Fatty acids routed from fresh grass to milk influence δ¹³C in milk

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Abstract
Dairy production systems vary widely in their feeding and livestock-keeping regimens. Both are well-known to affect milk quality and they are important topics for consumer perceptions. Here we examine whether carbon isotope composition (δ¹³C) in milk can be sufficiently predicted from feeding information, so that measurement of δ¹³C in milk could serve as authenticity proof. We obtained 671 milk samples from 40 farms distributed over Central Europe to measure δ¹³C and fatty acid composition. Feeding protocols by the farmers in combination with a model based on δ¹³C feed values from the literature were used to predict δ¹³C in feed and subsequently in milk. The model considered dietary contributions of C₃ and C₄ plants, contribution of concentrates, altitude of forage production, seasonal variation in 12/13CO₂, Suess’s effect, and diet-milk fractionation. Predicted and measured δ¹³C in milk correlated closely (r²=0.93). Analysing milk for δ¹³C allowed validation of a reported C₄ component with an uncertainty of <8% in 95% of all cases. This included the uncertainties of the method (measurement and prediction) and of the feeding information. However, the mismatch was not random but varied seasonally and correlated with the seasonal variation in long-chain fatty acids originating from fresh grass. This indicated routing of long-chain fatty acids from fresh grass to milk. In conclusion, δ¹³C in milk can be predicted in the authentication case but larger errors occur with high proportions of fresh-grass feeding, due to routing of long-chained fatty acids from fresh grass to milk.

Keywords: authentication, isotopes, unsaturated long-chain fatty acids, maize, grass

Introduction
Isotope analysis could be valuable for food authentication, proof of origin or proof of production system. In experiments showing that the isotopic composition of feed is forwarded to the product, both the feed and product are usually measured. This does not comply with the situation typical for authentication in which the isotopic composition of the feed is unknown and has to be estimated from the information of the reported feeding regimen or the livestock-keeping regimen. However, for milk there are no procedures in literature on how to make such a prediction or which parameters to include. We set up such a prediction model to examine the hypothesis that the designated feeding information can be verified by analysing δ¹³C in milk. We then quantified the prediction uncertainty that results even with correct feeding information. Further, we will analyse whether routing of fatty acids derived from fresh grass contributes significantly to the mismatch between expected and measured δ¹³C, because it is known that long-chained unsaturated fatty acids, which differ strongly in δ¹³C (Richter et al., 2012) are routed (Dewhurst et al., 2006). An extended version of this work can be found elsewhere (Auerswald et al., 2015).

Materials and methods
Sampling comprised 40 farms in Austria and Germany with detailed analysis of the feeding and livestock keeping regimes. Feeding protocols were obtained by interviews. In total 671 milk samples were collected year round and analysed for δ¹³C and fatty-acids. Prediction of δ¹³C in milk distinguished between roughage and concentrates derived from either C₃ or C₄ plants, thus:

\[ \delta^{13}C_{\text{feed}} = m_a \cdot \delta^{13}C_a + m_b \cdot \delta^{13}C_b + m_c \cdot \delta^{13}C_c + m_d \cdot \delta^{13}C_d \]
where \( m \) denotes the fraction of total dry feed mass and the indices a to d refer to the four components C\(_3\) or C\(_4\) roughage and C\(_3\) or C\(_4\) concentrates. The basic δ\(^{13}\)C values of the four components (-29.5‰, -12.7‰, -26.8‰, -12.1‰) have to be adjusted for the Suess effect (which is the change in atmospheric δ\(^{13}\)C\(_{CO_2}\)) mainly due to fossil fuel burning since 2003 when the basic δ\(^{13}\)C values for the feed components were determined; C\(_3\) roughage has additionally to be adjusted to the mean altitude of the individual farm and fresh C\(_3\) roughage for the seasonal variation of δ\(^{13}\)C in atmospheric CO\(_2\) (Table 1).

### Results and discussion

Measured δ\(^{13}\)C of milk correlated closely with the independently predicted δ\(^{13}\)C of milk \((r^2=0.932, n=671, P<0.001; \text{Figure 1A})\) with a root of the mean square error (RMSE) of 0.75‰. The RMSE comprised less than one tenth of the entire range of feed δ\(^{13}\)C. When restricting the data set to farms feeding no maize and less than 15% concentrates (12 farms; 397 milk samples), for which a mismatch between prediction and measurement could not result from errors in the reported feed ration, the percentage of fatty acids with a chain length of 18 and longer (long-chain fatty acids – LCFA; mainly unsaturated LCFA from grass) correlated with the unexplained variation of δ\(^{13}\)C in milk \((r^2=0.225, n=397, P<0.001)\). LCFA have a much more negative δ\(^{13}\)C than shorter fatty acids (Richter et al., 2012).

