New Parameters and Analytical Challenges for Milk Recording by Fourier-Transform Mid-Infrared Spectrometry (FTMIR)

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ICAR – 1st June 2010 – Riga – Latvia

Why mid-infrared?

- Advantages of **InfraRed Spectrometry** :
  - Fast
  - No destructive method
  - Environmentally friendly

- Near infrared *vs.* mid-infrared (MIR) :
  - **MIR** : high sensitivity to the chemical environment due to the fundamental absorptions of molecular vibrations (Belton, 1997)
  - **NIR** : much more complex structural information related to the vibration behavior of combination bonds (Cen and He, 2006)

MIR Spectrum

1700 – 1500 cm^{-1} : N-H

1200 – 900 cm-1 : C-O

- ***MIR spectrum*** : absorptions of IR at frequencies correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000)
- Typical chemical composition (Smith, 1996)

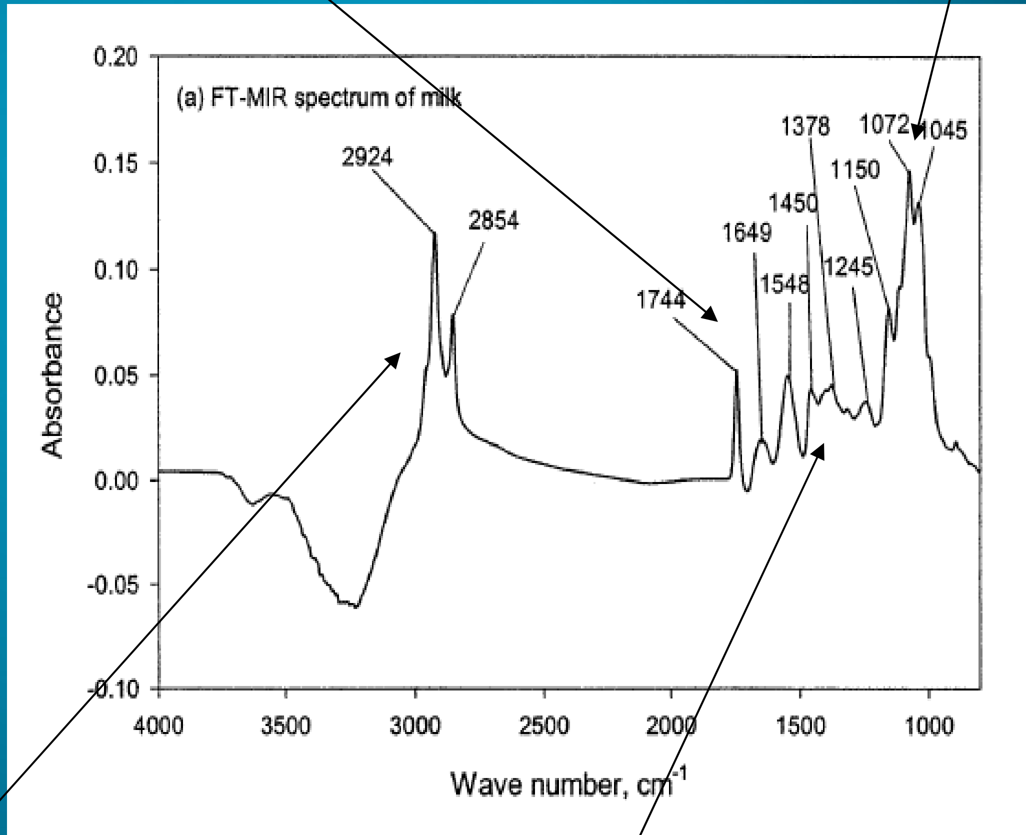

Figure 1 : MIR spectrum of milk (Sivakesava and Irudayaraj, 2002)

3000-2800 cm^{-1}: C-H

1450-1200 cm-1 : COOH

Why mid-infrared?

