



Opportunities of exploiting  
MIR in dairy farming, much more  
than simple phenotyping through  
the prediction of major milk components

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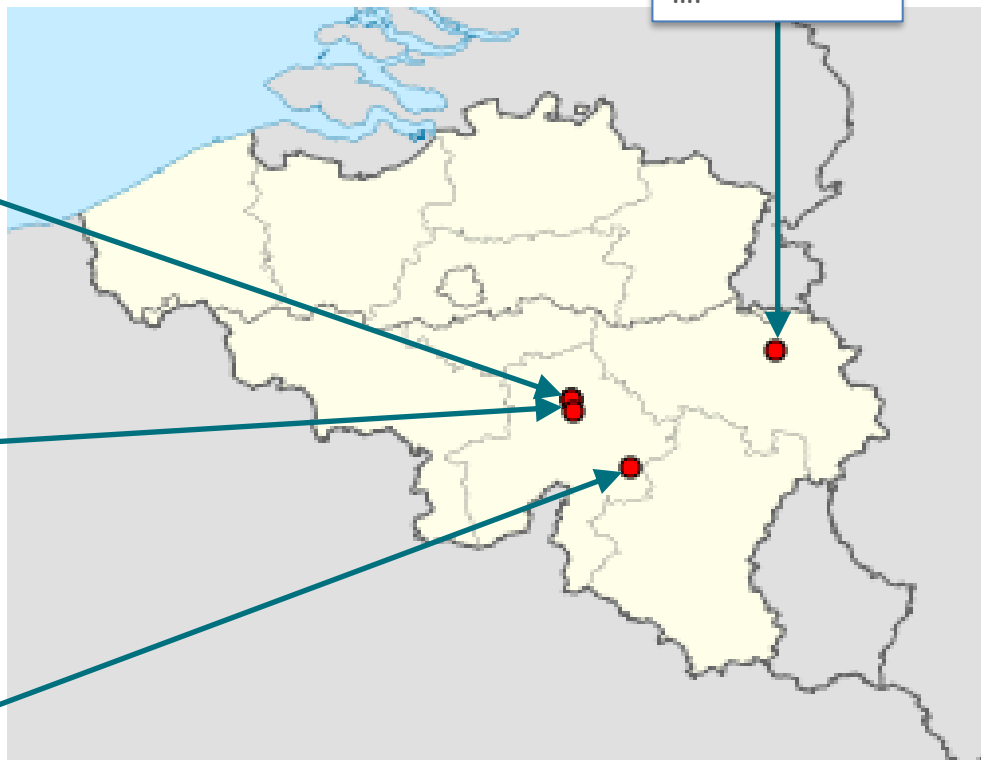
# FUTUROSPECTRE since 2008

P. Dardenne  
F. Dehareng  
C. Grelet  
A. Vanlierde  
....

H. Soyeurt  
N. Gengler  
....

C. Bertozzi  
C. Bastin  
....

E. Piraux  
D. Veselko  
....



# Outline of Presentation

Milk recording and  
MIR spectrometry

Usefulness of (fine)  
milk composition

MIR spectrometry  
and novel milk  
composition  
based traits

Recent innovations  
in the use of milk  
MIR spectral data



# Part I: Reminder: Milk Recording and MIR Spectrometry

# Reminder: Milk Recording

## ➤ Developed in the early 1900's

➤ .....

➤ France

- Normandy (76) in 1907

➤ Denmark

➤ Germany

➤ .....

Status  
in 1935



- Regular visits to farms by a technician (mostly)
  - Each week (experimental farms) to 4-6-8 weeks
  - Records quantity of milk and takes milk samples

# Milk Recording (MR)

- Early picture
  - Shows why in French milk recording agents often not called “contrôleurs” but “peseurs”
- Explains also
  - Tradition to express milk yields in kg (or lbs) of milk



