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Using (mid-)infrared spectroscopy methods to measure milk composition, energy balance and beyond…. in dairy cows

N. Gengler

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First: where I come from….

Gembloux Agro-Bio Tech and University of Liège (ULg)

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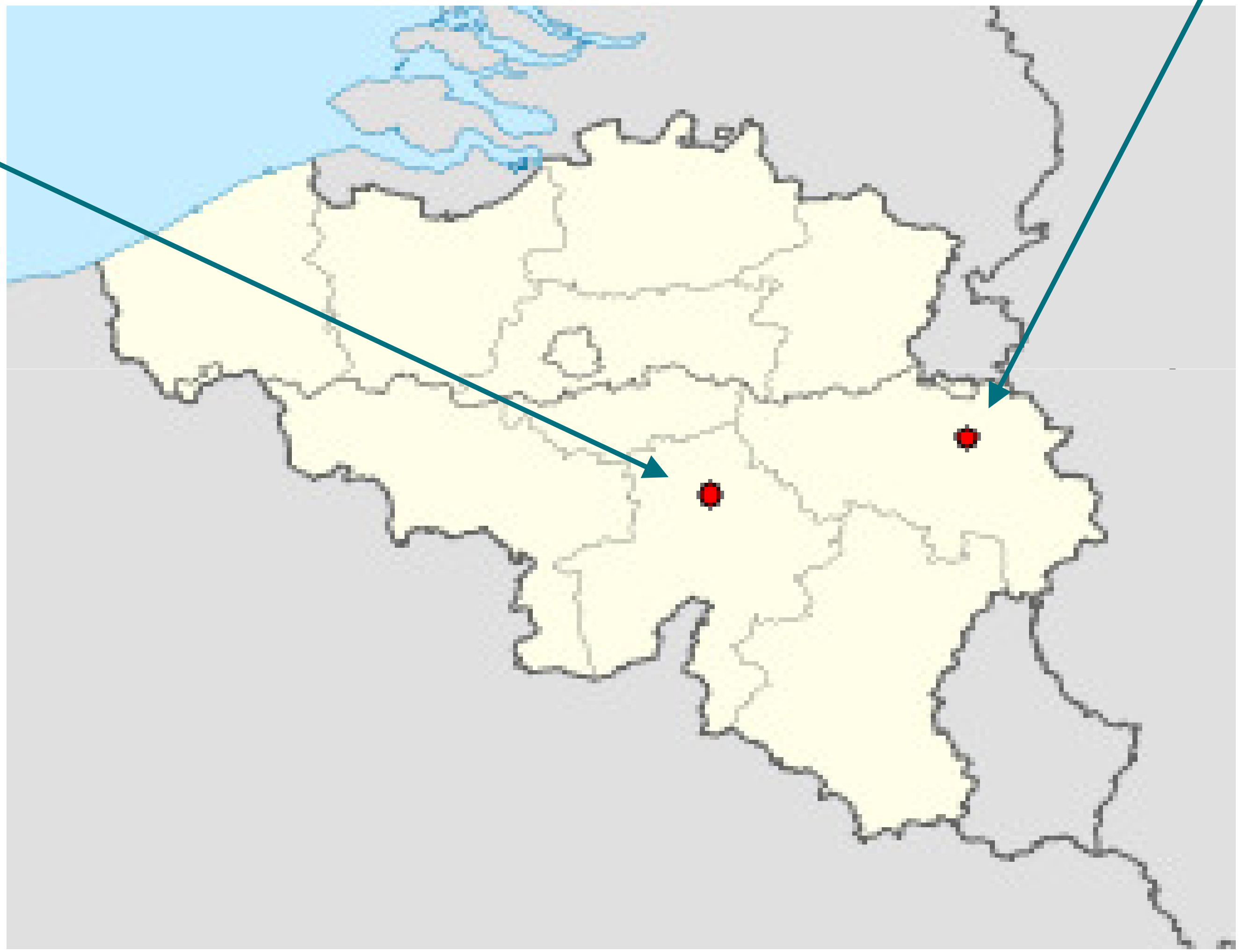


Gembloux and
Gembloux Agro-Bio Tech (GxABT)

