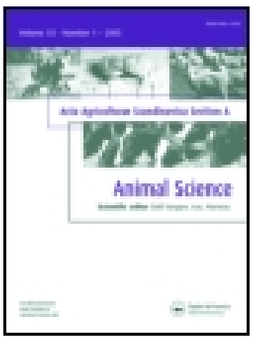

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Acta Agriculturae Scandinavica, Section A — Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/saga20

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To cite this article: Mikaela Lindberg (2020): Milk fatty acid profiles from inclusion of different calcium salts in dairy cow diets, Acta Agriculturae Scandinavica, Section A — Animal Science, DOI: 10.1080/09064702.2020.1781925

To link to this article: https://doi.org/10.1080/09064702.2020.1781925

Published online: 24 Jun 2020.

ACTA AGRICULTURAE SCANDINAVICA, SECTION A — ANIMAL SCIENCE
https://doi.org/10.1080/09064702.2020.1781925

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BRIEF REPORT

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Milk fatty acid profiles from inclusion of different calcium salts in dairy cow diets

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ABSTRACT

This experiment evaluated the effects of inclusion of three different types of calcium salts (CaS): mixed fatty acids, 70% palmitic acid-based and rapeseed oil-based, on milk production and milk fatty acid composition. Dietary treatments were randomly assigned to six mid-lactation dairy cows in a double Latin square experimental design with three 21-days experimental periods. All cows received a similar amount of grass-clover silage and concentrates and an additional 800 g of CaS daily. Analysis of milk fatty acids showed that a high proportion of palmitic acid in the feed resulted in milk fat with significantly higher levels of C16 fatty acids than with CaS based on rapeseed oil. Milk from cows on the diet with rapeseed oil based CaS contained significantly higher concentrations of C18 fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and trans fatty acids than milk from cows on the diet with palmitic acid CaS.

ARTICLE HISTORY
Received 24 February 2020
Accepted 4 June 2020

KEYWORDS
Rumen protected fat; rapeseed oil; palm oil; milk fatty acid

Introduction

Milk fat composition can be strongly affected by dairy feed components, especially if lipids are added to the diet. A typical fatty acid (FA) composition in cow's milk is around 70% saturated fatty acids (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA). If unsaturated FA (UFA) are included in the diet, a large proportion (60–90%) is hydrogenated in the rumen into SFA, mainly stearic acid C18:0 (Grummer, 1991). In calcium salts (CaS) of FA, the fat is largely protected from biohydrogenation and the UFA are taken up into the blood and transported to the udder, resulting in a higher proportion of UFA in the milk. Adding lipids to the feed of dairy cows has also been found to reduce the production of enteric methane (Knapp et al., 2014). Palm oil is often used as a fat source in animal feed, but the cultivation of oil palm is now being strongly questioned, not least for the impact of deforestation on greenhouse gas emissions (Zarin et al., 2016). Therefore, it is important to find other options for palm oil. The aim of the present study was to investigate feed consumption, milk production and milk FA composition when three different sources of CaS FA were added to a basal diet of cereal grain concentrate and grass-clover silage for dairy cows. The hypothesis was that dry matter intake (DMI) and milk yield would be similar regardless of FA source and that the milk FA composition resulting from diets containing CaS based on rapeseed oil would be improved in a human nutrition perspective compared with CaS based on palm oil. The products compared were the mixed FA AkoFeed Lac 45 (AAK, Karlshamn, Sweden), one with a high proportion of palmitic acid and one based on rapeseed oil FA.

Material and methods

Animals, treatments and experimental design

The experiment was conducted at Lövsta national research centre, Uppsala, Sweden, on March 4 to May 5 2013, and designed as a double Latin square with six cows of the Swedish Red breed, three treatments, and three 21-days periods. The first 14 days of each period were used for adaptation to the CaS, while data collection and sampling were performed during the last 7 days. The cows were multiparous and were 193 ± 19 days in milk (DIM) (mean ± SD) at the start of the experiment. They were tied in stalls during the whole experiment and milked twice daily, at 05.00 and 17.00 h. All cows were weighed and scored for body condition at the start and end of the experiment. At the start, they had body weights (BW) of 682 ± 64 kg and body condition score (BCS) of 3.5 ± 0.4 on a five-point scale (DEFRA, 2001). All cows were managed according to the Swedish Animal Welfare Act, Animal Welfare Ordinance (SFS 1988:534, 1988) and the European Communities Council Directive 86/609/EEC. The experiment was approved by Uppsala Local Ethics Committee C21/13.