ARS-USDA



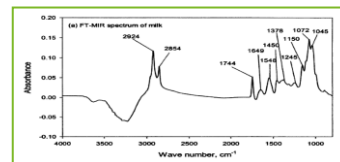
# Predicting Milk Components



**Milk samples**  
(milk payment, milk recording)



**MIR spectrometry analysis**



**Raw data = MIR spectra**

**Manufacturer's prediction**



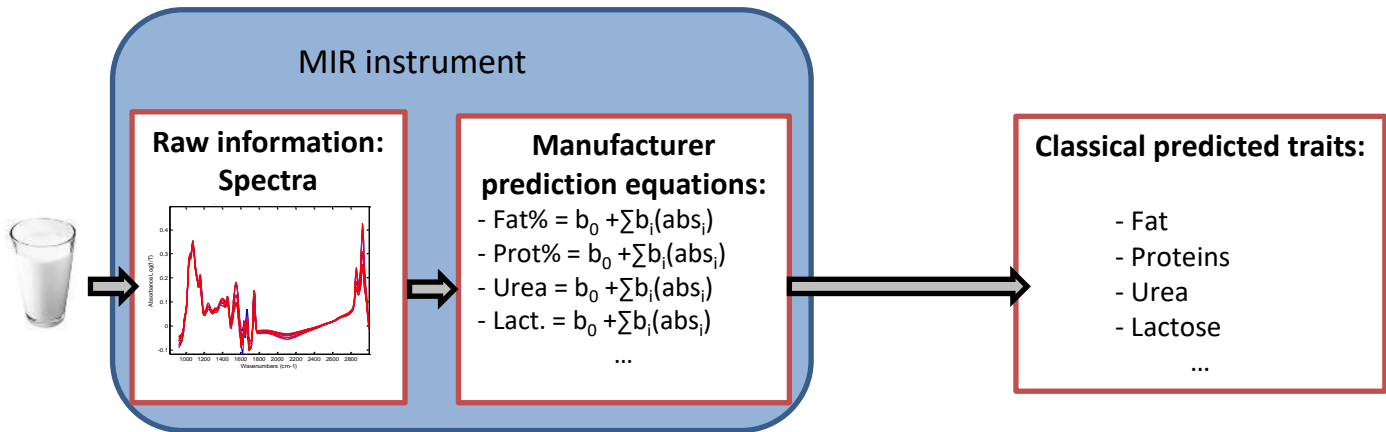
**equations**

**Quantification:**

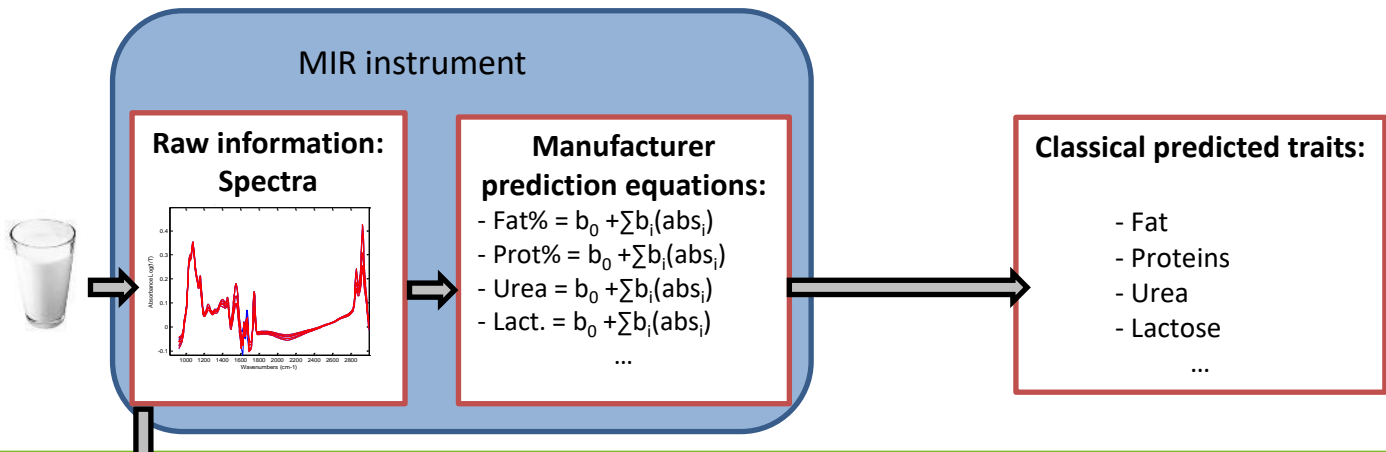
Fat, protein, urea, lactose....



## Classical Use



## Classical Use



**CdL**  
comité du lait

Extraction  
of the raw  
information

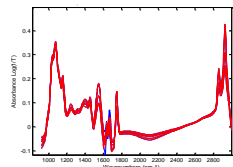
## Novel Uses

← still based on "simple" calibrations

Wallonie  
recherche  
CRA-W



**Raw information:  
Spectra**



**Standardized  
Spectra**

**More later**



**New prediction equations:**

- Equation 1 =  $b_0 + \sum b_i(abs_i)$
  - Equation 2 =  $b_0 + \sum b_i(abs_i)$
  - Equation 3 =  $b_0 + \sum b_i(abs_i)$
  - Equation 4 =  $b_0 + \sum b_i(abs_i)$
  - Equation 5 =  $b_0 + \sum b_i(abs_i)$
- ...

**New predicted traits:**

- Prediction 1
  - Prediction 2
  - Prediction 3
  - Prediction 4
  - Prediction 5
- ...

# New MIR Predicted Phenotypes

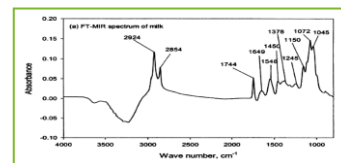
New



*Milk samples*  
(milk payment, milk recording)



*MIR spectrometry analysis*



*Raw data = MIR spectra*

*New prediction equations*



*Quantification:*

**New:** fatty acids, ...





## Part II: Usefulness of (Fine) Milk Composition

# Milk Composition

- Milk composition
  - Highly affected by feed and genetic background
- But also:
  - Health
  - And many other factors

⇒ **very intense interaction**  
**Blood circulation ⇔ Milk composition**

⇒ **Concept of Biomarkers**

# Concept of Biomarkers

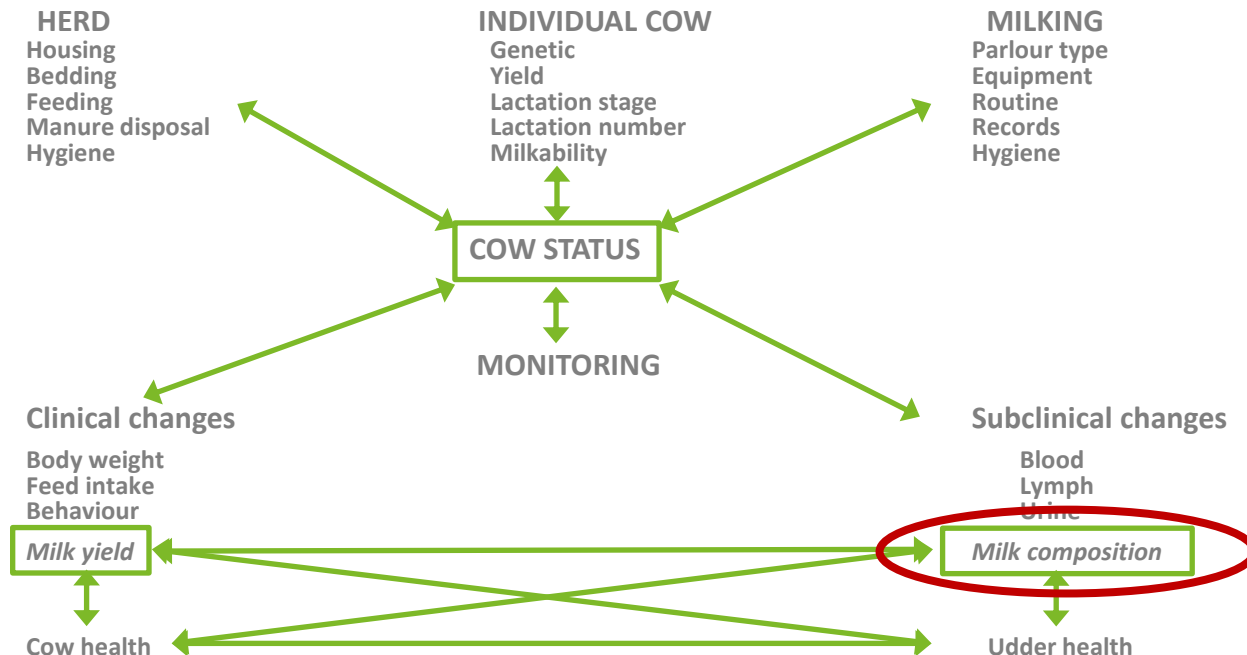
## ➤ Definition

- “... objectively measured and evaluated ... indicator of normal biological processes, pathogenic processes, or ... responses to an ... intervention”

