Improvement and validation of mid-infrared predictions of milk fatty acid

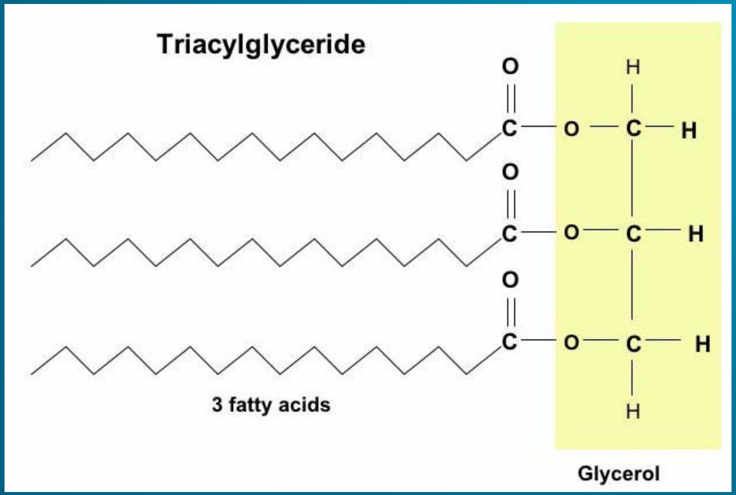
H. Soyeurt[1,2,*], S. McParland[3], D.P. Berry[3], E. Wall[4], N. Gengler[1,2], F. Dehareng[5], and P. Dardenne[5]

[1] University of Liège, Gembloux Agro-Bio Tech - GxABT, Gembloux, Belgium
[2] National Fund for Scientific Research, Brussels, Belgium
[3] Teagasc Moorepark Dairy Production Rsearch Centre, Cork, Ireland
[4] Scottish Agricultural College, Penicuik, UK
[5] Walloon Agricultural Research Center – CRA-W, Gembloux, Belgium

ADSA – 14th July 2010 – Denver - USA

Fatty Acids

- Generally, 2.5 to 7.0% of fat in bovine milk
- 96% of fat is composed by triglycerides



Langara, 2008

- Groups of fatty acids (FA):
  - **Saturated** (SAT): 70%
  - Unsaturated (UNSAT): 30%
    - **Monounsaturated** (MONO): 25%
    - **Polyunsaturated** (POLY): 5%

Measurement

- Gas chromatography:
  - Major advantage: accuracy
  - Major disadvantages:
    - Expensive reagents
    - Time consuming
    - Skilled staff

Measurement

- Gas chromatography:
  - Major advantage: accuracy
  - Major disadvantages:
    - Expensive reagents
    - Time consuming
    - Skilled staff

➔ Find an alternative method

Measurement

- Gas chromatography:
  - Major advantage: reliability
  - Major disadvantages:
    - Expensive reagents
    - Time consuming
    - Skilled staff

- Mid-InfraRed (MIR) spectrometry:
  - Fast analysis (up to 500 samples/hour)
  - Cheap analysis
  - Used in routine milk recording

Collection of samples

Collection of samples

High variability:

- Collected in Belgium, Ireland and Scotland
- Between March 2005 and August 2009
- From several breeds and cows
- Samples from individual cows and for milk payment

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=267)

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=267)

PLS approach was used to estimate the calibration equations

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file:
    - Spectra provided by different spectrometers for the same milk samples

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative applied to the spectra:
    - Correction of baseline drift

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative (DER1)
  - (4) PLS + DER1 + REP

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative (DER1)
  - (4) PLS + DER1 + REP
  - (5) PLS + second derivative (DER2)

Methodology

- 6 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative (DER1)
  - (4) PLS + DER1 + REP
  - (5) PLS + second derivative (DER2)
  - (6) PLS + DER2 + REP

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis — Mid-Infrared spectrum

CALIBRATION SET (N=267)

PLS approach was used to estimate the calibration equations

Internal validation by cross-validation

External validation by adding new samples gembloux agro bio tech

Université de Liège

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=267)

PLS approach was used to estimate the calibration equations

Internal validation by cross-validation

Cross-validation :

- 20 groups

Collection of samples

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis — Mid-Infrared spectrum

CALIBRATION SET (N=267)