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The treatments consisted of daily diet supplementation with 800 g of CaS as: (1) a mixed FA (MFA), (2) palmitic acid-based (P70) or (3) rapeseed oil-based (RO). The basal diet consisted of grass-clover silage and concentrates. During the first period, all cows were fed 10 kg dry matter (DM) silage and 12 kg concentrate per day. During the second and third periods, the daily feed ration was adjusted to the natural decline in milk yield by lowering it to 9 kg DM silage and 11 kg concentrate. The cows were supplied daily with 150 g minerals (Mg1.8, Kvarnbyfoder, Staffanstorp, Sweden). All feeds were given in four daily feedings, at 05.00, 09.00, 13.00 and 18:00 h. The CaS was mixed into the concentrate manually at the time of feeding and all feeds were weighed and fed manually. Orts were collected and weighed once daily, before the first feeding. The feed ration was calculated according to the Swedish feeding recommendations (Spörndly, 2003) to balance the energy, protein and mineral content according to the average requirement of the cows at the start of the experiment and at the start of the second period.

The silage used was a second cut harvest grass-clover mix consisting of timothy (*Phleum pratense L.*), meadow fescue (*Festuca pratensis L.*) and approximately 100 g/kg DM of red clover (*Trifolium pratense L.*). The concentrate (Vega 200, Johan Hansson AB, Uppsala, Sweden) was pelleted and had the following composition (% of feed): barley (27); rapeseed meal (16); molassed beet fibre (16); peas (12); rapeseed cake (10); wheat (8); wheat bran (8); minerals and vitamins (3). The chemical composition of the feeds is shown in Table 1.

All the CaS were composed of 85% FA and 15% $Ca(OH)_2$ with a DM content of 97% and 13% ash. The mixed FA product (MFA) contained FA from palm oil. The P70 product contained 70% palmitic acid (C16:0). The RO product contained FA from rapeseed oil and had a higher concentration of MUFA and PUFA than the other CaS (Table 2). All CaS contained 27.2 MJ ME per kg DM, tabulated value (Spörndly, 2003).

Samples of silage were collected five days per week, immediately frozen at −20°C and pooled into one sample per 14-days period. Concentrate was sampled twice per week and pooled into one sample per period. Forage feed samples were dried overnight at 60°C in a forced-air oven. Dry matter in concentrates was determined by drying at 103°C for 16 h. Ash was determined after incineration for 3 h at 550°C in a pre-heated furnace. Total N concentration was analysed using a fully automated Kjeldahl procedure (Technicon, Solna, Sweden) and CP was calculated as N × 6.25. Ether extract in the concentrate was analysed by prior hydrolysis (EC, 2009). Metabolisable energy in forages was calculated from in vitro digestibility (Lindgren, 1979) and starch in the concentrate was analysed enzymatically (Larsson & Bengtsson, 1983).

Milk samples were obtained from four consecutive milkings during the last week of each period. Samples for analysis of milk composition and SCC were treated with bronopol preservative and stored at +4°C until analysis. Concentrations of milk fat, protein and lactose were determined by infrared spectroscopy (MilkoScanTM FT120, Foss, Hillerød, Denmark) and ECM was calculated according to Sjaunja et al. (1990). Milk samples for analysis of FA profiles were immediately frozen at −80°C. Before milk fat extraction, the samples were thawed, pooled and weighed according to milk yield into one sample per cow and period.

Milk lipids were extracted in a modification of the method described by Nourooz-Zadeh and Appelqvist (1988). In brief, 10 mL hexane:isopropanol (3:2 vol/vol) were added to 2 mL milk in a Teflon tube and shaken vigorously. Then 5 mL Na_2SO_4 (6.67% solution) were added and the tubes were shaken and centrifuged using a Sorvall RC-5 (Thermo Scientific, Waltham, MA, USA) for 5 min at 4000 rpm +18°C. The hexane layer was transferred to a second tube and evaporated with N_2 to a final volume of 1 mL. The lipid was weighed and then stored at −80°C until methylation.

Table 1. Chemical composition of silage and concentrate, mean ± standard deviation (SD). Units g/kg DM unless otherwise stated.

	Silage *n*=4	Concentrate *n*=3
DM	295 ± 16.2	889 ± 1.0
ME, MJ/kg DM	10.8 ± 0.11	12.9*
CP	128 ± 1.8	196 ± 1.3
NDF	458 ± 7.6	203 ± 2.0
Ether extract	n.d.	61 ± 0.9
Ash	96 ± 3.2	68 ± 0.3
Starch	n.d.	301 ± 2.8

Note: *Obtained from feed manufacturer.
n.d, not determined.

Table 2. Fatty acid (FA) composition of the different calcium salts, in % of total FA (obtained from manufacturer). Mixed FA (MFA), 70% palmitic acid-based (P70) and rapeseed oil-based (RO).

