

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

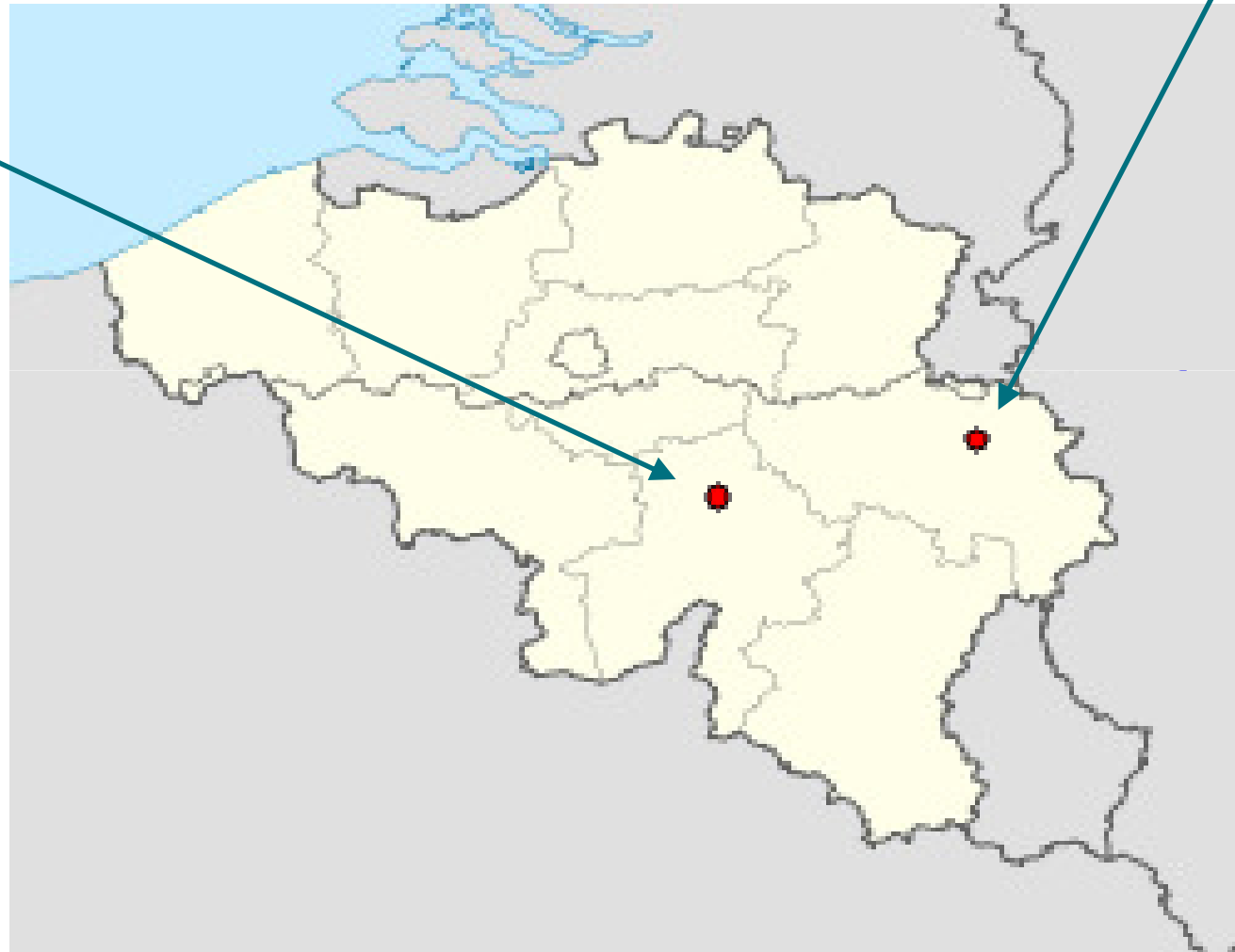
N. Gengler

University of Liège – Gembloux Agro-Bio Tech, Belgium

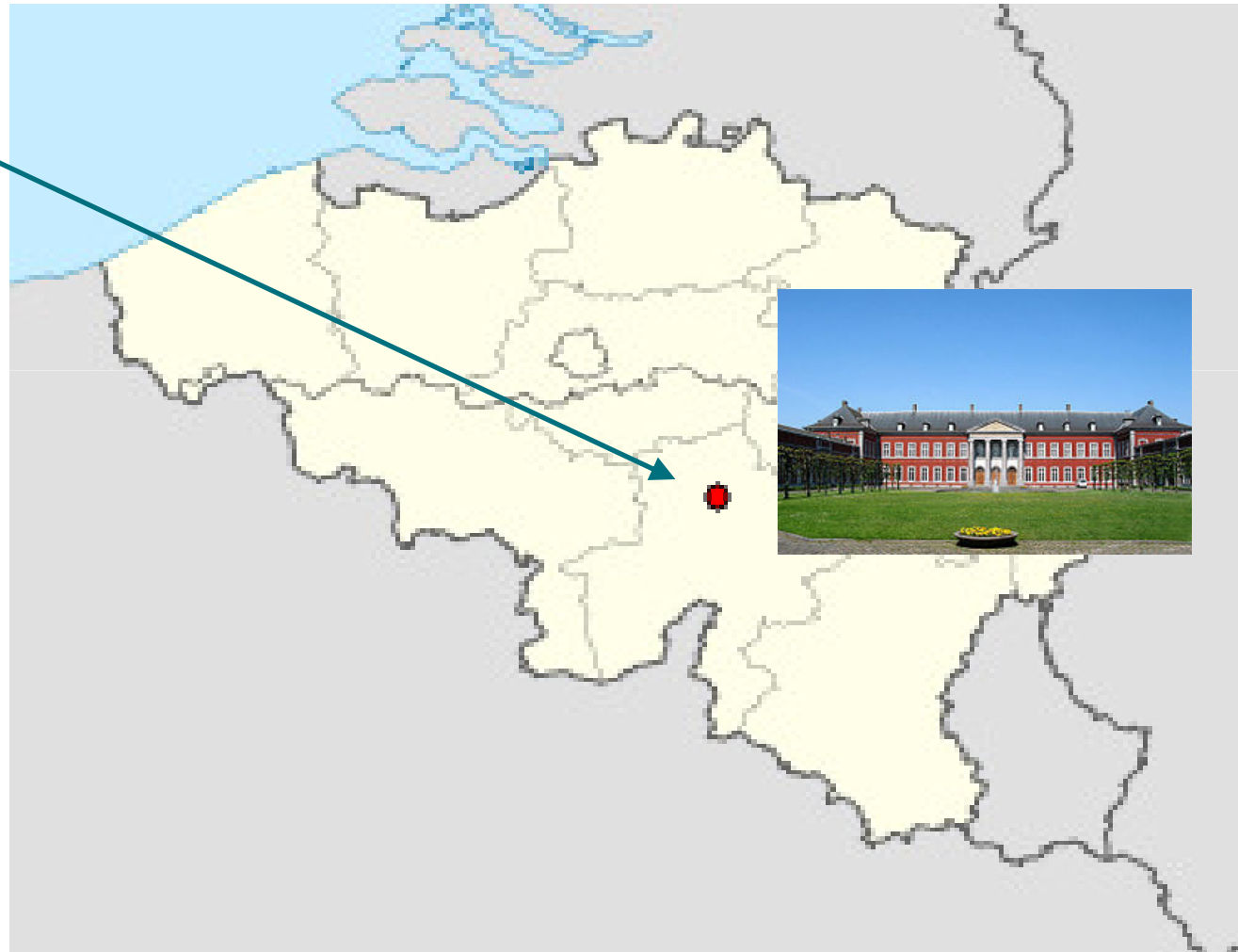
First: where I come from....