LIÈGE université
Gembloux
Agro-Bio Tech

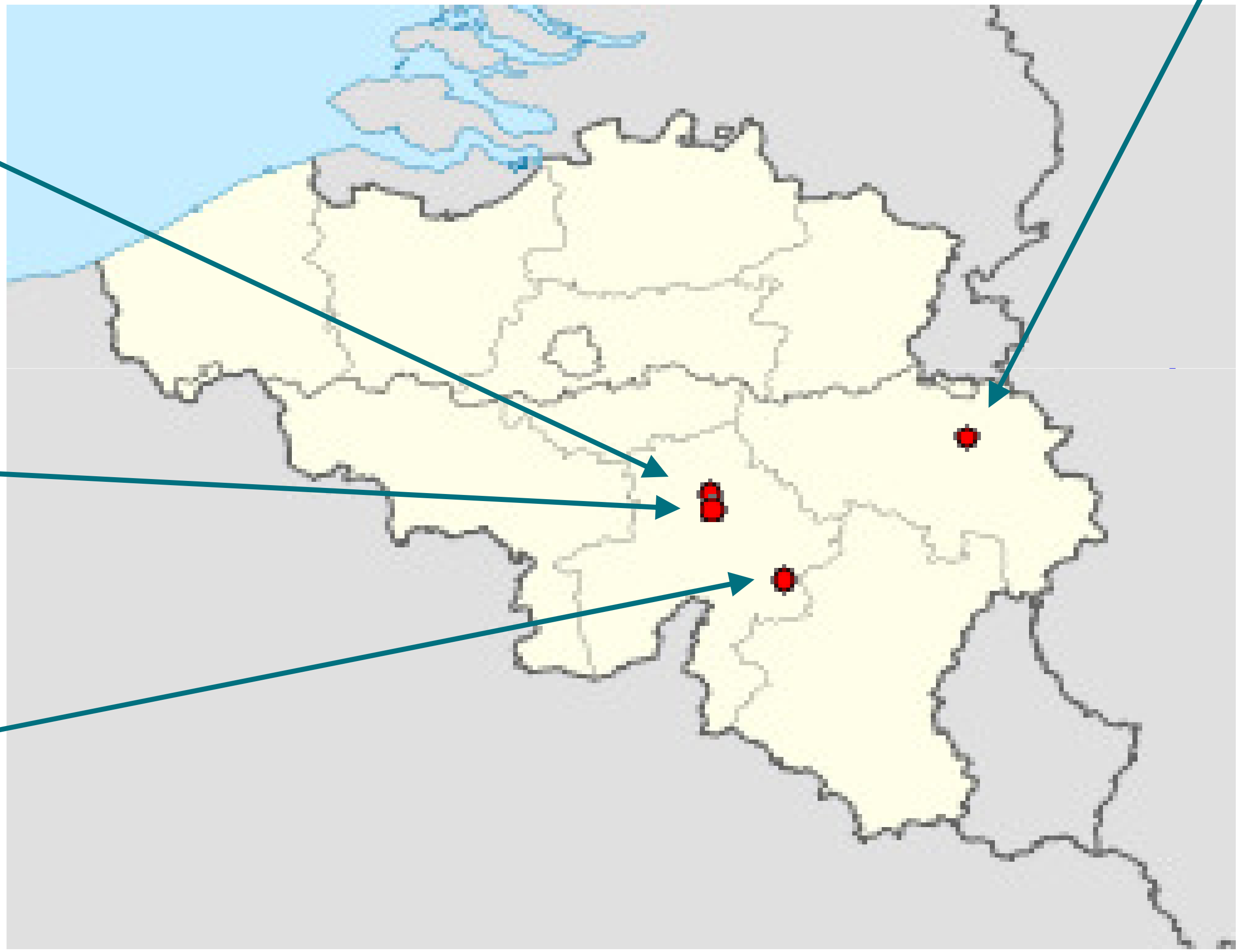
Collaborations inside Belgium

LIÈGE université

LIÈGE université Gembloux Agro-Bio Tech

cra-w

awe



Outline of Presentation

I. What is infrared (IR) spectroscopy?

II. Assessing fine milk composition from IR

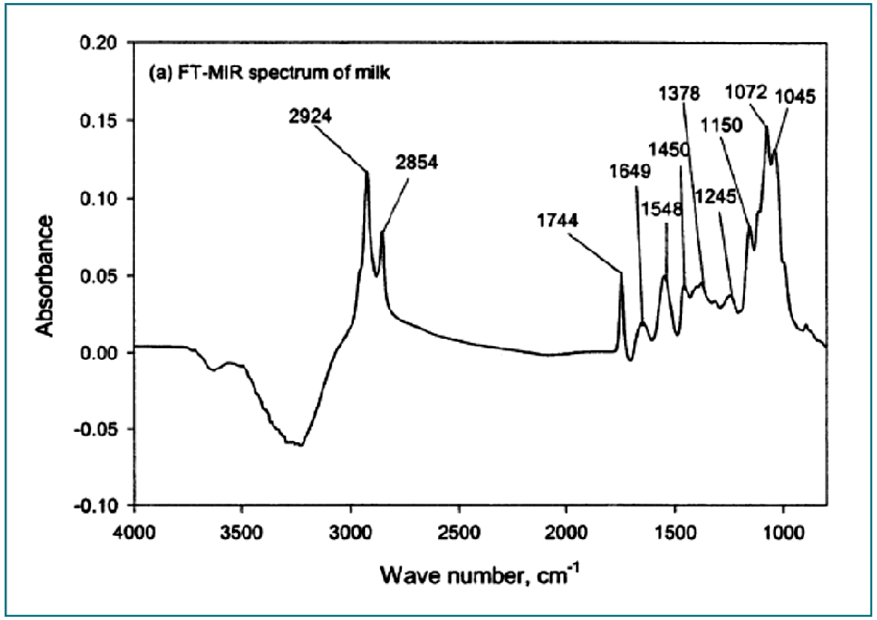
III. Beyond milk composition from IR

IV. Future of IR – ongoing research

What is Infrared (IR) Spectroscopy?

- IR spectroscopy or Vibrational spectroscopy
  - Interaction of infrared radiation with matter
- Large range of techniques, e.g.
  - Absorption spectroscopy (more liquids, gases)
  - Reflectance spectroscopy (more solids)
- Instruments called IR spectro(photo)meters
- Methods often called “Spectrometry”
  - As it is about quantification

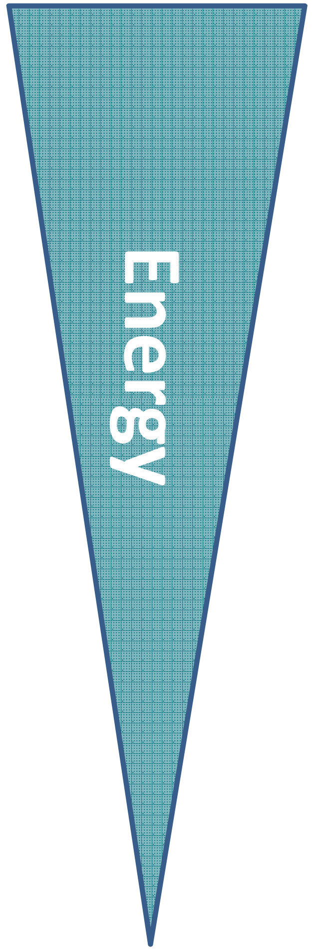
IR Spectrum



- ❑ IR light absorbances (or transmittances) for range of frequencies or wavelengths
  - ➢ Units of IR frequency → reciprocal cm (cm^{-1})
    - ❖ also called “wave numbers”
  - ➢ Units of IR wavelength → micrometers (µm)
    - ❖ also called microns
    - ❖ related to wave numbers in a reciprocal way
- ❑ Different IR ranges

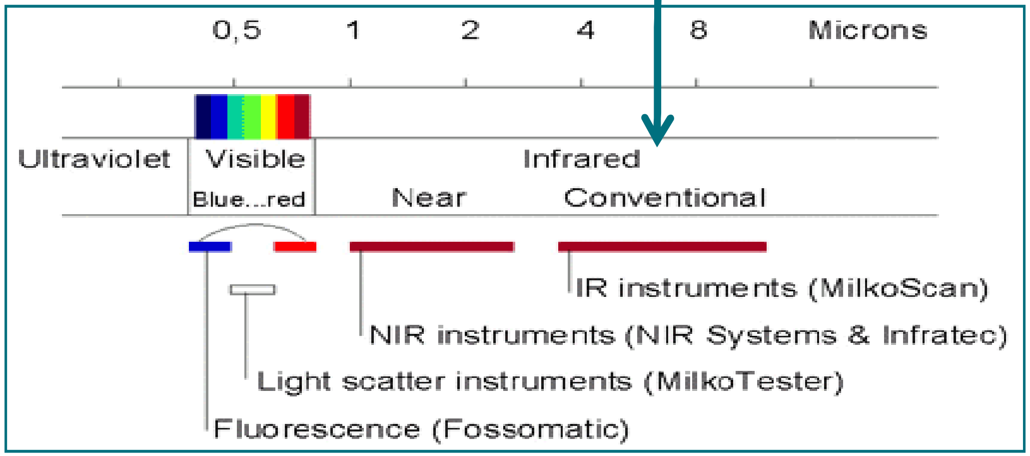
IR Spectral Ranges

- Near-IR (NIR)
  - Approximately 14000–4000 cm^{-1} (0.8–2.5 µm)
  - Can excite overtone or harmonic vibrations
- Mid-infrared (MIR)
  - Approximately 4000–400 cm^{-1} (2.5–25 µm)
  - May be used to study fundamental vibrations and associated rotational-vibrational structure
- Far-infrared (FIR)
  - Approximately 400–10 cm^{-1} (25–1000 µm)
  - Adjacent to the microwave region, low energy and may be used for rotational spectroscopy



IR Spectral Ranges

- Types of IR spectra ranges (here in milk applications)
  - Mid-Infrared (MIR)



FOSS

Typical MIR Spectrometers (milk testing)

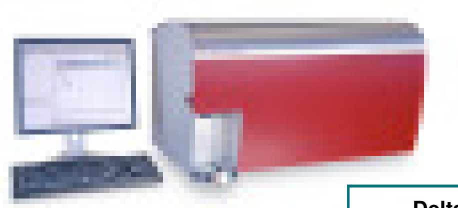
- **FOSS** MilkoScan™ 7

FOSS

- **Bentley Instruments**
  DairySpec FT automatic

Bentley Instruments

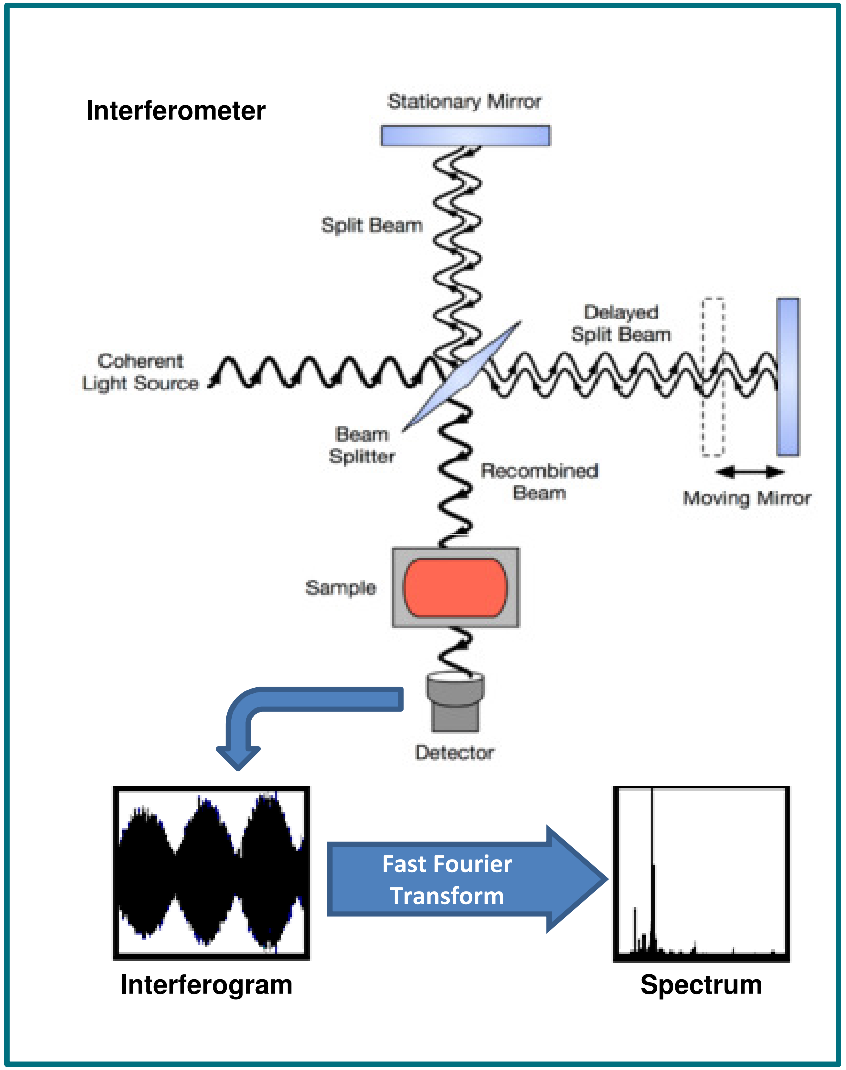
- **Delta Instruments**
  LactoScope FTIR Advanced