- Other advantages of **MIR spectrometry**:
  - Largely used by milk labs to quantify the major components of milk
  - Milk samples collected for the milk payment or for the routine milk recording are analyzed by MIR

General aim

- **Aim** : Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context

- ***How ?***
  - Direct use of the results obtained from calibration equations which predict the contents of specific milk components

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Interest

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MIR spectrum

Animal Health

Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone…

Environment

Fatty acids, methane emission through fatty acid contents, urea

Herd Management

Urea, fat, protein, lactose…

Biodiversity

Changes in milk composition

Nutritional Quality of Milk

Fatty acids, lactoferrin, minerals…

Hygienic Quality of Milk

Antibiotics, somatic cells… (less important for milk recording)

Technological Quality of Milk

Traits related to cheese making (casein, dornic acidity, coagulation time...)…

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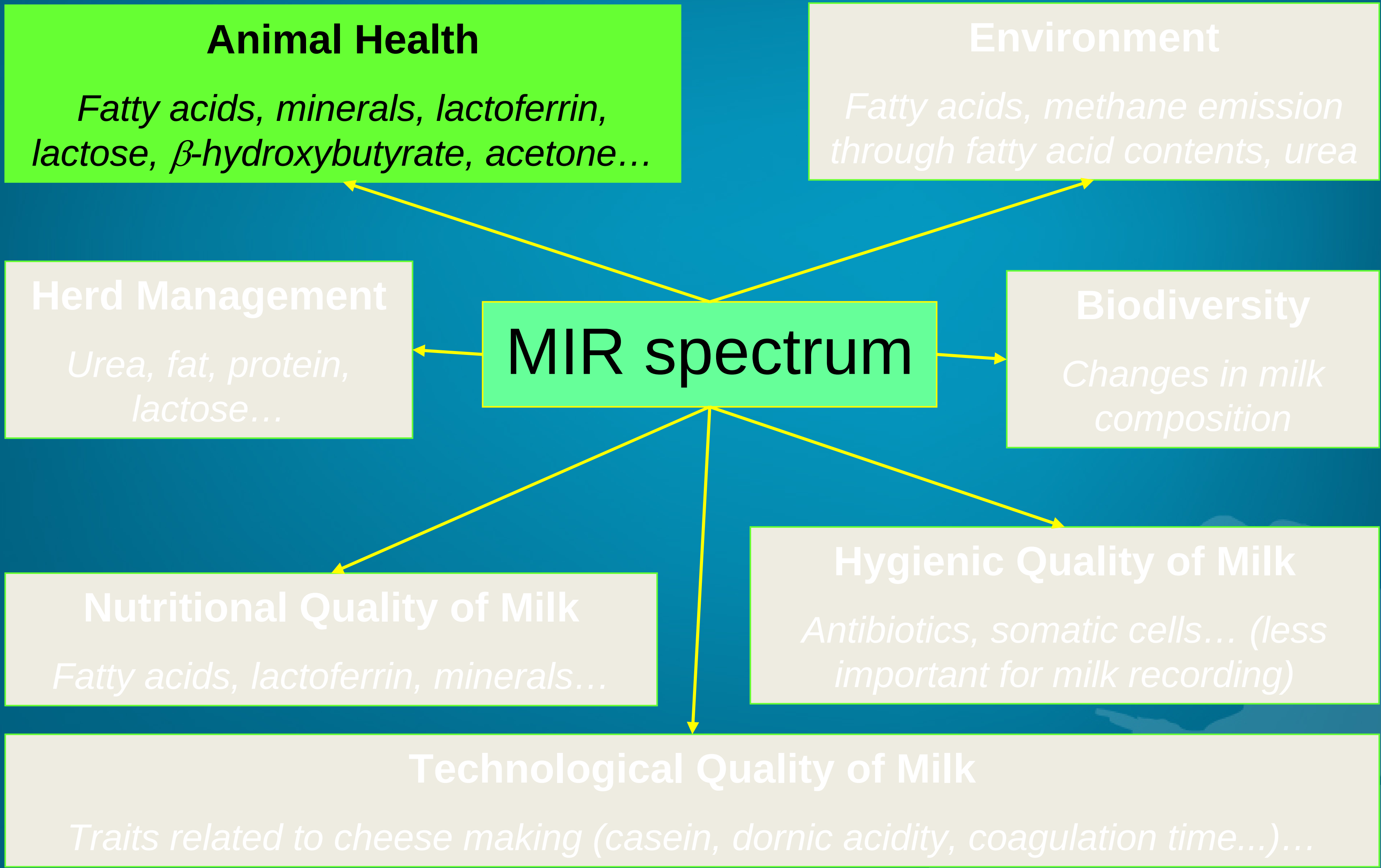