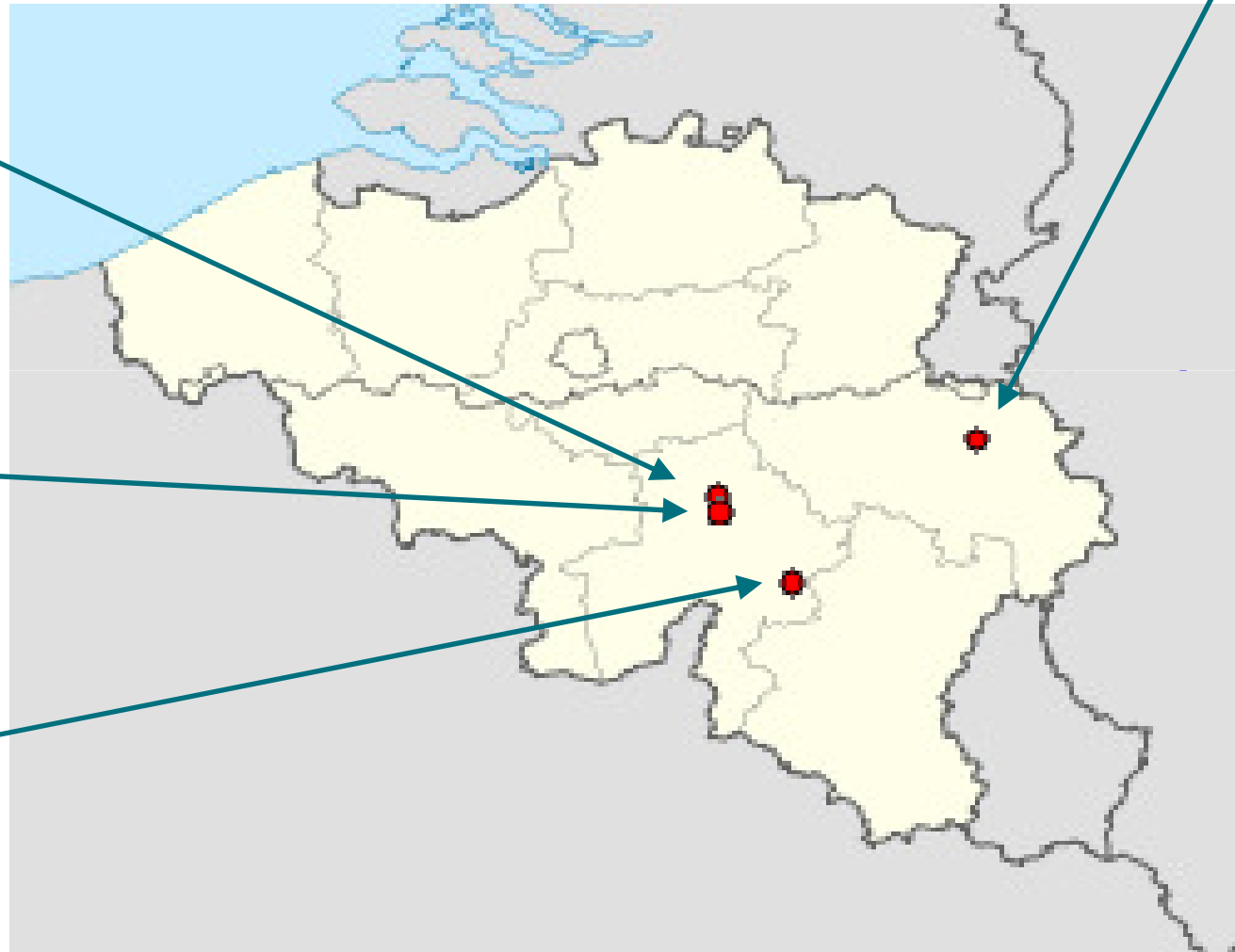
Gembloux Agro-Bio Tech and University of Liège (ULg)



Gembloux and Gembloux Agro-Bio Tech (GxABT)



Collaborations inside Belgium



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?

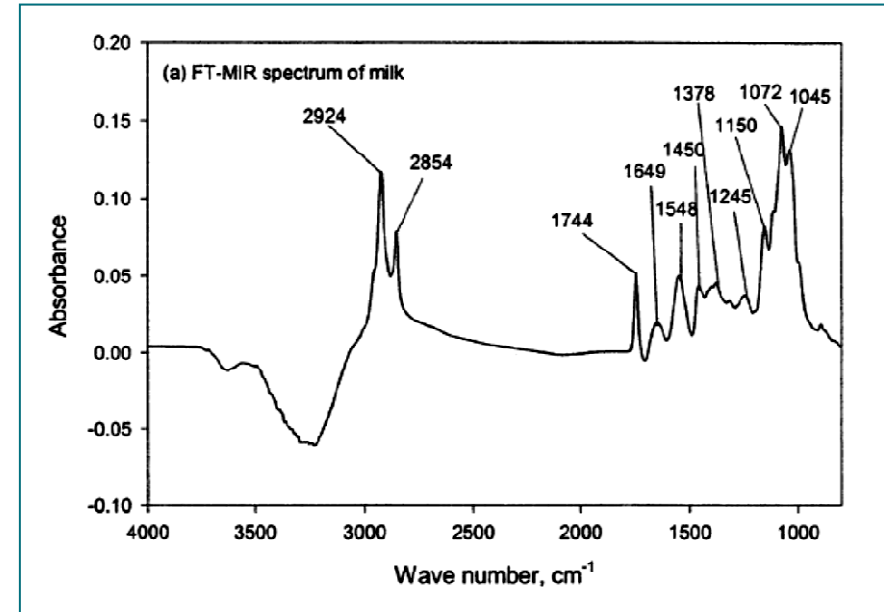
- ❑ IR spectroscopy or Vibrational spectroscopy
 - Interaction of infrared radiation with matter
- ❑ Large range of techniques, e.g.
 - Absorption spectroscopy (more liquids, gases)
 - Reflectance spectroscopy (more solids)
- ❑ Instruments called IR spectro(photo)meters
- ❑ Methods often called “Spectrometry”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

IR Spectrum

□ IR light absorbances (or transmittances) for range of frequencies or wavelengths

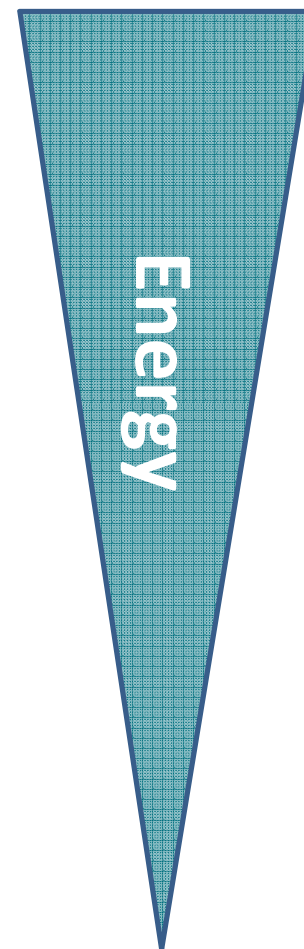
- Units of IR frequency → reciprocal cm (cm^{-1})
 - ❖ also called “wave numbers”
- Units of IR wavelength → micrometers (μm)
 - ❖ also called microns
 - ❖ related to wave numbers in a reciprocal way

