Short communication: Within-day variation in fatty acid composition of milk from cows in an automatic milking system

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ABSTRACT

Milk fatty acid composition is influenced by a range of conditions such as breed, feeding, and stage of lactation. Knowledge of milk fatty acid composition of individual cows would make it possible to sort milk at farm level according to certain fatty acid specifications. In the present study, 225 milk samples were taken from 84 cows in a herd equipped with an automatic milking system. Milk fat content varied depending on yield and time since last milking, whereas the milk fatty acid composition did not vary significantly within cow. It was concluded that random milk samples may be used for analysis of fatty acid composition.

Key words: fatty acid composition, automatic milking

Short Communication

Milk composition of individual cows is influenced by a range of conditions such as genetics, feeding, and stage of lactation, and by controlling certain conditions, it is possible to some extent to add value to milk at the farm level and make the milk more suitable for specific purposes. Some properties may be monitored on milk samples of individual cows at the farm level and, based on this information, milk may be directed into separate farm bulk milk tanks to obtain high-quality milk for special purposes and for standard milk, respectively.

Milk fat composition has received great attention due to effects on human health, and conditions such as feeding regimens, breeding, and stage of lactation are of great importance for the composition of milk FA (Dewhurst et al., 2006; Palmquist et al., 2006; Stoop et al., 2008, 2009). In addition, milk FA composition may be used to predict methane output of individual cows (Chilliard et al., 2009). A certain feed composition could be linked to specifications for milk FA composition and analysis of individual cow samples would

reveal whether milk from individual cows meet the specifications. Based on this knowledge, milk may be sorted into value-added milk or standard milk. Knowledge of milk FA composition of individual cows might also be used in breeding values if a specific pattern of milk FA is desired. The composition of FA in milk from individual cows can reliably be assessed by use of bronopol-preserved routine test samples for yield parameters (Butler and Stergiadis, 2011).

Use of automatic milking systems (AMS) has changed the workload for dairy farmers and in 2011, 22% of Danish dairy farms milked by AMS, and the share of AMS is still increasing (<http://www.landbrugsinfo.dk>). Sampling of milk from individual cows in AMS is more complex than manual systems that subsample morning and afternoon milkings. Such procedures may involve the sampling of all milkings within a specified period, such as 24 or 48 h, which creates large sample sets. Procedures that use fewer samples per individual cow require knowledge of how milk quality parameters are affected by factors such as milk yield and time since last milking, and several protocols are in use for estimation of the mean 24-h percentages of milk fat and milk protein from one or several samples (Hand et al., 2006). Such protocols are developed from large data sets and include parameters such as time since last milking, yield at last milking, as well as DIM and parity (Hand et al., 2006).

The variation in fat and protein contents between individual AMS milkings within a 48-h period was evaluated by Friggens and Rasmussen (2001), who found that the variation was related to total milk yield and time since last milking. Daily milk protein yield may be estimated from protein analysis of 1 sample and milk yield data, whereas determination of daily milk fat yield may require several samples (Friggens and Rasmussen, 2001).

Milk fat content increases during milking (Nielsen et al., 2005), so at higher milking frequencies, the residual high-fat milk from the previous milking contributes to a higher share of the cisternal milk, which may explain the higher milk fat content at short intervals between milkings (Nielsen et al., 2005). In contrast, milking half

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udders 2 or 4 times per day, respectively, shows no differences in fat content between milkings (Wiking et al., 2006). However, increased milking frequency reduces the concentration of PUFA in milk from udder halves milked 4 times daily compared with milk from udder halves milked twice daily (Wiking et al., 2006).

Composition of milk from morning and afternoon milkings is affected by differences in day and night feeding. Sahana et al. (2008) studied cows consuming the major part of their feed between the morning and afternoon milking and starving for the last 8 h before morning milking, and reported a higher fat content and a higher content of C18 unsaturated FA in the afternoon milk compared with the morning milk, whereas the content of de novo synthesized FA C6 to C16 was reported to be lower. In contrast, if cows are mainly grazing between morning and afternoon milkings and eat supplemental feed between afternoon and morning milkings, the differences in fat content and FA composition are not significant (Sahana et al., 2008).

These results show that milk FA composition under certain conditions varies during the day and may be affected by varying time intervals between milkings. Cows from AMS herds may be milked at all times during the day and night, and time intervals between milkings vary as well. The purpose of the present study was, therefore, to examine the FA composition of milk samples from several milkings of individual cows in an AMS herd and to determine the variation between milkings, and use this information to establish guidelines for sampling of milk from individual cows to analyze the FA composition in AMS herds.