Delta Instruments

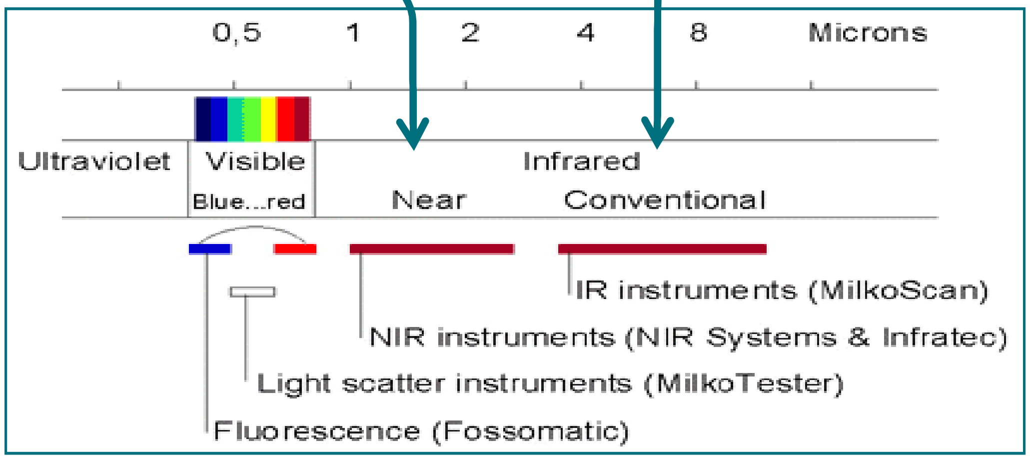
FTIR Spectrometry

- Use of Fourier-Transform (FT) based technology
  - (Fast) FT algorithm transforming an interferogram to a spectrum
- Generally associated to MIR ⇨ FT-MIR
- In commercial applications often called FTIR (= FT-MIR)
- But there is also FT-NIR etc.



IR spectral ranges

- Types of IR spectra ranges (here in milk applications)
  - Mid-Infrared (MIR)
  - Near-Infrared (NIR)



FOSS

NIR Spectrometry

- Often called NIRS
  - Can be absorbance or reflectance (often)
  - Often also FT based technologies
- NIR more energy then MIR
  - Often used on bulk material
  - Little preparation
    ➔ as feed stuff, cheese (as **FOSS** DairyScan™)
- NIR less "precise" then MIR
- NIR less sensitive ➔ ok for less controlled environments
  - Recently NIR started to be used in in-line on-farm applications (as AFILAB by **Afimilk**)

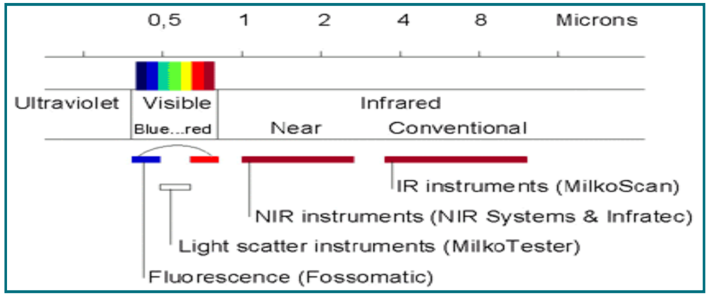
FOSS

Afimilk

II - Assessing fine milk composition from IR spectral data

Milk Composition from IR

- ❑ On-farm ➔ NIR (starting)
  - ➢ Useful for major components



FOSS

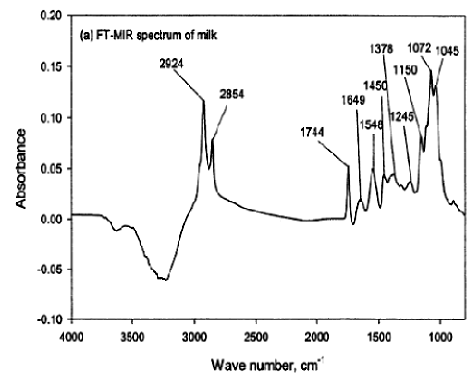
- ❑ More common: MIR in central milk test labs
  - ➢ Standard method for fat, protein, urea and lactose
  - ➢ Existing technology in (nearly) all milk testing labs
  - ➢ Used in milk payment and milk recording

MIR Spectrometry

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Calibration equations

Quantification:

fat

protein

urea

lactose

MIR Spectrometry

Milk samples

(milk payment, milk recording)

MIR analysis

Traditional data flow
(no MIR spectra stored)

Quantification:

fat

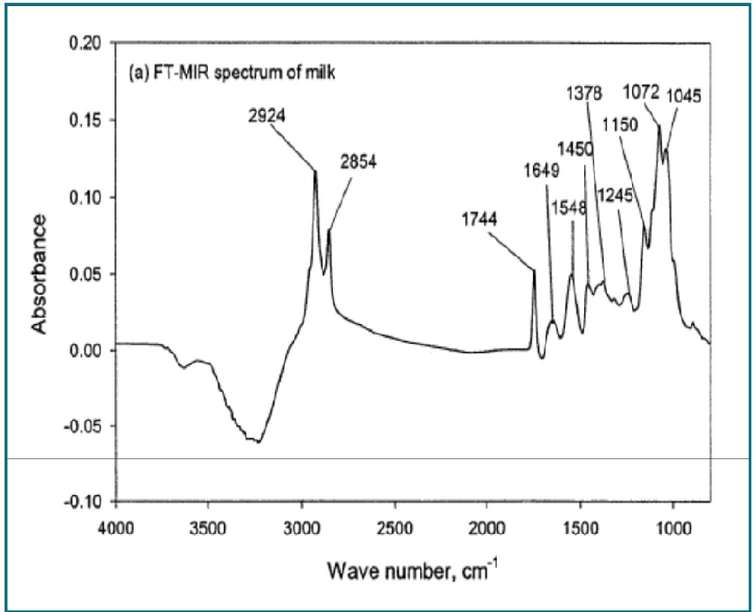
protein

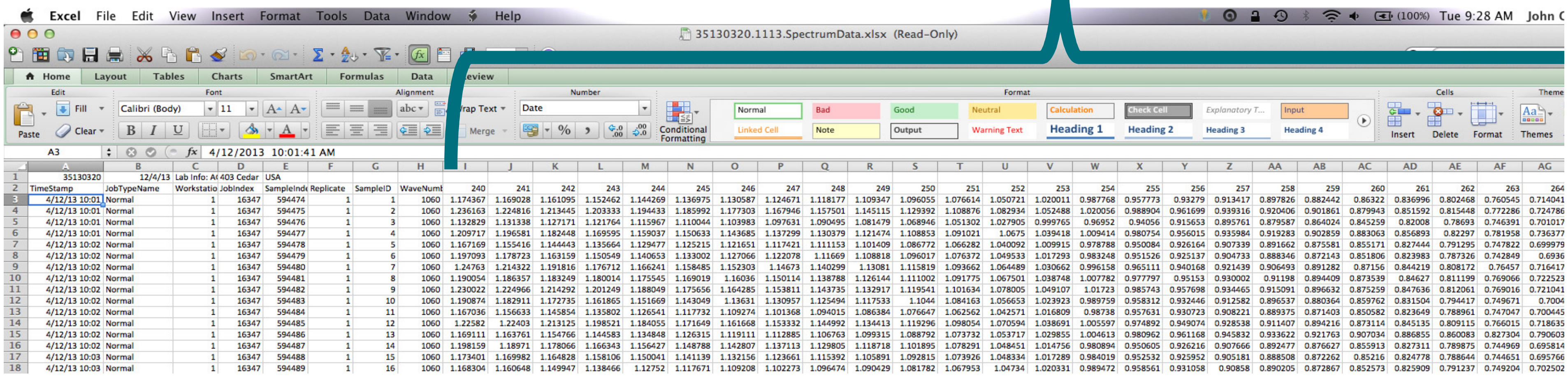
urea

lactose

MIR Spectrometry - Calibration

- Different between brands and models
  - Between 850 – 1060 absorptions values (abs)
- "Calibration"
  - Obtaining b coefficients
    e.g., Fat% = $b_0 + \sum b_i\,(abs)_i$