□ Different IR ranges



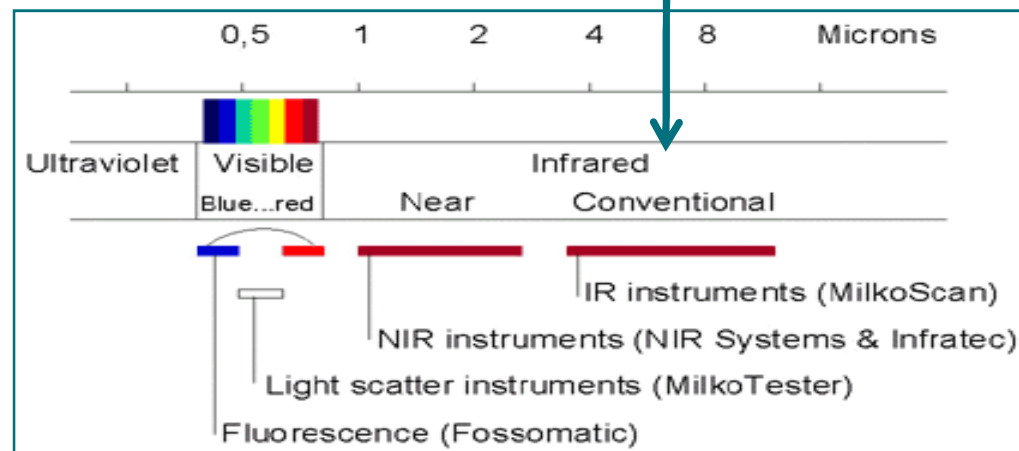
IR Spectral Ranges

- ❑ Near-IR (NIR)
 - Approximately $14000\text{--}4000\text{ cm}^{-1}$ ($0.8\text{--}2.5\text{ }\mu\text{m}$)
 - Can excite overtone or harmonic vibrations
- ❑ Mid-infrared (MIR)
 - Approximately $4000\text{--}400\text{ cm}^{-1}$ ($2.5\text{--}25\text{ }\mu\text{m}$)
 - May be used to study fundamental vibrations and associated rotational-vibrational structure
- ❑ Far-infrared (FIR)
 - Approximately $400\text{--}10\text{ cm}^{-1}$ ($25\text{--}1000\text{ }\mu\text{m}$)
 - 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**



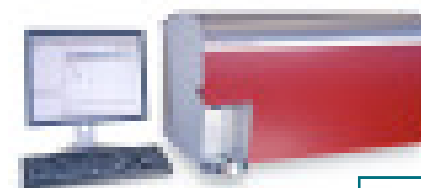
FOSS

❑ **Bentley Instruments DairySpec FT automatic**



Bentley Instruments

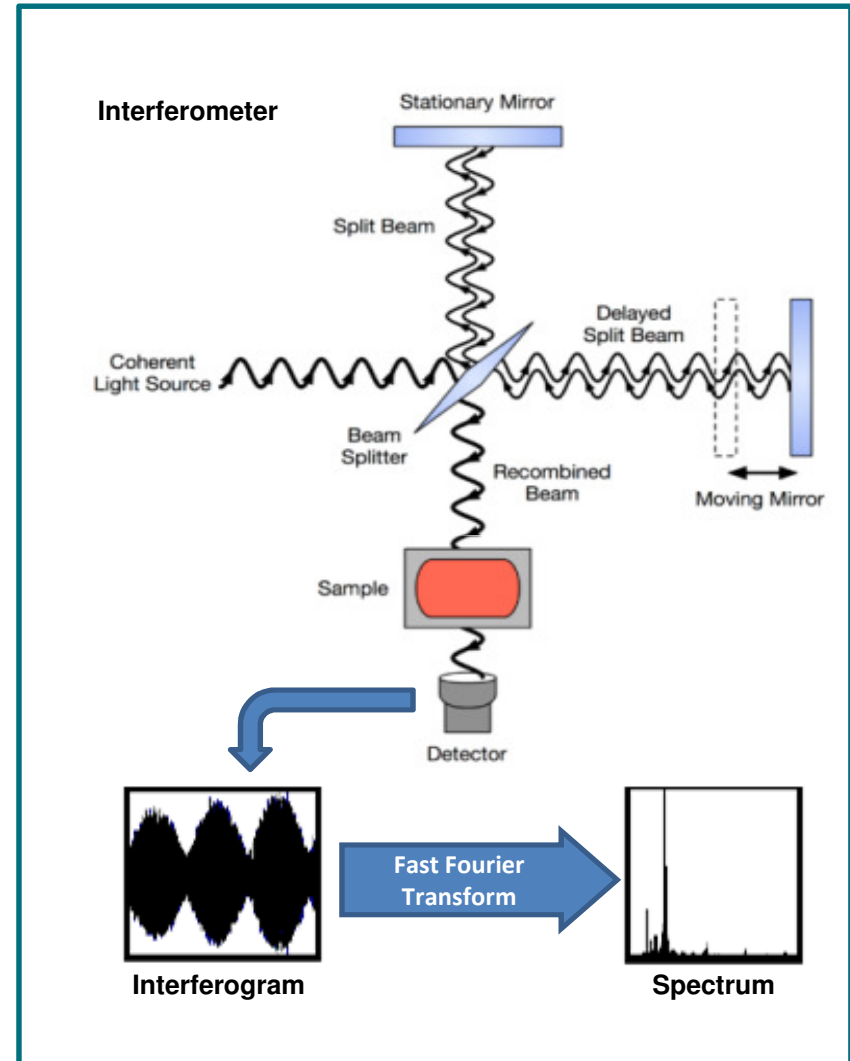
❑ **Delta Instruments LactoScope FTIR Advanced**



Delta Instruments

FTIR Spectrometry

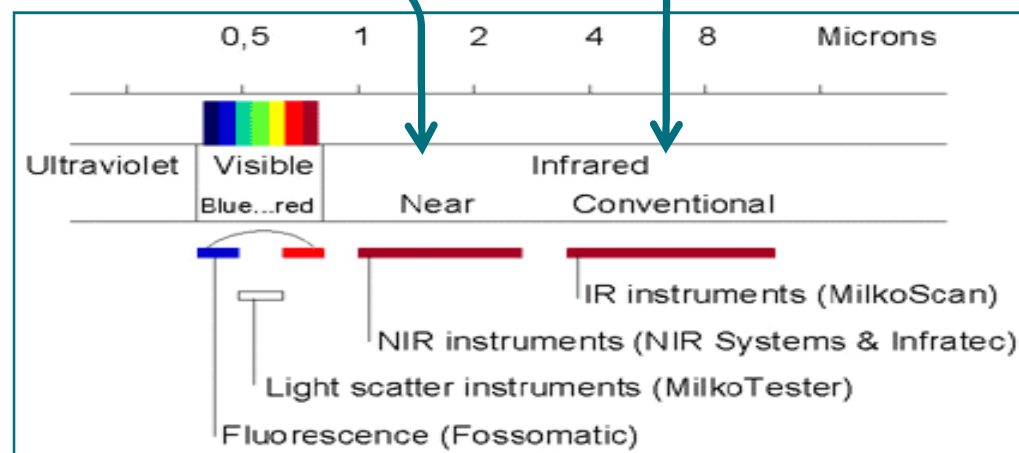
- ❑ Use of Fourier-Transform (FT) based technology
 - (Fast) FT algorithm transforming an interferogram to a spectrum
- ❑ Generally associated to MIR
⇒ FT-MIR
- ❑ In commercial applications often called FTIR (= FT-MIR)
- ❑ But there is also FT-NIR etc.



