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

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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 $\rightarrow$ reciprocal cm ($cm^{-1}$)
    - also called “wave numbers”
  - Units of IR wavelength $\rightarrow$ micrometers ($\mu m$)
    - 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)
Typical MIR Spectrometers (milk testing)

- FOSS MilkoScan™ 7
- Bentley Instruments  
  DairySpec FT automatic
- Delta Instruments  
  LactoScope FTIR Advanced
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)
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

- 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)

Quantification:
- fat
- protein
- urea
- lactose

Calibration equations

Raw data = MIR spectra
MIR Spectrometry

Milk samples
(milk payment, milk recording)

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$\% = b_0 + \sum b_i (abs)_i$
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!

Factors of influence determining cow health

**HERD**
- Housing
- Bedding
- Feeding
- Manure disposal
- Hygiene

**INDIVIDUAL COW**
- Genetic
- Yield
- Lactation stage
- Lactation number
- Milkability

**MILKING**
- Parlour type
- Equipment
- Routine
- Records
- Hygiene

**COW STATUS**

**MONITORING**
- Clinical changes
- Subclinical changes
  - Body weight
  - Feed intake
  - Behaviour
  - Milk yield
  - Cow health

**Milk composition**
- Blood
- Lymph
- Urine
- Udder health


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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
  - $\alpha$-lactalbumins
  - $\beta$-lactoglobulins
  - Other minor proteins (e.g., lactoferrin)

- **Other minor constituents**
  - $\beta$-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)

Quantification:
- fat
- protein
- urea
- lactose

Calibration equations

Raw data = MIR spectra
Novel Traits

Milk samples
(milk payment, milk recording)

Quantification:
Novel traits

MIR analysis

Novel Calibration equations

Raw data = MIR spectra
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

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

H. Soyeurt,†1 2 F. Deharenge,†1 N. Gengler,†1 S. McParland,§ E. Wall,† D. P. Berry,§ M. Coffey,# and P. Dardenne†

J. Dairy Sci. 94:1657–1667
doi:10.3168/jds.2010-3406
Examples of Successful Consortia

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⇒ increased robustness
Calibration equations were developed from at least 1,600 milk samples
R² ≥ 0.80 for all FA except for C14:1, C16:1cis, the individual polyunsaturated FA and the group of polyunsaturated FA

Calibration equations were developed from at least 1,600 milk samples
In conclusion, currently 18 FA MIR equations could be used in practice.

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

R² of internal validation

- R² ≥ 0.80 for all FA except for C14:1, C16:1cis, the individual polyunsaturated FA and the group of polyunsaturated FA

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- Milk mineral equations:
  - First equations developed in 2006
  - Improved through international collaborations:
    - Belgium, France, Germany, and Luxembourg
Examples of Successful Consortia

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- **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 $= 0.71$

$\Rightarrow$ MIR predictor of lactoferrin

- Estimation of Biomarker not without errors
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)

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MIR \rightarrow \text{Indicator} \rightarrow \text{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

Diagram:

- Animal
- Milk
- Corrective action
- Metabolic or Health status
- Prediction
- MIR spectra
- Error

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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
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 $\leftrightarrow$ FA $\leftrightarrow$ MIR Spectra
  - More details in article

- **Variable calibration equation coefficients**
  - Here Days in Milk (DIM) dependent
  - $P(\text{CH}_4) = f_{b0}(\text{DIM}) + \sum f_{bl}(\text{DIM}) \times (\text{abs})_l$

- **But can be used in many other situations**

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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, J. A. Fernández Pierna, P. Dardenne, V. Baeten, and F. Dehareng
  
  Wallonian Agricultural Research Center, Visualisation of Agricultural Products Department, 24 Chaussées de Namur, 5030 Gembloux, Belgium
IV - Future of IR – ongoing research
Trent in Animal Breeding: Direct Use of MIR

- 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) $\Rightarrow$ 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“
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  - Team CRA-W: P. Dardenne, F. Dehareng, C. Grelet, A. Vanlierde, E. Froidmont, ....
Thank you!