(National Institutes of Health)

# Milk Composition as Source of “Biomarkers” for Cow Status and Health

## Factors of influence determining cow health



# Milk Composition

- Milk composition
  - Highly affected by feeding and genetic background
- But also:
  - Health
  - And many other factors
- Very intense interaction:
  - Blood circulation ↔ Milk production
- Therefore: milk composition could be used to monitor
  - Status and health (wellbeing)
- But also
  - Diet
  - Environmental impact
  - Quality of milk and milk products

# Milk Composition

## ➤ Use of 5 major conventional components\*

- Fat content
- Protein content
- Somatic cell count\*\*
- Urea
- Lactose

**Milk payment +  
Milk recording**

**Certain milk  
recording plans**

\* Some variants between countries (e.g., in USA use of Solid-Non-Fat) or different definitions as “True protein” (e.g., France, USA) vs. others “Crude protein” (e.g., Belgium)

\*\* Different from the other components (no content but counting of somatic cells), use of different technology

# Major Components ↔ Status

- Known for long time and available on a large scale
  - ➔ Extensive research to establish usefulness
- Useful as indicators or proxies
  - ➔ Status of the cow ?
- Abundant literature
- Here only most important findings

# Somatic Cell Count (SCC)

- SCC not a typical component
  - Count of cells not a content of molecule(s) of interest
- From the beginning SCC developed as
  - Indicator or proxy for udder infection (mastitis)
  - Then entered payment systems
  - Difference to other components like fat content also in the used technology
- Inconvenience
  - Somatic cells are part of the udder defense strategy
  - Controversy ⇨ are low SCC good or bad?
  - Need for novel (MIR based) biomarkers!

# Use of Fat, Protein, Urea and Lactose

- Some examples of use of major milk components:
  - Fat content depression
    - ⇒ Potential subacute ruminal acidosis (SARA)
  - High fat / protein ratio
    - ⇒ Potential negative energy balance (and associated issues as ketosis ...)
  - Urea and protein content
    - ⇒ Indicator of ruminal balance between protein and energy
  - Reduction in lactose content
    - ⇒ Indicator of mastitis
  - However, many authors also showed
    - ⇒ Limitation in usefulness of major milk components
  - Need for **novel (MIR based) biomarkers!**

# Fine Milk Composition

- Until recently ~ 5 major major components
- However
  - Milk is a very complex substance
  - Large number of constituents
  - Some major components themselves
    - ⇒ complex groupings of minor constituents

**Therefore:**

⇒ **many potential Biomarkers**

# Fine Milk Composition

- Milk fat
  - Fatty acids (FA) mostly as triglycerides
  - Non-esterified fatty acids (NEFAs)
- Milk protein
  - Caseins
  - $\alpha$ -lactalbumins
  - $\beta$ -lactoglobulins
  - Other minor proteins (e.g., lactoferrin)
- Other minor constituents
  - $\beta$ -hydroxybutyrate (BHBA)
  - Acetone and acetoacetate
  - Minerals
  - Citrate
  - Vitamins
  - ....

# Fine Milk Composition

- Therefore: complexity of fine milk composition very useful to assess indirectly (some examples):
  - Animal (health) status  
(e.g., ketosis using BHBA, acetone, acetoacetate and citrate)
  - Milk and milk product quality, technological properties  
(e.g., FA, caseins)
  - Udder health  
(e.g., lactoferrin, minerals)
  - And even, as shown by recent research, feeding behavior under heat stress  
(e.g., FA linked to body fat reserve mobilization)



J. Dairy Sci. 98:4956–4968  
<http://dx.doi.org/10.3168/jds.2014-9148>  
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**Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows**

H. Hammami,\*†<sup>1</sup> J. Vandenplas,\*† M.-L. Vanrobays,\* B. Rekik,‡ C. Bastin,\* and N. Gengler\*

# Fine Milk Composition

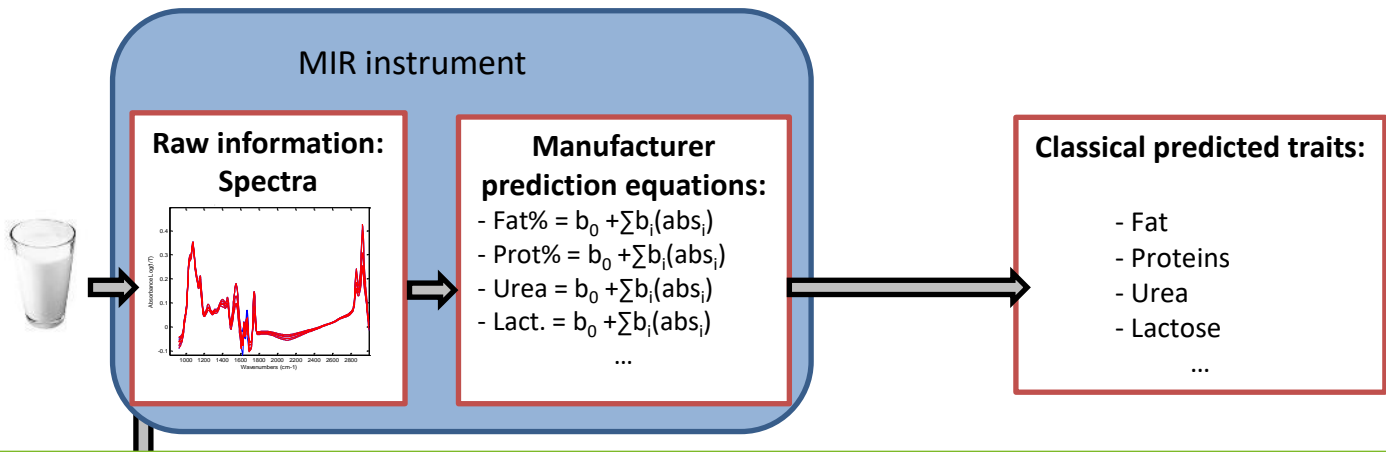
- Therefore, complexity of fine milk composition very useful to assess indirectly (some examples):
  - Animal (health) status
  - Milk and milk product quality, technological properties
  - Udder health
  - Feeding behavior under heat stress
- But also: environmental impact traits:
  - Phosphorus efficiency
  - Nitrogen efficiency
  - Energy efficiency and Methane