Fatty acid	MFA	P70	RO
C14:0	1	1	
C16:0	48	71	4
C18:0	4	2	29
C18:1	37	21	45
C18:2	9	4	14
C18:3	0	0	5
C20:0	0	0	1
C20:1	0	0	1
Other	1	1	1

The fatty acid methyl esters (FAME) were prepared using the method described by Luddy et al. (1968) and analysed using a Varian CP-3380 gas chromatograph equipped with a flame ionisation detector (Agilent Technologies Inc., Santa Clara, CA, USA). Separation was achieved using a fused silica capillary column (0.2 µm film, phase NS-351, 0.32 mm ID × 25 m; HNU-Nordion Ltd., Helsinki, Finland). After injection, the temperature was increased from 70°C to a final temperature of 220°C at a rate of 5°C/min and then kept at 220°C for 25 min, for a total run time of 55 min. Nitrogen was used as the carrier gas and N (30 mL/min), H (30 mL/min) and air (300 mL/min) as the make-up gas. The detector temperature was kept at 230°C. Peaks were routinely identified by comparison of retention times with FAME standards (GLC 463, Nu-Check Prep Inc., Elysian, MN, USA).

Statistical analysis

Statistical analysis was performed using Statistical Analysis Software (9.4, Cary, NC, USA). Data were analysed using procedure MIXED and the model:

$$Y_{ij(k)m} = \mu + \mathrm{SQ}_m + P(\mathrm{SQ})_{im} + C(\mathrm{SQ})_{jm} + \tau_k + \varepsilon_{ij(k)m}$$

where $Y_{ij(k)m}$ is the dependent variable, μ is the overall mean, SQ_m the effect of square (random) ($m = 1–2$), $P(\mathrm{SQ})_{im}$ the effect of period i within square m ($i = 1–3$), $C(\mathrm{SQ})$ the effect of cow j within square m (random) ($j = 1–6$), τ_k the effect of treatment ($k = 1–3$), and $\varepsilon_{ij(k)m}$ the residual error term.

Results and discussion

There were no significant differences in DMI between the treatments (Table 3) but the intake of CaS based on rapeseed oil was somewhat higher compared with the intake of CaS based on palm oil. Overall BW and BCS increased from the start values (682 ± 64 kg and 3.5 ± 0.4, respectively) to 708 ± 77 kg and 3.7 ± 0.6, respectively, during the whole experimental period. Milk yield and milk composition did not differ significantly between the treatments (Table 4). However, the low number of animals and the experimental design may have affected the ability to detect treatment differences. Previous studies have shown reduced levels of fat and protein in the milk in some cases when feeding CaS based on rapeseed oil (Kowalski et al., 1999), but this was not observed in this study.

The analyses of FA profiles showed significantly different concentrations of C10:0 for the MFA and P70 treatments, whereas the RO treatment did not differ from the other two. Concentration of C14:1 was significantly lower for the RO treatment than for MFA and P70. The concentration of C16:0 differed between all treatments, with the highest concentration for P70 and

Table 3. Least square means (LSM) of feed intake per cow and day (kg DM unless otherwise stated) for the different calcium salt treatments: mixed FA (MFA), 70% palmitic acid-based (P70) and rapeseed oil-based (RO).

	Treatment				
Intake	MFA	P70	RO	SEM	*P*-value
Total DMI	19.7	19.8	19.8	0.31	0.874
Silage	9.0	9.1	8.9	0.16	0.389
Concentrate + Ca salts	10.7	10.7	10.8	0.17	0.499
CP	3.2	3.3	3.3	0.06	0.809
NDF	6.3	6.3	6.3	0.11	0.739
Ether extract (Ee)	1.27^{b}	1.26^{b}	1.31^{a}	0.034	0.035
ME_{tot}, MJ	235	235	236	3.65	0.854
ME_{Ee}/ME_{tot}	0.082^{b}	0.081^{b}	0.086^{a}	0.0029	0.030

Note: $^{a–b}$LSM within rows with different superscripts differ ($P < 0.05$).

Table 4. Least square means (LSM) of milk yield, energy corrected milk yield (ECM), milk composition (%) and somatic cell count (SCC) per cow and day. Milk fatty acid (FA) profiles (g/100 g FA) on the different calcium salt treatments: mixed FA (MFA), 70% palmitic acid-based (P70) and rapeseed oil-based (RO).