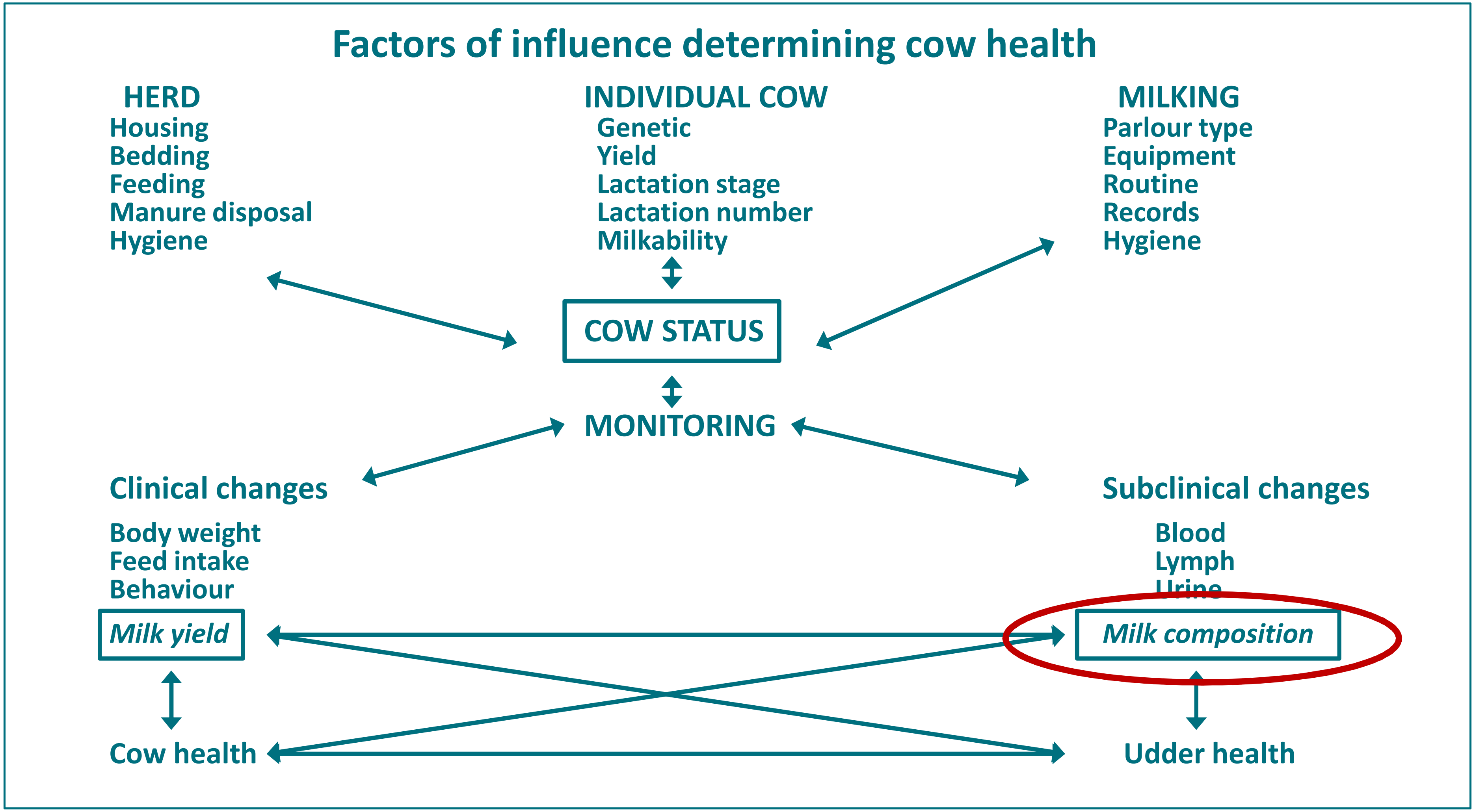
Major Challenge: Data

- Without data
  - No breeding or management possible!
- But data has also to be relevant
  - As close as possible to the processes we follow
  - But always also a cost-benefit issue
    (e.g., health and environmental traits)

Major Challenge: Relevant Data

- Without data
  - No breeding or management possible!
- But data has also to be relevant
  - As close as possible to the processes we follow
- Here enters relatively new concept of biomarkers defined as:
  - "… objectively measured and evaluated … indicator of normal biological processes, pathogenic processes, or … responses to an … intervention" (National Institutes of Health)

Usefulness of Milk Composition!



Hamann & Krömker 1997. Livest. Prod. Sci. 48: 201-208.

Milk Composition

- ❑ Until recently 5 major constituents
  - ➢ Milk fat, protein, urea nitrogen, lactose and somatic cell count (not IR !)
- ❑ However
  - ➢ Milk is a very complex substance with large number of constituents
  - ➢ Some major constituents themselves complex groupings of minor constituents

⇨ many potential Biomarkers

Fine Milk Composition

- Milk fat
  - Fatty acids mostly as triglycerides
  - Non-esterified fatty acids (NEFAs)
- Milk protein
  - Caseins
  - α-lactalbumins
  - β-lactoglobulins
  - Other minor proteins (e.g., lactoferrin)
- Other minor constituents
  - β-hydroxybutyrate (BHB or 3-hydroxybutyrate)
  - Acetone and acetoacetate
  - Minerals
  - Vitamins
  - ....

However Fundamental Problem

- How to get (fine) milk composition:
  - Fast and reliable
  - At reasonable costs

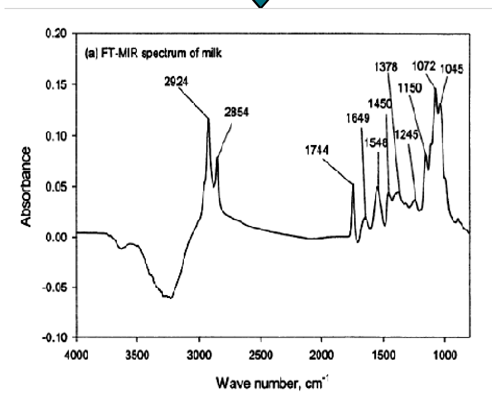
- Idea: following the example of major milk components
  - Using IR, in particular MIR as technology already widespread

Major Milk Components (except SCC)

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Calibration equations

Quantification:

fat

protein

urea

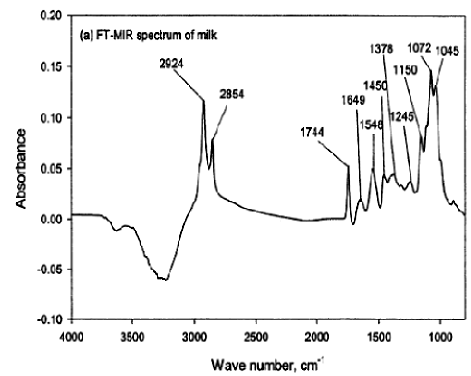
lactose

Novel Traits

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Novel
Calibration equations

Quantification:

Novel traits

Key Issue: Calibration

- Creating linear prediction equations from observed absorbances
  - P(trait of interest) = $b_0 + \sum b_i(\text{abs})_l$

- Calibration: Highly specialized field in itself

Calibration

- Important to assemble both
  - Reference phenotypic data (“Gold-standards“) and
  - Reference spectral data

- And to cover spectral and phenotypic variabilities
  - Expected range of phenotypes must be covered by range of reference data used in calibration
    - E.g., predicted values expected from 1 to 10, reference data used in calibration process needs to cover this range too
  - Multidimensional space defined by reference spectral data must cover the space expected in the field data
    - Often checked using the GH parameter (Global Standardized Mahalanobis Distance)

Calibration

- ❑ Computing spectral prediction equation coefficients
  - ➢ Field of “Chemometrics“
  - ➢ Numerous multivariate methods:
    - ❖ Partial Least Squares (often used),
      but also Ridge Regression, Bayesian methods, SVM, …
  - ➢ Also different pre-treatment of MIR data
  - ➢ Variable selection, etc….

- ❑ Very similar to genomic prediction
  - ➢ Spectral data ⇔ SNP Data
  - ➢ Methods
  - ➢ Variable selection
  - ➢ “Sample” selection….

Developing Calibrations - Collaborative Model

- Developing calibration equations through a concerted action
  - New partners join with data (reference ⇔ spectra) and help improve equations
  - Get in exchange access to equation + updates

- Until recently unknown in MIR
  - More usual in NIR ← feed composition
  - In collaboration with Walloon Agricultural Research Center (CRA-W)
  - Consortia were initiated for many novel traits

Indeed…

- Developed calibration equations
  - Have to be validated before use in new populations
  - Different breeds, feeding and production systems may influence prediction accuracies!

