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Repeatability of fatty acid composition in pm:am milk samples from cows

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ABSTRACT

The present study was designed to estimate the repeatability of fatty acid composition in cows' milk collected at a pm:am herd test. As part of a larger project, whole milk was collected during a routine herd test in March 2003 from 10 Friesian and 10 Jersey cows at Flock House, in the evening and again the following morning. Fat in the 40 samples was separated by centrifugation, fatty acids were converted to their methyl esters, and individual fatty acid percentages in each sample were assayed by gas chromatography. In general, the fatty acid composition of milk was very similar at the pm and am samplings. For the fatty acids making the largest contributions (10 to 29% of the total) to fatty acids present in milk (C14:0, C16:0, C18:0 and C18:1 cis-9 (oleic)) and for conjugated linoleic acid and the Mono-Unsaturated/Saturated ratio, pooled within-breed correlations of compositions between pm and am milk samples were high (0.76, 0.80, 0.88, 0.76, 0.75 and 0.74, respectively; standard errors 0.06 to 0.12). The pm:am correlation, averaged over the most abundant 15 fatty acids in milk, was 0.77 ± 0.10. It is concluded that a single milk sample or a pooled pm:am sample is adequate for estimating the composition of the predominant fatty acids.

Keywords: milk; fatty acid; cows; repeatability**.**

INTRODUCTION

In taking single samples of milk from cows for composition analysis, it is generally assumed that milk composition is repeatable over time. McLaren *et al.* (1998) analysed pm:am samples separately for the concentrations of α-lactalbumin, β-lactoglobulin, β-casein, lactose and total fat in milk from cows milked twice daily with 8 h:16 h (pm:am) intervals, and found for example that, on a bulked sample basis, α-lactalbumin concentrations were the same after 8 or 16 h intervals, whereas β-lactoglobulin concentrations differed by 50% (higher after the 16 h interval), regardless of stage of lactation, and total fat concentration was affected in the opposite direction to β-lactoglobulin. The present study was designed to measure the pm:am compositions and the repeatability of fatty acids in the milk of Friesian and Jersey cows.

MATERIALS AND METHODS

Animals

The animals in this study consisted of 10 Friesian-sired and 10 Jersey-sired cows in late lactation (March 2003) in AgResearch's herd at Flock House, Manawatu province. The cows were selected at random to represent 10 sires per breed, and they were part of a larger study of variation in the composition of cows' milk. Any cows with somatic cell counts above 800,000 cells/ml were excluded from the file from which cows were selected. All cows were grazing together, on a ryegrass/white clover diet. The 20 cows selected were aged 2 to 9 years in each breed, averaging 5.5 and 4.7 years in the Friesian and Jersey groups, respectively. Their milk was collected from a pm sampling and from the following am sampling during a routine herd-test, with an interval of 8 h:16 h (pm:am). Instead of pooling the milk, as in the normal herd-test practice, the two portions were analysed separately. Fat in the 40 samples was separated by centrifugation, fatty acids were converted to their methyl esters, and individual fatty acid percentages in each sample (including conjugated linoleic acid, CLA) were assayed with a gas chromatograph – flame ionisation detector. Standard deviations were determined on the gas chromatograph by running repeated subsamples of a single sample.

Data analyses

Fixed effects tested were for breed, time of sampling, and their interactions, using analyses of variance (SAS, 1995). The repeatability was estimated from the correlation between pm and am results, after adjusting for breed.

RESULTS

Time and breed effects

Table 1 shows the time of day and breed effects on fatty acid composition traits (ten pm and ten am samples per breed). Within-sample standard deviations for each fatty acid are also tabulated. Four fatty acids accounted for 69% of all fatty acids in the milk sampled, namely C14:0, C16:0, C18:0 and C18:1 cis-9 (oleic). In general, the fatty acid composition of milk was very similar at the pm and am samplings. The largest absolute differences between pm and am means were for C18:1 cis-9 with 21.72: 19.10% ($P < 0.01$), C16:0 with 28.17: 29.83% ($P < 0.05$), and C14:0 with 9.29: 10.05% ($P < 0.05$), which were respectively 12.8, 5.7 and 7.9% of the relevant means.

For comparison, fatty acid compositions were available from milk samples taken on the same dates from another 224 cows in the main herd, comprising

TABLE 1: Fatty acid percentages in milk (% of total fatty acids in each sample, averaged over all samples, for the 15 fatty acids with highest prevalence): from a Friesian herd (n = 130) and a Jersey herd (n = 114) with subgroups of 10 cows per breed where pm and am milk samples were analysed separately. Correlations between compositions of the pm and am samples are also shown (pooled within breed).

Trait	Standard deviation[1]	Friesian mean		Jersey mean		Mean pm	Mean am	Correlation between pm & am
Number of cows		120	10	104	10	20	20	20
C4:0	0.16	3.73	3.53	3.75	3.53	3.44	3.62	0.63
C6:0	0.08	2.11	1.96	2.17	2.06	1.94	2.08	0.73
C8:0	0.04	1.24	1.13	1.29	1.25	1.16	1.22	0.85
C10:0	0.07	2.69	2.43	2.87	2.78	2.53	2.68	0.89
C12:0	0.05	3.11	2.8	3.3	3.22	2.96	3.06	0.89
C14:0	0.09	10.01	9.23	10.25	10.1	9.29	10.05	0.76
C14:1	0.01	1.29	1.3	1.15	1.23	1.25	1.28	0.48
C15:0	0.01	1.27	1.18	1.26	1.23	1.18	1.23	0.78
C16:0	0.18	29.16	28.93	29.46	29.07	28.17	29.83	0.8
C16:1	0.06	1.73	1.84	1.47	1.67	1.79	1.72	0.82
C18:0	0.07	9.57	9.36	10.76	10.11	9.89	9.58	0.88
C18:1 trans-11	0.02	2.12	2.13	2.42	2.3	2.23	2.2	0.86
C18:1 cis-9 (oleic)	0.11	19.88	21.44	18.23	19.37	21.72	19.1	0.76
C18:2	0.01	1.39	1.55	1.4	1.44	1.51	1.48	0.72
CLA[2]	0.01	0.87	0.9	0.82	0.91	0.93	0.88	0.75

[1] Within-sample standard deviation.
[2] Conjugated linoleic acid.