The experiment was carried out at the Danish Cattle Research Centre with a capacity of approximately 110 Danish Holstein (**DH**) and 40 Danish Jersey (**DJ**) cows. The cows were kept in a loose-housing system with slatted floor and cubicles with mattresses. Lactating cows were allowed 3 kg of concentrate daily in the automatic milking unit (**AMU**) during the whole lactation, and they had 24-h ad libitum access to feed mixed rations

based on maize silage, grass silage, and concentrate. A free cow traffic system for access to the AMS (DeLaval AB, Tumba, Sweden) was used. Within the dairy herd, DH cows were divided into 2 groups and DJ cows made up 1 group, and each group had access to 1 AMU equipped with a device for automatic measurement of milk yield and an automatic milk sampling unit. Cows were not given access to the milking unit until a limit for expected yield or a time limit of 10 h was met. For first-parity cows, the limits for expected yield were 6 and 9 kg of milk for DJ and DH, respectively, whereas the limits were 7 and 10 kg of milk for second and higher parities.

Milk samples from all milkings during a period of 24 h were sampled, and samples from cows that were milked at least twice during the period were selected for analysis of FA composition. In total, 225 milk samples from 31 DJ and 53 DH cows were sampled and analyzed (see Table 1). Together with sampling time, the milk yield as kilograms of milk and time interval since last milking were recorded.

Fat and protein concentrations of all samples were analyzed by use of a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark). The FA composition of milk fat was analyzed after separation of milk fat by centrifugation, methylation of milk FA by sodium methylate, and separation and quantification of FA methyl esters by gas chromatography as described by Larsen et al. (2011). Fatty acids present in average concentrations below 1 mg/g of FA were not included. A reference milk sample was analyzed together with the collected milk samples at each analytical session to be able to quantify the day-to-day variation of all analyses.

Statistical analysis of data was done using SAS for Windows software (version 9.1; SAS Institute Inc., Cary, NC). For all parameters, average values were calculated for each individual cow. Within cow, the differences (**DIF**) between these average values and values of individual samples were calculated for each parameter (cow DIF values). For control samples, differences be-

Table 1. Overview of samples used for analysis of FA composition (means, with SD in parentheses)

Breed	Parameter	No. of milk samples during 24 h		
		2	3	4
Danish Jersey	Number of cows	12	10	9
	Average number of days in lactation	174 (73)	187 (96)	95 (76)
	Average parity	2.2 (1.2)	2.0 (1.2)	1.4 (0.5)
	Average time since last milking (h)	12.8 (3.5)	7.8 (1.2)	7.2 (1.2)
	Average milk yield per milking (kg)	9.7 (3.6)	7.1 (1.6)	7.0 (1.7)
Danish Holstein	Number of cows	30	17	6
	Average number of days in lactation	184 (121)	162 (113)	65 (42)
	Average parity	1.8 (0.9)	1.4 (0.7)	2.0 (1.2)
	Average time since last milking (h)	11.8 (4.0)	7.9 (1.6)	6.8 (1.3)
	Average milk yield per milking (kg)	12.5 (5.3)	11.1 (3.2)	10.1 (2.7)

Table 2. Overall mean values and 2 sample tests of variances of SAS (SAS Institute Inc., Cary, NC) of differences between values of individual samples and average values¹

Item	Amount	F-value	Pr < F	%CV of the control samples
Fat (g/kg of milk)	49.2	55.8	0.0013	2.2
Protein (g/kg of milk)	37.0	51.3	0.0015	0.7
FA (mg/g of FA)				
C4	50.5	0.46	NS	13.4
C6	27.8	0.30	NS	14.2
C8	14.6	0.29	NS	11.1
C10	30.9	0.43	NS	7.7
C11	3.0	0.64	NS	6.5
C12	32.7	0.84	NS	4.3
C13	1.7	1.67	NS	3.9
C14	99.8	0.85	NS	2.6
C14:1	7.4	1.29	NS	3.9
C15	10.8	2.66	NS	2.0
C16:0	261.6	1.42	NS	1.8
C16:1	15.4	3.57	NS	1.7
C17	6.1	0.92	NS	7.6
C17:1	1.9	0.28	NS	31
C18:0	141.6	0.65	NS	4.4
<i>trans</i> -9 C18:1	3.8	1.19	NS	4.9
<i>trans</i> -11 C18:1	21.1	1.86	NS	3.1
<i>cis</i> -9 C18:1	224.7	2.24	NS	2.6
<i>cis</i> -9, <i>cis</i> -12 C18:2	18.8	0.81	NS	4.3
C20	2.3	1.44	NS	9.6
C20:1	1.4	1.23	NS	27
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	7.6	1.02	NS	10.0
<i>cis</i> -9, <i>trans</i> -11 C18:2	6.4	3.02	NS	5.1

