Milk biomarkers to detect ketosis and negative energy balance using MIR spectrometry

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³ French Livestock Institute (IDELE), France
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⁷ AWE, Belgium

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Negative energy balance and ketosis

In early lactation:
energy intake $<$ energy output

- fertility
- health

(Collard et al., 2000; Butler, 2003)
In early lactation: energy intake < energy output

Negative energy balance

Body fat mobilisation

If excessive
- imbalance in hepatic carbohydrate and fat metabolism
- ↑ of ketone bodies in blood

= Ketosis type I

↓ fertility
↓ health
(Collard et al., 2000; Butler, 2003)

Prevalence: 7 to 43% (Suthar et al., 2013)

↓ milk yield
↓ reproductive performances
↓ displaced abomasum

... (Duffield, 2000)
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¶ displaced abomasum...

(Dufffield, 2000)

BHB and Acetone known as biomarkers
(Enjalbert et al., 2001)
Citrate?

- Krebs cycle molecule
- Present in milk
Citrate?

- Krebs cycle molecule
- Present in milk

Induced nutrient restriction
Citrate?

- Krebs cycle molecule
- Present in milk
- BHBA in milk

Induced nutrient restriction

NEFAs in blood

Citrates in milk
• Bjerre-Harpoth (2012)
  « ...greatest increase (58%) during restriction for all cows »
  « ...promising early indicator of physiological imbalance »

• Baticz et al. (2002)
  « Sodium citrate should be measured by easy and automated method such as FT-MIR technology to evaluate the energy status of cows »
Mid Infra Red (MIR)

- MIR spectrum reflect milk composition
- World-wide used for milk recording, payment
- Fast, cheap
  - 1 sample → X predicted values
    - Fatty acids
    - Minerals
    - Methane
    - Cows state
    - Technical properties
    - ...
- Limit of detection : 100 ppm (Dardenne, 2015)
### Previous studies in link with MIR

**Acetone: ketosis biomarker**

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### Citrate: energy status of cow/physiological imbalance

- Not very well documented, no target values or thresholds in the literature
- No published MIR calibration (existing FOSS calibration)
Goals of the study

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Body fat mobilisation

If excessive

⇒ imbalance in hepatic carbohydrate and fat metabolism
⇒ ↑ of ketone bodies in blood = Ketosis type I
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(1) Realize Optimir own MIR calibrations for BHB and acetone, with validation step
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(2) Evaluate possibility to predict citrate via MIR

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(2) Evaluate possibility to predict citrate via MIR

(3) Use samples and spectra from several countries
   → robust equations
Collect of samples

**CLASEL: MRO**
- France
- Hostein
- Maize silage or fresh grass
- DIM 7-305
- 200 samples

**CONVIS: MRO**
- Luxembourg
- Hostein
- Maize silage supplemented by grazing in summer
- DIM 5-60
- 110 samples

**Poisy: experimental farm**
- France (Montain area)
- Abundance and Montbéliarde
- Fresh grass or hay and maize silage
- DIM 7-56
- 174 samples

**Neumühle: experimental farm**
- Germany
- Hostein
- Maize silage
- DIM 7-56
- 82 samples

- Harmonized protocol by IDELE
- ICAR approved sampling systems
- Morning and evening samples pooled
- 566 * 2 identical samples generated → MIR and chemical analysis
Analysis of samples

- Chemical analysis at CRA-W (Belgium)
- Continuous flow analyzer (Skalar, The Netherlands)
- Enzymatic/chemical reactions
Analysis of samples

- Chemical analysis at CRA-W (Belgium)
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- Spectral analysis locally
- Foss and Bentley
- Standardization of spectra enabling a common database and a common use
Results of chemical analysis

- 566 samples in total
- Removing of missing values
- Same ranges than litterature (Denis-Robichaud et al., 2014; Garnsworthy et al., 2006)

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• Limit of detection with MIR: 100 ppm

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- Unbalanced distribution for BHB and Acetone
  ➔ Use of Log (10) transformation
• Unbalanced distribution for BHB and Acetone
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  → Artificial removing of low values (randomly)
Editing of data