PLS approach was used to estimate the calibration equations

<u>250 new samples</u> :

- Collected in Belgium, Ireland and Scotland
- Between April 2008 and August 2009
- From several breeds and cows

External validation on independent new samples

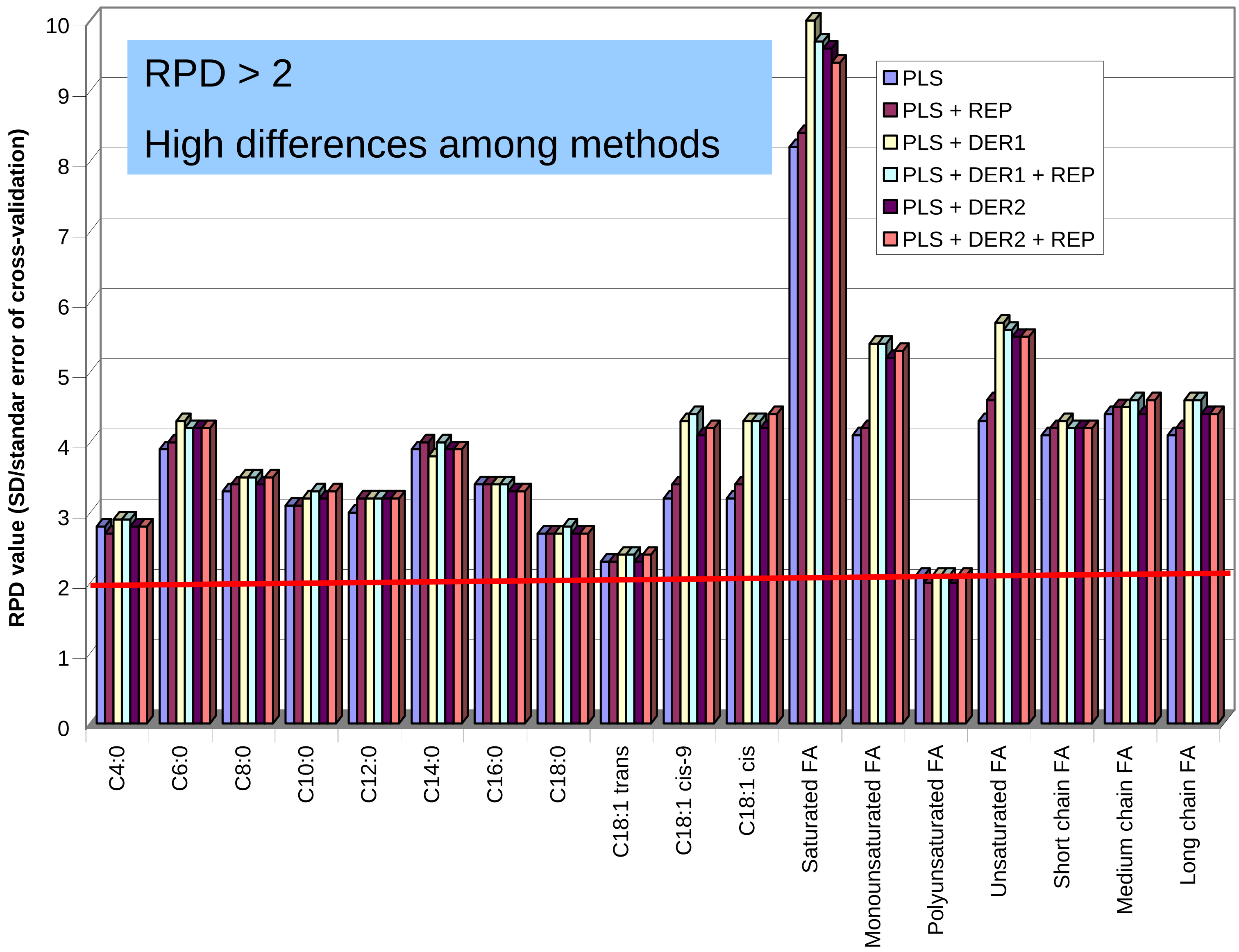
Most Interesting Results

Constituents (g/dl of milk)	Mean	SD	CV
C4:0	0.11	0.03	31.27
C6:0	0.08	0.02	31.93
C8:0	0.05	0.02	34.82
C10:0	0.11	0.04	39.91
C12:0	0.14	0.06	41.17
C14:0	0.45	0.15	32.07
C14:1	0.04	0.02	45.15
C16:0	1.23	0.43	35.02
C16:1 cis	0.07	0.03	45.48
C18:0	0.45	0.20	45.30