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Common traits

- Fat content
- Protein content
- Urea
- Lactose
- Casein
- Free fatty acids

Fat content, Protein content → Milk payment + milk recording

Urea, Lactose → Milk recording

Casein, Free fatty acids → Few milk recording

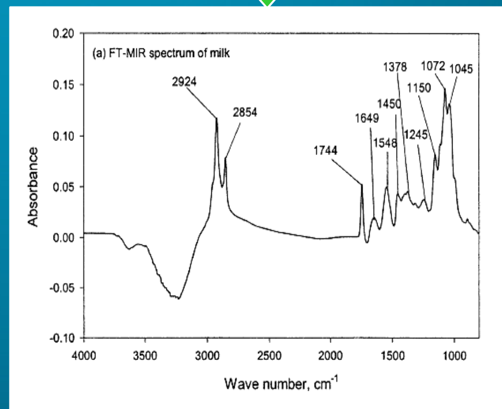
→ Recent studies showed that the MIR spectrometry is currently under-used

Principle

Collection of milk samples

(Foss, 2008)

MIR spectrometer



Raw data = Spectra

Calibration equations

Prediction:

- Fat
- Protein
- Lactose
- …

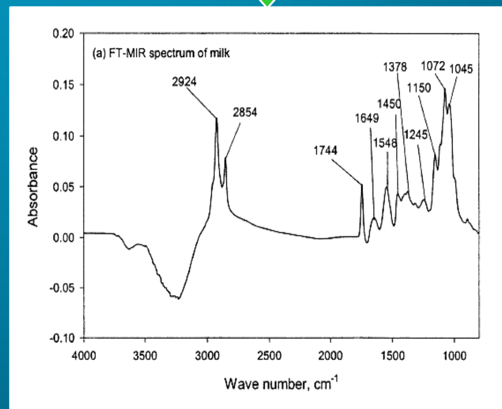
Principle

Collection of milk samples

(Foss, 2008)

MIR spectrometer

Development of new equations



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Few examples…

Fatty acids, minerals, lactoferrin, ketone bodies, cheese-making properties…

Fatty Acids (FA)

- Recent studies confirmed the ability of MIR to predict FA in milk (g/dl of milk):
  - Soyeurt et al. (2006, 2008, 2009), Rutten et al. (2009)
- Lower ability to predict FA content in fat (g/100g of fat)
- New results obtained in the RobustMilk project (www.robustmilk.eu)
  - Multi-breeds, multiple countries and multiple production systems
- All studied FA have a RPD (SD/SECV) greather than 2

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Fatty Acids

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Constituent (g/dl of milk)	N	Mean	SD	RPD	SECV
Saturated FA	496	2.40	0.80	15.7	0.0513
Monounsaturated FA	491	1.06	0.37	8.9	0.0411
Polyunsaturated FA	499	0.16	0.05	2.6	0.0204
Unsaturated FA	492	1.22	0.41	9.6	0.0428
Short chain FA	486	0.31	0.11	6.7	0.0165
Medium chain FA	496	1.78	0.60	6.5	0.0928
Long chain FA	495	1.52	0.57	6.5	0.0875

H. Soyeurt, F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne. 2010. J. Dairy. Sci. Submitted.

This study will be presented in details at ADSA conference in July at Denver (USA)

Minerals

- First results were published by Soyeurt et al., 2009

mg/l de lait	N	Mean	SD	SECV	RPD
Ca	87	1,333	260	95	2.74
K	61	1,336	168	136	1.24
Mg	61	110	18	11	1.68
Na	87	403	107	64	1.68
P	87	1,093	127	50	2.54

- Current study confirmed these results with a larger database (more than 100 samples)

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Lactoferrin

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mg/l de lait	N	Mean	SD	SECV	RPD
Lactoferrin	57	253	206	86	2,39

