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Variation of milk composition traits among Holstein-Friesian-sired cows

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Abstract
A pooled afternoon and morning milk sample was obtained from each of 3,789 cows of known pedigree during a standard herd-test carried out in 21 North Island spring-calving herds between November and February. The cows were all sired by one of 11 Holstein-Friesian bulls, in a sampling design intended to sample milk from many daughters per sire, for segregation studies. Routine herd-test data consisted of 24 hour milk yield, protein %, fat % and somatic cell count. Samples were measured at Ruakura for fatty acid composition, fat melting point, and the concentrations of the following components, lactoferrin, β-lactoglobulin, β-casein, κ-casein, and total carotenoids. After liaison between AgResearch Ruakura staff and each dairy farmer a variate describing the current grazing groups of cows were included in analyses. Coefficients of variation were calculated for each milk component, and ranged from 0.04 to 0.35. Phenotypic correlations among components were estimated. The correlations were negative between the percentage of mono-unsaturated fatty acids present in milkfat and both the protein % (-0.26 ± 0.03 (standard error)) and fat % (-0.38 ± 0.02) of the milk. Some implications of selection mainly for yield from cows at pasture are discussed in terms of likely effects on milk composition.

Keywords: lactation; composition; cow; milk; variation

Introduction
Three milk composition traits are generally derived from dairy cows herd-tested in New Zealand, namely protein and fat percentages, and somatic cell count (SCC). The genetic parameters for the milk protein and fat yields (Johnson et al. 2000) of cows managed under these conditions are well known. Johnson et al. (2000) also reported parameters for yields of casein, whey and lactose. However, there are many other components of cows’ milk which remain less well described. All components are important for determining the healthiness of milk to consumers.

It is useful to know whether changing the fatty acid (FA) composition of milk, and the melting point of fat, is consistent with breeding cows for higher productivity. These milk traits have risen in importance as consumer interest has developed in soft fat, mono-unsaturated fatty acids (MUFA) and melting point. Solid fat content at 10°C, a factor related to the melting point of fat, has been reported in New Zealand by Mackle et al. (1997). Likewise it is useful to know whether the main protein components in milk will change with selecting for production.

The objective of our present study was to report the variability of some of these other components, and their inter-relationships, in milk from New Zealand dairy cows grazing pasture, and to monitor sire-to-sire variation in milk compositional traits. These included fat and protein components, as well as fat colour. Part of the trial design was reported by Morris et al. (2002) in describing a study of sire effects on anti-parasite antibody concentrations in milk from the same cows.