C18:1 trans	0.13	0.07	51.95
C18:1 cis-9	0.85	0.34	39.63
C18:1 cis	0.92	0.35	38.30
C18:2	0.09	0.03	32.69
C18:2 cis-9,cis-12	0.06	0.03	39.80
C18:3 cis-9,cis-12,cis-15	0.02	0.01	49.63
C18:2 cis-9,trans-11	0.03	0.02	56.42
Saturated	2.82	0.87	31.02
Monounsaturated	1.20	0.41	34.29
Polyunsaturated	0.18	0.06	32.35
Unsaturated	1.37	0.46	33.16
Short chain (C4-C10)	0.36	0.12	32.00
Medium chain (C12-C16)	2.08	0.67	32.28
Long chain (C17-C22)	1.74	0.63	36.08
Omega-3	0.03	0.02	52.40
Omega-6	0.11	0.03	31.91

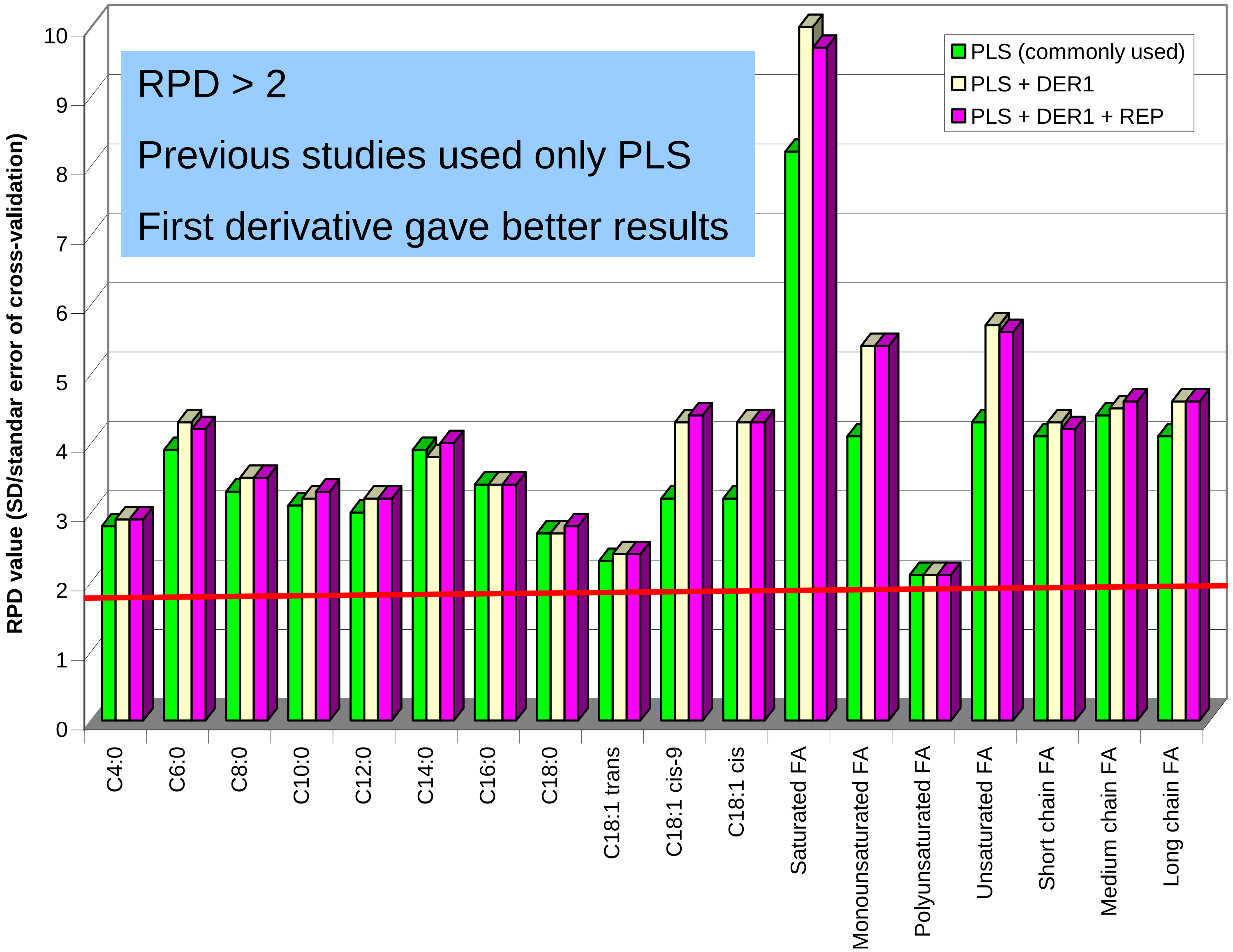
High variability of FA :

