Biomarkers del latte per la valutazione dello stato sanitario della mammella in vacche ad elevata produzione

Cinzia Marchitelli¹, Federica Signorelli¹, Francesco Napolitano¹, Clément Grelet², Nicolas Gengler³, Frédéric Dehareng², Hélène Soyeurt³, Klaus Lønne Ingvartsen⁴, Martin Tang Sørensen⁴, Torben Larsen⁴, Mark Crowe⁵ and GplusE consortium

¹ Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di ricerca Zootecnia e Acquacoltura (CREA-ZA), Monterotondo (Roma), Italy; ²Centre Wallon De Recherches Agronomiques, Gembloux, Belgium; ³University of Liège, Gembloux, Belgium; ⁴Aarhus University, Dept. of Animal Science, Tjele, Denmark; ⁵UCD School of Veterinary Medicine, Dublin, Ireland

cinzia.marchitelli@crea.gov.it
Genotype and Environment contributing to the sustainability of dairy cow production systems through the optimal integration of genomic selection and novel management protocols based on the development and exploitation of genomic data and supporting novel phenotyping approaches.

Duration: 60 months

Total estimated eligible cost: 11,607,551.40 euro
Total requested EU contribution: 8,997,235.00 euro

GplusE aims to identify those genotypes that control biological variation of the important phenotypes of dairy cows, to appreciate how these are influenced by environmental and management factors, and thus to allow more informed and accurate use of Genomic Selection (GS).
The Perfect Cow

- Few metabolic disorders, maintains body condition
- Shows heat and conceives when bred
- Produces a live calf without assistance
- High milk yield, correct composition, inexpensive ration, low maintenance costs
- Walks and stands comfortably, rarely needs trimming
- Resists mastitis, avoids injury
What are functional traits?

- The **ICAR** Functional Traits Working Group currently is working on:
  - General health traits
  - Female fertility
  - Feet and legs problems
  - Udder health
  - Workability

*International Commitee for Animal Recording*
Functional traits are being used

<table>
<thead>
<tr>
<th>Country</th>
<th>Type</th>
<th>Longevity</th>
<th>Udder Health</th>
<th>Fertility</th>
<th>Other man. &amp; health traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan - NTP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel - PD11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand - BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France - ISU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy - PFT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany - RZG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switzerland - ISEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada - LPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain - ICO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium (Walloon) - VEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia - APR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States - TPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa - BVI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Britain - PLI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nordic Countries - TMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland - EBI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States - NM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands - NVI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Miglior et al., 2012
GplusE vs functional traits

- Milk biomarkers include metabolites, mastitis parameters, hormones, glycan profile and infrared spectra obtained using the MIR technology.

- The biomarkers measured in milk will be compared with the production, health and welfare traits observed in the same animals.

- A potential biomarker will be identified as a valid when an observed trait can be predicted with good precision from that particular biomarker measured in milk.
1. Materials and methods