• Unbalanced distribution for BHB and Acetone
  → Use of Log (10) transformation
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558 → 433 samples for BHB
548 → 224 samples for acetone
MIR calibrations

• Spectral pretreatment:

  Absorbance, Standardized, First derivative gap 5, Autoscale

  Area used: 968.1 - 1577.5, 1731.8 - 1762.6, 1781.9 - 1808.9 and 2831.0 - 2966.0 cm\(^{-1}\)
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- **Partial Least Square (PLS) regression**

- **Cross-validation using 10 subsets**

- **Validation ¾ - ¼**

- **Use of Matlab and the PLS toolbox**
MIR calibrations

- Criteria observed
  - $R^2$ (but dependent of the range)
  - RMSE (Root Mean Square Error)

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} |e_i|^2}{n}}
\]
MIR calibrations

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  - $R^2$ (but dependent of the range)
  - RMSE (Root Mean Square Error)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} |e_i|^2}{n}}$$
MIR calibrations

- **Criteria observed**
  - R² (but dependent of the range)
  - RMSE (Root Mean Square Error)
    
    \[ \text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} |e_i|^2}{n}} \]
  - RPD = SD (calibration) / RMSE

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MIR calibrations

- Criteria observed
  - $R^2$ (but dependent of the range)
  - RMSE (Root Mean Square Error)
    \[
    \text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} |e_i|^2}
    \]
  - $\text{RPD} = \frac{\text{SD (calibration)}}{\text{RMSE}}$

- Classification
  - 0.20 mmol/L for BHB
  - 0.15 mmol/L for acetone

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## Results – BHB

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Validation dataset

- $y = 1.0042x + 0.0071$
- $R^2 = 0.625$
Results – BHB

- **Statistics**

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Allows discriminate high or low levels
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<td>0.193</td>
<td>0.109</td>
<td>0.71</td>
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<td>-</td>
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<td>0.755</td>
<td>0.204</td>
<td>0.136</td>
<td>0.083</td>
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</tr>
</tbody>
</table>

y = 1.0042x + 0.0071
R² = 0.625

Validation dataset

<table>
<thead>
<tr>
<th>Low BHB content (&lt;0.200mmol/l)</th>
<th>High BHB content (&gt;0.200mmol/l)</th>
<th>Global good classification</th>
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</thead>
<tbody>
<tr>
<td>Validation</td>
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<td>Predicted low</td>
<td>90.90%</td>
<td>9.40%</td>
</tr>
<tr>
<td>Predicted high</td>
<td>9.10%</td>
<td>90.60%</td>
</tr>
</tbody>
</table>

Allows discriminate high or low levels
### Results – Acetone

#### Statistics

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>No. of LV</th>
<th>No. of Outliers</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>RMSE</th>
<th>$R^2$</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetone (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-validation</td>
<td>168</td>
<td>7</td>
<td>2</td>
<td>0.02</td>
<td>3.355</td>
<td>0.19</td>
<td>0.397</td>
<td>0.248</td>
<td>0.73</td>
<td>1.6</td>
</tr>
<tr>
<td>Validation</td>
<td>56</td>
<td>-</td>
<td>-</td>
<td>0.021</td>
<td>1.968</td>
<td>0.179</td>
<td>0.306</td>
<td>0.196</td>
<td>0.67</td>
<td>2.03</td>
</tr>
</tbody>
</table>

**Validation dataset**

\[
y = 1.5033x - 0.0384 \\
R^2 = 0.6723
\]
## Results – Acetone

### Statistics

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>No. of LV</th>
<th>No. of Outliers</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>RMSE</th>
<th>R²</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetone (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</tr>
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<td>0.179</td>
<td>0.306</td>
<td>0.196</td>
<td>0.67</td>
<td>2.03</td>
</tr>
</tbody>
</table>

The validation dataset allows to discriminate high or low levels.