- Reasons why new reference data needed:
  1. Validation of existing equations
  2. Introduction of novel variability in calibration datasets

- Shows interest of gradual process with new “populations” joining calibration consortium leading over time to:
  - Variability represented in the calibration data ↗
  - Capacity of equations to adapt to novel circumstances ↗
  - Therefore: general ”Robustness” of equations ↗

Examples of Successful Consortia

- Milk fatty acid (FA) equations:
  - First equations developed in 2005
  - Improved through international collaborations:
    - Belgium, France, Germany, Ireland, UK, Luxembourg, Finland, ….
    - Developed and validated in multiple breeds, countries and production systems

J. Dairy Sci. 94:1657–1667
doi:10.3168/jds.2010-3408

Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries

H. Soyeurt,*†[1,2] F. Dehareng,‡[1] N. Gengler,*† S. McParland,§ E. Wall,‡ D. P. Berry,§ M. Coffey,# and P. Dardenne‡

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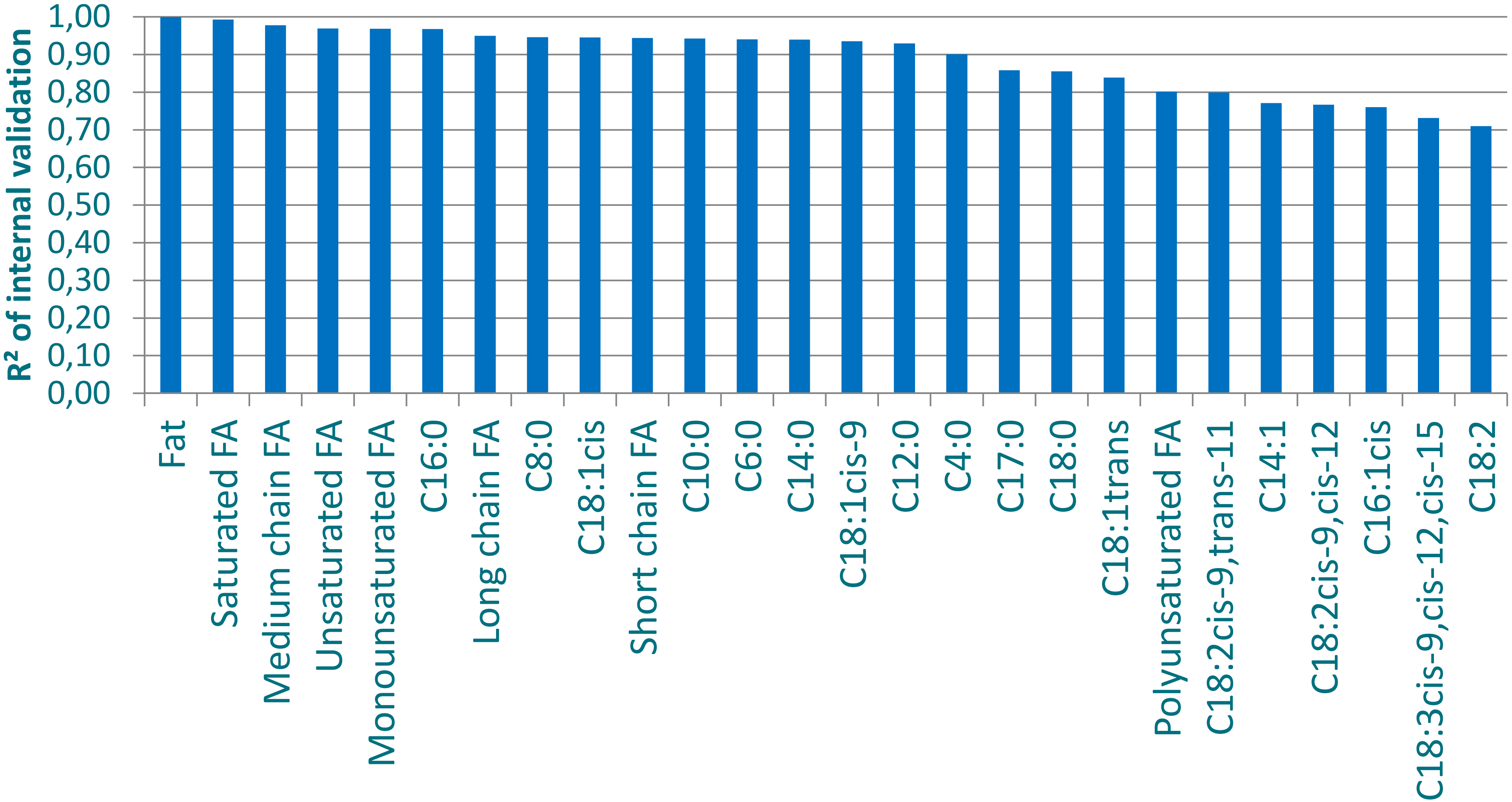
⇨ increased robustness

Accuracy of Fatty Acids Calibration Equations



Calibration equations were developed from at least 1,600 milk samples

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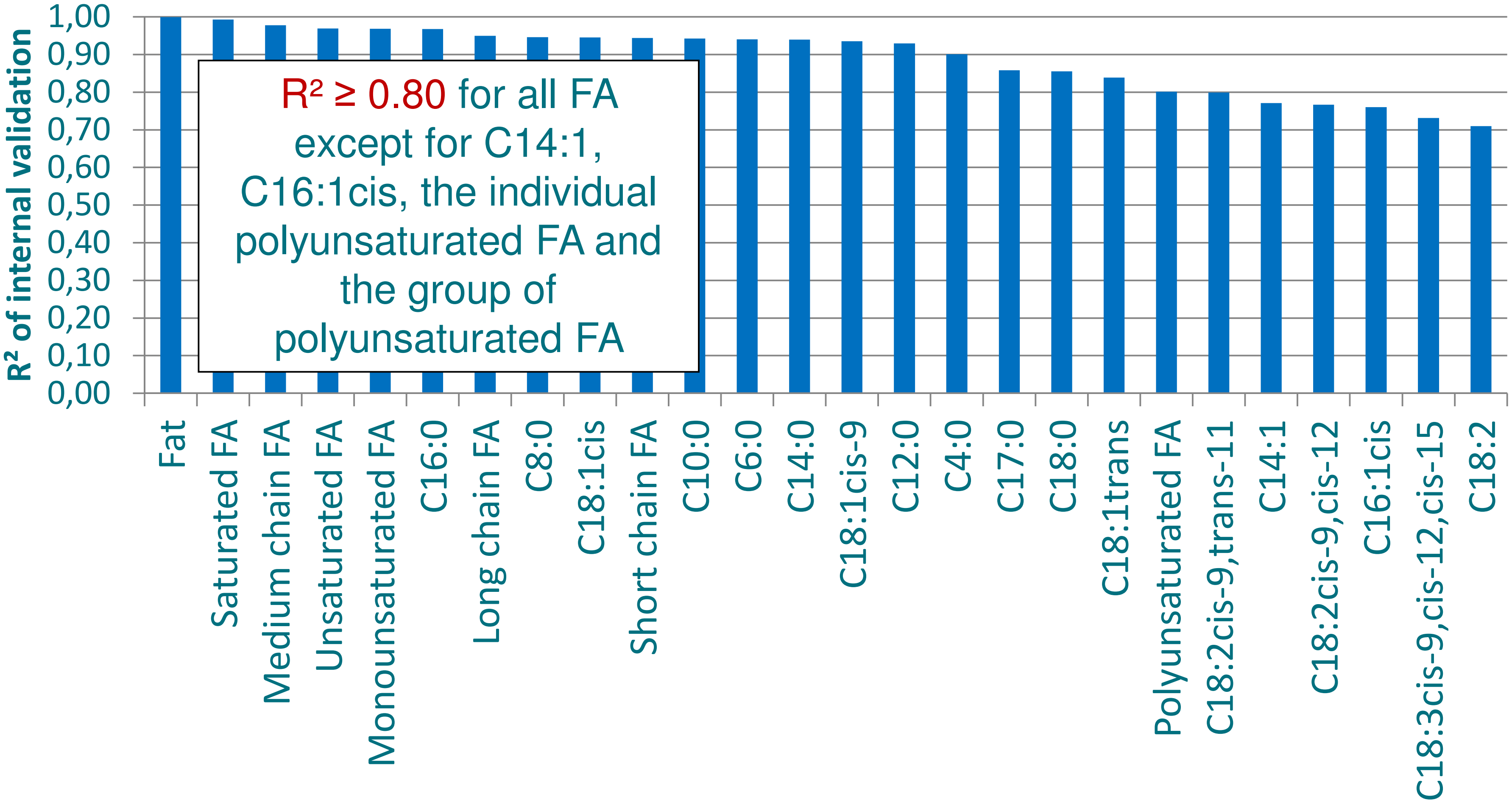

