Genotype plus Environment
Integration for a more sustainable dairy production system

On the use of novel milk phenotypes as predictors of difficult-to-record traits in breeding programs

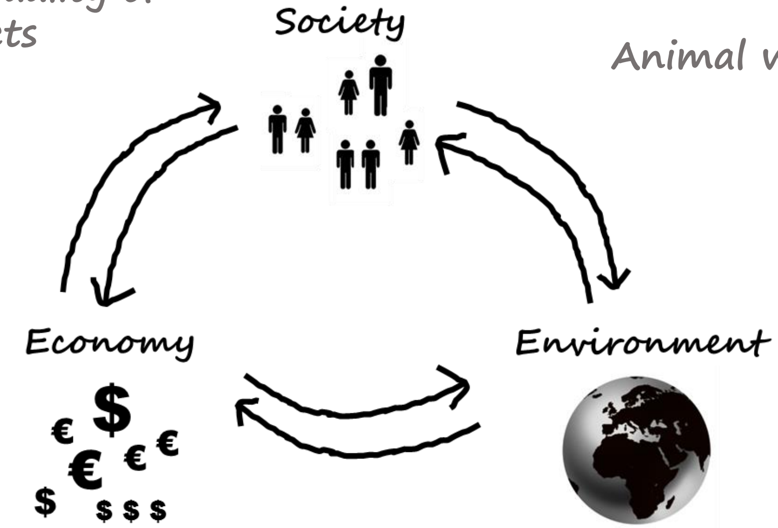
Catherine Bastin[1], F. Colinet[1], F. Dehareng[2], C. Grelet[2], H. Hammami[1], A. Lainé[1], H. Soyeurt[1], A. Vanlierde[2], M-L. Vanrobays[1] & N. Gengler[1]

[1] University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium
[2] Walloon Agricultural Research Centre, Gembloux, Belgium

66th EAAP Annual Meeting, Warsaw, Poland, 2015

Dairy production faces the challenge of sustainability

- Nutritional quality of dairy products
- Reduced labor
- Breeders' profitability
- Supply the expanding world population



- Animal welfare
- Reduced use of antibiotics
- Efficiency in the use of resources
- Reduction of cattle environmental footprint
- Resilience to climate change

New phenotypes will address new breeding goals

Breeding is part of the response to the sustainability challenge

Even in the genomic era, phenotypes are required for:

- ☐ health and fertility
- ☐ environmental footprint
- ☐ welfare
- ☐ efficiency
- ☐ resilience
- ☐ quality of products

New phenotypes might be difficult to obtain

These new phenotypes are often:

- not (readily) available or only for a few animals
- difficult and/or expensive to record
- subject to poor quality or censoring

⇨ **Milk biomarkers could be used as predictors of these difficult-to-record phenotypes.**

Why are milk biomarkers useful?

☑ Mirror of the cow's physiological status

☑ Non invasive measurement

☑ Easy to collect (even routinely)

☑ Especially if they can be measured by cost-effective and high-throughput methods

Some biomarkers are already used

Phenotype of interest		**Milk biomarker**
Udder health	⟷	Somatic cell count
Fertility	⟷	Progesterone
Nutritional imbalance	⟷	Fat / protein

Outline

Can we get more out of milk?

Novel biomarkers for key phenotypes

Are mid-infrared predicted traits useful in breeding programs?

- ☐ Fertility
- ☐ Health (mastitis & ketosis)
- ☐ Environmental footprint

Novel biomarkers for key phenotypes?

3 groups of phenotypes investigated in the frame of GplusE:

- ☑ Metabolites
- ☑ Glycan profiles
- ☑ Mid-infrared predicted traits

Novel biomarkers for key phenotypes?

3 groups of phenotypes investigated in the frame of GplusE:

- ☑ **Metabolites** ➔ **“Phenotypic interrelationships between parameters predominantely in milk” by K. Ingvartsen**
- ☑ **Glycan profiles**
- ☑ **Mid-infrared predicted traits**

Novel biomarkers for key phenotypes?