<table>
<thead>
<tr>
<th>Country</th>
<th>N° COW</th>
<th>N° MILK SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>AU</td>
<td>14</td>
<td>56</td>
</tr>
<tr>
<td>CRAITA</td>
<td>45</td>
<td>173</td>
</tr>
<tr>
<td>CRAW</td>
<td>31</td>
<td>121</td>
</tr>
<tr>
<td>FBN</td>
<td>13</td>
<td>43</td>
</tr>
<tr>
<td>UCD</td>
<td>38</td>
<td>151</td>
</tr>
<tr>
<td>TOTAL COW</td>
<td>162</td>
<td>623</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARITY</th>
<th>Country</th>
<th>AFBI</th>
<th>AU</th>
<th>CRAITA</th>
<th>CRAW</th>
<th>FBN</th>
<th>UCD</th>
<th>TOTAL cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST</td>
<td></td>
<td>6</td>
<td>2</td>
<td>7</td>
<td>13</td>
<td>1</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>SECOND</td>
<td></td>
<td>2</td>
<td>9</td>
<td>15</td>
<td>9</td>
<td>3</td>
<td>11</td>
<td>49</td>
</tr>
<tr>
<td>≥THIRD</td>
<td></td>
<td>13</td>
<td>3</td>
<td>23</td>
<td>9</td>
<td>9</td>
<td>24</td>
<td>81</td>
</tr>
<tr>
<td>TOTAL cows</td>
<td></td>
<td>21</td>
<td>14</td>
<td>45</td>
<td>31</td>
<td>13</td>
<td>38</td>
<td>162</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARITY</th>
<th>Country</th>
<th>AFBI</th>
<th>AU</th>
<th>CRAITA</th>
<th>CRAW</th>
<th>FBN</th>
<th>UCD</th>
<th>N° MILK SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST</td>
<td></td>
<td>22</td>
<td>8</td>
<td>31</td>
<td>52</td>
<td>4</td>
<td>12</td>
<td>129</td>
</tr>
<tr>
<td>SECOND</td>
<td></td>
<td>8</td>
<td>36</td>
<td>58</td>
<td>34</td>
<td>9</td>
<td>44</td>
<td>189</td>
</tr>
<tr>
<td>≥THIRD</td>
<td></td>
<td>49</td>
<td>12</td>
<td>84</td>
<td>35</td>
<td>30</td>
<td>95</td>
<td>305</td>
</tr>
<tr>
<td>N° MILK SAMPLES</td>
<td></td>
<td>79</td>
<td>56</td>
<td>173</td>
<td>121</td>
<td>43</td>
<td>151</td>
<td>623</td>
</tr>
</tbody>
</table>
# 1. Materials and Methods

## Day Post Partum

<table>
<thead>
<tr>
<th>DAY POST PARTUM</th>
<th>Country</th>
<th>N° MILK SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFBI</td>
<td>AU</td>
</tr>
<tr>
<td>7±3</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>14±3</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>21±3</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>35±3</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>N° MILK SAMPLES</td>
<td>79</td>
<td>56</td>
</tr>
</tbody>
</table>

## Season

<table>
<thead>
<tr>
<th>SEASON</th>
<th>Country</th>
<th>N° MILK SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFBI</td>
<td>AU</td>
</tr>
<tr>
<td>SPRING</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SUMMER</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>AUTUMN</td>
<td>78</td>
<td>54</td>
</tr>
<tr>
<td>WINTER</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>N° MILK SAMPLES</td>
<td>79</td>
<td>56</td>
</tr>
</tbody>
</table>
2. Materials and Methods

**Milk Recording**
- Milk
- Protein
- Fat
- Lactose
- SCC

**MIR DATA**

<table>
<thead>
<tr>
<th>MIR DATA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>SAT</td>
</tr>
<tr>
<td>C6:0</td>
<td>MONO</td>
</tr>
<tr>
<td>C8:0</td>
<td>POLY</td>
</tr>
<tr>
<td>C10:0</td>
<td>INSAT</td>
</tr>
<tr>
<td>C12:0</td>
<td>SCFA: C4 to C10</td>
</tr>
<tr>
<td>C14:0</td>
<td>MCFA: C12 to C16</td>
</tr>
<tr>
<td>C14:1 cis</td>
<td>LCFA: C17 and more</td>
</tr>
<tr>
<td>C16:0 iso + anteiso</td>
<td></td>
</tr>
<tr>
<td>C16:1 cis</td>
<td>omega 3</td>
</tr>
<tr>
<td>C17:0</td>
<td>omega 6</td>
</tr>
<tr>
<td>C18:0 ODD</td>
<td></td>
</tr>
<tr>
<td>Tot18_1trans Total_Trans</td>
<td></td>
</tr>
<tr>
<td>C18:1 cis9</td>
<td>BHB</td>
</tr>
<tr>
<td>Tot18_1cis</td>
<td>Acetone</td>
</tr>
<tr>
<td>C18_2c9c12</td>
<td>Citrates</td>
</tr>
<tr>
<td>C18_3c9c12c15</td>
<td>Na</td>
</tr>
<tr>
<td>C18_2c9t11</td>
<td>Ca</td>
</tr>
<tr>
<td>SAT</td>
<td>P</td>
</tr>
<tr>
<td>MONO</td>
<td>Mg</td>
</tr>
<tr>
<td>POLY</td>
<td>K</td>
</tr>
<tr>
<td>INSAT</td>
<td>Lactoferrin</td>
</tr>
</tbody>
</table>

**Milk Metabolite**
- Glu6P
- GluFree
- BOHB
- IsoC
- Urea
- NA Gase
- Uric acid
- Progesteron

Protocols of milk standard laboratory
- Protocols of Larsen, 2005;
- Larsen and Nielsen, 2005;
- Chagunda et al, 2006;
- Larsen and Moyes, 2010;
- Larsen 2014.