Materials and methods

Trial design
The study was carried out on 21 large dairy herds in the upper North Island of New Zealand. Eleven widely used Holstein-Friesian artificial-insemination bulls from the Livestock Improvement Corporation team of ‘Premier Sires’ with daughters in many herds, were evaluated by sampling as many of their daughters as possible in each herd. Over all herds, between 138 and 939 daughters per sire were sampled for the study with an average of 372 daughters per sire. Over all sires, between 37 and 420 daughters per herd were sampled with an average of 195 per herd. In all, 4,105 cows were sampled. Complete analyses are described here for samples from 3,789 cows. The herds were spring calving, grazing mainly pasture, year-round. Cows were in mid-lactation, averaging about four months post-calving. Based on the findings of Johnson et al. (2000) for protein and casein yield, the heritabilities and coefficients of variation were not affected by stage of lactation between early, mid or late. As a consequence we were able to concentrate on one stage of lactation, namely mid-lactation. The average size of the herds in our study was approximately 750 cows, or over three times the national average of the day. A restricted number of cows were included here from 11 pre-selected Holstein-Friesian sire groups where the sire groups were approximately a 50% sample of all cows of known pedigree, excluding Jerseys and Jersey-crosses. Over all herds, percentages of cows in each age group were: two-year-olds (16%), three-year-olds (21%), four-year-olds (15%), five-year-olds (12%), six-year-olds (7%), seven-year-olds (13%), eight-year-olds (11%), and nine-, 10- and 11-year-olds (6%). The unusual age
Table 1 Mean, residual standard deviation and coefficient of variation (residual standard deviation/mean) for components measured in up to 3,789 samples of milk. A total of 3,760 samples were measured for fatty acids and 1,026 were measured for lactoferrin. C18:1 cis and C18:1 trans refer to the cis- and trans-isomers of this molecule. CLA = Conjugated linoleic acid.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>Residual standard deviation</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (L/day)</td>
<td>18.5</td>
<td>2.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Protein% (kg/L)</td>
<td>3.46</td>
<td>0.26</td>
<td>0.08</td>
</tr>
<tr>
<td>Fat% (kg/L)</td>
<td>4.37</td>
<td>0.57</td>
<td>0.13</td>
</tr>
<tr>
<td>Somatic cell count (log_{10}(count/mL))</td>
<td>4.97</td>
<td>0.46</td>
<td>0.09</td>
</tr>
<tr>
<td>Fat melting point (°C)</td>
<td>33.5</td>
<td>1.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4:0 (%)</td>
<td>2.7</td>
<td>0.3</td>
<td>0.11</td>
</tr>
<tr>
<td>C6:0 (%)</td>
<td>2.2</td>
<td>0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>C8:0 (%)</td>
<td>1.6</td>
<td>0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>C10:0 (%)</td>
<td>3.8</td>
<td>0.4</td>
<td>0.11</td>
</tr>
<tr>
<td>C12:0 (%)</td>
<td>4.3</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>C14:0 (%)</td>
<td>12.6</td>
<td>0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>C15:0 (%)</td>
<td>1.4</td>
<td>0.2</td>
<td>0.11</td>
</tr>
<tr>
<td>C16:0 (%)</td>
<td>30.5</td>
<td>2.7</td>
<td>0.09</td>
</tr>
<tr>
<td>C16:1 (%)</td>
<td>1.3</td>
<td>0.3</td>
<td>0.22</td>
</tr>
<tr>
<td>C17:0 (%)</td>
<td>0.6</td>
<td>0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>C18:0 (%)</td>
<td>9.5</td>
<td>1.3</td>
<td>0.14</td>
</tr>
<tr>
<td>C18:1 cis (%)</td>
<td>15.7</td>
<td>1.8</td>
<td>0.12</td>
</tr>
<tr>
<td>C18:1 trans (%)</td>
<td>3.5</td>
<td>0.8</td>
<td>0.24</td>
</tr>
<tr>
<td>C18:2 (%)</td>
<td>1.4</td>
<td>0.2</td>
<td>0.15</td>
</tr>
<tr>
<td>C18:2 (CLA) (%)</td>
<td>0.8</td>
<td>0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>C18:3 (%)</td>
<td>0.8</td>
<td>0.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Lactoferrin (µg/mL)</td>
<td>149</td>
<td>52</td>
<td>0.35</td>
</tr>
<tr>
<td>β-lactoglobulin (µg/mL)</td>
<td>3.1</td>
<td>0.7</td>
<td>0.22</td>
</tr>
<tr>
<td>β-casein (µg/mL)</td>
<td>14.5</td>
<td>1.5</td>
<td>0.10</td>
</tr>
<tr>
<td>κ-casein (µg/mL)</td>
<td>3.5</td>
<td>0.7</td>
<td>0.20</td>
</tr>
<tr>
<td>Total carotenoids (Optical density at 450 nm)</td>
<td>0.32</td>
<td>0.09</td>
<td>0.28</td>
</tr>
</tbody>
</table>

distribution of few six-year-olds, reflected the particular choice of the eleven sires for the study and the years in which they were heavily used after selection as proven sires.

A single sub-sample of 40 to 100 mL of milk was taken at one of Livestock Improvement Corporation’s Herd-Test Collection Depots from each selected cow’s sample, obtained during a routine herd test in the period from 17 November 2000 to 6 February 2001. Sire data and other parameters such as correlations were then analysed on a within-herd basis, and pooled across herds.

Sample analyses
Concentrations of protein, fat and SCC in the milk samples on herd-test day were analysed by Livestock Improvement Corporation, using a Milkoscan FT120 (Johnson et al. 2000).

Fatty acids (C4:0 to C18:3) were analysed as their methyl esters using a gas chromatograph/flame ionisation detector, as described by Morris et al. (2007). The identity of each FA was determined by comparing the retention time of each peak with known standards. The largest FA measured was C18:3, because of the long experimental time required to run the chromatography column for over 4,000 samples, and difficulties with standards for reading the area under the curve at larger carbon-sizes. Melting point was measured by reading the temperature at which each melted fat sample solidified again, a procedure which was then automated by recording the temperature at which the optical density changed whilst scanning warmed 200 µL samples in a 96-well plate.