3 groups of phenotypes investigated in the frame of GplusE:

- ☑ Metabolites
- ☑ Glycan profiles
- ☑ Mid-infrared predicted traits

Why looking at glycan?

- Biomolecular glycosylation has fundamental roles in many biological recognition events
- Oligosaccharides of glycoconjugates are rapidly responsive to disease and physiological state
- Functional glycomics
  - ✓ looks at glycan (sugar) structure and function
  - ✓ identifies glycoproteins associated with disease or physiological state
  - ✓ studies their biological function

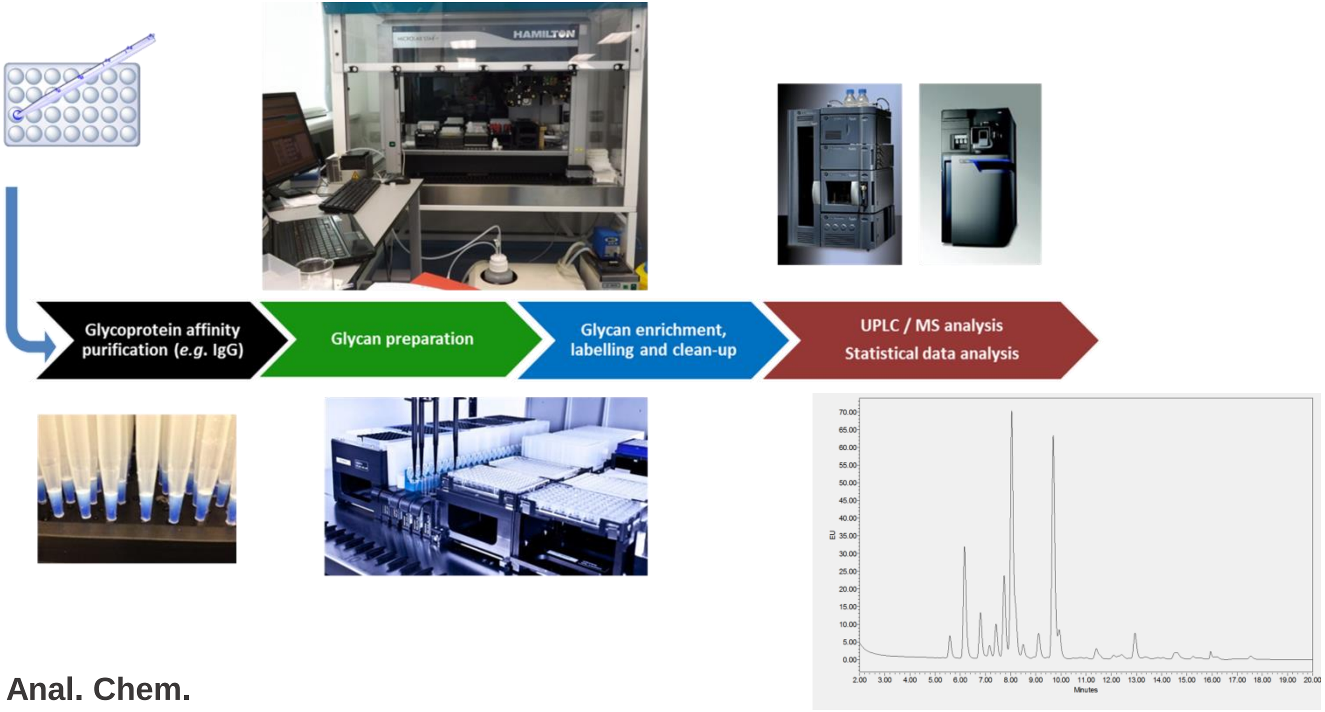
⇨ **Functional glycomics on IgG**

✓ IgG are central players of the immune system

Stöckmann et al., 2013, Anal. Chem.
Tharmalingam et al., 2013, Glycoconj J.
Taniguchi et al., 2009, J. Proteome R.

Glycoprofiling of IgG

The glycoprofiling of IgG can be performed by an automated, accurate, high-throughput and cost efficient N-glycan analysis platform



Stöckmann et al., 2013, Anal. Chem.