Protocols of Larsen, 2005;
- Larsen and Nielsen, 2005;
- Chagunda et al, 2006;
- Larsen and Moyes, 2010;
- Larsen 2014.

Fourier transform mid-infrared (FT-MIR) spectroscopy according to
3. Materials and methods
Statistical analysis

<table>
<thead>
<tr>
<th>SCC class</th>
<th>Country</th>
<th>N° MILK SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>low (≤100,000)</td>
<td>AFBi</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>AU</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>CraitA</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Craw</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>FBN</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>UCD</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>413</td>
</tr>
<tr>
<td>medium (101,000≤X≤400,000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AFBi</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AU</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>CraitA</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Craw</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>FBN</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>UCD</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>123</td>
</tr>
<tr>
<td>high (≥401,000)</td>
<td>AFBi</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>AU</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CraitA</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Craw</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>FBN</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>UCD</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>87</td>
</tr>
</tbody>
</table>

Shapiro-Wilks test for normality

Correlation

Canonical discriminat analysis

MIXED GLM model

\[ Y_{ijklmn} = \mu + P_i + S_j + C_k + H_l + \alpha_m + \beta(MY)_{ijklmn} + e_{ijklmn} \]

- \( Y_{ijklmn} \) = observed value of milk parameters
- \( \mu \) = overall mean
- \( P_i \) = fixed effect of parity
- \( S_j \) = fixed effect of season of calving
- \( C_k \) = fixed effect of the days in milk
- \( H_l \) = fixed effect of the SCC
- \( \alpha \) = random effect
- \( \beta(MY)_{ijklmn} \) = regression effect of milk yield with the regression coefficient
- \( e_{ijklmn} \) = random error

DairyCare Conference- 19-21th March-Thessaloniki
Results 1

Differences between country
Results 1

Differences between country

---

**Na for country**

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAITA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Ca for country**

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAITA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**P for country**

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAITA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

DairyCare Conference- 19-21th March-Thessaloniki
Results 1

Differences between country

Mg for country

K for country

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAITA</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAW</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBN</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCD</td>
<td>96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFBI</td>
<td>1400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td>1420</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAITA</td>
<td>1440</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRAW</td>
<td>1460</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBN</td>
<td>1480</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCD</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results

Differences between sampling day

SCC for sampling day

Lactoferrin for sampling day

NAGase for sampling day

LDH for sampling day

1 = 7±3
2 = 14±3
3 = 21±3
4 = 35±3
Results 1

Differences between sampling day

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>Na (mg/Kg)</th>
<th>Ca (mg/Kg)</th>
<th>P (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>340</td>
<td>1040</td>
<td>900</td>
</tr>
<tr>
<td>2</td>
<td>360</td>
<td>1060</td>
<td>920</td>
</tr>
<tr>
<td>3</td>
<td>380</td>
<td>1080</td>
<td>940</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>1100</td>
<td>960</td>
</tr>
</tbody>
</table>

1 = 7 ± 3
2 = 14 ± 3
3 = 21 ± 3
4 = 35 ± 3
Results 1

Differences between sampling day

**Results**

1. Differences between sampling day:

   - **Mg for sampling day**
     - Mean
     - Mean±SE
     - Mean±1.96*SE
     - 1 = 7 ± 3
     - 2 = 14 ± 3
     - 3 = 21 ± 3
     - 4 = 35 ± 3

   - **K for sampling day**
     - Mean
     - Mean±SE
     - Mean±1.96*SE
     - 1460
     - 1480
     - 1500
     - 1520
     - 1540
     - 1560
     - 1580
     - 1600

   - [Charts showing Mg and K distribution with mean, standard error, and 1.96 standard error for each sampling day.]
Results 1

Differences between SCC group

NAGase for group SCC

LDH for SCC group

Lactoferrin for SCC group

low (≤100,000)
medium (101,000≤X≤400,000)
high (≥401,000)
Results 1

Differences between SCC group

**Na for SCC group**

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ca for SCC group**

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>1120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1130</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**P for SCC group**

