ration}. However, it could also be due to a direct

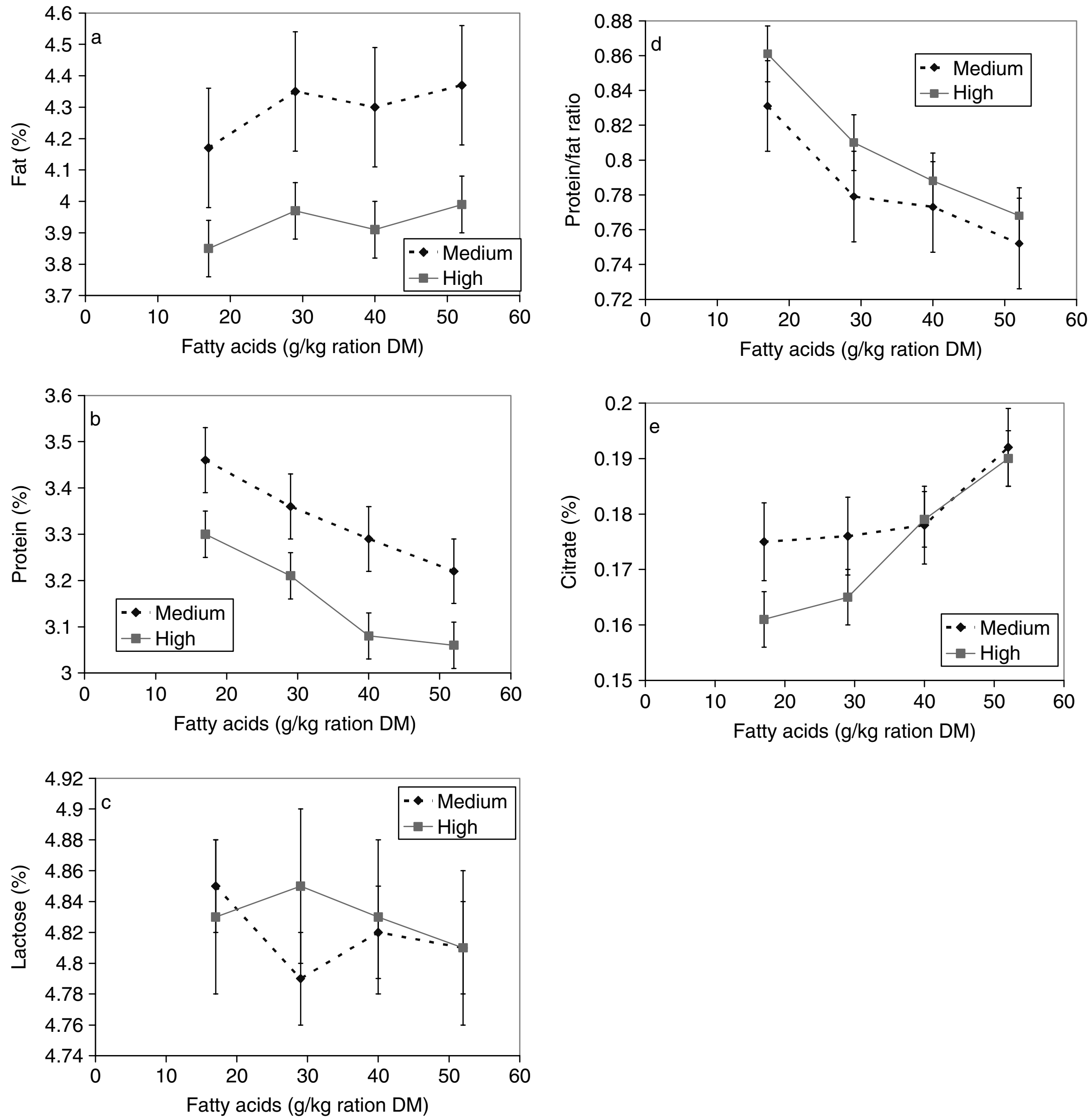

Fig. 3. Effect of FA intake on milk composition for the high and medium yielding cows.
Significant linear effects except for fat % and lactose % (*P* for a 0·3, b 0·0002, c 0·7, d 0·0002, e 0·0008). Quadratic effects, and interactions between linear and quadratic FA_{ration} and production level were non significant for all shown milk composition measures.

effect on protein synthesis, as DePeters & Palmquist (1990) found that fat supplementation reduced casein concentration more than concentration of non protein and whey proteins.