Novel biomarkers for key phenotypes?

3 groups of phenotypes investigated in the frame of GplusE:

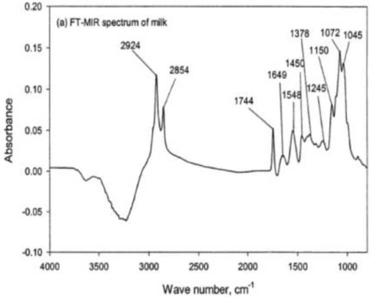
- ☑ Metabolites
- ☑ Glycan profiles
- ☑ Mid-infrared predicted traits

MIR spectrometry is already used worldwide

Milk samples

milk payment, milk recording

MIR analysis



Spectrum

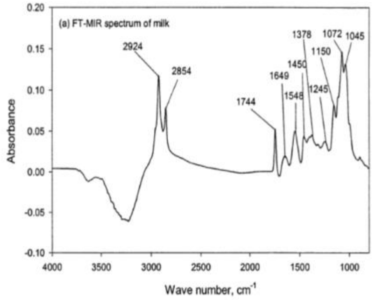
= fingerprint of milk composition

MIR spectrometry is already used worldwide

Milk samples

milk payment, milk recording

MIR analysis



Equations of prediction

Classical components:

Fat & protein + urea, lactose, casein

+

Fatty acids, feed efficiency, minerals, ketone bodies, protein fractions, milk technological properties, methane emissions, etc.

On the opportunities of MIR analysis

☑ A wide range of traits

<u>Milk composition</u>

- Fatty acids
- Protein fraction
- Minerals
- Ketone bodies
- Citrate
- Melamine
- …

<u>Phenotypes related to milk composition</u>

- Milk technological properties
- Methane emission
- Body energy status
- Feed efficiency
- …

De Marchi et al., 2014, J. Dairy Sci.

On the opportunities of MIR analysis

☑ A wide range of traits

☑ High throughput and cost efficient

☑ At population level, several times over the lactation

through milk recording

☑ Even retrospectively

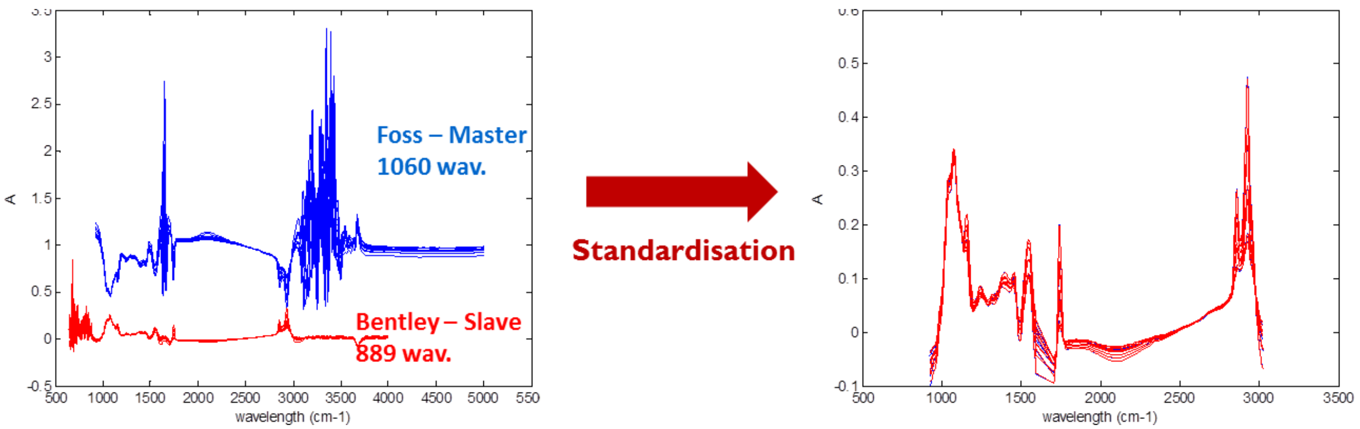
thanks to spectral databases

De Marchi et al., 2014, J. Dairy Sci.