Assays for β-lactoglobulin, β-casein and κ-casein were carried out on de-fatted milk by nephelometry, as described by Collin et al. (2002). Lactoferrin concentration was analysed using an ELISA method (Bethyl Laboratories, Montgomery, Texas, USA). The total carotenoid content was assayed by spectrophotometry, using absorbance at a wavelength of 450 nm.

Statistical analyses
The overall data consisted of milk yield and its composition from a mid-lactation herd test, for 3,789 cows from a total of 21 herds sampled at one herd-test. The data were analysed first using SAS (1995). Fixed effects for each trait included: breed of cow (% Holstein-Friesian (three classes), age of cow (two-, three-, four-....eight+ years-old), grazing group on the milk-sampling day which was generally mob within
Table 2 Heritability estimates calculated by a sire model based on 11 sires ± standard error in bold on the diagonal and phenotypic correlations ± standard error above the diagonal for milk yield and ten composition traits. MY = Milk yield; P = Protein; F = Fat; SCC = Somatic cell count; MPt = Melting point of fat; MUFA = Mono-unsaturated fatty acid (total); Lf = Lactoferrin; Lg-β = β-lactoglobulin; Cas-β = β-casein; Cas-κ = κ-casein. FatCol = Fat colour, equivalent to Total carotenoids.

<table>
<thead>
<tr>
<th>Trait</th>
<th>MY</th>
<th>P%</th>
<th>F%</th>
<th>SCC</th>
<th>MPt</th>
<th>MUFA</th>
<th>Lf</th>
<th>Lg-β</th>
<th>Cas-β</th>
<th>Cas-κ</th>
<th>FatCol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY</td>
<td>0.23 ± 0.11</td>
<td>-0.49 ± 0.02</td>
<td>-0.43 ± 0.02</td>
<td>-0.03 ± 0.02</td>
<td>-0.23 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>-0.04 ± 0.04</td>
<td>-0.22 ± 0.03</td>
<td>-0.34 ± 0.03</td>
<td>-0.27 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>P%</td>
<td>0.52 ± 0.21</td>
<td>0.60 ± 0.02</td>
<td>0.04 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td>-0.26 ± 0.03</td>
<td>-0.02 ± 0.05</td>
<td>0.48 ± 0.03</td>
<td>0.70 ± 0.02</td>
<td>0.54 ± 0.02</td>
<td>0.00 ± 0.03</td>
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<tr>
<td>F%</td>
<td>0.27 ± 0.12</td>
<td>0.01 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>-0.38 ± 0.02</td>
<td>-0.02 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.01 ± 0.03</td>
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<tr>
<td>SCC</td>
<td>0.08 ± 0.05</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>-0.00 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>-0.17 ± 0.03</td>
<td>-0.03 ± 0.02</td>
<td>0.03 ± 0.02</td>
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<tr>
<td>MPt</td>
<td>0.11 ± 0.06</td>
<td>-0.54 ± 0.01</td>
<td>-0.01 ± 0.03</td>
<td>0.15 ± 0.02</td>
<td>0.14 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>-0.10 ± 0.02</td>
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<tr>
<td>MUFA</td>
<td>0.16 ± 0.08</td>
<td>-0.04 ± 0.04</td>
<td>-0.17 ± 0.03</td>
<td>-0.25 ± 0.03</td>
<td>-0.14 ± 0.02</td>
<td>0.13 ± 0.02</td>
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<tr>
<td>Lf</td>
<td>0.30 ± 0.16</td>
<td>0.04 ± 0.04</td>
<td>-0.06 ± 0.05</td>
<td>0.07 ± 0.04</td>
<td>-0.07 ± 0.04</td>
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<tr>
<td>Lg-β</td>
<td>0.45 ± 0.19</td>
<td>0.28 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>-0.02 ± 0.03</td>
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<tr>
<td>Cas-β</td>
<td>0.57 ± 0.23</td>
<td>0.48 ± 0.03</td>
<td>-0.04 ± 0.03</td>
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<tr>
<td>Cas-κ</td>
<td>0.27 ± 0.12</td>
<td>0.01 ± 0.03</td>
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<tr>
<td>FatCol</td>
<td>0.21 ± 0.10</td>
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</tr>
</tbody>
</table>

Table 3 Heritability estimates calculated by a sire model based on 11 sires ± standard error in bold on the diagonal and phenotypic correlations ± standard error above the diagonal for milk yield and nine fatty acid components. MY = Milk yield. C18:1 cis and C18:1 trans refer to the cis- and trans-isomers of this molecule.