IR spectral ranges

Types of IR spectra ranges (here in milk applications)

- Mid-Infrared (MIR)
- Near-Infrared (NIR)



FOSS

NIR Spectrometry

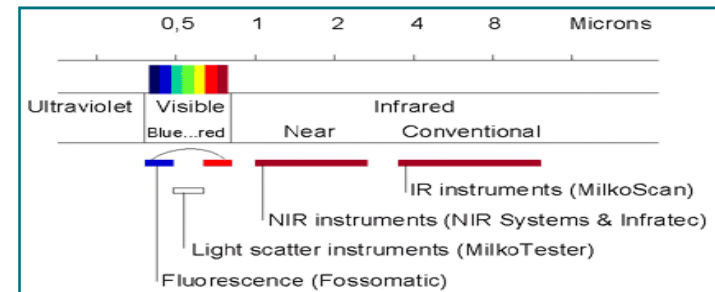
- ❑ Often called NIRS
 - Can be absorbance or reflectance (often)
 - Often also FT based technologies
- ❑ NIR more energy than MIR
 - Often used on bulk material
 - Little preparation
 - ➔ as feed stuff, cheese (as **FOSS DairyScan™**)
- ❑ NIR less “precise” than MIR
- ❑ NIR less sensitive ➔ ok for less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



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

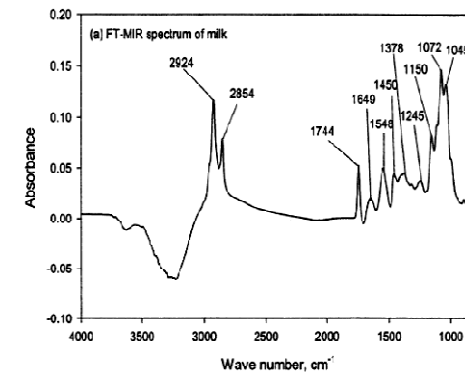
MIR Spectrometry



Milk samples
(milk payment, milk recording)



MIR analysis



Raw data = MIR spectra



Quantification:
fat
protein
urea
lactose

Calibration equations



MIR Spectrometry

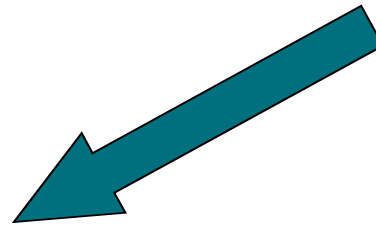


Milk samples

(milk payment, milk recording)



MIR analysis



Quantification:

- fat
- protein
- urea
- lactose

*Traditional data flow
(no MIR spectra stored)*

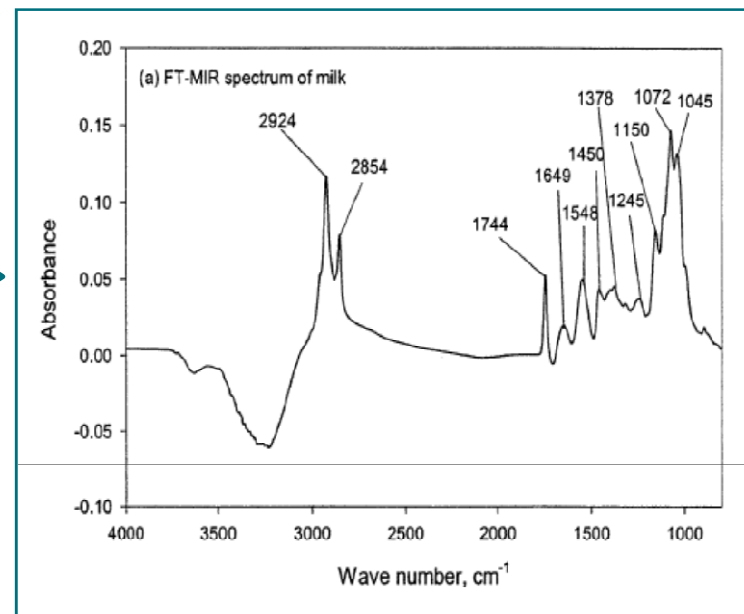
MIR Spectrometry - Calibration

□ Different between brands and models

➤ Between 850 – 1060 absorptions values (abs)

□ “Calibration”

➤ Obtaining b coefficients
e.g., Fat% = $b_0 + \sum b_i (\text{abs})_i$



Excel File Edit View Insert Format Tools Data Window Help

35130320.1113.SpectrumData.xlsx (Read-Only)

Home Layout Tables Charts SmartArt Formulas Data Review

Font:Calibri (Body) 11

Number:Normal, Bad, Good, Neutral, Calculation, Check Cell, Explanatory T..., Input

Cells:Insert, Delete, Format, Themes

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4	4/12/13 10:01	Normal	1	16347	594475	1	2	1060	1.236163	1.224816	1.213445	1.203333	1.194433	1.185992	1.177303	1.167946	1.157501	1.145115	1.129392	1.108876	1.082934	1.052468	1.020056	0.988904	0.961699	0.939316	0.920406	0.901861	0.879943	0.851592	0.815448	0.772286	0.724786	
5	4/12/13 10:01	Normal	1	16347	594476	1	3	1060	1.132829	1.131338	1.127171	1.121764	1.115967	1.110044	1.103983	1.097631	1.090495	1.081479	1.068946	1.051302	1.027905	0.999765	0.969552	0.940056	0.915653	0.895761	0.879587	0.864024	0.845259	0.82008	0.78693	0.746391	0.701017	
6	4/12/13 10:01	Normal	1	16347	594477	1	4	1060	1.209717	1.196581	1.182448	1.169955	1.159037	1.150633	1.143685	1.137299	1.130379	1.121474	1.108853	1.091021	1.0675	1.039418	1.009414	0.980754	0.956015	0.935984	0.919283	0.902859	0.883063	0.856893	0.82297	0.781958	0.736377	
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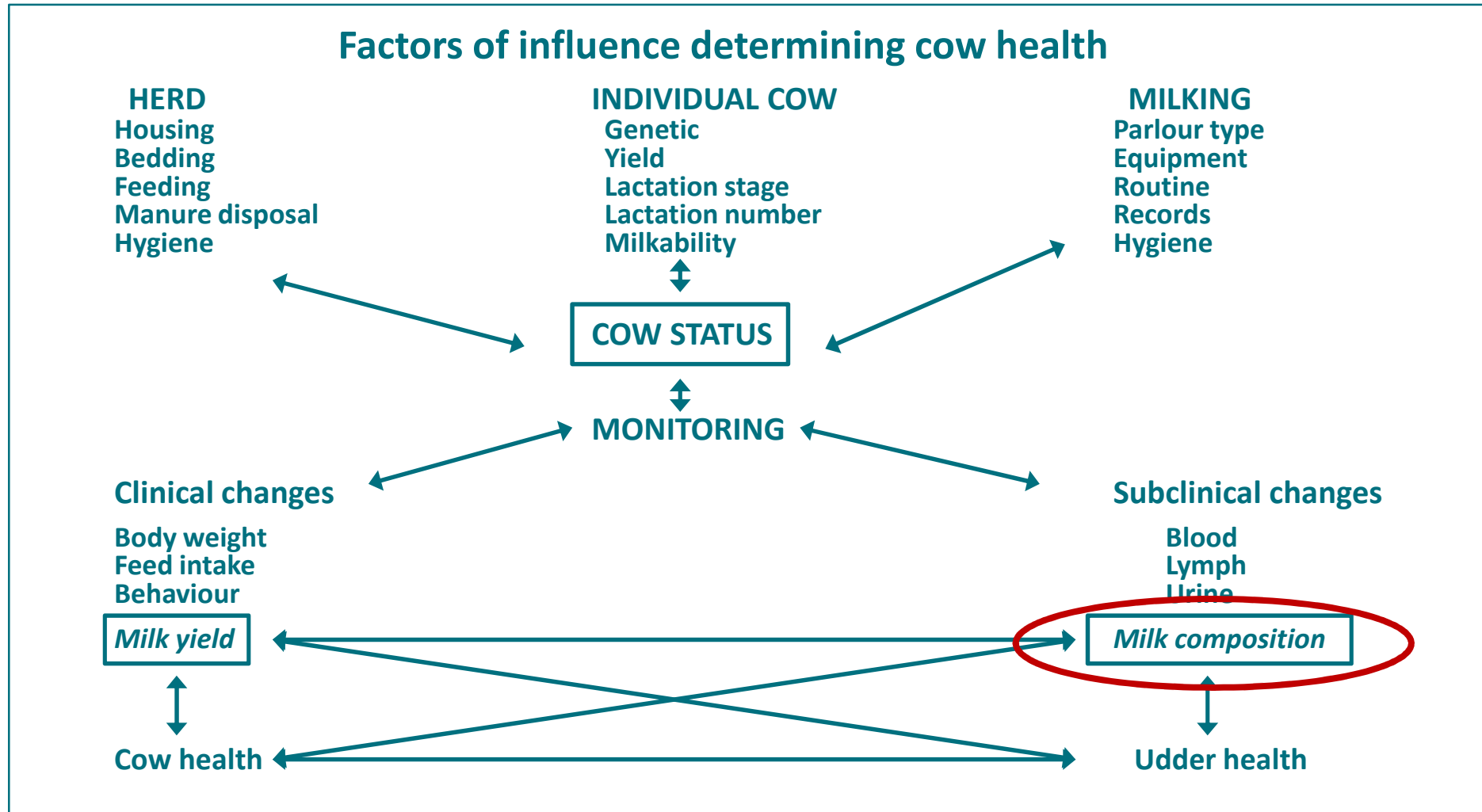
Major Challenge: Data