Table 1. Prediction model based on published δ\(^{13}\)C for specific feed components. Total feed composition is given from the mass fractions of the four components (a: C\(_3\) roughage; c: C\(_3\) concentrate; b: C\(_4\) roughage; d: C\(_4\) concentrate) and their isotopic composition. For C\(_3\) roughage (fresh or conserved), an altitude effect was included. Parameter \( a \) denotes altitude above sea level in m. The Suess effect since 2003 has to be added to the δ\(^{13}\)C of all components. Parameter \( t \) denotes the year of interest. The δ\(^{13}\)C of fresh grass has to be adjusted for the seasonal variation of δ\(^{13}\)C in CO\(_2\), \( \delta\text{DoY} \), is day of the year (unit: d). For references see Auerswald et al. (2015).

<table>
<thead>
<tr>
<th>Model component</th>
<th>δ(^{13})C (‰)</th>
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<tbody>
<tr>
<td>C(_3) roughage</td>
<td>( \delta^{13}C_a = -29.5 + 1.1 \times \frac{a}{1000} )</td>
</tr>
<tr>
<td>C(_3) concentrate</td>
<td>( \delta^{13}C_c = -26.8 )</td>
</tr>
<tr>
<td>C(_4) roughage</td>
<td>( \delta^{13}C_b = -12.7 )</td>
</tr>
<tr>
<td>C(_4) concentrate</td>
<td>( \delta^{13}C_d = -12.1 )</td>
</tr>
<tr>
<td>Suess effect(^1)</td>
<td>( \delta^{13}C_a = \delta^{13}C_a - 0.02644 \times t + 52.95 )</td>
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<tr>
<td>Seasonal δ(^{13})C(_{CO_2}) variation</td>
<td>( \delta^{13}C_a = \delta^{13}C_a \times \frac{0.51}{\sin\left(\frac{\delta\text{DoY} - 121}{365} \times 2 \pi\right)} - 0.35 )</td>
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<tr>
<td>Diet-milk fractionation</td>
<td>( \delta^{13}C_{milk} = \frac{(0.4 - \delta^{13}C_{feed})}{\delta^{13}C_{feed} - 1} )</td>
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</tbody>
</table>

\(^1\) Due to the irregular change in δ\(^{13}\)C\(_{CO_2}\) that impedes any prediction, the difference in δ\(^{13}\)C\(_{CO_2}\) between the year of interest and the reference year (2003) should better be obtained from published measurements at: http://www.esrl.noaa.gov/gmd/dv/data/.

Figure 1. (A) Predicted and measured δ\(^{13}\)C in bulk milk (line denotes unity). (B) Seasonal change of predicted minus measured δ\(^{13}\)C of milk (only farms without maize feeding). The line is a parabolic regression \((r^2=0.101, n=397, P<0.001)\). (C) Seasonal change in the proportion of fatty acids with a chain length of 18 and longer, LCFA, in total fat. The line is a parabolic regression \((r^2=0.360, n=397, P<0.001)\).
and are not synthesized by the mammalian milk gland (Dewhurst et al., 2006). The concentration of LCFA showed a distinct seasonality with a maximum in July and minima in March and December (Figure 1C). This seasonality in LCFA concentration paralleled the seasonal pattern of the unexplained variation of δ¹³C (Figure 1B). This similar seasonal behaviour of LCFA and the unexplained variation of δ¹³C were even more obvious when individual farms with exceptionally low contributions of concentrates were analysed separately. The regression equations between the percentage of LCFA and the residuals indicated that the δ¹³C of LCFA should be more depleted than the average carbon in milk (by 6 ‰ to 8 ‰), which agrees with data from literature (e.g. Richter et al., 2012).

In summary, a prediction model for authentication purposes was set up and proved to be suitable. Deviations between predicted and measured δ¹³C in milk larger than 1.4‰ indicate that the error in the feeding information is larger than the uncertainty of this information on experimental farms. With large proportions of fresh grass in the diet, the routing of LCFA from grass to milk causes additional uncertainty in the prediction.

References


The multiple roles of grassland in the European bioeconomy

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Volume 21
GRASSLAND SCIENCE IN EUROPE

EGF 2016
4-8 September 2016 | TRONDHEIM, NORWAY

EGF 2016

EGF 2016

EGF 2016