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean</th>
<th>Mean±SE</th>
<th>Mean±1.96*SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>980</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Levels**

- Low (≤100,000)
- Medium (101,000≤X≤400,000)
- High (≥401,000)
Results 1

Differences between SCC group

**Mg for SCC group**

- Low (≤100,000)
- Medium (101,000 ≤ X ≤ 400,000)
- High (≥ 401,000)

**K for SCC group**
## Results 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Obs</th>
<th>MSE</th>
<th>Country</th>
<th>Parity</th>
<th>Control</th>
<th>LevelSCC</th>
<th>LevelSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (Kg/d)</td>
<td>623</td>
<td>35.6</td>
<td>*** ***</td>
<td>*** ***</td>
<td>*** ***</td>
<td>27.7 c</td>
<td>29.9 b</td>
</tr>
<tr>
<td>Fat (g/100mL)</td>
<td>623</td>
<td>0.93</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>4.42 AB</td>
<td>4.55 A</td>
</tr>
<tr>
<td>Protein (g/100mL)</td>
<td>623</td>
<td>0.06</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>3.28</td>
<td>3.24</td>
</tr>
<tr>
<td>Lactose (g/100mL)</td>
<td>548</td>
<td>0.04</td>
<td>↑</td>
<td>*** ***</td>
<td>*** ***</td>
<td>4.56 b</td>
<td>4.71 a</td>
</tr>
<tr>
<td>Glu6P (mM)</td>
<td>623</td>
<td>0.004</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>0.245 a</td>
<td>0.204 b</td>
</tr>
<tr>
<td>GluFree (mM)</td>
<td>623</td>
<td>0.005</td>
<td>***</td>
<td>*** ***</td>
<td>*** ***</td>
<td>0.185 bB</td>
<td>0.201 bA</td>
</tr>
<tr>
<td>BOBH (Ln mM)</td>
<td>623</td>
<td>0.18</td>
<td>*** ***</td>
<td>-</td>
<td>-</td>
<td>4.01</td>
<td>4.01</td>
</tr>
<tr>
<td>IsoC (mM)</td>
<td>623</td>
<td>0.002</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>0.178 B</td>
<td>0.193 A</td>
</tr>
<tr>
<td>Urea (mM)</td>
<td>619</td>
<td>0.95</td>
<td>***</td>
<td>*</td>
<td>↑</td>
<td>2.88 b</td>
<td>3.13 a</td>
</tr>
<tr>
<td>NAGase (U/L)</td>
<td>623</td>
<td>1.83</td>
<td>***</td>
<td>**</td>
<td>*** ***</td>
<td>4.79 a</td>
<td>2.75 b</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>623</td>
<td>8.17</td>
<td>*** ***</td>
<td>*** ***</td>
<td>*** ***</td>
<td>8.89 a</td>
<td>4.27 b</td>
</tr>
<tr>
<td>Uric acid (mM)</td>
<td>622</td>
<td>2429</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>162</td>
<td>165</td>
</tr>
<tr>
<td>Prog (U/L)</td>
<td>622</td>
<td>4.14</td>
<td>*</td>
<td>-</td>
<td>***</td>
<td>4.02 b</td>
<td>4.18 ab</td>
</tr>
</tbody>
</table>