On the challenges of MIR analysis

☑ **Spectra should be collected, stored and standardized**

Harmonizing the spectra
- ✓ over time
- ✓ among spectrometers (several brands)

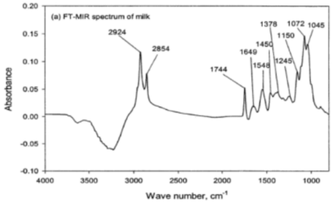


Grelet et al., 2015, J. Dairy Sci.

On the challenges of MIR analysis

☑ Equations of prediction should be created

Calibration dataset

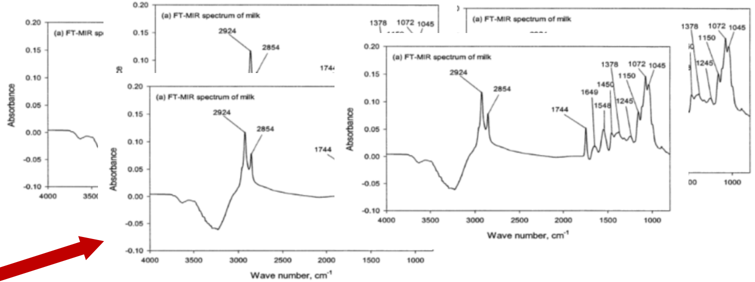


+

"Reference" analysis

Equations of prediction

Population



Phenotypes for the whole population

On the challenges of MIR analysis

☑ **Equations of prediction should be created**

- ✓ **The calibration dataset should be representative of the population in which the equation will be used: breeds, lactation stage, feeding, etc.**
- ✓ **Errors on the reference analysis should be limited**
- ✓ **Limit of detection: starting from 100 ppm**
- ✓ **Accuracy of prediction should be considered in relation to the use of the equation (milk payment, genetics, management, etc.)**

Range of RPD (min-max)		Class	Application	Symbol
0	2	Very poor	Allows to compare groups of cows, distinguish high or low values	-
2	3	Poor	Rough screening	0
3	5	Fair	Screening	+
5	6.5	Good	Quality control	++
6.5	+	Excellent	As precise as reference value	+++

Dardenne, 2015
Grelet, 2015

Outline

Can we get more out of milk?

Novel biomarkers for key phenotypes

Are mid-infrared predicted traits useful in breeding programs?

- ☐ Fertility
- ☐ Health (mastitis & ketosis)
- ☐ Environmental footprint

Why are milk biomarkers useful?
… in the frame of breeding

- ☑ **Mirror of the cow's physiological status**
- ☑ **Non invasive measurement**
- ☑ **Easy to collect (even routinely)**
- ☑ **Especially if they can be measured by cost-effective and high-throughput methods**

➔ **Milk biomarkers can be used as indicator trait of difficult-to-record lowly heritable phenotypes**

if
- ✓ easier to record
- ✓ heritable
- ✓ genetically correlated with the phenotype of interest

Which MIR predicted traits as fertility indicators?

In early lactation:
energy intake < energy output

↓

Negative energy balance
Body fat mobilization

↓

Poor fertility

Changes in milk composition

- ↑ fat
- ↓ protein

} ↑ fat to protein ratio

- ↑ urea
- ↑ ketone bodies
- Changes in milk fatty acids profile
  - ↑ long-chain FA
  - ↓ de novo synthesized FA

Are these traits heritable?

Are they genetically correlated with fertility?

de Vries & Veerkamp, 2000; Reist et al., 2002; Reksen et al., 2002; König et al., 2008; Martin et al., 2015, J. Dairy Sci.; Gross et al., 2011, J. Dairy Res.

Some MIR predicted traits are good candidates as fertility indicators

- Traits measured in early lactation are the most interesting
- Some estimates from the literature

Milk based trait in early lactation	h^2	Fertility trait	r_g
Average milk urea from two 1st test-days	0.13	Calving to 1st service	0.29
Fat to protein ratio at 30 DIM	0.16	Calving to 1st service	0.28
Content in milk of C10:0 at 5 DIM	0.28	Days open	-0.37
Content in milk of C18:1 *cis*-9 at 5 DIM	0.13	Days open	0.39
Log (BHBA in milk) from 5 to 20 DIM	0.14	Calving to 1st service	0.21
MIR predicted direct energy balance at 5 DIM	0.20	Days open	-0.20

König et al., 2008; Negussie et al., 2013; Bastin et al., 2012;
Koeck et al., 2014; Bastin et al., 2013, J. Dairy Sci.

