

## Detailed milk fatty acid profiling of the Danish dairy cattle population

L. Hein<sup>1</sup>, A. J. Buitenhuis<sup>2</sup>, S. B. Gregersen<sup>3</sup>, L. B. Larsen<sup>3</sup>, D. Demirovic<sup>4</sup>, S. Warnecke<sup>5</sup>, N. H. Nielsen<sup>6</sup>, Arne Munk<sup>1</sup>, L. P. Sørensen<sup>1</sup>, J. Pedersen<sup>1</sup> & M. Kargo<sup>1,2</sup>

<sup>1</sup>Seges, Agro Food Park 15, 8200 Aarhus N, Denmark ([lihe@seges.dk](mailto:lihe@seges.dk), [arm@seges.dk](mailto:arm@seges.dk), [laps@seges.dk](mailto:laps@seges.dk), [jop@seges.dk](mailto:jop@seges.dk), [mks@seges.dk](mailto:mks@seges.dk))

<sup>2</sup>Aarhus University, Department of Molecular Biology and Genetics - Center for Quantitative Genetics and Genomics, Blichers Allé 20, 8830 Tjele, Denmark ([bart.buitenhuis@mbg.au.dk](mailto:bart.buitenhuis@mbg.au.dk), [morten.kargo@mbg.au.dk](mailto:morten.kargo@mbg.au.dk))

<sup>3</sup>Aarhus University, Department of Food Science - Food Chemistry and Technology, Blichers Allé 20, 8830 Tjele, Denmark ([sandrab.gregersen@food.au.dk](mailto:sandrab.gregersen@food.au.dk), [lbl@food.au.dk](mailto:lbl@food.au.dk))

<sup>4</sup>Eurofins, Ladelundvej 85, 6600 Vejen, Denmark ([DinoDemirovic@eurofins.dk](mailto:DinoDemirovic@eurofins.dk))

<sup>5</sup>Foss, Foss Allé 1, 3400 Hillerød, Denmark ([swa@foss.dk](mailto:swa@foss.dk))

<sup>6</sup>RYK, Agro Food Park 15, 8200 Aarhus N, Denmark ([nhn@ryk-fonden.dk](mailto:nhn@ryk-fonden.dk))

### Abstract

The fatty acid (FA) composition of bovine milk may be related to human health and technological properties of dairy products. The FA composition can be altered both through management and breeding. Large scale FA profiling of the Danish dairy cattle population has now been implemented for more than a year. For the first time access to millions of FA observations across an entire dairy population enables in depth analysis of the factors affecting the FA profile of milk. This was the aim of this study. For 12 months more than 3.5 million milk samples from four Danish dairy breeds had been analyzed using mid infrared spectroscopy and application of FOSS Application Note 64 for predicting content of seven FA groups and four individual FA. The results showed significant ( $P \leq 0.05$ ) effects of dairy breed, parity and lactation stage on all 11 FA categories. Also, significant effects of production system (organic vs. conventional) was found. In months, where cows were feed fresh grass, the proportion of unsaturated FA increased, whereas the proportion of saturated FA decreased. This was seen in both organic and to a lesser extent in conventional milk. Comparison of FA profiles from daughters of bulls also showed clear genetic variation for all 11 FA. In conclusion this study showed that FA profiles of milk can be altered and depends on the choice of breed, parity, lactation stage, production system and genetics.

*Keywords: Fatty acid profile, milk recording, dairy cows, mid-infrared spectroscopy, organic*

### Introduction

The composition of bovine milk fat is complex. It consists of both saturated fat (65-70 %), monounsaturated fat (27-33 %) and polyunsaturated fat (3-5 %). Increased intake of unsaturated fat has been positively associated with human health whereas the negative effect of saturated fat is debatable (Bier, 2015). Thus, the effect of bovine milk fat on human health cannot generally be described as favorable or unfavorable, and the biological function of each fatty acid (FA) should be considered separately (Arnold and Jahreis, 2011). Changing the FA composition of milk towards a healthier profile from a human health perspective or a profile that improves technological properties of dairy products may prove beneficial for both dairy farmers and the dairy industry, i.e. increased income. The FA composition of milk can be altered through dietary changes or through breeding when selecting animals with a more preferable FA profile. However, in both cases large scale data gathering is required to monitor and potentially change individual FA profiles. Until recently, this has not been possible because the current gold standard for FA profiling, gas chromatography, is

too laborious and relatively expensive to use. Now, mid infrared (MIR) spectroscopy provides an alternative for fast and cheap large scale FA profiling of bovine milk (De Marchi et al., 2014). This method is already implemented to measure total fat, protein and lactose content of milk through the Danish herd testing scheme. The addition of a module (Foss Application Note 64, Foss Electric, Hillerød, Denmark) for measuring groups of FA and a specific number of specific FA has now also been implemented in the Danish herd control scheme.