When FA_{ration} was increased from 17 to 52 g/kg ration DM, the combined effect of a reduced protein and a slightly increased fat concentration heavily reduced ($P<0·0001$) the protein/fat ratio in milk, which was reduced from 0·83 to 0·75 and 0·86 to 0·77 for medium and high yielding cows, respectively (Fig. 3d). The general linear response in protein/fat ratio was −0·024 per 10 g increase in FA_{ration} ($P<0·0001$) (Table 5). Thereby fat supplementation seriously affects the composition of the value solids in milk (protein and fat).

Citrate concentration was highly affected by increased FA_{ration}. Increased FA_{ration} from 17 to 29 g FA/kg ration DM affected only citrate concentration slightly (Fig. 3e). However, neither quadratic FA_{ration} effect nor interactions between production level and FA_{ration} were significant. The general linear response (percent units) per 10 g increase in FA_{ration} was 0·0064 ($P<0·0001$), and the response was not correlated to cows' actual citrate concentration (Table 5). A higher citrate concentration reflects a decreased de novo FA synthesis in the mammary gland, as

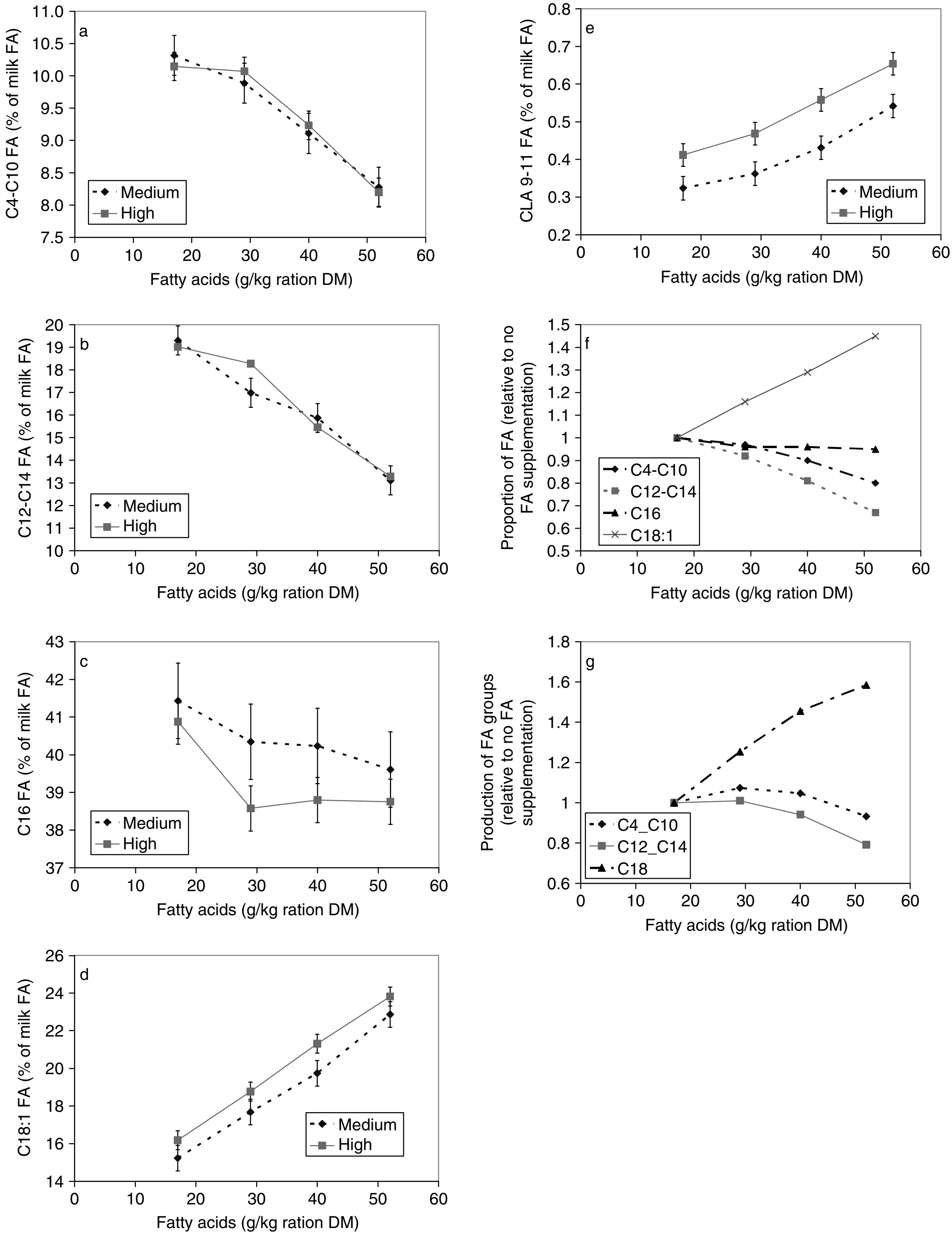

Fig. 4. Effect of FA intake on milk FA composition (% of total FA) for the high and medium yielding cows (a, b, c, d, e), and relative changes in proportions (f) and in daily FA production for some FA groups (g).
Significant linear effect for all shown FA composition measures (*P* for a <0·0001, b <0·0001, c 0·04, d <0·0001, e <0·0001), and significant quadratic effect on C4–C10 (*P*=0·02). Quadratic effects (except $C_{4:0}$–$C_{10:0}$), and interactions between linear and quadratic FA_{ration} and production level were non significant for all shown milk FA composition measures.

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Table 6. FFA in milk fat (mmol/100 g fat)

	FA-17†	FA-29	FA-40	FA-52	SEM	*P* value‡	*P* value§	*P* value¶
FFA	0·603	0·583	0·605	0·608	0·017	0·7	0·6	0·6

† FA-17, FA-29, FA-40 and FA-52 refer to 17, 29, 40 and 52 g FA/kg ration DM, respectively
‡ Proc GLM, model including period and treatment as class variables. Only composite samples over treatments per period were analysed
§ Linear contrast
¶ Quadratic contrast

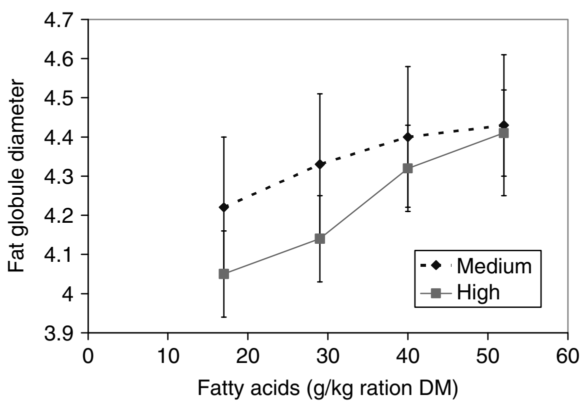

Fig. 5. Effect of FA intake on average milk fat globule diameter (μm) for the high and medium yielding cows.
Significant linear effect ($P=0{\cdot}03$). Quadratic effects, and interactions between linear and quadratic FA_{ration} and production level were non significant.