- Milk glycoprotein involved in the immume system defenses
- Preliminary results published in 2007
- Validation in the RobustMilk project (www.robustmilk.eu) on more than 3,000 data

Ketone Bodies

- Acetone: Hansen (1999) and Heuer et al. (2001)
- De Roos et al. (2007) studied also 2 other ketone bodies (N spectral data > N sample)

mMol	N	Mean	SECV	R^2c
Acetone	1,063	0.146	0.184	0.72
β-hydroxybutyrate	1,069	0.078	0.065	0.62

De Roos et al., 2007

Cheese-Making

		N	Mean	SD	R^2cv	SECV
Titrable acidity (SH°/50ml)	De Marchi et al., 2009	1,063	3.26	0.43	0.66	0.25
Rennet coagulation time (min)	De Marchi et al., 2009	1,049	14.96	3.84	0.62	2.36
	Dal Zotto et al., 2008	74	15.05	3.78	0.73	0.80
pH	De Marchi et al., 2009	1,064	6.69	0.12	0.59	0.07
Titrable acidity (D°)	Colinet et al., 2010(*)	203	16.22	2.01	0.90	0.64
Curd firmness (mm)	Dal Zotto et al., 2008	74	32.43	7.95	0.45	5.49

(*) These results will be presented by Colinet at «New Technologies » session on Friday at 10:50 am

General aim

- **Aim** : Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context

- ***How ?***
  - **Direct use** of the results obtained from calibration equations which predict the contents of specific milk components
  - Integration of these infrared predictions in specific models taken into account the variability of these values in order to extend the number of possible valorizations

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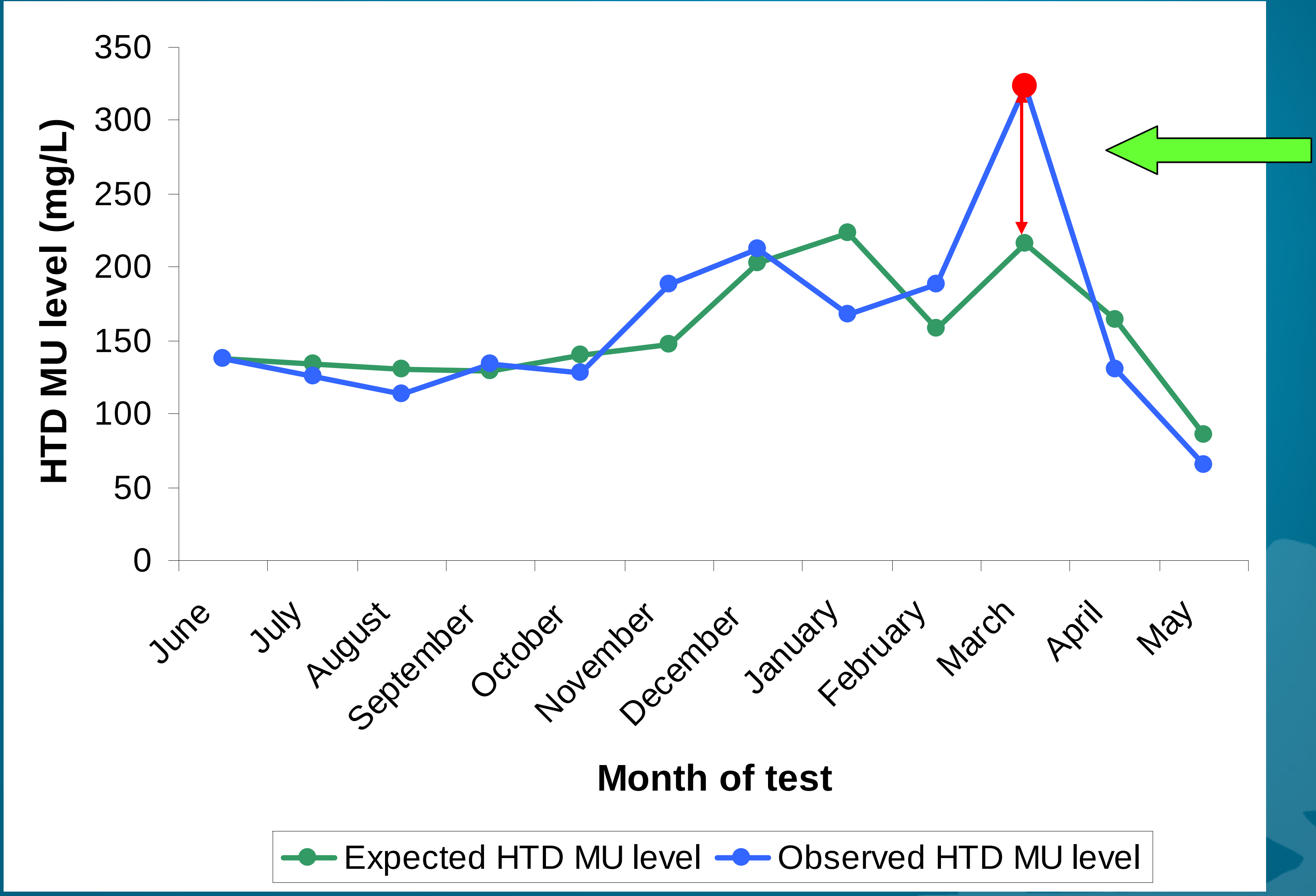
Few examples…