The aim of this study is to present results from one year of routine FA profiling of the Danish dairy cattle population using MIR technology. This provides for the first time, to our knowledge, an unique opportunity to study effects of cow specific factors such as breed and parity but also dietary effects, for example by comparing organic and conventionally fed cows, on the FA profile of milk. Genetic effects on FA composition will also be studied briefly by comparing milk profiles from daughters of bulls used on Danish dairy farms.

## Materials and methods

Observations of FA contents of raw milk were collected from dairy cows participating in the Danish herd control scheme from May 2015 to May 2016. The raw milk samples were analyzed using Milkoscan equipment based on Fourier-transformed infrared spectra analysis, and 11 different FA categories (Table 1) were subsequently predicted using Foss Application Note 64 (Foss Electric, Hillerød, Denmark). All FA were expressed as the proportion (%) of the sum of SFA, PUFA and MUFA.

*Table 1. Groups of fatty acids and individual fatty acids predicted using Foss Application Note 64.*

Fatty acid group	Characteristics	Abbreviation
short chain fatty acids	C4-C10, saturated	SCFA
medium chain fatty acids	C12-C16, mostly saturated	MCFA
long chain fatty acids	C18, both saturated and unsaturated	LCFA
saturated fatty acids	C4-C20	SFA
mono unsaturated fatty acids	C18:1 (C16:1, C14:1)	MUFA
poly unsaturated fatty acids	C18:2 <i>n</i> 6, C18:3 <i>n</i> 3, CLA	PUFA
<i>trans</i> fatty acid	C18:1 <sub>tr</sub> , CLA	<i>trans</i> FA
<b>Individual fatty acids</b>		
C14:0		C14:0
C16:0		C16:0
C18:0		C18:0
C18:1		C18:1

CLA: conjugated linoleic acid

After editing, a total of 3,517,272 FA measurements from Danish Holstein cows (DH; N obs. = 2,480,387), Danish Jersey (DJ; N obs. = 435,583), Red Dairy Cattle (RDC; N obs. = 232,955) and crossbred cows (N obs. = 368,347) in 1<sup>st</sup> to 6<sup>th</sup> lactation were left. Each lactation, 8-305 days in milk (DIM), was divided into 30 d intervals. Data was analyzed using the GLM procedure in SAS (Version 9, SAS Institute Inc., Cary, NC, USA) using the following model including only fixed and residual effects:

$$\text{FA} = \text{breed} + \text{parity} + \text{production system} + \text{season} + \text{production system} \times \text{season} + \text{lactation interval} + \text{residual}$$

where breed = 4 levels (DH, DJ, RDC and crossbreeds); parity = 3 levels (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>+); season = 12 levels (Jan – Dec); production system = 2 levels (organic and conventional), production system × season = 24 levels; and lactation interval = 11 levels.

The model was run including all effects to estimate effect of breed. Subsequently, it was run without breed effects to estimate the remaining effects for DH only. All estimates are presented as least square means. Effects were considered significant when  $P \leq 0.05$ . To show possible genetic variation for FA, genetic effects of the five most used sires (most FA observations) within the data set is shown as means of daughters.

## Results and discussion

Breed differences for individual and groups of FA (Figure 1) were all significant. The proportions of SFA, MCFA, SCFA and C16:0 were higher for DJ compared with the remaining breeds whilst the lowest proportions were seen in DH. Moreover, the highest proportion of MUFA, PUFA, LCFA and C18:1 were seen in DH. These FA are thought to have a positive effect on human health (Bhupathiraju and Tucker, 2011; Flock and Kris-Etherton, 2013), whereas C16:0 may have a negative effect (Givens, 2010). This indicates that even though DJ cows have the highest proportion of SCFA they may have a less healthy FA composition.

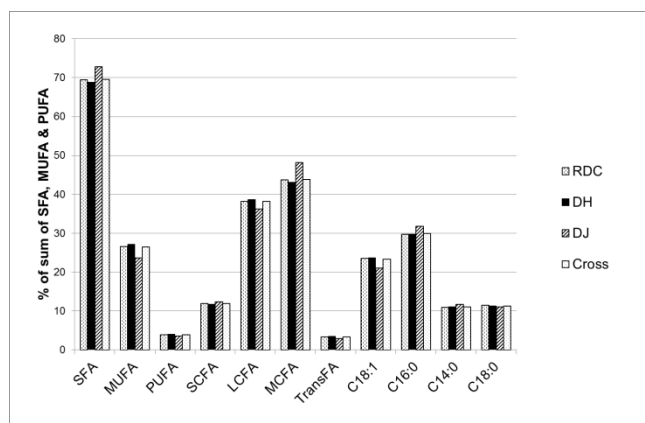


Figure 1. Effect of breed on fatty acid composition of bovine milk. RDC: Red Dairy Cattle; DH: Danish Holstein; DJ: Danish Jersey; Cross: Crossbreed dairy cows.