Coefficient of variation (CV) (100/mean * SD) ranged from 31.02% to 56.42%.

Most Interesting Results



Most Interesting Results



Most Interesting Results

Constituent (g/dl of milk)	R^2validation (250 new samples)
C4:0	0.83
C6:0	0.88
C8:0	0.90
C10:0	0.90
C12:0	0.90
C14:0	0.91
C16:0	0.86
C18:0	0.74
C18:1 trans	0.84
C18:1 cis-9	0.90
C18:1 cis	0.91
Saturated FA	0.98
Monounsaturated FA	0.96
Polyunsaturated FA	0.82
Unsaturated FA	0.96
Short chain FA	0.91
Medium chain FA	0.92
Long chain FA	0.93

R^2v confirms the ability of MIR to predict some FA directly in bovine milk

Complete dataset

- Validation samples were added to the calibration set (517 samples)
  - 267 calibration samples + 250 validation samples
- Thanks to the good mid-infrared predictions of fatty acids, the critical T test was used to detect abnormal gas chromatographic values
- Thanks to the increase of samples in the dataset the use of repeatability file was less interesting
  - The best method was PLS + DER1

Constituent (g/dl of milk)	N	Mean	SD	SECV	**R^2cv**	**RPD**
C4:0	490	0.10	0.03	0.01	**0.94**	**4.1**
C6:0	492	0.07	0.02	0.00	**0.97**	**5.7**
C8:0	490	0.04	0.02	0.00	**0.97**	**6.1**
C10:0	495	0.10	0.04	0.01	**0.96**	**5.1**
C12:0	495	0.12	0.05	0.01	**0.96**	**5.2**
C14:0	494	0.39	0.13	0.02	**0.97**	**5.4**
C14:1	493	0.04	0.01	0.01	**0.68**	**1.8**
C16:0	494	1.02	0.37	0.08	**0.95**	**4.6**
C16:1 cis	493	0.07	0.02	0.01	**0.71**	**1.9**
C17:0	484	0.03	0.01	0.00	**0.89**	**3.1**
C18:0	492	0.37	0.17	0.05	**0.90**	**3.2**
C18:1 trans	502	0.14	0.07	0.02	**0.88**	**2.9**
C18:1 cis-9	494	0.73	0.28	0.05	**0.97**	**5.9**
C18:1 cis	495	0.79	0.30	0.05	**0.97**	**6.0**
C18:2	503	0.08	0.03	0.01	**0.73**	**1.9**
C18:2 cis9,cis-12	502	0.05	0.02	0.01	**0.74**	**2.0**
C18:3 cis9,cis-12,cis-15	489	0.02	0.01	0.01	**0.71**	**1.8**
C18:2 cis9,trans-11	488	0.04	0.02	0.01	**0.74**	**2.0**
Saturated FA	496	2.40	0.80	0.05	**1.00**	**15.7**
Monounsaturated FA	491	1.06	0.37	0.04	**0.99**	**8.9**
Polyunsaturated FA	499	0.16	0.05	0.02	**0.85**	**2.6**
Unsaturated FA	492	1.22	0.41	0.04	**0.99**	**9.6**
Short chain FA	486	0.31	0.11	0.02	**0.98**	**6.7**
Medium chain FA	496	1.78	0.60	0.09	**0.98**	**6.5**
Long chain FA	495	1.52	0.57	0.09	**0.98**	**6.5**
Branched FA	492	0.09	0.03	0.01	**0.83**	**2.4**
Omega-3	485	0.03	0.01	0.01	**0.75**	**2.0**
Omega-6	504	0.10	0.03	0.02	**0.74**	**2.0**