<table>
<thead>
<tr>
<th>Trait</th>
<th>MY</th>
<th>C4:0</th>
<th>C6:0</th>
<th>C10:0</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1 cis</th>
<th>C18:1 trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY</td>
<td>0.23 ± 0.11</td>
<td>0.18 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>-0.08 ± 0.02</td>
<td>-0.13 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>-0.24 ± 0.02</td>
<td>0.14 ± 0.03</td>
<td>0.20 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>C4:0</td>
<td>0.10 ± 0.06</td>
<td>0.73 ± 0.01</td>
<td>-0.13 ± 0.02</td>
<td>-0.26 ± 0.02</td>
<td>-0.16 ± 0.02</td>
<td>-0.12 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>-0.04 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C6:0</td>
<td>0.07 ± 0.04</td>
<td>0.37 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>-0.05 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>-0.19 ± 0.02</td>
<td>-0.17 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
<td>0.15 ± 0.08</td>
<td>0.96 ± 0.00</td>
<td>0.74 ± 0.01</td>
<td>-0.07 ± 0.02</td>
<td>-0.09 ± 0.02</td>
<td>-0.49 ± 0.02</td>
<td>-0.15 ± 0.02</td>
<td></td>
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</tr>
<tr>
<td>C12:0</td>
<td>0.17 ± 0.08</td>
<td>0.76 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>-0.23 ± 0.02</td>
<td>-0.54 ± 0.02</td>
<td>-0.17 ± 0.02</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.19 ± 0.09</td>
<td>0.09 ± 0.02</td>
<td>-0.28 ± 0.03</td>
<td>-0.51 ± 0.02</td>
<td>-0.19 ± 0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>0.17 ± 0.08</td>
<td>-0.52 ± 0.02</td>
<td>-0.65 ± 0.01</td>
<td>-0.46 ± 0.02</td>
<td></td>
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</tr>
<tr>
<td>C18:0</td>
<td>0.27 ± 0.12</td>
<td>0.43 ± 0.02</td>
<td>-0.01 ± 0.02</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C18:1 cis</td>
<td>0.14 ± 0.07</td>
<td>0.05 ± 0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C18:1 trans</td>
<td></td>
<td>0.17 ± 0.08</td>
<td></td>
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</tbody>
</table>
herd, and a covariate for days in milk. Additionally,
for the fatty acid traits, ‘analysis group’ of rack
within herd, was included. Somatic cell count (SCC)
was transformed to logarithms (base 10). Protein %
and log(SCC) were added as covariates for analysis
of lactoferrin concentration.

Estimates of heritability for each trait, and
phenotypic correlations among traits, were obtained
from a sire-model restricted maximum likelihood
analysis (Gilmour et al. 2009), with a relationship
matrix for the 11 sires. The same fixed effects as
above were included in the maximum likelihood
analyses.

**Results**

The mean, residual standard deviation after fitting
fixed effects, and coefficient of variation for the
measured milk components are shown in Table 1.
The coefficients of variation ranged from 0.04 to
0.35. The smallest coefficient of variation was for fat
melting point; nevertheless there were 5% of values
more than 2.6°C above or below the mean.

Phenotypic correlations among protein and other
components of milk are shown in Table 2, along with
estimates of heritability based on 11 sire groups.
Phenotypic correlations between levels of MUFA and
protein % and fat % in milk were both negative: -0.26
± 0.03 and -0.38 ± 0.02, respectively. Phenotypic
correlations among the main protein components
analysed were positive, and ranged from 0.20 to 0.48.

Fat colour had phenotypic correlations of only
0.04 ± 0.02 with milk yield or fat yield, and with fat
melting point the correlation was also close to zero (-
0.09 ± 0.02).