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001  A, B: P < 0.10; a, b, c: P < 0.05  -  MSE: Mean Standard Error
### Results 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Obs</th>
<th>MSE</th>
<th>Country</th>
<th>Parity</th>
<th>Control</th>
<th>LevelSC</th>
<th>LevelSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/100mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>C4 : 0</td>
<td>458</td>
<td>0.11</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>-</td>
<td>2.89</td>
</tr>
<tr>
<td>C6 : 0</td>
<td>458</td>
<td>0.04</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>†</td>
<td>1.70&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C8 : 0</td>
<td>458</td>
<td>0.04</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>1.03&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>C10 : 0</td>
<td>458</td>
<td>0.46</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>2.12</td>
</tr>
<tr>
<td>C12 : 0</td>
<td>458</td>
<td>0.68</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>2.46</td>
</tr>
<tr>
<td>C14 : 0</td>
<td>458</td>
<td>2.67</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>9.58</td>
</tr>
<tr>
<td>C14 : 1 cis</td>
<td>458</td>
<td>0.03</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>0.66</td>
</tr>
<tr>
<td>C16 : 0</td>
<td>458</td>
<td>14.2</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>28.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16 : 1 cis</td>
<td>458</td>
<td>0.10</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>-</td>
<td>1.76&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>C17 : 0</td>
<td>458</td>
<td>0.002</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>-</td>
<td>0.685</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>458</td>
<td>1.96</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>12.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tot C18 : 1 trans</td>
<td>458</td>
<td>0.79</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>†</td>
<td>3.12&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18 : 1 cis-9</td>
<td>458</td>
<td>20.7</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>23.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tot C18 : 1 cis</td>
<td>458</td>
<td>23.2</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>25.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001  
A, B: P < 0.10;  
a, b, c: P < 0.05  
-MSE: Mean Standard Error
<table>
<thead>
<tr>
<th>Item</th>
<th>Obs</th>
<th>MSE</th>
<th>Country</th>
<th>Parity</th>
<th>Control</th>
<th>LevelSCC</th>
<th>LevelSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/100mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>C18 : 2</td>
<td>458</td>
<td>0.10</td>
<td>***</td>
<td>†</td>
<td>***</td>
<td>-</td>
<td>2.51</td>
</tr>
<tr>
<td>C18 : 2 cis-9,cis-12</td>
<td>458</td>
<td>0.07</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>1.76</td>
</tr>
<tr>
<td>C18 : 3 cis-9,cis-12,cis-15</td>
<td>458</td>
<td>0.01</td>
<td>***</td>
<td>-</td>
<td>**</td>
<td>-</td>
<td>0.54</td>
</tr>
<tr>
<td>C18 : 2 cis-9,trans-11</td>
<td>458</td>
<td>0.05</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>0.47</td>
</tr>
<tr>
<td>Saturated FA</td>
<td>458</td>
<td>25.7</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>†</td>
<td>64.8&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>MUFA</td>
<td>458</td>
<td>27.1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>31.0</td>
</tr>
<tr>
<td>PUFA</td>
<td>458</td>
<td>0.47</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>3.97</td>
</tr>
<tr>
<td>Unsaturated FA</td>
<td>458</td>
<td>31.0</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>34.8</td>
</tr>
<tr>
<td>Short chain</td>
<td>458</td>
<td>1.28</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>†</td>
<td>7.95&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium chain</td>
<td>458</td>
<td>30.1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>44.2</td>
</tr>
<tr>
<td>Long chain</td>
<td>458</td>
<td>41.4</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>46.8</td>
</tr>
<tr>
<td>Isoanteiso</td>
<td>458</td>
<td>0.05</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>1.88</td>
</tr>
<tr>
<td>Omega3</td>
<td>458</td>
<td>0.02</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>0.69</td>
</tr>
<tr>
<td>Omega6</td>
<td>458</td>
<td>0.22</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>2.68</td>
</tr>
<tr>
<td>ODD</td>
<td>458</td>
<td>0.11</td>
<td>***</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>3.39</td>
</tr>
<tr>
<td>Total trans</td>
<td>458</td>
<td>1.07</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>†</td>
<td>3.96&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tot C18 : 1</td>
<td>458</td>
<td>26.1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>27.9</td>
</tr>
</tbody>
</table>

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001  A, B: P < 0.10; a, b, c: P < 0.05  -  MSE: Mean Standard Error
<table>
<thead>
<tr>
<th>Item</th>
<th>Obs</th>
<th>MSE</th>
<th>Country</th>
<th>Parity</th>
<th>Control</th>
<th>LevelSCC</th>
<th>LevelSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>Acetone (mM)</td>
<td>458</td>
<td>0.01</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Citrates (mM)</td>
<td>458</td>
<td>3.16</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>9.13</td>
</tr>
<tr>
<td>Na (mg/Kg)</td>
<td>458</td>
<td>2069</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>423&lt;sup&gt;a&lt;/sup&gt; 379&lt;sup&gt;b&lt;/sup&gt; 380&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca (mg/Kg)</td>
<td>458</td>
<td>6863</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>-</td>
<td>1189</td>
</tr>
<tr>
<td>P (mg/Kg)</td>
<td>458</td>
<td>6159</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>1048</td>
</tr>
<tr>
<td>Mg (mg/Kg)</td>
<td>458</td>
<td>73</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>103</td>
</tr>
<tr>
<td>K (mg/Kg)</td>
<td>458</td>
<td>10343</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>1533</td>
</tr>
<tr>
<td>Lactoferrin (Ln mg/L)</td>
<td>458</td>
<td>0.83</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>5.14&lt;sup&gt;a&lt;/sup&gt; 4.54&lt;sup&gt;b&lt;/sup&gt; 4.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001  
A, B: P < 0.10;  a, b, c: P < 0.05  
MSE: Mean Standard Error
### Results 3