RPD was globally ≥ 2 for all studied FA

RPD ranged from 1.8 to 15.7

R^2cv ranged from 0.71 to 1.00

Constituent (g/dl of milk)	N	Mean	SD	**SECV**	R^2cv	RPD
C4:0	490	0.10	0.03	**0.01**	0.94	4.1
C6:0	492	0.07	0.02	**0.00**	0.97	5.7
C8:0	490	0.04	0.02	**0.00**	0.97	6.1
C10:0	495	0.10	0.04	**0.01**	0.96	5.1
C12:0	495	0.12	0.05	**0.01**	0.96	5.2
C14:0	494	0.39	0.13	**0.02**	0.97	5.4
C14:1	493	0.04	0.01	**0.01**	0.68	1.8
C16:0	494	1.02	0.37	**0.08**	0.95	4.6
C16:1 cis	493	0.07	0.02	**0.01**	0.71	1.9
C17:0	484	0.03	0.01	**0.00**	0.89	3.1
C18:0	492	0.37	0.17	**0.05**	0.90	3.2
C18:1 trans	502	0.14	0.07	**0.02**	0.88	2.9
C18:1 cis-9	494	0.73	0.28	**0.05**	0.97	5.9
C18:1 cis	495	0.79	0.30	**0.05**	0.97	6.0
C18:2	503	0.08	0.03	**0.01**	0.73	1.9
C18:2 cis9,cis-12	502	0.05	0.02	**0.01**	0.74	2.0
C18:3 cis9,cis-12,cis-15	489	0.02	0.01	**0.01**	0.71	1.8
C18:2 cis9,trans-11	488	0.04	0.02	**0.01**	0.74	2.0
Saturated FA	496	2.40	0.80	**0.05**	1.00	15.7
Monounsaturated FA	491	1.06	0.37	**0.04**	0.99	8.9
Polyunsaturated FA	499	0.16	0.05	**0.02**	0.85	2.6
Unsaturated FA	492	1.22	0.41	**0.04**	0.99	9.6
Short chain FA	486	0.31	0.11	**0.02**	0.98	6.7
Medium chain FA	496	1.78	0.60	**0.09**	0.98	6.5
Long chain FA	495	1.52	0.57	**0.09**	0.98	6.5
Branched FA	492	0.09	0.03	**0.01**	0.83	2.4
Omega-3	485	0.03	0.01	**0.01**	0.75	2.0
Omega-6	504	0.10	0.03	**0.02**	0.74	2.0

Conclusion

- MIR can be used to quantify FA directly on milk
- Previous studies used only PLS to develop calibration equations → the obtained results showed the advantage of using a method which combines PLS and the first derivative applied to the spectral data.

Interest

- Implementation of these equations directly in milk lab
  - Useful for dairy industries to develop **dairy products with differentiated nutritional quality**
    - Since 2008, the MIR predictions of FA are implemented in our Walloon milk lab
    - Used by one dairy company to give subsidies to the farmers who produce more unsaturated FA in milk
  - Milk recording organisations: **improvement of FA profile**
    - Management tools: feeding…
    - Selection tools: quantitative genetics, molecular genetics

Acknowledgement

- Walloon Breeding Association (AWE) and Milk Committee of Battice
- National Fund for Scientific Research (FNRS): 2.4.623.08.F
- Ministry of Agriculture of the Walloon Region of Belgium (projects D31-1207 and D31-1224/S1)
- European Commission, Directorate-General for Agriculture and Rural Development, under Grant Agreement 211708 (project Robustmilk).

This study has been carried out with financial support from the Commission of the European Communities, FP7, KBBE-2007-1. It does not necessarily reflect its view and in no way anticipates the Commission's future policy in this area.

www.robustmilk.eu