Urea



Bastin et al., 2009

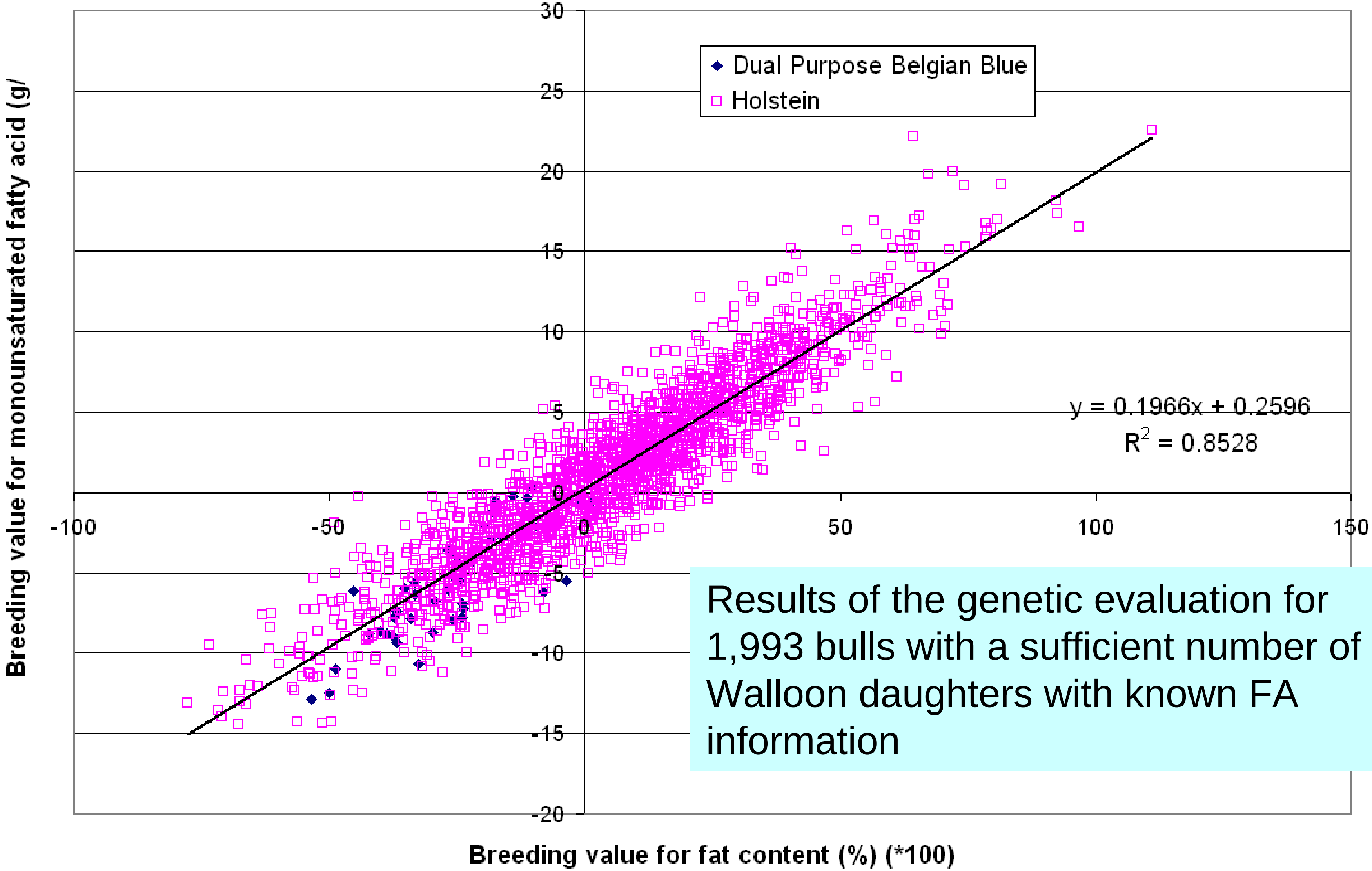
Fatty Acids

- Potential used of FA predictions (*milk labs*):
  - FA predicted from bulk tank milk:
    - Separate scheme of milk collecting
    - Subsidy given in Belgium by a dairy company for milk with higher unsaturated FA
  - FA predicted from individual cows (*Milk recording*)
    - To discard cows
    - Animal selection programs
      - Most interesting bulls and dams
      - Possible internationalization based on relationships among animals