⇐ **example link to FA**



## Part III: MIR spectrometry and novel milk composition based traits

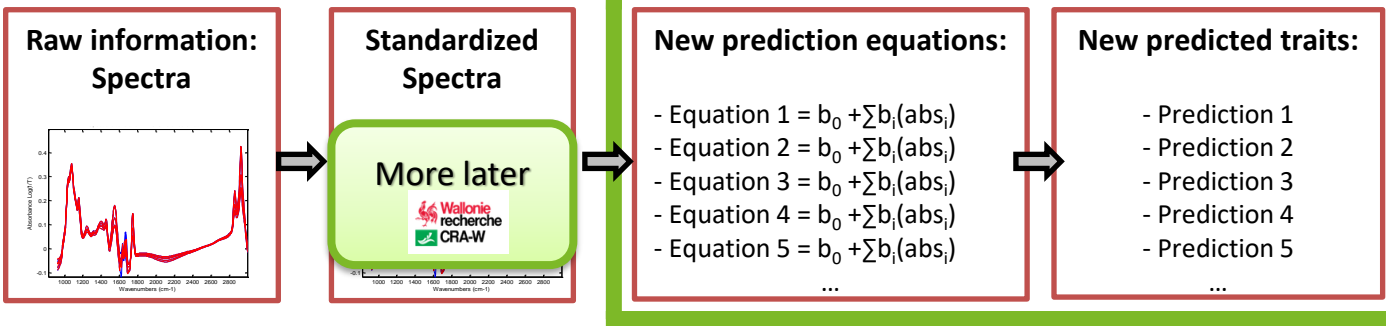
## Classical Use



Extraction  
of the raw  
information

## Novel Uses

← still based on “simple” calibrations



# Examples of Applications

## Milk quality



### Estimating Fatty Acid Content in Cow Milk Using Mid-Infrared Spectrometry

H. Soyeurt,<sup>1,2</sup> P. Dardenne,<sup>3</sup> F. Deh P. Mayeres,<sup>4</sup> and N. Gengler<sup>1</sup>

### Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry

H. Soyeurt,<sup>1</sup> D. Bruwier,<sup>2</sup> J.

Prediction of individual milk proteins including free amino acids in bovine milk using mid-infrared spectroscopy and their correlations with milk processing characteristics

A. McDermott,<sup>1</sup> G. Visentin,<sup>2</sup> M. De Marchi,<sup>3</sup> D. P. Berry,<sup>4</sup> M. A. Fenelon,<sup>5</sup> P. M. O'Connor,<sup>6</sup> O. A. Kenny,<sup>7</sup> and S. McParland<sup>8</sup>

## Technological properties



### Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy

M. De Marchi,<sup>1</sup> C. C. Fagan,<sup>2</sup> C. P. O'Donnell,<sup>3</sup> A. Cecchinato,<sup>4</sup> R. Dal Zotto,<sup>5</sup> M. Cassandro,<sup>6</sup> M. Penasa,<sup>7</sup> and G. Bittante<sup>8</sup>

### Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows

### Mid-infrared prediction of lactoferrin content in bovine milk: potential indicator of mastitis

F. Dehareng,<sup>1</sup> A. Vanlierde<sup>2</sup>

### The potential of Fourier transform infrared spectroscopy of milk samples to predict energy intake and efficiency in dairy cows<sup>1</sup>

H. Soyeurt,<sup>1,2</sup> F. Dehareng,<sup>3</sup> M. Coffey,<sup>4</sup> L.

S. McParland

Development of Fourier transform mid-infrared calibrations to predict acetone,  $\beta$ -hydroxybutyrate, and citrate contents in bovine milk through a European dairy network

C. Grelet,<sup>1</sup> C. F. G. Colinet

Prediction and validation of residual feed intake and dry matter intake in Danish lactating dairy cows using mid-infrared spectroscopy of milk

N. Shetty,<sup>1</sup> P. L.

Assessing the effect of pregnancy stage on milk composition of dairy cows using mid-infrared spectra

A. Lainé,<sup>1</sup> C. Bastin,<sup>2</sup> C. Grelet,<sup>3</sup> H. Hammami,<sup>4</sup> F. G. Colinet,<sup>5</sup> L. M. Dale,<sup>6,7</sup> A. Gillon,<sup>8</sup> J. J. Vandenplas,<sup>9</sup> F. Dehareng,<sup>10</sup> and N. Gengler<sup>11</sup>

## Cow phenotypes



## Outliers, detection of contaminants



Use of a multivariate moving window PCA for the untargeted detection of contaminants in agro-food products, as exemplified by the detection of melamine levels in milk using vibrational spectroscopy

J.A. Fernández Pierna, D. Vincke, V. Baeten, C. Grelet, F. Dehareng, P. Dardenne<sup>\*</sup>

## Milk origin determination



Building of prediction models by using Mid-Infrared spectroscopy and fatty acid profile to discriminate the geographical origin of sheep milk

Marco Caredda<sup>1</sup>, Margherita Addis<sup>2</sup>, Ignazio Idda<sup>3</sup>, Riccardo Leardi<sup>4</sup>, Maria Francesca Scintu<sup>5</sup>, Giovanni Piredda<sup>6</sup>, Gavino Sanna<sup>6,7</sup>