Which MIR predicted traits as mastitis and ketosis indicators?

Mastitis

- ↑ SCS
- ↑ lactoferrin
- ↓ casein
- ↑ Na
- ↓ K
- ↑ pH
- ↓ lactose
- ↓ citrate

Ketosis

- ↑ fat to protein ratio
- ↑ ketone bodies
- Changes in milk fatty acids profile
  ↑ long-chain FA

Are these traits heritable?

Are they genetically correlated with health?

Hamann & Krömker, 1997, Livest. Prod. Sci.; Brandt et al., 2010, J. Dairy Sci.; Le Maréchal et al., 2011, Dairy Sci. Technol.
Heuer, 1999; Van Healst, 2008, Van der Drift, 2012, J. Dairy Sci.

Some MIR predicted traits are of interest to select for udder health

- Genetic parameters of traits derived from SCS and MIR predictions with clinical mastitis

Milk based trait	h^2	r_g
Average SCS from 5 to 305 DIM	0.13	0.79
Standard deviation of Na from 5 to 305 DIM	0.01	0.83
Standard deviation of citrate from 5 to 305 DIM	0.04	0.77
Average acetone from 5 to 65 DIM	0.11	0.60

Bastin et al. 2015, Unpublished results

MIR predicted ketone bodies as indicators of ketosis

- Gold standard = plasma BHBA

r_g	Milk acetone	Milk BHBA
Blood BHBA	0.52	0.52

- Genetic correlations with clinical ketosis

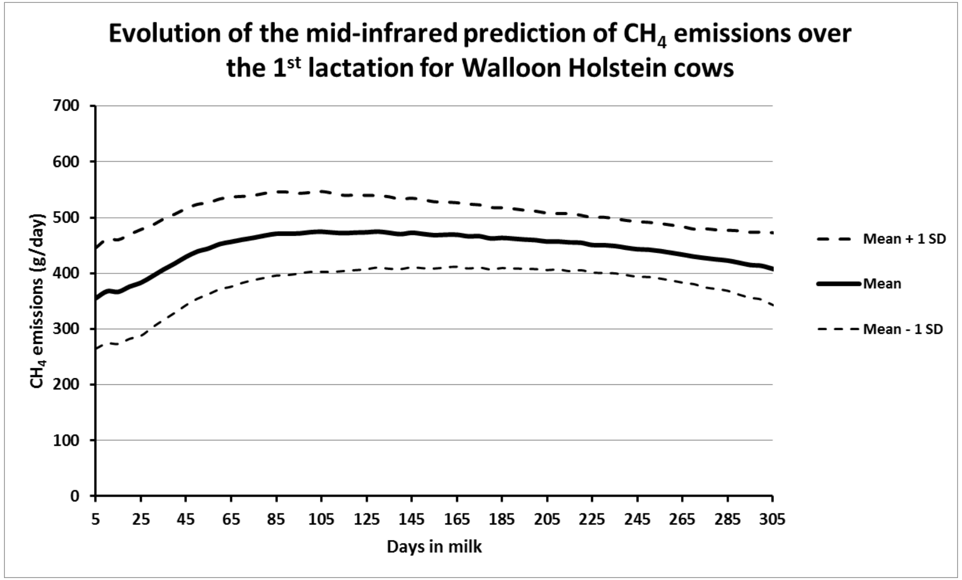
Some estimates from the literature

Milk based trait in early lactation	h^2	r_g
Log (BHBA in milk) at 1st test-day	0.12	(0.48)
Fat to protein ratio > 1.5 at 1st test-day	0.07	0.35
Fat to lactose ratio at 1st test-day	0.19	-0.25

Van der Drift et al., 2012; Koeck et al., 2013 & 2014, J. Dairy Sci.; Ederer et al., 2014, Proc. ICAR meeting

Which MIR predicted traits as environmental footprint indicators?