- ❑ 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

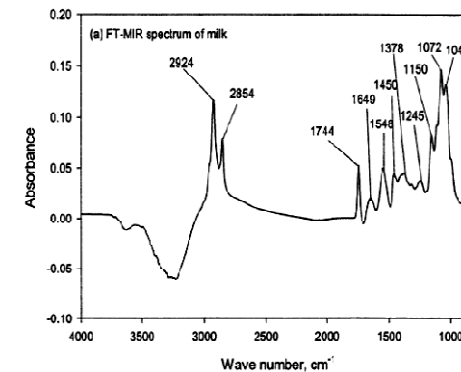
Major Milk Components (except SCC)



Milk samples
(milk payment, milk recording)



MIR analysis



Raw data = MIR spectra



Quantification:

- fat
- protein
- urea
- lactose

Calibration equations



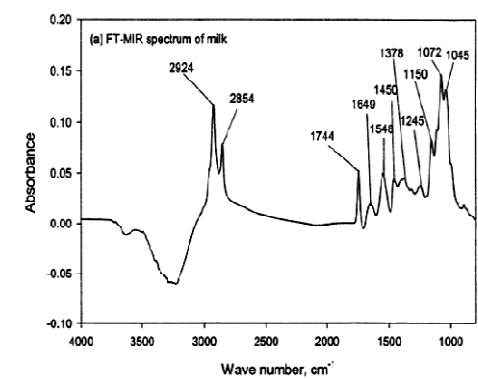
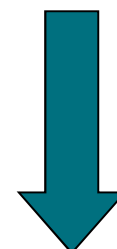
Novel Traits



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
 - $P(\text{trait of interest}) = b_0 + \sum b_i(\text{abs})_i$
- ❑ 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
© American Dairy Science Association®, 2011.

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

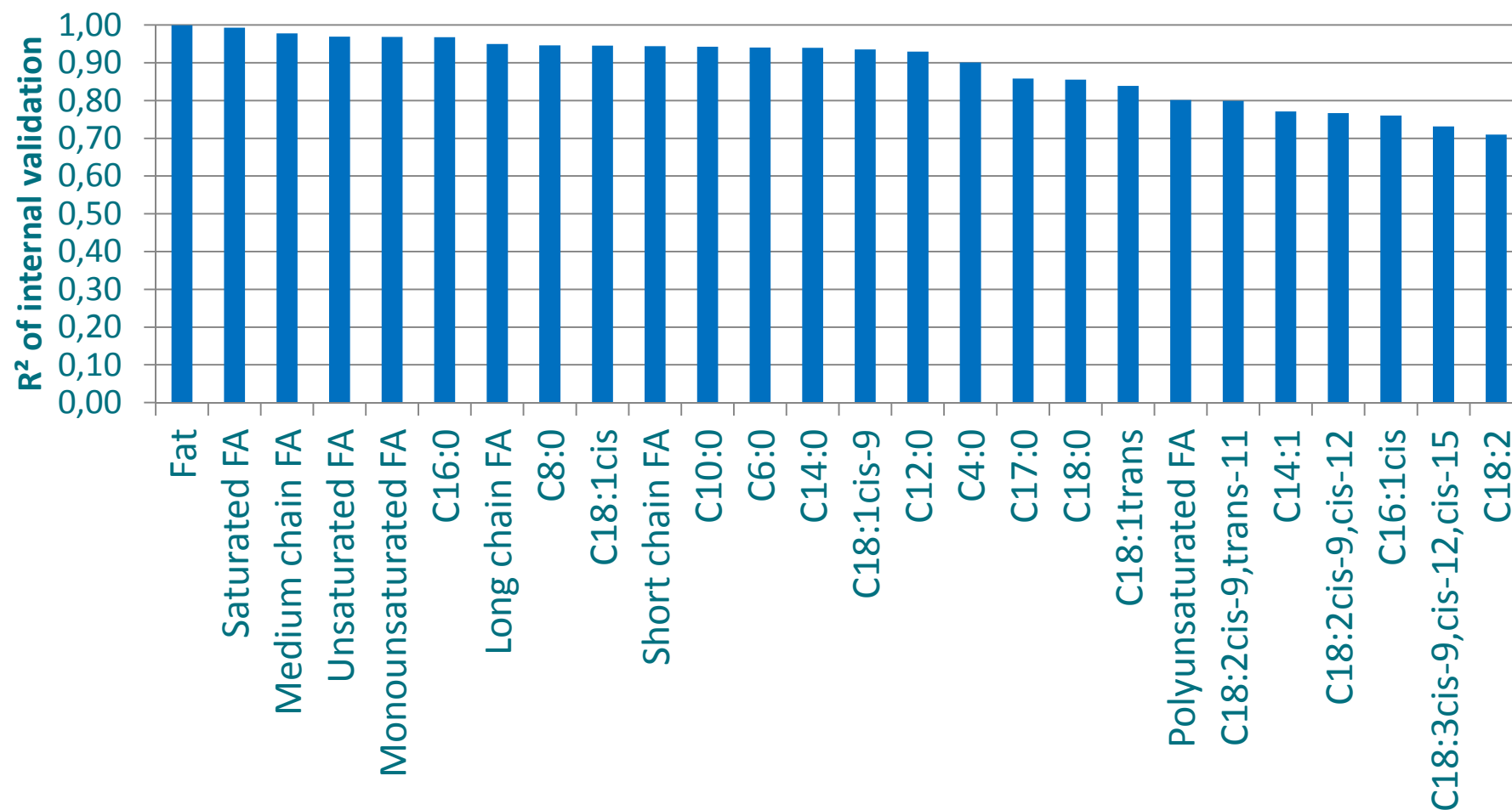
H. Soyeurt,^{*†1,2} F. Dehareng,^{‡1} N. Gengler,^{*†} S. McParland,[§] E. Wall,[‡] D. P. Berry,[§] M. Coffey,[#] and P. Dardenne[‡]