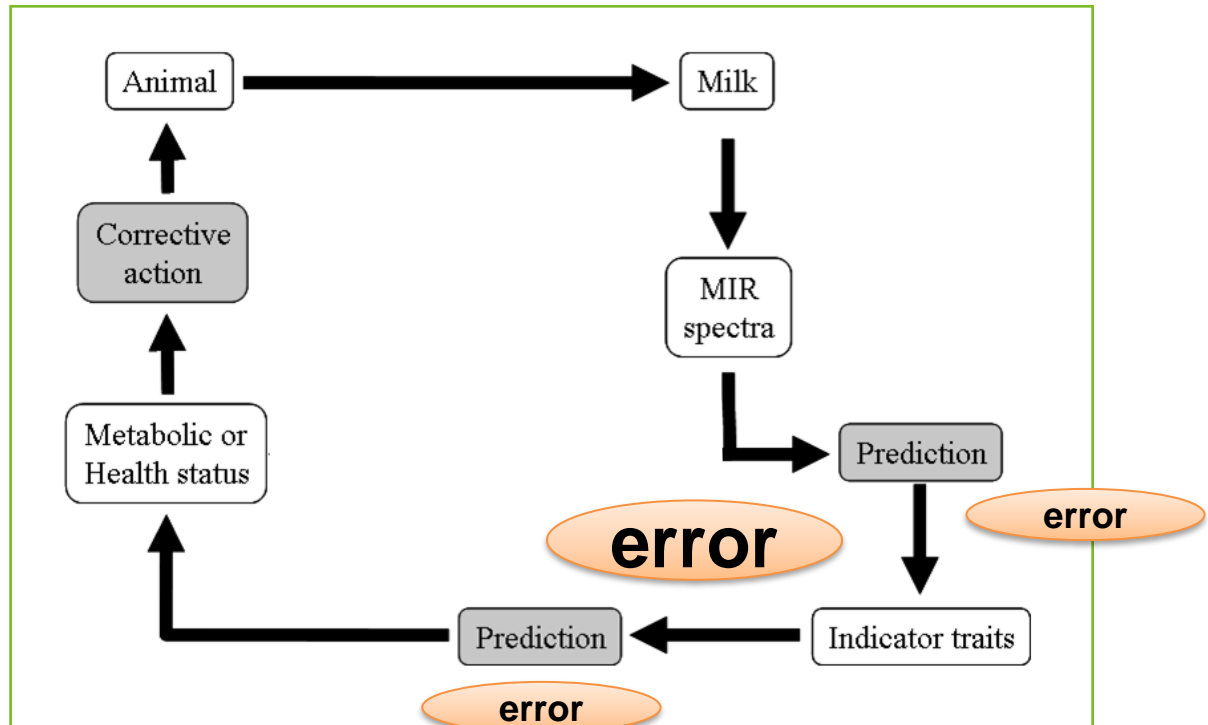


## Part IV: Recent innovations in the use of milk MIR spectral data

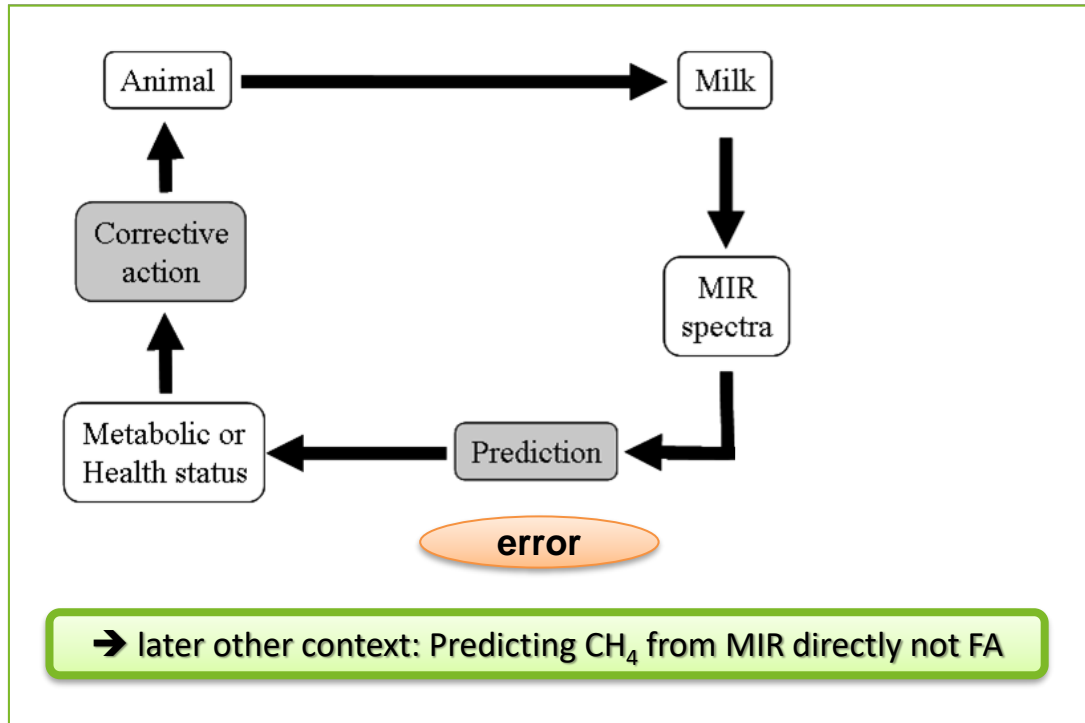
## ***1st Innovation:***

# **Direct Prediction of Traits of Interest from MIR Spectra**

# MIR ⇒ Components ⇒ Trait of Interest



## Directly: MIR $\Rightarrow$ Trait of Interest



## *2nd Innovation:*

**Adjusting calibrations for  
longitudinal traits: example CH<sub>4</sub>**

# Context – Measuring CH<sub>4</sub>

- CH<sub>4</sub> ← large scale phenotyping
  - Difficult, time consuming, expensive
  - Proxies ?
- Use of a milk mid-infrared (MIR) spectra based proxy
  - Was illustrated as being a real opportunity

Animal, page 1 of 8 • The Animal Consortium 2012  
doi:10.1017/S1751101312000496



## Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows

F. Dehareng<sup>1,†</sup>, C. Delfosse<sup>1,†</sup>, E. Froidmont<sup>2</sup>, H. Soyeurt<sup>3,4</sup>, C. Martin<sup>5</sup>, N. Gengler<sup>3,4</sup>, A. Vanlierde<sup>1</sup> and P. Dardenne<sup>1</sup>

<sup>1</sup>Valization of Agricultural Products Department, Walloon Agricultural Research Centre, B-5030 Gembloux, Belgium; <sup>2</sup>Department of Production and Sector, Agricultural Research Centre, B-5030 Gembloux, Belgium; <sup>3</sup>Animal Science Unit, Gembloux Agro-Bio-Tech, University of Liège, B-5030 Gembloux, Belgium; <sup>4</sup>Unit for Scientific Research, B-1000 Brussels, Belgium; <sup>5</sup>ULB213 Herbivores, INRA/UMRI1295 Research Centre, F-63122 Saint-Genès-Champagnelle, France



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## Hot topic: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra

A. Vanlierde,<sup>1</sup> M.-L. Vanrobays,<sup>1</sup> F. Dehareng,<sup>2</sup> E. Froidmont,<sup>3</sup> H. Soyeurt,<sup>4</sup> S. McParland,<sup>5</sup> E. Lewis,<sup>6</sup> M. H. Deighton,<sup>7</sup> F. Grandi,<sup>8</sup> M. Kreuzer,<sup>9</sup> B. Gredler,<sup>10</sup> P. Dardenne,<sup>1</sup> and N. Gengler<sup>1</sup>  
<sup>1</sup>Walloon Agricultural Research Centre, Valization of Agricultural Products Department, 5030 Gembloux, Belgium  
<sup>2</sup>Agriculture, Bio-engineering and Chemistry Department, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium  
<sup>3</sup>Walloon Agricultural Research Centre, Production and Sector Department, 5030 Gembloux, Belgium  
<sup>4</sup>Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland  
<sup>5</sup>Agriculture Research Division, Department of Economic Development, Jobs, Transport and Resources, Ellinbank Centre, 3521 Victoria, Australia  
<sup>6</sup>ETH Zürich, Institute of Agricultural Sciences, 8052 Zürich, Switzerland  
<sup>7</sup>Qualitas AG, 6300 Zug, Switzerland



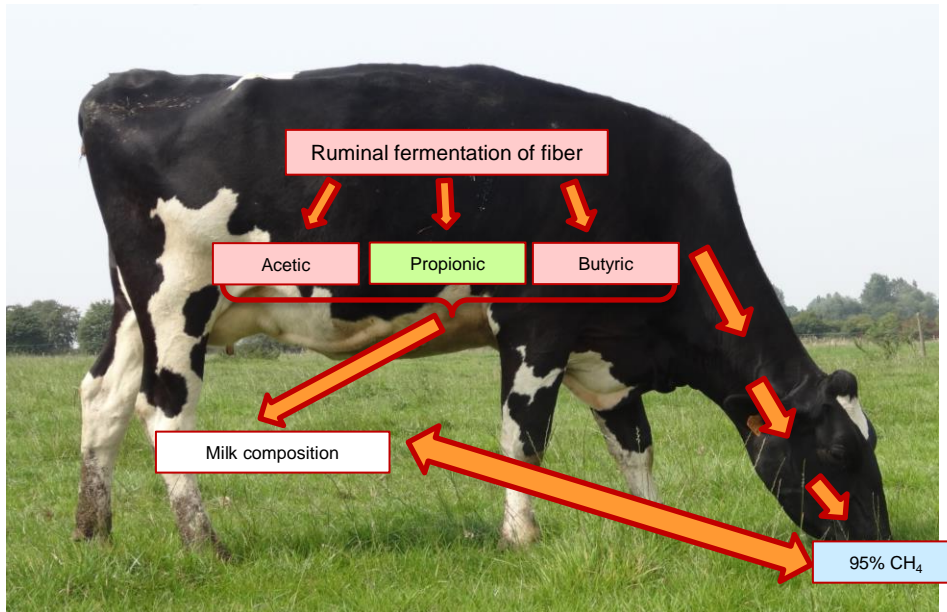
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**Short communication:** Development of an equation for estimating methane emissions of dairy cows from milk Fourier transform mid-infrared spectra by using reference data obtained exclusively from respiration chambers

A. Vanlierde,<sup>1</sup> H. Soyeurt,<sup>4</sup> N. Gengler,<sup>1</sup> F. G. Colinet,<sup>4</sup> E. Froidmont,<sup>3</sup> M. Kreuzer,<sup>9</sup> F. Grandi,<sup>8</sup> M. Belli,<sup>10</sup> P. Lund,<sup>11</sup> D. W. Olijhoek,<sup>12</sup> M. Eugène,<sup>13</sup> C. Martin,<sup>14</sup> B. Kuhla,<sup>15</sup> and F. Dehareng<sup>1</sup>  
<sup>1</sup>Walloon Agricultural Research Centre, Valization of Agricultural Products, 5030 Gembloux, Belgium  
<sup>2</sup>Gembloux Agro-Bio Tech, University of Liège, Agroalchimie Department and Research and Teaching Centre (TERRA), 5030 Gembloux, Belgium  
<sup>3</sup>Walloon Agricultural Research Centre, Production and Sector Department, 5030 Gembloux, Belgium  
<sup>4</sup>ETH Zürich, Institute of Agricultural Sciences, 8052 Zürich, Switzerland  
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<sup>6</sup>Agri-Food and Biosciences Institute, Large Park, Hillsborough, BT26 6DR, United Kingdom  
<sup>7</sup>Department of Animal Science, AU Forum, Aarhus University, 8630 Tjele, Denmark  
<sup>8</sup>UMR Herbivores, INRA, VetAgro Sup, Université Clermont Auvergne, 63122 Saint-Genès-Champagnelle, France  
<sup>9</sup>Leibniz Institute for Farm Animal Biology (FBN), Institute of Nutritional Physiology, 18186 Dummerstorf, Germany

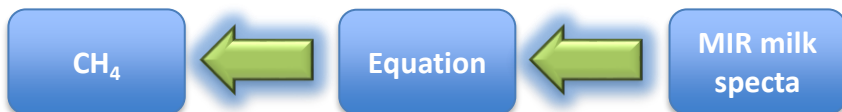
# $\text{CH}_4$ $\leftrightarrow$ Milk Composition



# CH<sub>4</sub> Equation – First Ideas

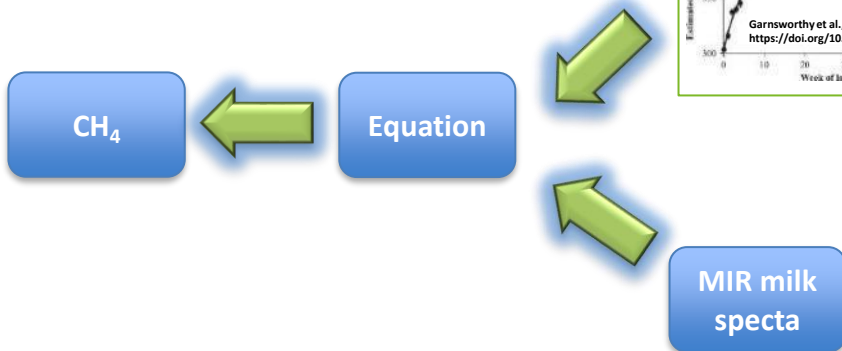
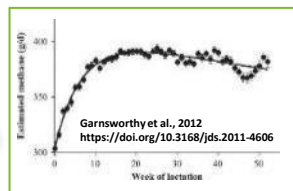