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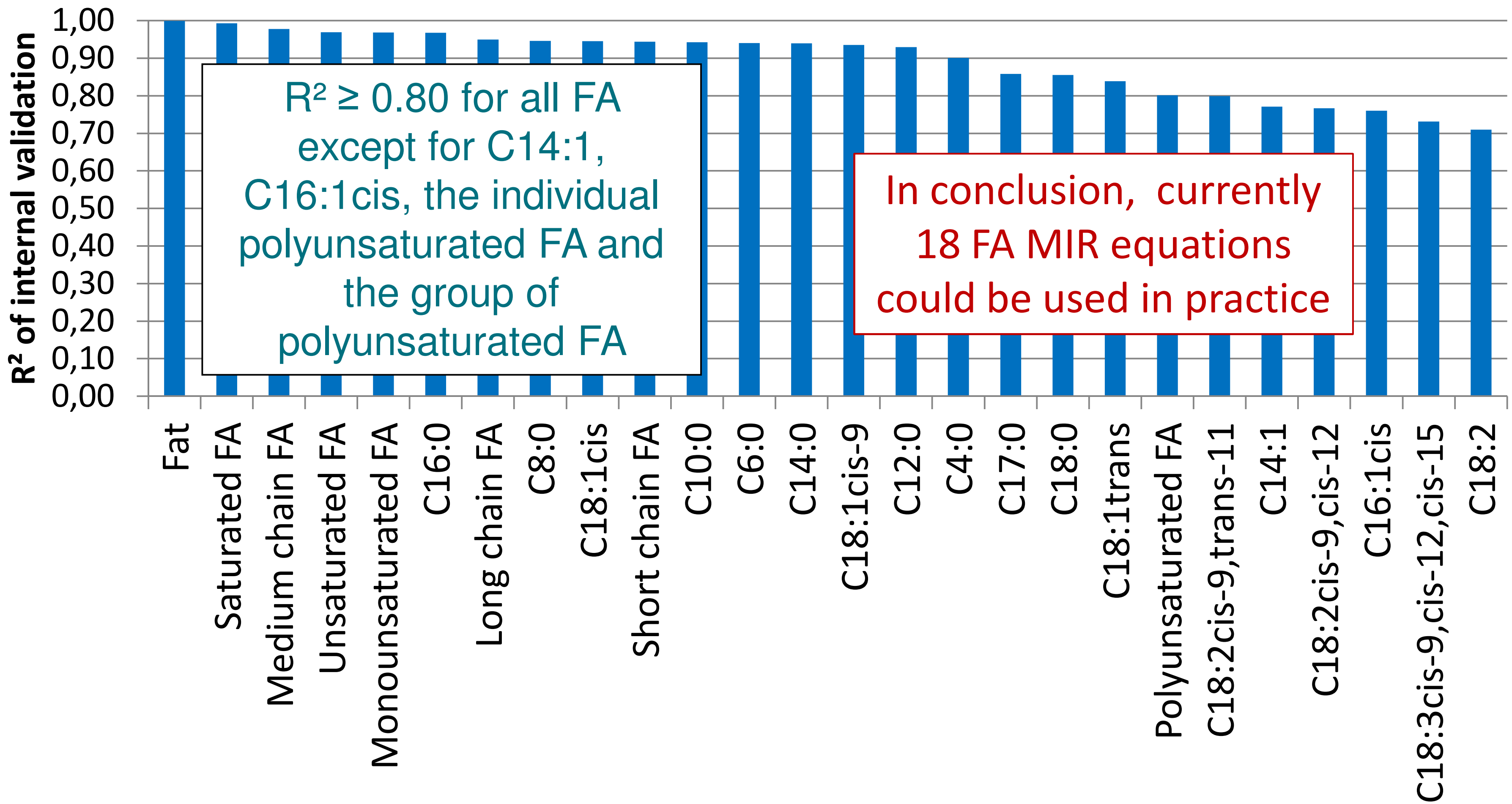

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- Milk mineral equations:
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Examples of Successful Consortia

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  - Multiple breeds, countries and production systems
- Milk mineral equations:
  - First equations developed in 2006
  - Improved through international collaborations:
    - Belgium, France, Germany, and Luxembourg
- Lactoferrin equations:
  - Cooperative effort of Belgium, Ireland and UK ← France

Lactoferrin

- Glycoprotein present naturally in milk
- Involved in the immune system
- Interests:
  - Potential indicator of mastitis
  - Help to maintain a good immune system in Humans
- However R^2 of internal validation = 0.71

⇨ MIR predictor of lactoferrin

- Estimation of Biomarker not without errors

Fine Milk Composition → Biomarkers → “Status”

- Therefore complexity of fine milk composition very useful to assess (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

Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows

H. Hammami,*†[1] J. Vandenplas,*† M.-L. Vanrobays,* B. Rekik,‡ C. Bastin,* and N. Gengler*

III - Beyond milk composition from IR

Biomarker and Indicator Traits

- “Classical” objective of milk MIR spectrometry
  ➔ predicting “perfectly” the component

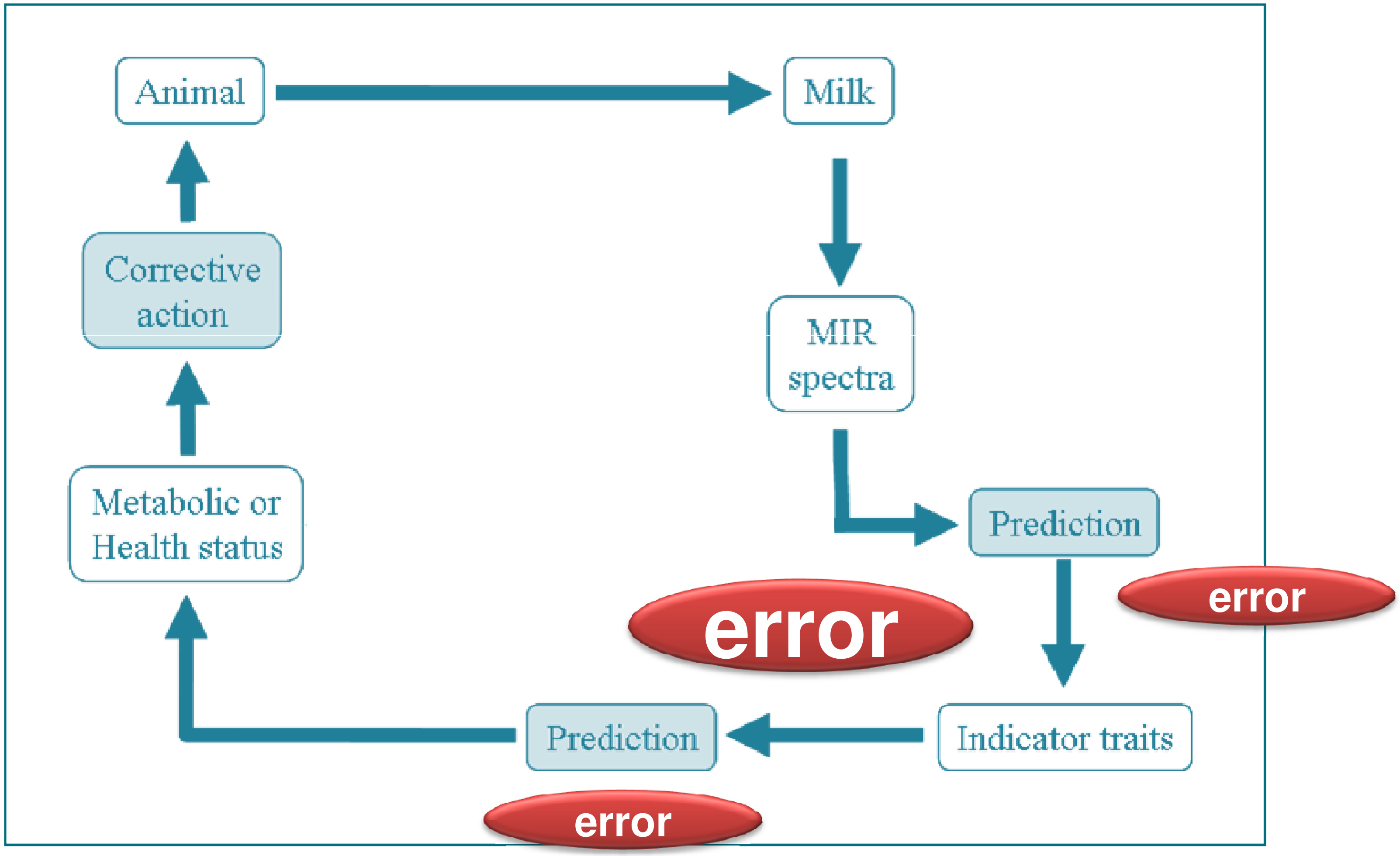
- However, many biomarkers or indicator traits can only be predicted rather imperfectly

Therefore Proposed Alternative

- Defining traits closer to “real” trait of interest
- Example from dairy cattle
  - Currently: MIR → BHB, acetone → Ketosis
  - Proposal: MIR → Ketosis
- Concept of
  “Management (Information) Trait”
  → OptiMIR project (www.optimir.eu)