¹F-values were calculated as within-cow variance/variance of analytical variation. Pr < F = test of probability of analytical variance > within-cow variance. The %CV of the control samples was calculated as $100 \times (\text{SD of control samples})/(\text{average value of control samples})$.

tween average values and values of individual samples were calculated in the same way (control DIF values).

For each parameter, the cow DIF values and the control DIF values were analyzed by the 2 sample tests for variances of SAS. If the variance of cow DIF values was similar to the variance of control DIF values for a particular parameter, it was concluded that for this parameter, the within-cow variation was similar to the analytical variation. Parameters, where the variance of cow DIF values was wider than the variance of control DIF values, were examined more closely, and possible relations between parameters were evaluated.

Results of the 2 sample tests of variances are shown in Table 2 together with the percent coefficient of variation of the control samples and overall mean concentrations. For fat and protein concentrations, the variation within cows was wider than the analytical variation, whereas the variation within cows of all FA as proportion of total FA was not wider than the analytical variation. The percent coefficient of variation increased at low concentrations of individual FA. Between individual cows, significant differences were found for all parameters (data not shown).

The milk yield at each milking increased with increasing intervals between milkings, but the milk yield was still affected by individual cow differences. These dif-

ferences were eliminated by calculation of DIF values, and the relation between milk yield DIF and time since last milking DIF is shown in Figure 1. The correlations were high ($r = 0.70$ for all data, $r = 0.67$ for DH, and $r = 0.84$ for DJ cows). These results are similar to results reported previously (Friggens and Rasmussen, 2001). Figure 2 shows how the DIF values of fat content and time since last milking were negatively correlated ($r = 0.48$ for all data, $r = 0.49$ for DH, and $r = 0.46$ for DJ), which means that shorter intervals between milkings resulted in a higher milk fat content, which is similar to results from other studies (Friggens and Rasmussen, 2001; Nielsen et al., 2005). Figures 1 and 2 show that results for the 2 breeds were similar. The variation in protein content in milk samples within cows was wider than the analytical variation and this was, to some extent, due to very low analytical variation in the analysis of the control samples (0.7% coefficient of variation). Within cows, the variation in protein was neither related to variation in milk yield nor to variation in time since last milking, and results were similar to results reported previously (Friggens and Rasmussen, 2001).

As the variation in milk FA content was not wider within cows than the analytical variation, all milk samples taken under conditions as described in the present study should be regarded as representative samples for

Figure 1. Within-cow differences (DIF) between values of individual samples and average values for hours since last milking and milk yield (kg) at the actual milking. Open symbols: Danish Holsteins; solid symbols: Danish Jerseys.

analysis of milk FA composition. As cows in the present study had ad libitum access to feed 24 h/d, a time-dependent variation in milk FA composition was not expected, whereas other studies have shown that milk FA composition changes when the cows have restricted access to feed (Sahana et al., 2008).

In the present study, we related the variation in time since last milking to the variation in content of individual FA, and no significant correlations were found. The time since last milking did not vary much within cows, and the deviation in time since last milking from individual cows average was 1 h or less for 67% of the observations and not greater than 3 h for 95% of the observations. This low variation in time between milkings was most likely due to the restrictions in access to milking where cows were not milked until a time limit or a limit for expected yield was met. Due to

Figure 2. Within-cow differences (DIF) between values of individual samples and average values for hours since last milking and milk fat content at the actual milking. Open symbols: Danish Holsteins; solid symbols: Danish Jerseys.

the low variation in time between milkings, it was not possible to confirm previous results where a lower proportion of PUFA has been reported in milk from udder halves milked 4 times daily compared with udder halves milked twice daily (Wiking et al., 2006).

In conclusion, the present study confirms that especially fat concentration varies between individual milk samples from individual cows. In contrast, the FA composition is not affected, and these results document that random samples may be used for analysis of such components, provided that cows have satisfactory access to feed and that time limits or limits for expected yield (or both) control access to the milking unit. This is especially useful when individual cow samples of AMS herds have to be used. However, the information is useful in herds with manual milking systems as well.

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