# MIR CH<sub>4</sub> Equation (1<sup>st</sup> innovation)



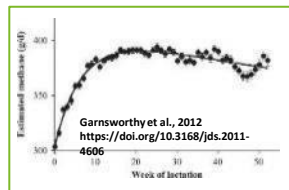
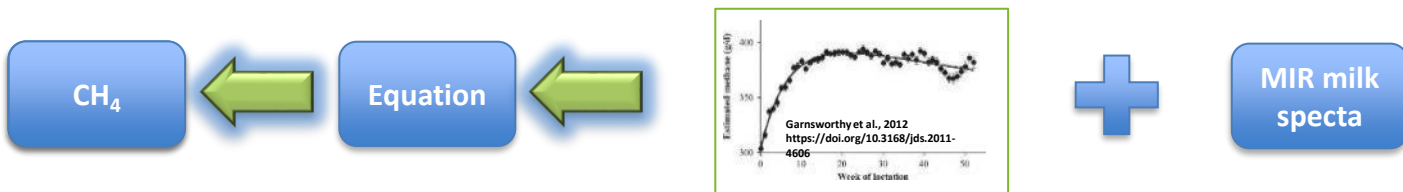
# CH<sub>4</sub> Equation ← Calibration MIR x Lact. curve

→ Lactation Stage Dependent



# CH<sub>4</sub> Equation ← Calibration MIR x Lact. curve

→ Lactation Stage Dependent



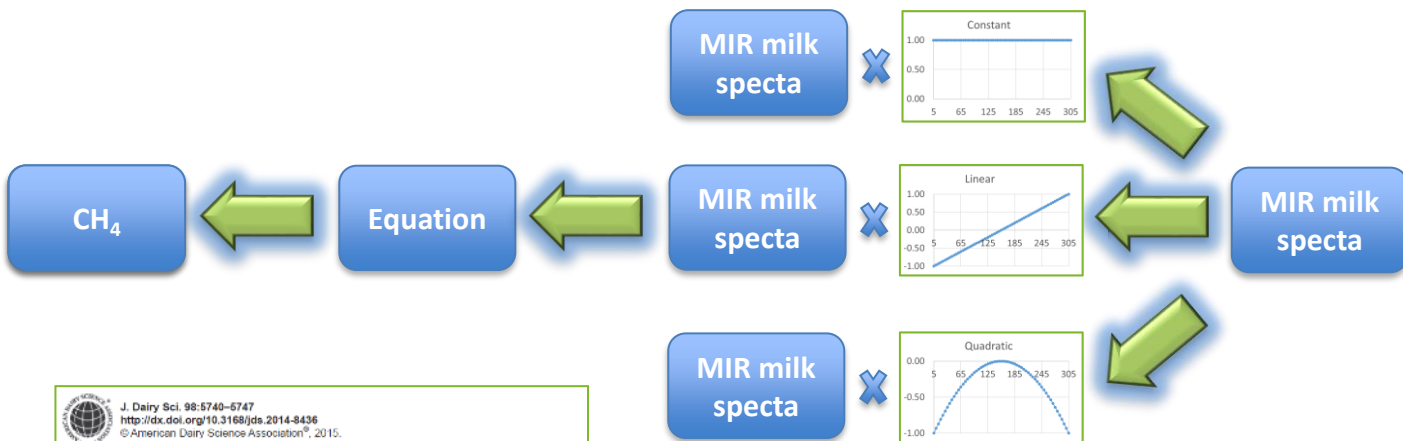
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**Hot topic: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra**

A. Vanlierde,<sup>1</sup> M.-L. Vanrobays,<sup>1</sup> F. Dehareng,<sup>2</sup> E. Froidmont,<sup>3</sup> H. Soyeurt,<sup>4</sup> S. McParland,<sup>5</sup> E. Lewis,<sup>6</sup> M. H. Deighton,<sup>7</sup> F. Grandi,<sup>8</sup> M. Kreuzer,<sup>9</sup> B. Gredler,<sup>10</sup> P. Dardenne,<sup>11</sup> and N. Gengler<sup>12</sup>

# CH<sub>4</sub> Equation ← Calibration MIR x Lact. curve

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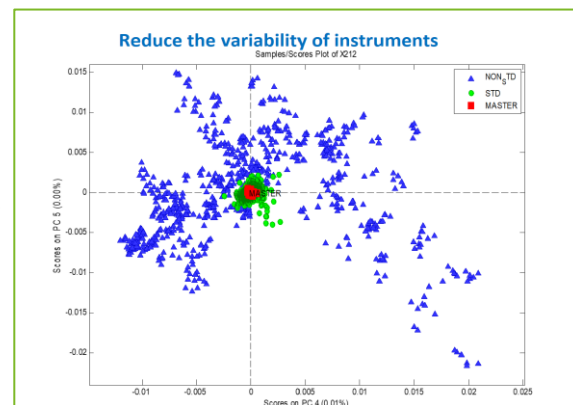
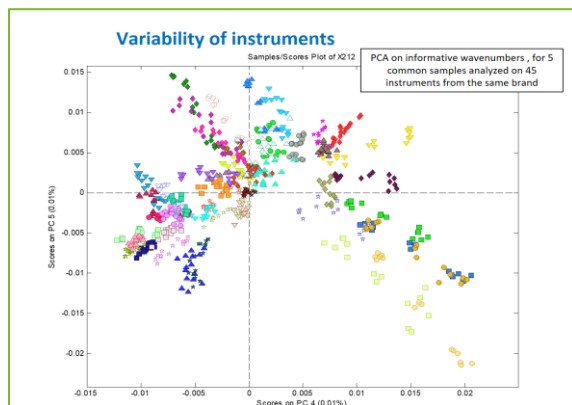
**Hot topic: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra**

A. Vanlierde,<sup>1</sup> M.-L. Vanrobays,<sup>1</sup> F. Dehareng,<sup>2</sup> E. Froidmont,<sup>3</sup> H. Soyeurt,<sup>4</sup> S. McParland,<sup>5</sup> E. Lewis,<sup>6</sup> M. H. Deighton,<sup>7</sup> F. Grandl,<sup>8</sup> M. Kreuzer,<sup>9</sup> B. Gredler,<sup>10</sup> P. Dardenne,<sup>11</sup> and N. Gengler<sup>12</sup>

***3rd Innovation (less in the NIRS world) :***

**Standardizing Spectra....**

# Standardization of MIR Spectra...



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## Standardization of milk mid-infrared spectra from a European dairy network