The effect of parity (DH only) on FA composition was significant for all FA and parity levels. The proportion of SFA and SCFA increased with increasing lactation number whereas the proportion of MUFA and C16:0 decreased (Figure 2). PUFA had the lowest proportion in 2<sup>nd</sup> parity and the highest in 1<sup>st</sup> parity. The increasing proportion of SCFA and decreasing proportions of MUFA and 16:0 indicated clearly that milk from first parity have a higher unsaturated fat content in the milk compared to second parity cows.

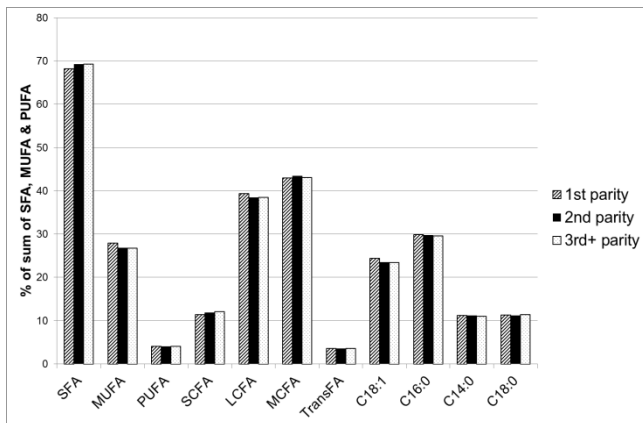


Figure 2. Effect of parity on fatty acid composition of DH milk.

The FA composition changed through lactation. All FA groups and the proportion of the specific FA and groups of FA were either highest or lowest in the first 30 DIM and increased or decreased to around 150-180 DIM (Figure 3). After this and throughout the remainder of the lactation, the FA composition was largely unchanged.

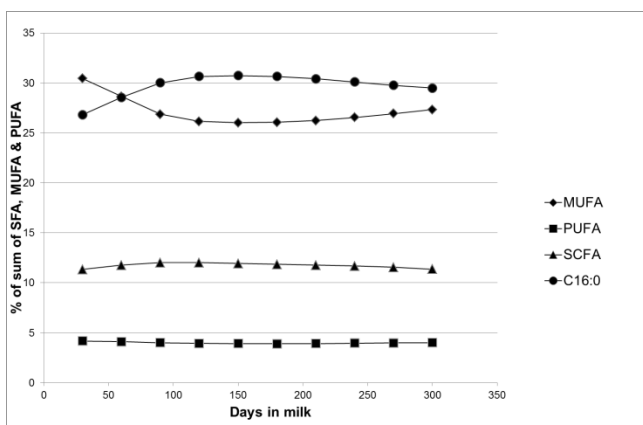


Figure 3. Effect of lactation stage on fatty acid composition of DH milk

The proportion of SFA, PUFA, SCFA, C16:0 and C14:0 were generally highest in organic milk compared to conventional milk, whereas MUFA, LCFA, C18:1 and C18:0 were highest in conventional milk then in organic milk. With the exception of SCFA, the proportions of FA were not always higher or lower in one production system but changed with season. In May and June the proportion of SFA was highest in conventional milk. In the same months the proportions of MUFA (Figure 4a) and C18:1 were highest in organic milk. The proportion of C16:0 was highest in conventional milk from April to August (Figure 4b). The difference between organic and conventional milk was very clear in May. This is shortly after the cows are put on pasture. Some conventional cows were also put on pasture; thus, the difference between feed ration including fresh grass and a ration without fresh grass may be even more distinctive than shown here. For conventional milk the proportion of MCFA (results not shown) and C16:0 decreased in May.