WITH THE SUPPORT OF

MIR ⇨ Indicator ⇨ Management Trait



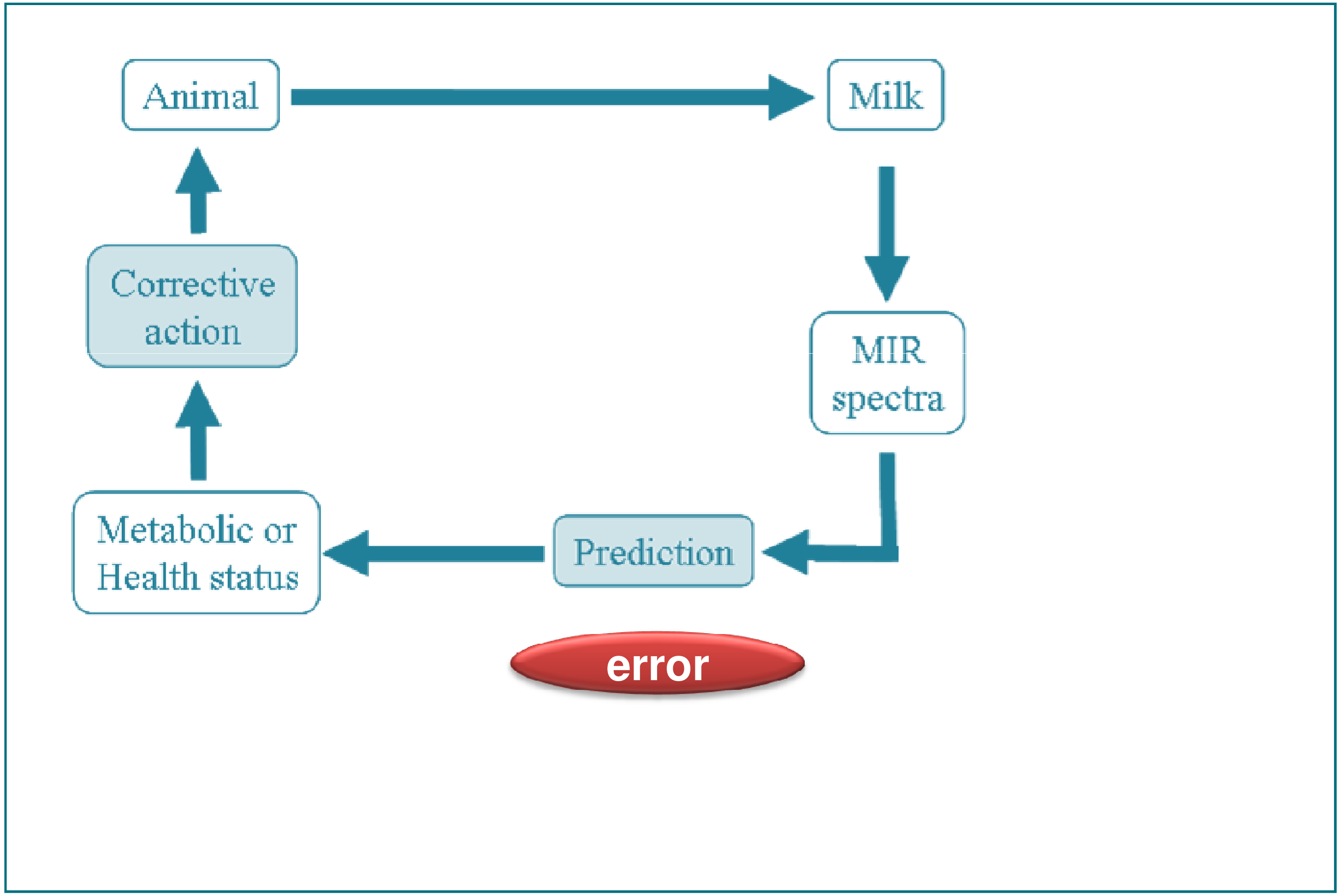
Direct Prediction of Traits of Interest

- "Classical" objective of milk MIR spectrometry
  ➔ predicting "perfectly" the component

- However many biomarkers or indicator traits can only be predicted rather imperfectly
  - Double "error"

- 1st Innovation
  - Direct prediction of "Management" Traits from MIR spectra
  - Not the direct component, but directly related to process/status

MIR ⇨ Management Trait

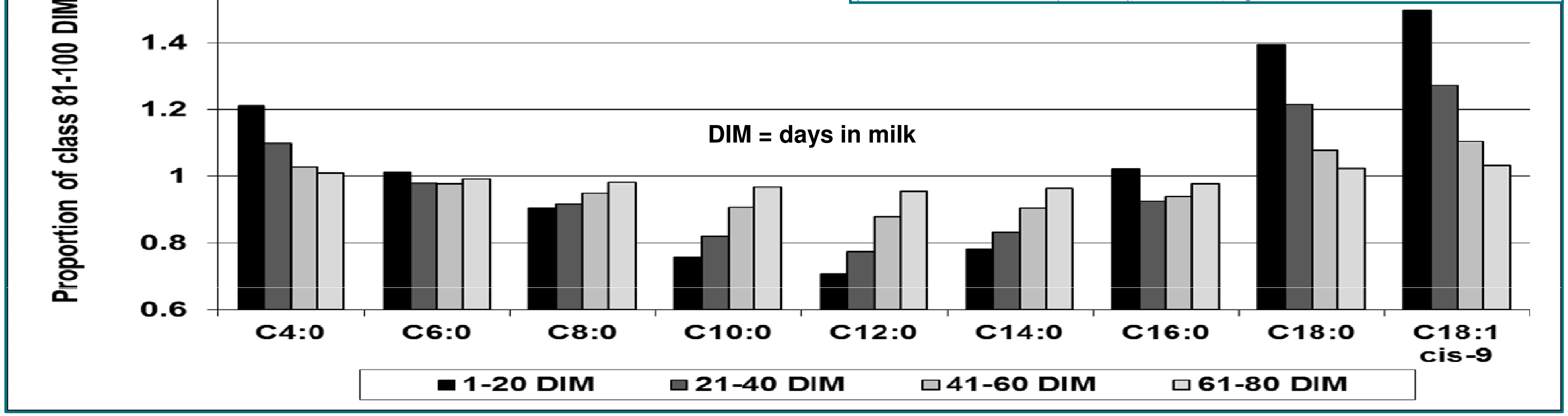


FA Profile Variable Throughout the Lactation

J. Dairy Sci. 94:4152–4163
doi:10.3168/jds.2010-4108

Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows

C. Bastin,*[1] N. Gengler,*† and H. Soyeurt*†
*University of Liège, Gembloux Agro-Bio Tech, Animal Science Unit, B-5030 Gembloux, Belgium
†National Fund for Scientific Research (F.R.S.-FNRS), B-1000 Brussels, Belgium



- Indirect: reflecting equilibrium between:
  Body fat mobilization ⇔ Feed intake
  - Body fat mobilization → also heat stress
  - Feed intake → driving force for CH_4

J. Dairy Sci. 98:4956–4968
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Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows

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*Animal Science Unit, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium
†National Fund for Scientific Research, 1000 Brussels, Belgium
‡School of Higher Education in Agricultural of Mateur, TN-7030 Mateur, Tunisia

- Direct calibration of energy balance and related traits

J. Dairy Sci. 99:4056–4070
http://dx.doi.org/10.3168/jds.2015-10051

The potential of Fourier transform infrared spectroscopy of milk samples to predict energy intake and efficiency in dairy cows[1]

S. McParland[2] and D. P. Berry
Animal and Grassland Research and Innovation Center, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Other Sources of Variation Added to Calibration

- 2nd Innovation
  - Adding other sources of variation into calibration process
- Example for MIR predicted methane
  - Methane (⇔ FA) ⇔ MIR Spectra

Other Sources of Variation Added to Calibration

- 2nd Innovation
  - Adding other sources of variation into calibration process
- Example for MIR predicted methane
  - Methane (⇔ FA) ⇔ MIR Spectra
  - More details in article

J. Dairy Sci. 98:5740–5747
http://dx.doi.org/10.3168/jds.2014-8436

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

A. Vanlierde,*[1] M.-L. Vanrobays,†[1] F. Dehareng,* E. Froidmont,‡ H. Soyeurt,† S. McParland,§ E. Lewis,§ M. H. Deighton,# F. Grandl,‖ M. Kreuzer,‖ B. Gredler,¶ P. Dardenne,* and N. Gengler†[2]