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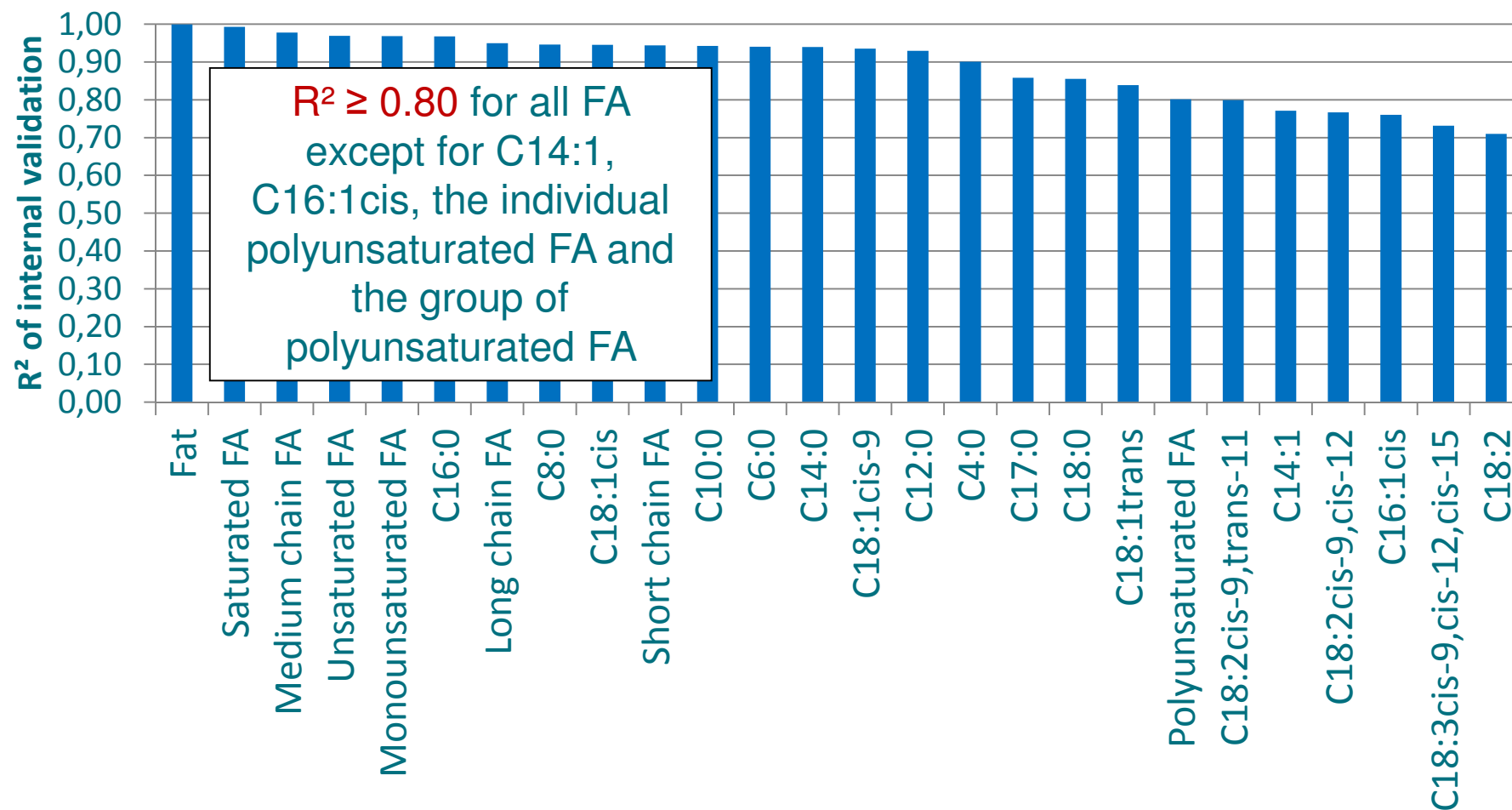
⇒ increased robustness

Accuracy of Fatty Acids Calibration Equations



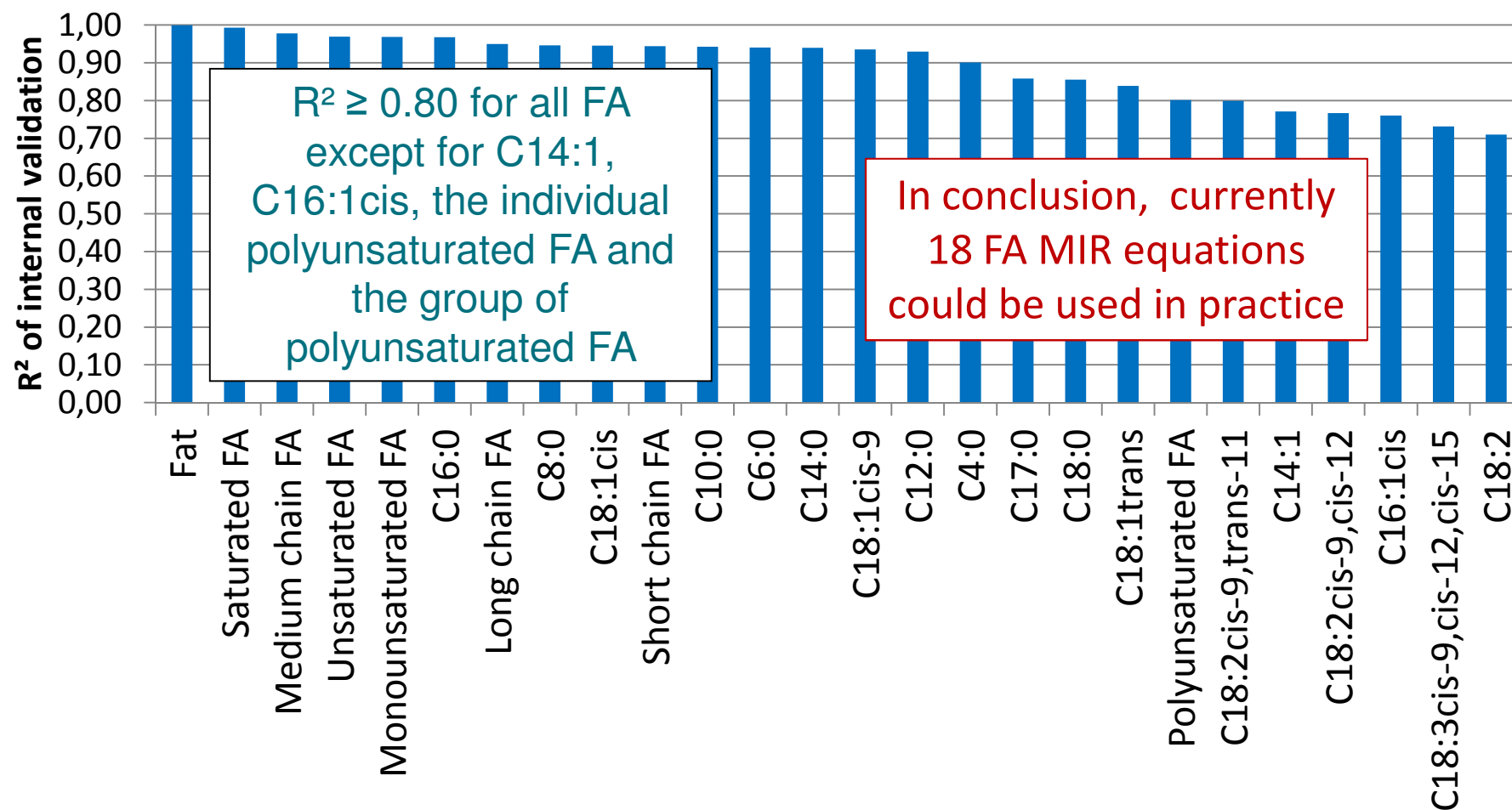
Calibration equations were developed from at least 1,600 milk samples

Accuracy of Fatty Acids Calibration Equations



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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

Examples of Successful Consortia

- ❑ Milk fatty acid (FA) equations:
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- ❑ Milk mineral equations:
 - First equations developed in 2006
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 - ❖ Belgium, France, Germany, and Luxembourg
- ❑ **Lactoferrin equations:**
 - **Cooperative effort of Belgium, Ireland and UK ← France**

Lactoferrin

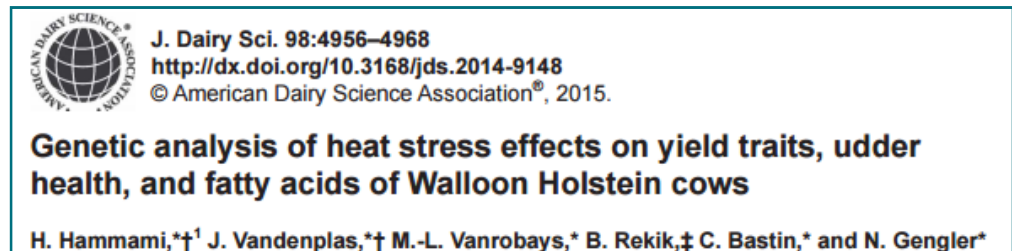
- ❑ Glycoprotein present naturally in milk
- ❑ Involved in the immune system
- ❑ Interests:
 - Potential indicator of mastitis
 - Help to maintain a good immune system in Humans
- ❑ However R^2 of internal validation = 0.71