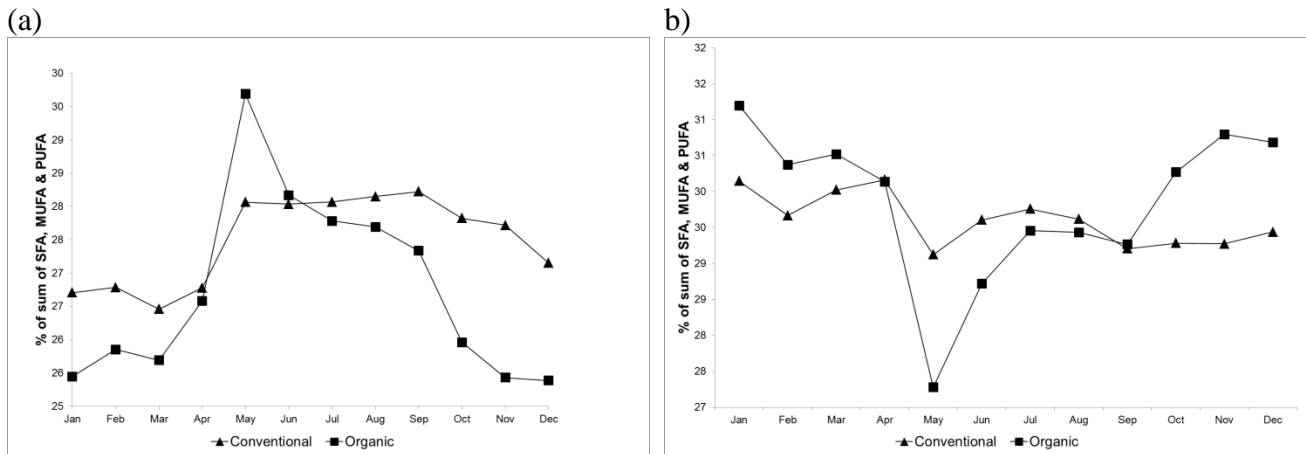


Figure 4. Effect of production system  $\times$  season on the proportion MUFA (a) and C16:0 (b).

Genetic effects on FA composition are shown in Figure 5 as progeny means from the five DH bulls with the most daughter observations in the data set. The bulls had between 61,531 and 89,858 daughter observations. Clear differences between these bulls, not selected for FA, were seen for all FA. This indicates that genetic variation exists for the different FA groups and specific FA and that it is possible to alter FA composition through breeding as already pointed out by e.g. Bilal et al. (2012). However, more research is necessary to confirm this, especially because genetic progress for the each FA group depends on the genetic correlation with other FA groups.

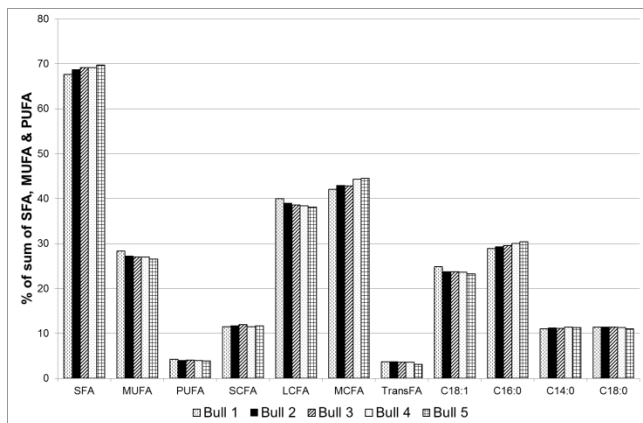


Figure 5. Genetic effects on FA composition shown as progeny means from the five bulls with the most daughter observations in the data set.

## Conclusion

This study confirms that FA composition of bovine milk is affected by breed, parity and lactation stage. Also, differences between production systems, organic vs. conventional, were clear. Finally, genetic variation between bulls was found. These results confirm that it is possible to alter the FA composition of milk by choosing the right dairy breed, using the right food ration and using the right bulls.

## Acknowledgements

This research was carried out in the project Specialized Organic Breeding Goals and Breeding Schemes for Dairy cattle (SOBcows). The project is coordinated by ICROFS and part of The Or-

ganic Research, Development and Demonstration Programme Organic RDD 2, supported by GUDP under Ministry of Environment and Food of Denmark.

### **List of references**

- Arnold, C. and G. Jahreis. 2011. *Ernahrungs Umschau* 58, 177-181.
- Bhupathiraju, S. N., K. L. Tucker. 2011. *Clin. Chim. Acta* 412, 1493-1514
- Bier, D.M. 2015. *Crit. Rev. 2015. Food Sci. Nutr.* 56, 1943-1946.
- Bilal, G., R. I. Cue, A. F. Mustafa, J. F. Hayes. 2012. *J.*
- De Marchi, M., V. Toffanin, M. Cassandro, M. Penasa. 2015. *J. Dairy Sci.* 97, 1171-1186.
- Flock, M. R., P. M. Kris-Etherton. 2013. *Curr. Opin. Clin. Nutr. Metab. Care* 16, 133-140.
- Givens, D. I. 2010. *Animal* 4, 1941-1952.