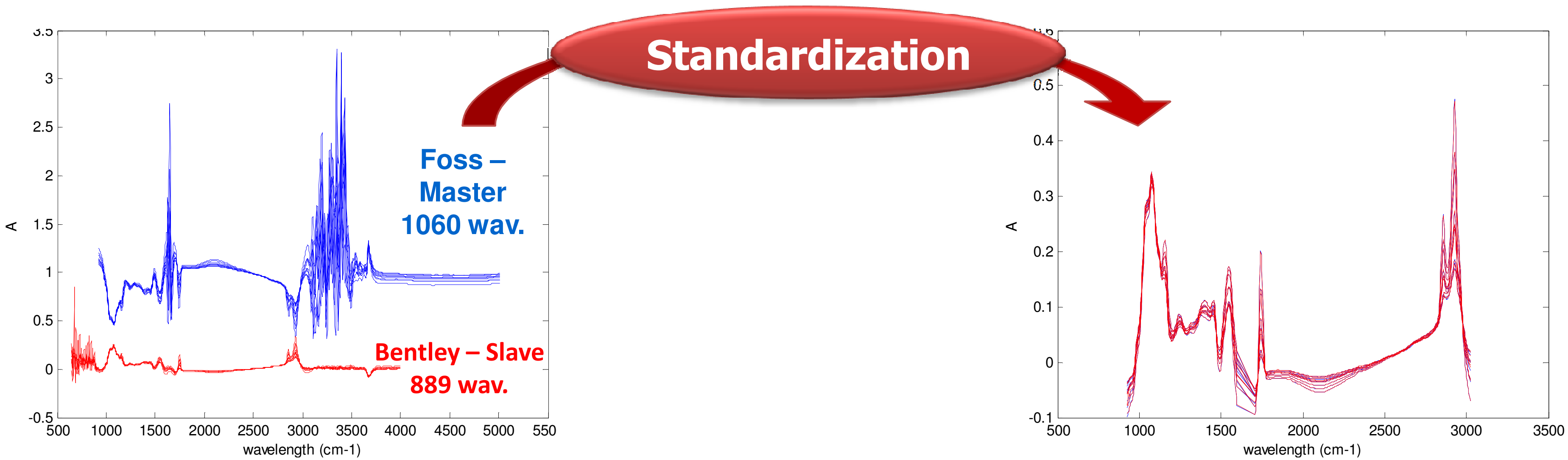
- Variable calibration equation coefficients
  - Here Days in Milk (DIM) dependent
  - $P(CH_4) = f_{b0}(DIM) + \sum f_{bi}(DIM) \times (abs)_i$
- But can be used in many other situations

Other Issues…

- Each calibration equation
  - Normally only for the instruments used for the calibration
- At least two issues
  - Different brands ⇨ different spectral wavelength ranges
  - Individual spectrometers ⇨ over time generated MIR data not 100% stable
- In context of traditional calibrations
  - Brand specific equations (“Black box”)
  - Manufacturers using different “tricks” like “Standardization Solutions”
  - Post-prediction adjustments for “Bias” and “Slope” using reference samples with known values

⇨ but for novel trait, traits with no obvious reference samples?

3rd Innovation: Spectra Standardization



- Two steps to generate "standardized" (harmonized) spectral data
    1. Transforming from different ranges of wavelength to a common one
    2. Applying "bias" and "slope" corrections for each wavelength
- Recent publication:

J. Dairy Sci. 98:2150–2160
http://dx.doi.org/10.3168/jds.2014-8764

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

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

IV - Future of IR – ongoing research

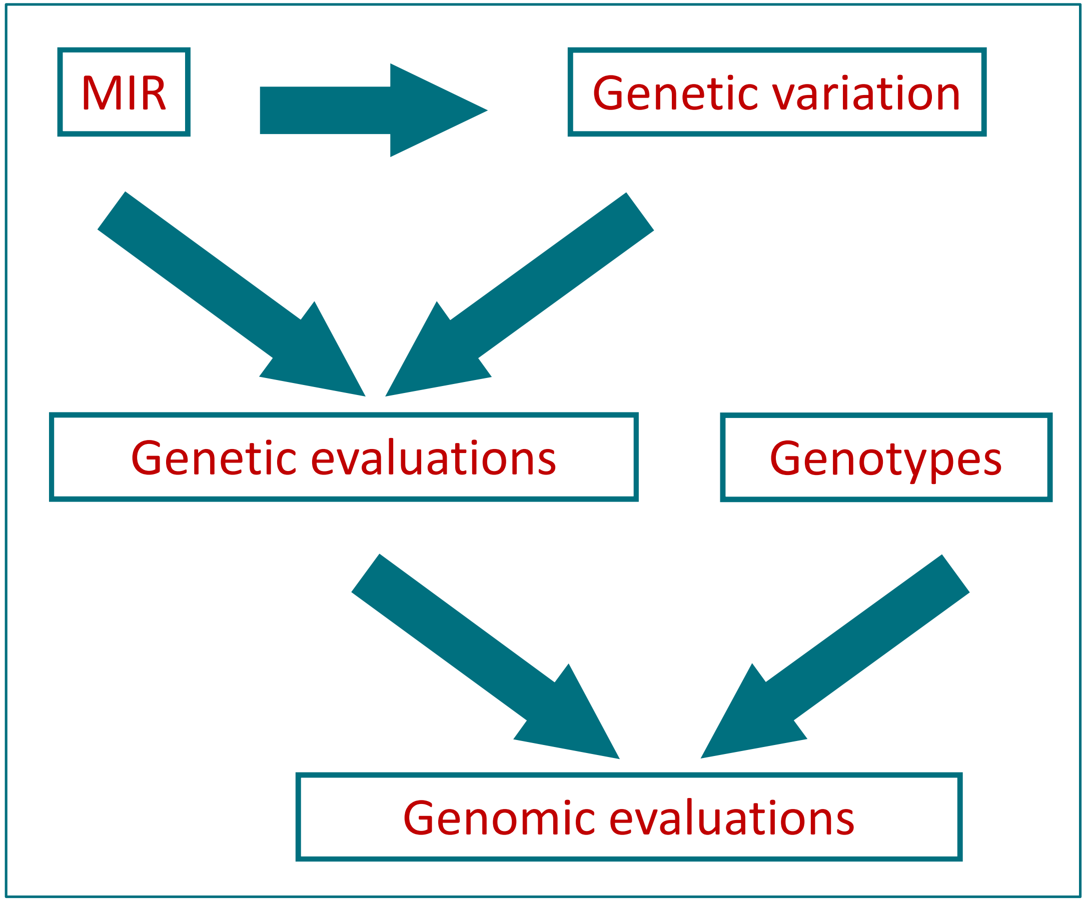
Trent in Animal Breeding: Direct Use of MIR

J. Dairy Sci. 93:1722–1728
doi:10.3168/jds.2009-2614

Genetic variability of milk components based on mid-infrared spectral data

H. Soyeurt,*†[1] I. Misztal,‡ and N. Gengler*†

- Traits:
  absorbance values at given wave numbers
- Avoiding "phenotypic" calibration and risk of low R^2_{CV}
- Problem of high nb of dimensions (many MIR traits) ➔ targeted combination of traits
  (My presentation at ICAR 2017 on the 15th of June)



Development ⇨ International MIR Projects

⇨ important to develop international collaborations

- Leading to several European projects
  - RobustMilk (FP7 – KBBE) – finished
    - FA and lactoferrin predictions
  - GreenHouseMilk (FP7 – Marie Curie – ITN) – finished
    - Methane predictions
  - OptiMIR (INTERREG-IVB North-West Europe) – finished
    - MIR tools implementation technology and management use
  - GplusE (FP7 – KBBE) – ongoing
    - Mostly health traits
- Collaboration in local projects in other countries (Germany, Australia)
- Continuing interested in other collaborations

MIR Spectral Databases and Standardization

- Creation of spectral databases related to milk recording needed
  - Already in Walloon Region of Belgium and in Luxembourg since several years

- At member milk recording organizations
  - European Milk Recording www.milkrecording.eu
  - Organizing "Standardization"

⇨ development of breeding and management tools

Conclusions

- Many opportunities in (M)IR based methods:
  - Illustrated by examples
  - Context of breeding and management of dairy cattle
    - But IR not only milk ➔ not elaborated in this talk
- Help to avoid:
  - Bottleneck of getting relevant data ➔ collaborations
- Simplifying concepts:
  - Researching direct link: MIR ⬄ "Management Information Traits"
  - In animal breeding: skipping phenotypic calibration
- Several other innovations
- Challenges (and opportunities ahead)
  - Integration into "Precision Livestock Farming"

Acknowledgments

- Support through the Futurospectre partnership:
  - awé – Milkcomite – CRA-W – ULg-GxABT

- Two core teams
  - Team ULg-GxABT: H. Soyeurt, C. Bastin, F.G. Colinet, H. Hammami, M.-L. Vanrobays, A. Lainé, S. Vanderick, ...
  - Team CRA-W: P. Dardenne, F. Dehareng, C. Grelet, A. Vanlierde, E. Froidmont, ...

Thank you!