citrate is involved in the de novo FA synthesis (Faulkner & Peaker, 1982; Garnsworthy et al. 2006). Therefore, the increased citrate concentration indicates a decreased de novo FA synthesis in the mammary gland as FA_{ration} is increased, which also milk FA composition indicates.

Milk FA composition was affected by FA_{ration} as shown in Fig. 4. Short and medium chain FA ($<C_{16}$) proportion of FA decreased considerably (Fig. 4a,b). Short chain FA ($<C_{12}$) did not change when FA_{ration} was increased from FA-17 to FA-29, but FA-40 and FA-52 resulted in more severe decreases (quadratic FA_{ration} effect significant, $P=0{\cdot}02$). This indicates, that the increase in FA_{ration} from FA-17 to FA-29 mainly resulted in a dilution of the de novo synthesised FA, whereas FA-40 and FA-52 both diluted and reduced de novo synthesised FA. Also, daily FA production indicates that *de novo* synthesis is not reduced by FA-29, and when de novo synthesis was reduced, it was mainly due to reduced $C_{12:0}$–$C_{14:0}$ synthesis, whereas daily production of $C_{4:0}$–$C_{10:0}$ was more constant (Figure 4g). However, no significant quadratic effects of FA_{ration} were found for the respective milk FA groups. The negligible effect of FA addition on the proportion of short chain FA in total milk FA is in accordance with Avila et al. (2000), who only found a minor decrease in C_4–C_8 proportion of milk FA when supplementing rations with fat differing in degree of saturation. The negative effect of increased FA_{ration} on de novo FA synthesis, seen in the FA proportions and daily productions, is in accordance with the positive effect on citrate concentration (Fig. 3e), which also mainly was affected by FA-40 and FA-52. Proportion of C_{18} FA in total milk FA increased with increased FA_{ration}, whereas $C_{16:0}$ FA (palmitic acid) only decreased slightly, indicating that increased supply by the feed was counteracted by decreased de novo synthesis of $C_{16:0}$ (Figure 4c,d,f). The negligible effect of increased FA_{ration} on $C_{16:0}$ concentration in milk, although C_{16} proportion of added FA was high, is in accordance with prediction equations proposed by Hermansen (1995). According to these equations predicting $C_{16:0}$ proportion of milk FA based on FA concentration and proportion of $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ in the feed ration, $C_{16:0}$ proportion in milk should have been 33·0, 35·3, 35·6 and 34·9 (weight %), where measured $C_{16:0}$ proportion was 41·2, 39·5, 39·5 and 39·2 (weight %) for FA-17, FA-29, FA-40 and FA-52, respectively. The higher proportion of $C_{16:0}$ was especially on the expence of $C_{4:0}$–$C_{10:0}$ FA.