	Treatment				
	MFA	P70	RO	SEM	*P*-value
Milk yield, kg	25.6	25.8	24.6	3.78	0.415
ECM, kg	25.7	26.0	24.6	2.24	0.665
Milk fat	4.05	4.17	3.91	0.33	0.354
Milk protein	3.43	3.42	3.43	0.244	0.947
Lactose	4.48	4.47	4.47	0.100	0.980
SCC (log)	1.95	1.84	1.90	0.232	0.366
Milk FA					
C4:0	2.77	2.83	2.74	0.126	0.734
C6:0	1.53	1.66	1.57	0.049	0.143
C8:0	0.95	0.97	0.94	0.031	0.678
C10:0	1.95^{b}	2.11^{a}	2.06^{ab}	0.104	0.039
C12:0	2.45	2.62	2.58	0.134	0.107
C14:0	8.87	9.30	9.08	0.408	0.236
C14:1	0.72^{a}	0.76^{a}	0.65^{b}	0.044	0.006
C15:0	0.73	0.78	0.68	0.031	0.128
C16:0	28.83^{b}	31.54^{a}	20.00^{c}	0.579	<0.0001
C16:1	1.50^{a}	1.64^{a}	1.18^{b}	0.092	<0.0001
C17:0	0.40	0.46	0.42	0.017	0.089
C17:1	0.20	0.20	0.18	0.010	0.419
C18:0	10.43^{b}	9.90^{b}	13.20^{a}	0.710	0.002
C18:1	31.28^{b}	28.12^{c}	35.58^{a}	0.576	<0.0001
C18:2	3.37^{b}	3.05^{c}	4.32^{a}	0.073	<0.0001
C18:3	0.62^{b}	0.61^{b}	0.85^{a}	0.035	0.001
C18:4	0.93^{a}	0.82^{b}	0.78^{b}	0.035	0.011
C18iso*	0.47^{b}	0.42^{b}	0.54^{a}	0.020	0.004
C20:0	0.20	0.20	0.24	0.019	0.077
C20:1	0.30^{b}	0.30^{b}	0.46^{a}	0.021	0.004
Trans	5.77^{b}	5.21^{c}	6.73^{a}	0.180	0.003
SFA	59.12^{b}	62.34^{a}	53.70^{c}	0.670	0.002
MUFA	34.00^{b}	31.00^{c}	38.05^{a}	0.640	0.003
PUFA	4.92^{b}	4.48^{c}	5.95^{a}	0.100	<0.0001

Notes: $^{a–c}$LSM within rows with different superscripts differ ($P < 0.05$).
*C18iso are peaks that are considered to belong to the C18 group but which are not identified against standards.

the lowest for RO. The concentration of C16:1 was lowest for RO and highest for P70. The concentrations of C18:0, C18:3 and C20:1 were significantly higher for RO than for the other two treatments. The concentrations of C18:1 and C18:2 differed between all treatments, with the highest level for RO and lowest for P70. The concentration of C18:4 was highest for MFA and lowest for RO. Trans FA content was highest for RO and lowest for P70. The SFA content also differed between treatments, being highest for P70 and lowest for RO, whereas MUFA and PUFA concentrations were highest for RO and lowest for P70 (Table 4).

The increased C16:0 content in the milk in treatments P70 and MFA is consistent with previous findings for palm oil-based CaS (Palmquist et al., 1993). Higher concentrations of C18:0 and C18:1 for treatment RO compared with STA and P70 were expected (Ferlay et al., 1992; Kowalski et al., 1999; Bayourthe et al., 2000). One interesting finding was that the milk from cows in treatment RO had a significantly higher content of C18:3 than milk from cows in the other treatments. When unprotected, C18:3 is biologically hydrogenated to a large extent in the rumen. The proportion of PUFA is higher in CaS based on rapeseed oil than in CaS based on palm oil, and it has been shown that PUFA are more easily released from the salt than MUFA (Sukhija and Palmquist, 1990). The milk from cows in treatment RO also contained significantly higher levels of trans FA than milk in the other treatments. Industrial trans FA have been shown to increase the incidence of cardiovascular disease in humans, but similar effects of milk fat intake have not been demonstrated (Tardy et al., 2011). This is partly because the amount of trans fat consumed as milk and meat is significantly lower than the amount consumed as hardened vegetable fats. The lower content of C16:0 and the higher C18:1 content in the milk in treatment RO compared with other treatments was positive, as C16:0 can raise blood cholesterol levels in humans. Contrary, an increased content of MUFA such as C18:1 in milk fat has beneficial effects on blood cholesterol (reviewed by e.g. Givens, 2010). An increased content of C18:1 in milk fat also improves the processing properties by providing a more spreadable butter (Bayourthe et al., 2000). In conclusion, feeding CaS based on rapeseed oil to dairy cows is an interesting alternative to palm oil, since it has positive FA properties from a human nutritional perspective and may be a more environmentally sustainable option.

Acknowledgements

The author wishes posthumously to thank Professor Emeritus Jan Bertilsson, SLU, for helpful advice in experiment planning and data analysis. Thanks also to technician Gunilla Helmersson and other staff for practical assistance at the research facility.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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