⇒ 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)



III - Beyond milk composition from IR

Biomarker and Indicator Traits

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

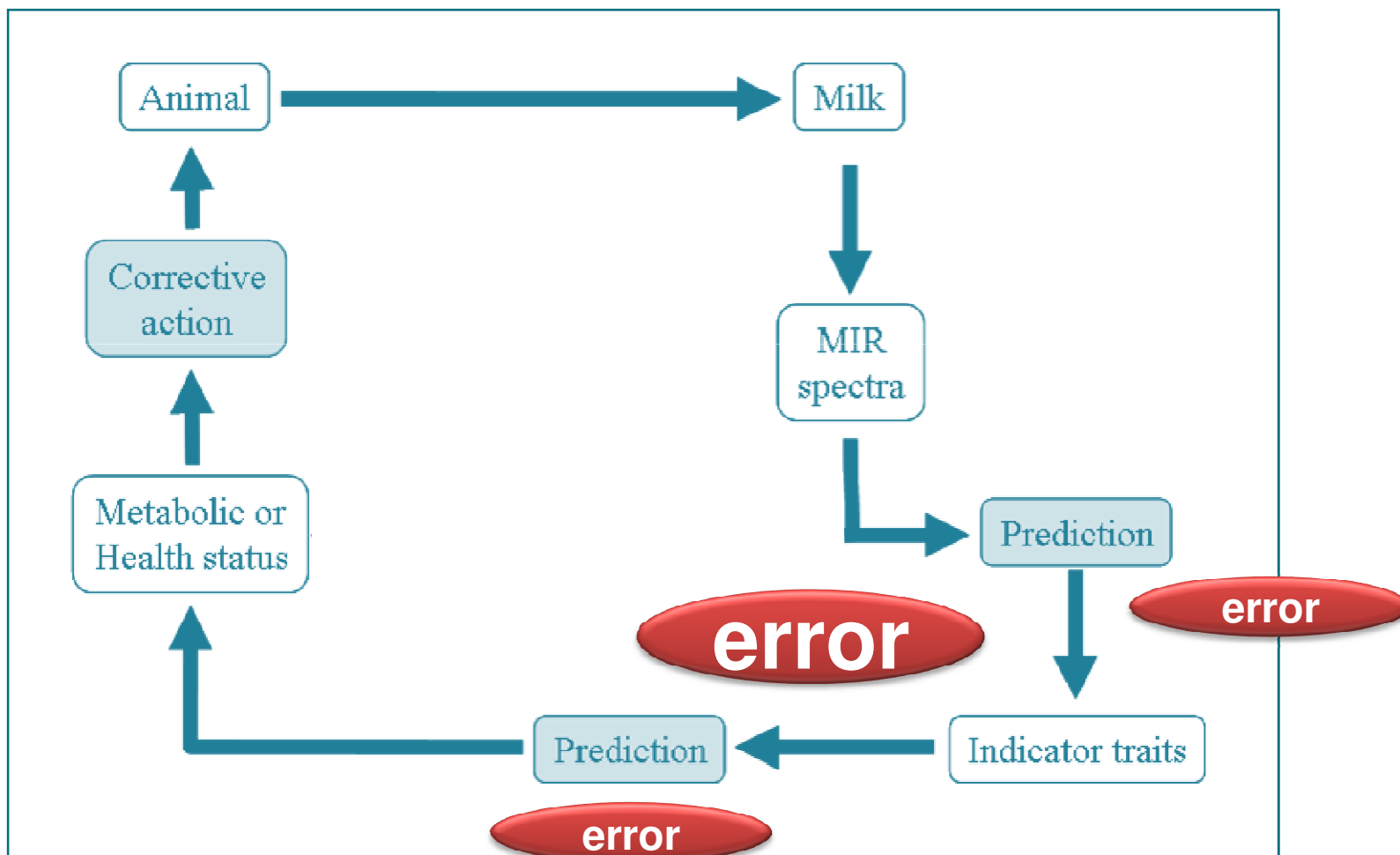
- ❑ However, many biomarkers or indicator traits can only be predicted rather imperfectly

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)