120 Friesians and 104 Jerseys in late lactation, grazing with the 20 selected animals. The pm and am samples from cows in the main herd were pooled for each cow on a 1: 1 basis by volume, and C14:0, C16:0, C18:0 and C18:1 cis-9 means of the 224 pooled samples were 10.0, 29.2, 9.6 and 19.9%, respectively for Friesians, and 10.3, 29.5, 10.8 and 18.2%, respectively for Jerseys. The two breeds were quite similar, and the subgroups of 10 cows per breed were not significantly different from their main herd breed means, although the subgroup means for C18:1 cis-9 ($P < 0.08$) and C14:0 ($P < 0.06$) approached significance.

Repeatability

Pooled within-breed correlations between pm and am composition for fatty acid components are also shown in Table 1. For those fatty acids making the largest contributions (10 to 29%) to total fatty acids present in milk (C14:0, C16:0, C18:0 and C18:1 cis-9), correlations were all high (0.76 ± 0.11, 0.80 ± 0.09, 0.88 ± 0.06 and 0.76 ± 0.11, respectively). For the 14 fatty acids whose percentages were at least 1% of the total, plus CLA, the correlation between pm and am samples averaged 0.77 ± 0.10, range 0.63 ± 0.15 to 0.89 ± 0.05 with one exception, C14:1, where the correlation was considerably lower at 0.48 ± 0.19. For C14:1, the pair of samples from a single cow appeared to differ greatly, but the elution data for these were re-checked and confirmed.

For C14:1/C14:0, C16:1/C16:0, C18:1 cis-9/C18:0 and the Mono-Unsaturated/Saturated ratio, pooled within-breed correlations were 0.55 ± 0.17, 0.56 ± 0.17, 0.95 ± 0.03 and 0.74 ± 0.12, respectively.

For fatty acids with a lower frequency than those shown in Table 1, C13:0, C15:1, C17:0, C17:1, C18:1 cis-11, C18:2 trans-9 trans-12, gamma-C18:3, C18:3, C20:0, C20:1, C20:2/C21:0 (two components co-eluting), C20:3n6, C20:3n3, C20:5, C22:0, C22:5 and C24:0, pooled within-breed correlations were 0.88, 0.77, 0.89, 0.69, 0.18, 0.52, 0.64, 0.83, 0.55, 0.77, 0.07, 0.33, 0.54, 0.10, -0.08, 0.24 and -0.14. Of these, the ten fatty acids with highest frequencies had correlations averaging 0.61 ± 0.16; the minor (i.e. least frequent) fatty acids were quite variable.

DISCUSSION

Means for pm versus am

The first conclusion was that the pm:am means of fatty acid composition traits were similar to each other. The residual fat in the udder after milking would have contributed to buffering any pm:am differences between means. McLaren *et al.* (1998) found, for four milk components in 60 and 48 New Zealand Holstein-Friesians at pasture, that protein% and lactose% were not affected by pm:am sample time (which was assumed to represent a milking interval effect, rather than a time of day effect). However fat% was significantly higher, and β-lactoglobulin% was significantly lower, in pm samples than in am samples. Sbodio *et al*. (1985), with samples collected over complete lactations from 18 Argentinian Holstein cows grazing at pasture with supplementation, found significant differences for fat% between pm and am milk (higher in evening milk), but not for casein%, protein%, lactose% or ash%.

Breed means

Stull & Brown (1964) and Beaulieu & Palmquist (1995) have reported that percentages of the short-chain and medium-chain fatty acids (up to C14:1) were increased in Jerseys relative to Friesians, and thus C16 and C18 and other long-chain fatty acids were relatively less prevalent in Jerseys. There was the same trend for the breed result in Table 1 (overall means of 24.8% short-chain and medium-chain fatty acids in the Jerseys and 24.2% in the Friesians; least squares mean difference, $P < 0.01$; age of cow was also a significant effect, $P < 0.001$).

Repeatabilities

The second conclusion from the present study was that repeatabilities in pm versus am milk were high (0.77 on average), for the 15 most abundant fatty acids in Friesians or Jerseys. Akerlind *et al*. (1999) reported a 'close correlation' between am and pm samples from all fatty acids studied except C18:2, in data from a Swedish thesis by Emanuelson (1989) with cows' milk sampled at 15 h (am milking) and 9 h (pm milking) intervals. In our data, the lower repeatability for C14:1 suggests that some other fatty acid may be co-eluting under the same peak, and the importance of any such effect is magnified because of the low prevalence of C14:1 in the sample (relative to, say, the prevalence of C16:0).

From the size of the correlations in Table 1, it is confirmed that a single milk sample or a pooled pm:am sample are adequate for estimating the composition of the predominant fatty acids.

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