- Methane emission (g/day) can be predicted by MIR



- Average daily h^2 = 0.25

Vanlierde et al., 2015, J. Dairy Sci.
Vanrobays et al., 2015, Unpublished results

MIR predicted traits are useful indicators in breeding programs

☑ To replace “direct” phenotypes (when not available)

- MIR prediction of methane emission
- Fatty acids as early indicators of fertility
  - ✓ when fertility phenotypes are not readily available

☑ To supplement “direct” phenotypes

- MIR predicted traits as indicators of mastitis
  - ✓ they might cover various aspects of udder health
  - ✓ also related to subclinical variations

⇨ What would be the benefit of including these traits in selection indices?

Fatty acids to improve days open

- Breeding goal = days open
- Accuracy of a fertility index for a bull with 100 daughters

Trait(s) in the index	Accuracy
Days open	0.75
C18:1 *cis*-9 at 5 DIM	0.35
C10:0 at 5 DIM	0.35
C10:0 + C18:1 *cis*-9 at 5 DIM	0.47
Days open + C10:0 + C18:1 *cis*-9 at 5 DIM	0.78

⇨ Fatty acids are of interest when days open is not available

⇨ Combining traits to achieve the best accuracy

Bastin et al., 2014, Proc. WCGALP2015

Combining traits to improve udder health

- Breeding goal = clinical mastitis
- Accuracy of a fertility index for a bull with 100 daughters

Trait(s) in the index	Accuracy
Clinical mastitis (CM)	0.71
SCS_{m305}	0.67
SCS_{m305} + Na_{sd305} + $Citrate_{sd305}$ + $Acetone_{m65}$	0.78
CM + SCS_{m305}	0.78
CM + SCS_{m305} + Na_{sd305} + $Citrate_{sd305}$ + $Acetone_{m65}$	0.84

⇨ MIR predicted traits are useful to supplement SCS

⇨ Combining traits to cover various aspects of udder health

Take home message

☑ Milk biomarkers (e.g., MIR predicted traits) are useful in breeding programs

- to supplement or replace difficult-to-record phenotypes
- given their underlying relationship with the physiology of the cow
- if they are heritable and genetically correlated with the phenotype of interest

☑ Further studies are warranted to

- grasp the underlying relationship among phenotypes
- estimate the genetic parameters of these traits
- further evaluate the use of these traits in genetic and genomic selection

More information about MIR at EAAP?

Monday

15:30 Can chamber and SF6 CH_4 measurements be combined in a model to predict CH_4 from milk MIR spectra?
A. Vanlierde, M.-L. Vanrobays, F. Dehareng, E. Froidmont, N. Gengler, S. Mcparland, F. Grandl, M. Kreuzer, B. Gredler, H. Soyeurt and P. Dardenne

Wednesday

14:00 Overview of possibilities and challenges of the use of infrared spectrometry in cattle breeding
Invited *N. Gengler, H. Soyeurt, F. Dehareng, C. Bastin, F.G. Colinet, H. Hammami and P. Dardenne*

14:45 Milk biomarkers to detect ketosis and negative energy balance using MIR spectrometry
C. Grelet, C. Bastin, M. Gelé, J.B. Davière, R. Reding, A. Werner, C. Darimont, F. Dehareng, N. Gengler and P. Dardenne

Thursday

10:00 Genetic variability of MIR predicted milk technological properties in Walloon dairy cattle
F.G. Colinet, T. Troch, V. Baeten, F. Dehareng, P. Dardenne, M. Sindic and N. Gengler

Thank you!

- Partners of research are acknowledged.

- Research conducted through OptiMIR, NovaUdderHealth (D31-1273), COMPOMILK (Grant 2.4604.11) and GplusE (Grant FP7-KBBE-613689) projects.

The content of the presentation reflects only the view of the authors; the Community is not liable for any use that may be made of the information contained in this presentation.

catherine.bastin@ulg.ac.be