MIR ⇒ Indicator ⇒ Management Trait



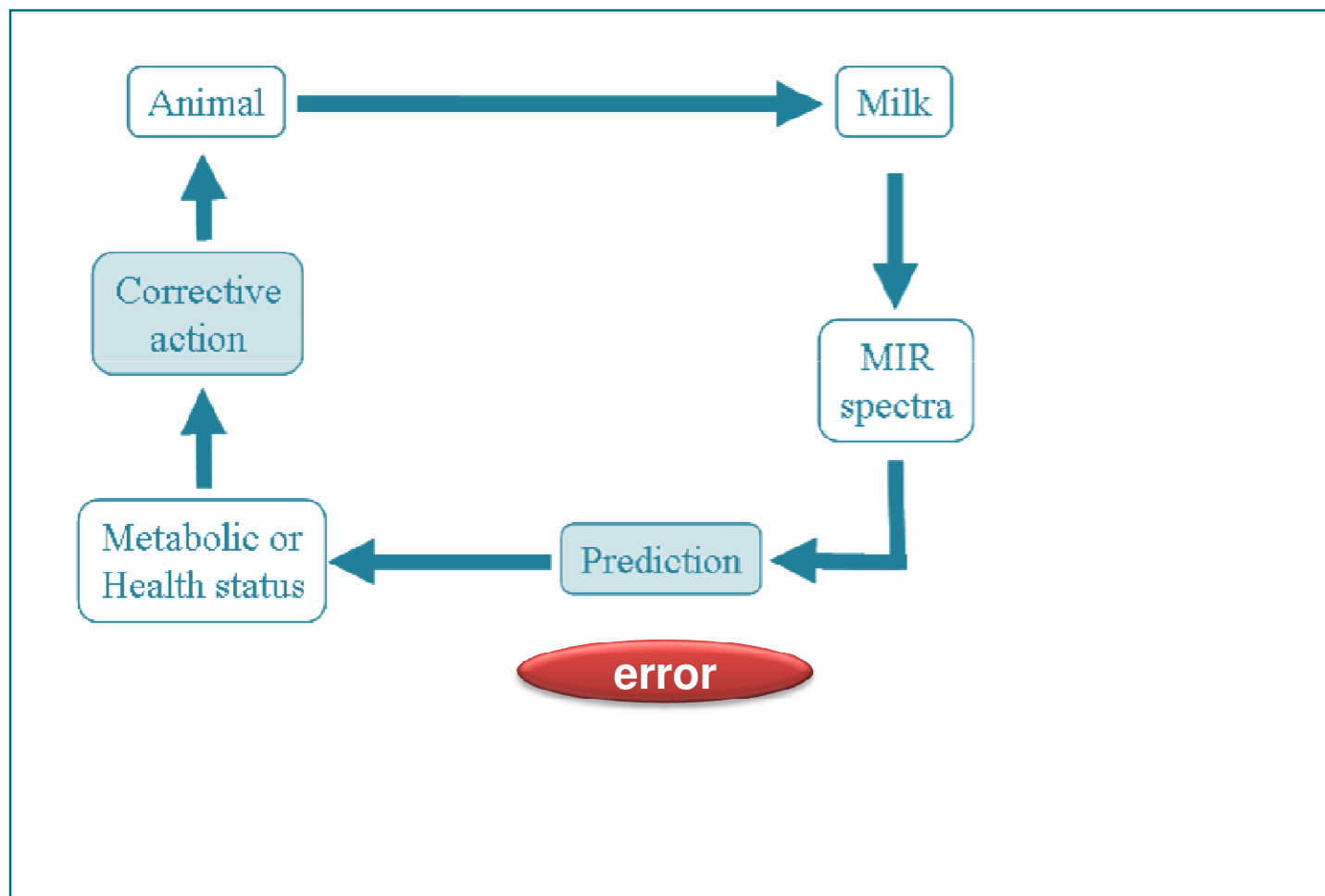
Direct Prediction of Traits of Interest

- ❑ “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

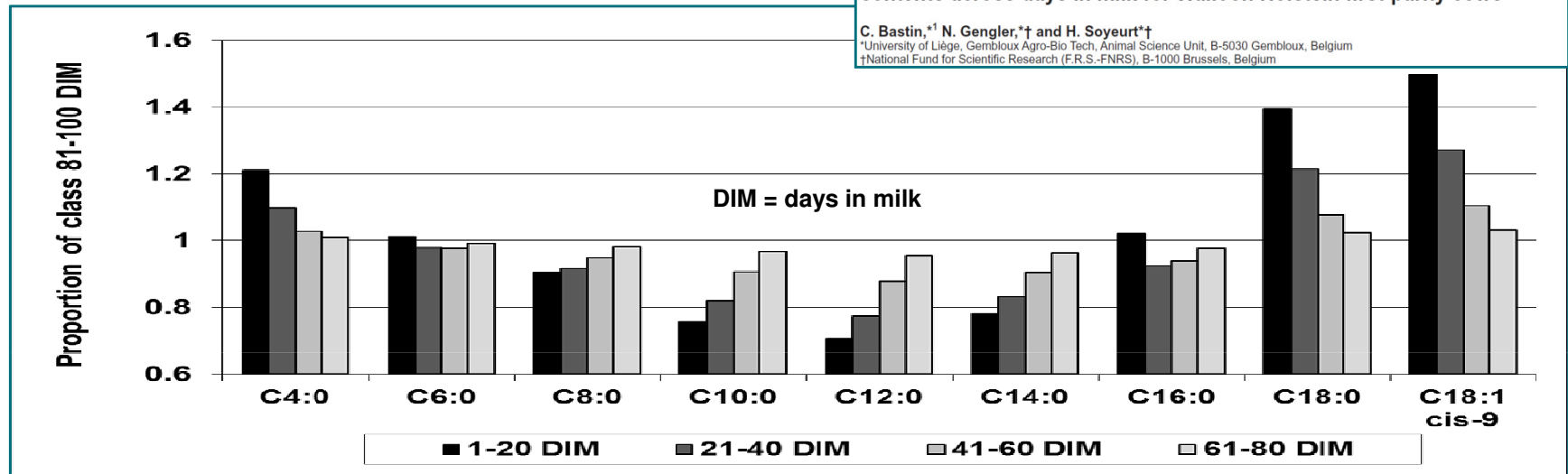


FA Profile Variable Throughout the Lactation


J. Dairy Sci. 94:4152–4163
doi:10.3168/jds.2010-4108
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Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows

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□ Indirect: reflecting equilibrium between:

Body fat mobilization ↔ Feed intake


- Body fat mobilization → also heat stress
- Feed intake → driving force for CH₄

□ Direct calibration of energy balance and related traits


J. Dairy Sci. 98:4956–4968
http://dx.doi.org/10.3168/jds.2014-9148
 © American Dairy Science Association®, 2015.

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

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J. Dairy Sci. 99:4056–4070
http://dx.doi.org/10.3168/jds.2015-10051
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The potential of Fourier transform infrared spectroscopy of milk samples to predict energy intake and efficiency in dairy cows¹

S. McParland² 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 (\Leftrightarrow FA) \Leftrightarrow 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 (\Leftrightarrow FA) \Leftrightarrow MIR Spectra
- More details in article



J. Dairy Sci. 98:5740–5747
<http://dx.doi.org/10.3168/jds.2014-8436>
© American Dairy Science Association®, 2015.

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

A. Vanlierde,^{*1} M.-L. Vanrobays,^{†1} F. Dehareng,^{*2} 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
- $P(\text{CH}_4) = f_{b0}(\text{DIM}) + \sum f_{bi}(\text{DIM}) \times (\text{abs})_i$

□ 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>
© American Dairy Science Association®, 2015.

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. Dehareng²
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IV - Future of IR – ongoing research

Trent in Animal Breeding: Direct Use of MIR

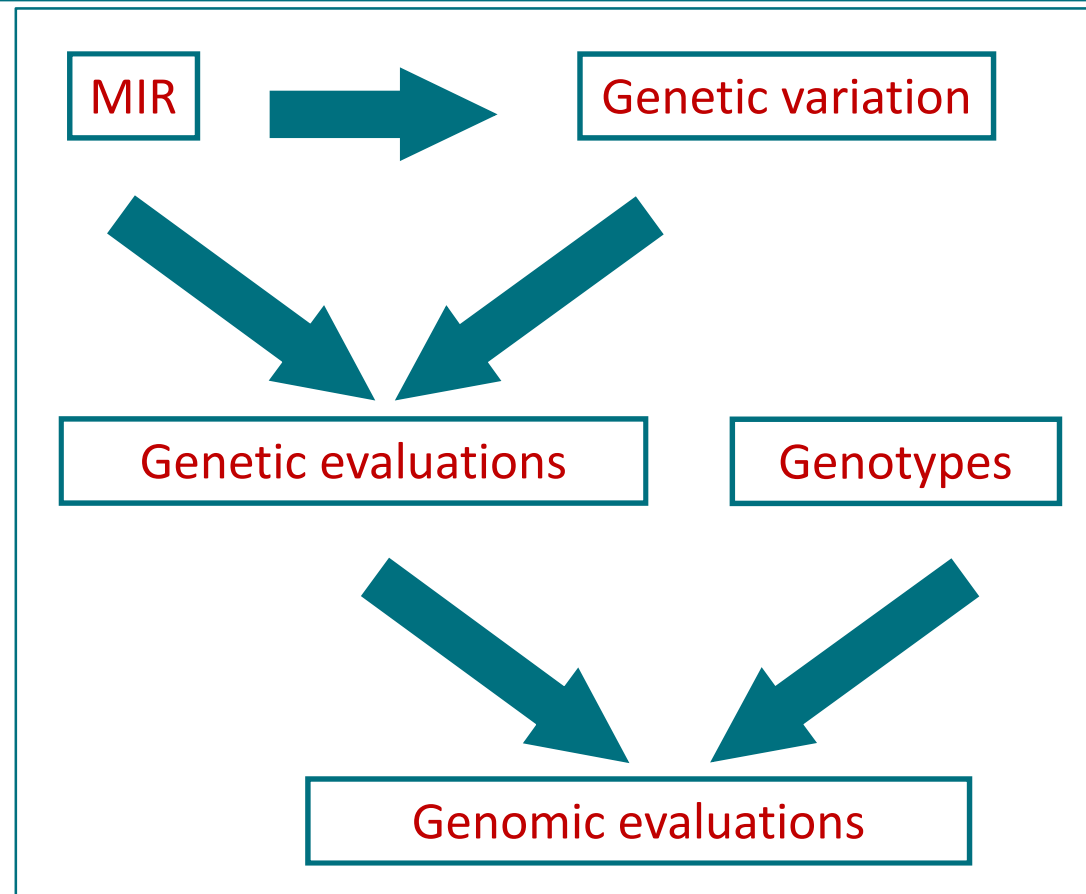


J. Dairy Sci. 93:1722–1728
doi:10.3168/jds.2009-2614
© American Dairy Science Association®, 2010.

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

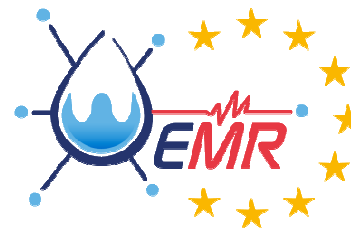
⇒ important to develop international collaborations

- ❑ 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

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 - **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!

SBMA Meeting (Ribeirão Preto - Sao Paulo, Brazil, June 12 to 13, 2017)