Fatty Acids

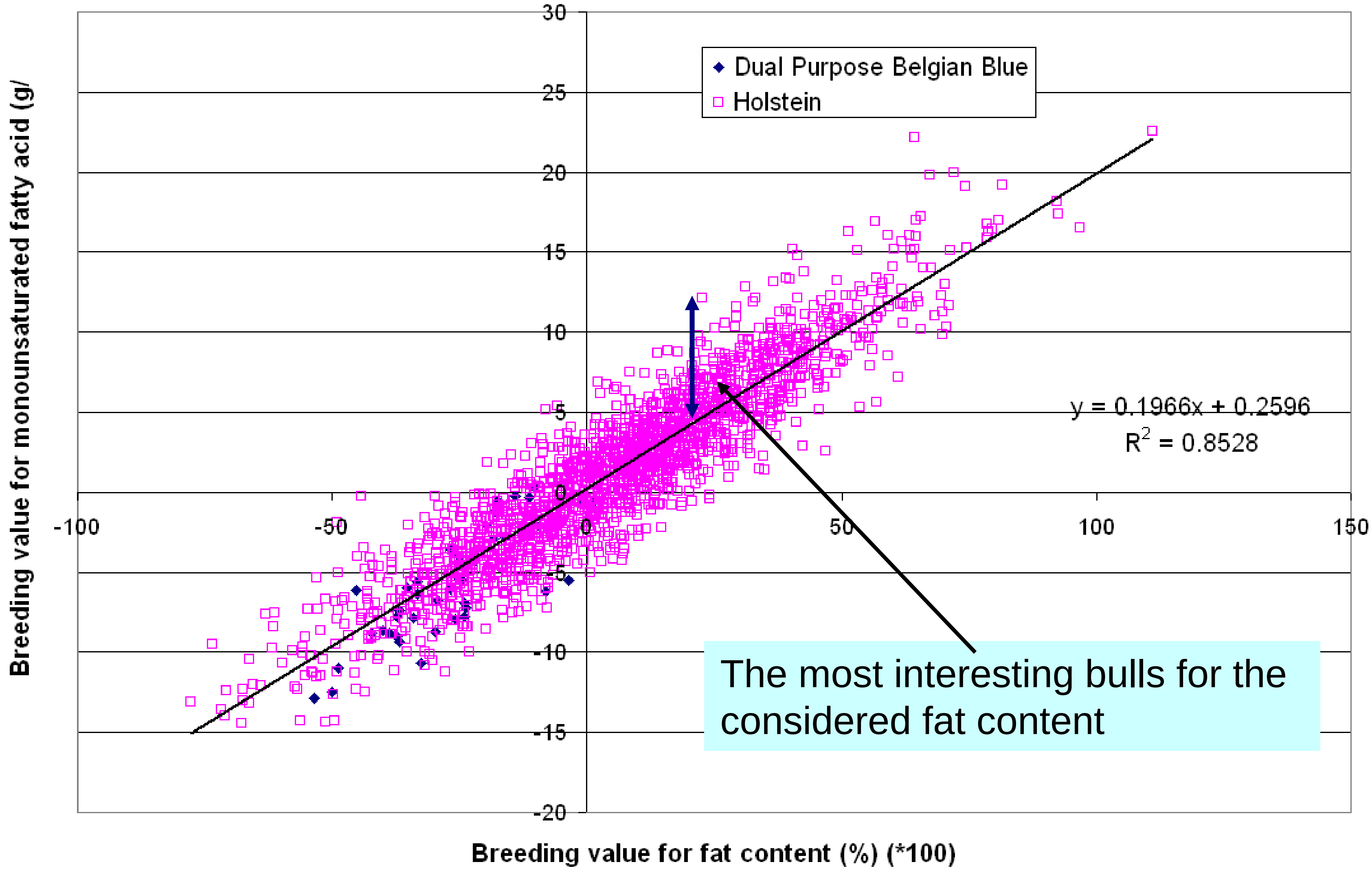
- Animal selection program for FA
  - RobustMilk project (www.robustmilk.eu)
  - Heritable trait with sufficient genetic variability
    - Saturated FA: +/- 44% (more than fat content)
    - Monounsaturated FA: +/- 22%
  - Feasability of genetic selection
    - Genetic evaluation for cows in first lactation
    - Results will be presented at INTERBULL session (Nicolas Gengler on Wednesday at 8:00 am) and at ADSA conference in Denver