The heritabilities of FAs and phenotypic
correlations among them are shown in Table 3.
Heritabilities for the more highly-represented FAs
(C14:0, C16:0, C18:0 and C18:1) were low to
intermediate in size (0.14 to 0.27). Phenotypic
correlations among the FA measures included
estimates of C16:0 with other FAs. They were 0.09 ±
0.02, -0.52 ± 0.02, -0.65 ± 0.01 and -0.46 ± 0.02, for
C14:0, C18:0, C18:1 and C18:1trans, respectively.
For milk yield, the phenotypic correlation with C16:0
concentration was -0.24 ± 0.02.

**Discussion**

The milk composition data analysed were from
samples originating in commercial herds, at around
mid-lactation. This study was designed to maximise
numbers of sire-daughter records for molecular
analyses, rather than maximising numbers of sires
and optimising progeny group size. It must be noted
that the 11 Holstein-Friesian sires involved had been
selected for widespread use by Livestock
Improvement Corporation, on Breeding Index which
is closely related to yield, so that the heritabilities for
yield may be underestimated in Table 2. Although
these heritabilities were obtained from 11 sires only
(Table 2), most estimates were at least as large as that

for milk yield (0.23 ± 0.11), for which genetic
parameters are commonly estimated. For the same
reasons of trial design as above, correlation estimates
are phenotypic rather than genetic.

The fact that FA concentrations of milkfat from
cows grazing New Zealand pasture were affected by
season (Thomson & van der Poel 2000) was not
important in this study, because our calculations were
made effectively on a within-management-group basis,
in order to test for between-sire FA differences.
Genetic source effects on FAs were found in earlier
studies with grazing cows in New Zealand (Wales et
al. 2009).

Concentrations of C16:0 and longer-chain FAs
are affected by diet (Salter et al. 2007). The shorter-
chain FAs of C14 and shorter, are derived from de
novo synthesis, whereas longer-chain FAs of C16 and
longer, are derived from diet and tissue mobilisation.
From the likely herd and diet effects noted above, it
was not surprising that the overall FA means in our
data with cows on pasture did not match closely with
Dutch data (Stoop et al. 2008), where cows were
concentrate-fed. In addition, animal factors may have
differed between the New Zealand and Dutch studies.

In our data, there was still a phenotypic
correlation (0.67 ± 0.01) between fat melting point
and C16:0, because melting point was a downstream
consequence of FA composition. This is explained
because the longer-chain saturated FAs would have
still been solid at body temperature, and thus C16:1
and C18:1 would have been synthesised from C16:0
and C18:0, respectively, to maintain the fluidity of
milk, for transport within the animal (Garnsworthy et
al. 2010). In our data, the phenotypic correlation
between fat melting point and fat % was positive
(0.51 ± 0.02), and it was negative between fat
melting point and MUFA (-0.54 ± 0.01).

If genetic and phenotypic correlations were the
same sign as each other, increases in fat yield or fat
% are expected to increase saturated fat levels and fat
melting point. Genetic selection to increase protein
yield per cow is likely to make little difference to the
concentrations of β-lactoglobulin, lactoferrin and the
caseins measured (-β and -κ), whereas increasing
protein % in milk by genetic selection is likely to
increase the concentrations of β-lactoglobulin and the
caseins measured (-β and -κ), but to have little effect
on lactoferrin concentration. Fat colour is unlikely to
be affected by selection mainly for yield. SCC
generally had phenotypic correlations close to zero
with yield traits, but any genetic correlations need to
be investigated further.

Heritability estimates from the milk samples of
New Zealand cows on pasture were compared with
those from Dutch cows fed concentrate rations for
protein components (Schopen et al. 2009), and the
main FAs (Stoop et al. 2008), including milk yield
itself as a control. Our estimates were about a half to
two-thirds of the size of the Dutch estimates, except
those for β-casein which were higher in New Zealand
at 0.57 versus 0.25, and C18:0 which was similar in each country (0.27 vs 0.23).

In conclusion, these data give a snapshot of the relationships between milk production and component traits in cows under the production systems in force in the early 2000s. However, continued selection since then for milk production traits, and the increasing use of supplementary feeding, which is now relatively common throughout lactation, may require that these parameters are re-evaluated with today’s cows and management.

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