The relationship between acetone reference values and predicted values is given by the equation:

\[ y = 1.5033x - 0.0384 \]

The coefficient of determination, \( R^2 \), is 0.6723.
## Results – Acetone

### Statistics

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>No. of LV</th>
<th>No. of Outliers</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
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<th>RMSE</th>
<th>$R^2$</th>
<th>RPD</th>
</tr>
</thead>
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<td>Acetone (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>0.306</td>
<td>0.196</td>
<td>0.67</td>
<td>2.03</td>
</tr>
</tbody>
</table>

**Low acetone content (<0.150mmol/l)**

**High acetone content (>0.150mmol/l)**

**Global good classification**

<table>
<thead>
<tr>
<th>Validation</th>
<th>n=43</th>
<th>n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted low</td>
<td>93.00%</td>
<td>23.10%</td>
</tr>
<tr>
<td>Predicted high</td>
<td>7.00%</td>
<td>76.90%</td>
</tr>
</tbody>
</table>

Allows discriminate high or low levels

\[ y = 1.5033x - 0.0384 \]

$R^2 = 0.6723$
## Results – Citrate

### Statistics

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>No. of LV</th>
<th>No. of Outliers</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>RMSE</th>
<th>R²</th>
<th>RPD</th>
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</thead>
<tbody>
<tr>
<td>Sodium citrate (mmol/L)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cross-validation</td>
<td>380</td>
<td>9</td>
<td>2</td>
<td>3.88</td>
<td>16.12</td>
<td>9.03</td>
<td>2.26</td>
<td>0.7</td>
<td>0.9</td>
<td>3.21</td>
</tr>
<tr>
<td>Validation</td>
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<td>-</td>
<td>-</td>
<td>4.44</td>
<td>15.16</td>
<td>9.08</td>
<td>2.03</td>
<td>0.76</td>
<td>0.86</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Validation dataset

\[
y = 0.9919x + 0.0582 \\
R^2 = 0.8575
\]
# Results – Citrate

## Statistics

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>No. of LV</th>
<th>No. of Outliers</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
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<th>RMSE</th>
<th>$R^2$</th>
<th>RPD</th>
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<tbody>
<tr>
<td>Sodium citrate (mmol/L)</td>
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<tr>
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<td>9.08</td>
<td>2.03</td>
<td>0.76</td>
<td>0.86</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Validation dataset

- $y = 0.9919x + 0.0582$
- $R^2 = 0.8575$

Allows screening, quantitative information
Exemple of use by MROs (Baugnies, 2015)

- Walloon breeding association (AWE) tool
- BHB, acetone, citrate, C18:1 cis 9
- Relative approach
- Cow value compared to population values at same DIM

• Score 0,1 or 2 for each component
• Global score from 0 to 8 as a global approach of metabolic disorders

<table>
<thead>
<tr>
<th>Exploitation</th>
<th>DATE CTRL</th>
<th>n° animal</th>
<th>n² lactation</th>
<th>JEL</th>
<th>Production (dl)</th>
<th>Cellules (*1000/ml)</th>
<th>Urée (mg/l)</th>
<th>Rapport TB/TP</th>
<th>Indice BHB</th>
<th>Indice acétone</th>
<th>Indice citrate</th>
<th>Indice c18:1cis9</th>
<th>Indice GLOBAL</th>
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<td>1</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

• Complex interpretation (ketosis, fat mobilization, fattening, feed effect, mastitis…)
• Preliminary tests in 4 farms
• Good feedback from breeders
• Cows to follow
Conclusions/Implications

• Calibrations for BHB and acetone → distinctions between high and low levels
• Citrate by MIR → good accuracy
• Standardisation of spectra: usable by all Optimir MROs
Conclusions/Implications

- Calibrations for BHB and acetone $\rightarrow$ distinctions between high and low levels
- Citrate by MIR $\rightarrow$ good accuracy
- Standardisation of spectra: usable by all Optimir MROs

- USE ON FIELD
  - Complex interpretation
  - Different way to use it by MROs
  - Interest from breeders
  - Already used in France and Luxembourg
  - Tests in Germany, Belgium
Thank you for your attention


c.grelet@cra.wallonie.be