The proportion of CLA (9, 11) increased substantially with increased FA_{ration} (Fig. 4e).

Desaturase activity in the mammary gland estimated as $C_{14:1}/(C_{14:1}+C_{14:0})$ as recently proposed by Garnsworthy et al. (2006), decreased ($P<0{\cdot}0001$) with increased FA_{ration} from 0·099 to 0·081 (values not shown), which is comparable to the range 0·081–0·087 found by Garnsworthy et al. (2006).

Milk fat quality

Recently, studies have shown that cheese produced by small MFG is less firm compared with cheese produced from large MFG (Michalski et al. 2003 & 2004). In the present study, MFG diameter for the lowest FA_{ration} levels seemed to be lower for high than for medium yielding cows, accordingly to a lower fat concentration (Fig. 5 & Fig. 3a). However, interaction between production level and FA_{ration} was not significant. The average diameter of MFG in general increased by 0·092 μm ($P<0{\cdot}0001$), when FA_{ration} was increased by 10 g/kg DM (Table 5). Recent studies have shown that feeding a diet with a high level of saturated fatty acids results in milk with MFG having a large average diameter (Wiking et al. 2003 & 2005), and further the average diameter of MFG is positively correlated with daily fat yield (Wiking et al. 2004).

Within production level, positive correlation between average diameter of MFG and daily fat yield was

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confirmed by the present study, and in accordance with the findings of Carroll et al. (2006). Large MFGs have been associated with high concentration of FFA in milk (Wiking et al. 2003 & 2005). In the present study, no effect on FFA was observed when FA_{ration} was increased (Table 6). This indicates that the use of moderately saturated dietary FA has no effect on spontaneous lipolysis in milk, in contrast to highly saturated dietary FA, which increases the FFA level in milk (Astrup et al. 1980; Wiking et al. 2003). The reason could be that the increase in the average diameter of MFG is lower compared with the other studies.

High levels of saturated FA can impair the milk quality by increasing FFA in milk, which can result in rancid flavour in dairy products. In studies, which used high levels of palmitic acid as fat supplements, the result was milk with high FFA content both spontaneous (Astrup et al. 1980) and after pumping (Wiking et al. 2003). In general, the FA supplement used in the present study increased the milk production without impairing the milk fat quality. Furthermore, the study demonstrates that it is feasible to design the distribution of MFG through feeding.

In conclusion, energy corrected milk yield increased with increased FA_{ration}, and milk protein concentration and especially protein/fat ratio in milk decreased. In contradiction to the hypothesis, the response to increased FA_{ration} in yield of energy corrected milk was negatively correlated to the individual cows' actual milk production level.

Proportion of short and medium chain FA in total milk FA decreased and C18 FA increased with increased FA_{ration}, whereas palmitic acid (C_{16}) proportion only decreased slightly.

Based on milk FA composition and production, as well as milk citrate concentration, increased FA_{ration} up to 29 g/kg DM seemed to dilute de novo synthesised FA in the mammary gland only, whereas increasing FA_{ration} up to 40 and 52 g/kg DM also decreased de novo synthesis itself, especially of $C_{12:0}$–$C_{14:0}$.

Milk fat globule diameter increased with increased FA_{ration}, whereas FFA level was unaffected.

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