Fatty Acids



Results of the genetic evaluation for 1,993 bulls with a sufficient number of Walloon daughters with known FA information

Fatty Acids



Dual Purpose Belgian Blue
Holstein
y = 0.1966x + 0.2596
R2 = 0.8528
The most interesting bulls for the considered fat content
Breeding value for monounsaturated fatty acid (g/
Breeding value for fat content (%) (*100)
30
25
20
15
10
5
0
-5
-10
-15
-20
-100
-50
0
50
100
150

Conclusions

- MIR is currently under used in practice
- New parameters predictable by MIR exist with potential interests for milk recording:
  - Directly MIR predictions
  - Models to offer specific valorizations for dairy industry (farmers, dairy companies, breeding associations…)

But … (analytical challenges)

- The MIR equation used should be validated on the considered cow population:
  - Breed differences can appear
  - Milk from bulk tank is less variable than milk samples collected from individual cows
- Currently, it is possible to implement externally the new equations thanks to the recording of spectra
  - Make sure that the variability of the spectral data used for the prediction was taken into account in the calibration set used to build the calibration equation
- The accuracy of the MIR prediction should be tested regularly by the use of reference samples
  - Since January 2008, MIR FA predictions is implemented in the Walloon milk lab → a maintenance is realized using milk samples with known contents of FA

But … (computational challenges)

- The number of studied traits will increase
  - Some traits are correlated → for the development of specific valorizations for breeders, it will be important to know the relationships among studied traits
    - e.g., fatty acids vs. protein, …
  - The optimum of content for the studied trait can be different following the considered aim
    - e.g., high lactoferrin in milk interesting for human health vs. Milk sample with high content of lactoferrin can be produced by a sick cow → take into account the natural variation of each studied trait

→ multiple traits models → high computational cost

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A lot of work to do …

Collaborators for our researches

- GxABT :
  - Nicolas Gengler - Valérie Arnould – Catherine Bastin - Alain Gillon - Sylvie Vanderick
- CRA-W :
  - Frédéric Dehareng - Pierre Dardenne
- Comité du Lait :
  - Didier Veselko – Emile Piraux
- AWE :
  - Carlo Bertozzi – Laurent Laloux – Xavier Massart

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Thank you for your attention

MIR spectrometer

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