C. Grelet,<sup>1</sup> J. A. Fernández Pierna,<sup>1</sup> P. Dardenne, V. Baeten, and F. Dehareng<sup>2</sup>  
<sup>1</sup>Walloon Agricultural Research Center, Valorisation of Agricultural Products Department, 24 Chaussée de Namur, 5030 Gembloux, Belgium

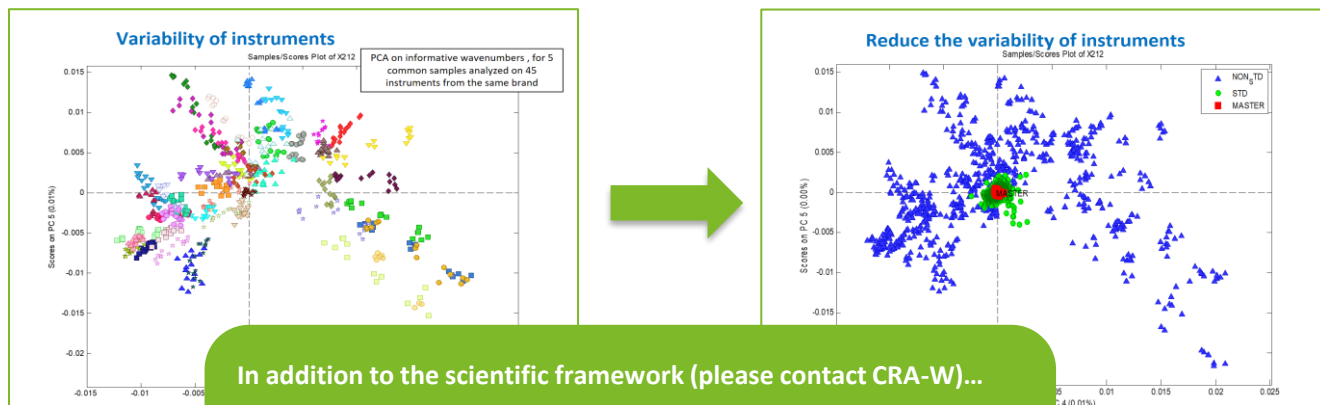


J. Dairy Sci. 100:7910–7921  
<https://doi.org/10.3168/jds.2017-12720>  
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## Standardization of milk mid-infrared spectrometers for the transfer and use of multiple models

C. Grelet,<sup>1</sup> J. A. Fernández Pierna,<sup>1</sup> P. Dardenne,<sup>1</sup> H. Soyeurt,<sup>1</sup> A. Vanlierde,<sup>1</sup> F. Colinet,<sup>1</sup> C. Bastin,<sup>1</sup> N. Gengler,<sup>1</sup> V. Baeten,<sup>1</sup> and F. Dehareng<sup>1</sup>  
<sup>1</sup>Valorization of Agricultural Products Department, Walloon Agricultural Research Center, 5030 Gembloux, Belgium  
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<sup>3</sup>Walloon Breeding Association, B-5590 Ciney, Belgium

# Standardization of MIR Spectra....



In addition to the scientific framework (please contact CRA-W)...

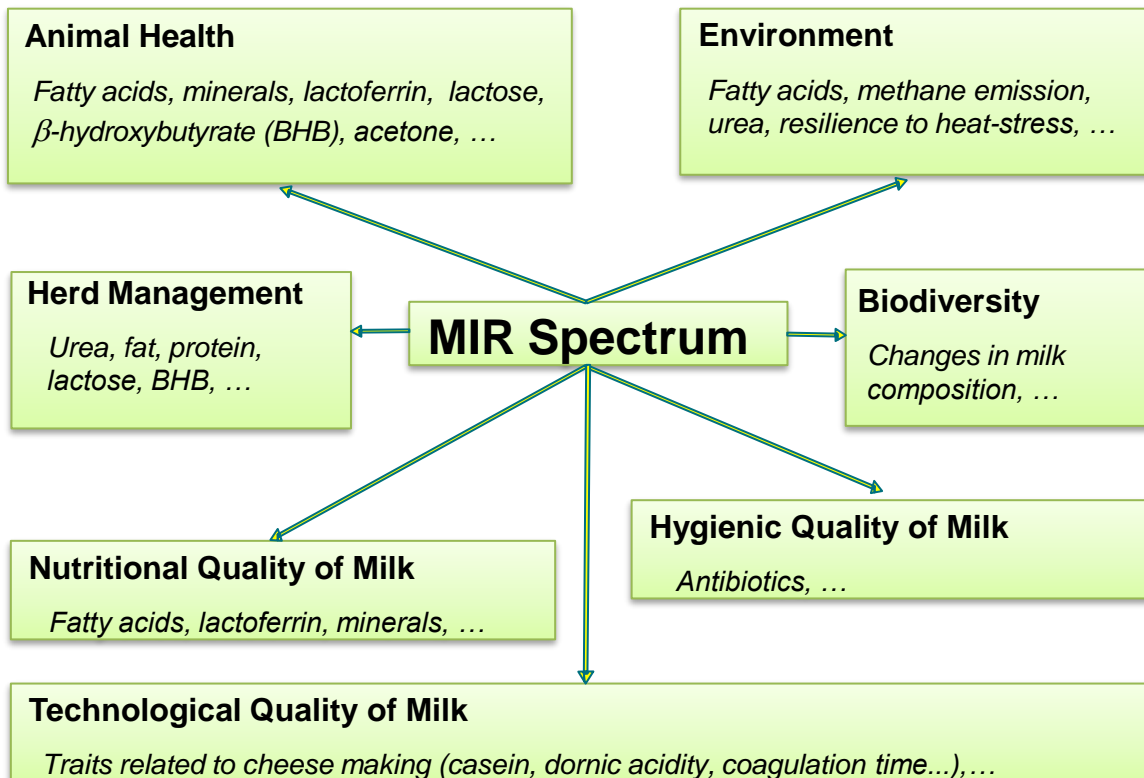
**Commercial services were started by**  
**EMR (European Milk Recording)**  
 please visit: [www.milkrecording.eu](http://www.milkrecording.eu)




 J. Dairy Sci. 98:21  
<http://dx.doi.org/10.3181/jdsci.2015-09-21>  
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**Standardization of milk**  
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 Wallonia Agricultural Research Center,

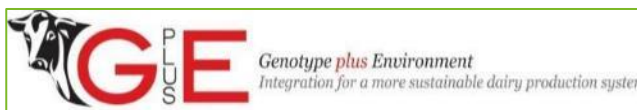
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# Conclusions



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# Thank you!

## Any questions?

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