#### Canonical discriminant analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAN1</th>
<th>CAN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>-0.561</td>
<td>0.045</td>
</tr>
<tr>
<td>Glu6P</td>
<td>0.350</td>
<td>0.217</td>
</tr>
<tr>
<td>GluFree</td>
<td>-0.335</td>
<td>-0.169</td>
</tr>
<tr>
<td>NAGase</td>
<td>0.772</td>
<td>0.1640</td>
</tr>
<tr>
<td>LDH</td>
<td>0.754</td>
<td>0.119</td>
</tr>
<tr>
<td>UA</td>
<td>0.157</td>
<td>0.332</td>
</tr>
<tr>
<td>C6</td>
<td>-0.158</td>
<td>-0.323</td>
</tr>
<tr>
<td>C8</td>
<td>-0.259</td>
<td>-0.352</td>
</tr>
<tr>
<td>C10</td>
<td>-0.284</td>
<td>-0.303</td>
</tr>
<tr>
<td>C12</td>
<td>-0.300</td>
<td>-0.292</td>
</tr>
<tr>
<td>C14</td>
<td>-0.288</td>
<td>-0.332</td>
</tr>
<tr>
<td>C16_1c</td>
<td>0.300</td>
<td>0.351</td>
</tr>
<tr>
<td>C17</td>
<td>0.325</td>
<td>0.466</td>
</tr>
<tr>
<td>SCFA</td>
<td>-0.242</td>
<td>-0.355</td>
</tr>
<tr>
<td>Na</td>
<td>0.626</td>
<td>0.029</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.457</td>
<td>0.054</td>
</tr>
</tbody>
</table>

#### Means of canonical variables

<table>
<thead>
<tr>
<th>Level of SCC</th>
<th>CAN1</th>
<th>CAN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>2.465</td>
<td>0.328</td>
</tr>
<tr>
<td>Low</td>
<td>-0.590</td>
<td>-0.261</td>
</tr>
<tr>
<td>Medium</td>
<td>-0.091</td>
<td>1.0580</td>
</tr>
</tbody>
</table>

#### Eigenvalue

<table>
<thead>
<tr>
<th></th>
<th>Proportional variance</th>
<th>Cumulative variance</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAN1</td>
<td>1.134</td>
<td>0.795</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CAN2</td>
<td>0.292</td>
<td>0.205</td>
<td>0.0258</td>
</tr>
</tbody>
</table>

#### Canonical value for each parameter
Results 3

Canonical discriminant analysis

DairyCare Conference - 19-21th March - Thessaloniki
Conclusion

- GLM confirmed NAGase, LDH, Lactoferrin, Na, Lactose

- Using discriminant analysis same parameters discriminate better High vs (Medium&Low), other parameters discriminate Medium vs (Low&High)

- How do these parameters interact?

- Which are the better to diagnose health status of mammary gland?
Future remarks

- Analysis of parameters obtained by milk samples of thousand cows
- GEBV
- GWAS
- The better milk parameters to detect or prevent diseases
- Use GplusE results in new management strategies and in new breeding schemes to obtain “the perfect cow”!
Acknowledgement

Napolitano Francesco Signorelli Federica Luca Buttazzoni

Klaus Lønne Ingvartsen Martin Tang Sørensen Torben Larsen

Clément Grelet Nicolas Gengler Frédéric Dehareng Hélène Soyeurt Miel Hostens

Mark Crowe, coordinator of GplusE
Acknowledgement