Milk biomarkers to detect ketosis and negative energy balance using MIR spectrometry

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Negative energy balance and ketosis

In early lactation:
energy intake < energy output

Negative energy balance

↘ fertility
↘ health
(Collard et al., 2000; Butler, 2003)

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If excessive
⇨ imbalance in hepatic carbohydrate and fat metabolism
⇨ ↗ of ketone bodies in blood

= Ketosis type I

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(Collard et al., 2000; Butler, 2003)

Prevalence : 7 to 43% (Suthar et al., 2013)

↘ milk yield
↘ reproductive performances
↗displaced abomasum
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(Duffield, 2000)

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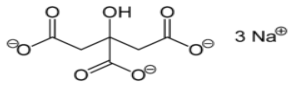
BHB and Acetone known as biomarkers

(Enjalbert et al., 2001)

Citrate ?

- **Krebs cycle molecule**
- **Present in milk**



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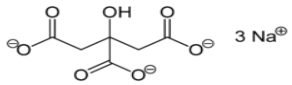

J. Dairy Sci. 95:2362–2380
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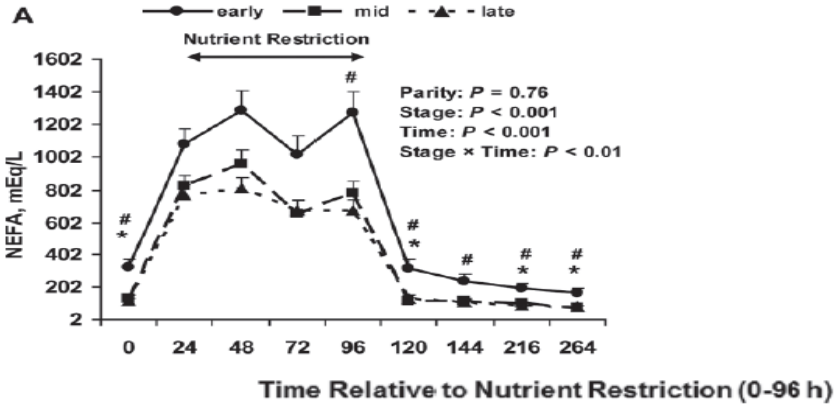
Induced nutrient restriction

NEFAs in blood



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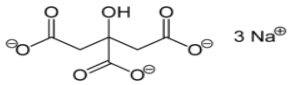

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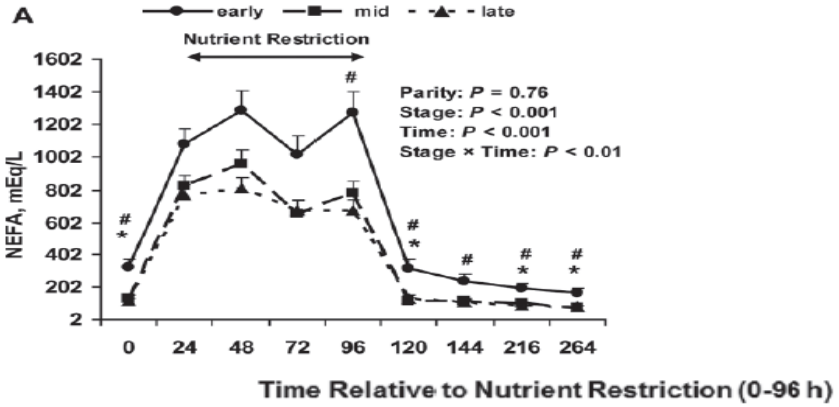
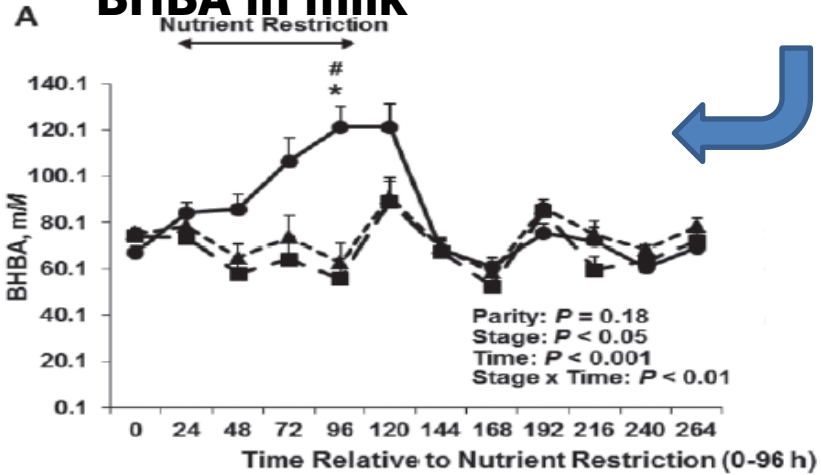
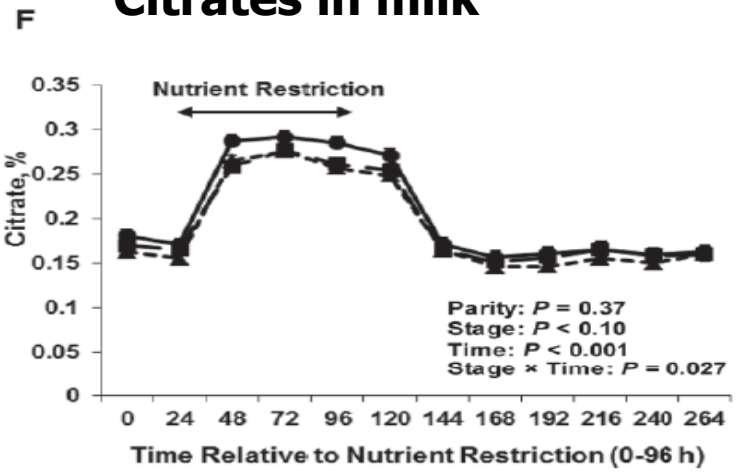


BHBA in milk



Citrates in milk



Citrate ?

- **Bjerre-Harpoth (2012)**

  « …greatest increase (58%) during restriction for all cows »

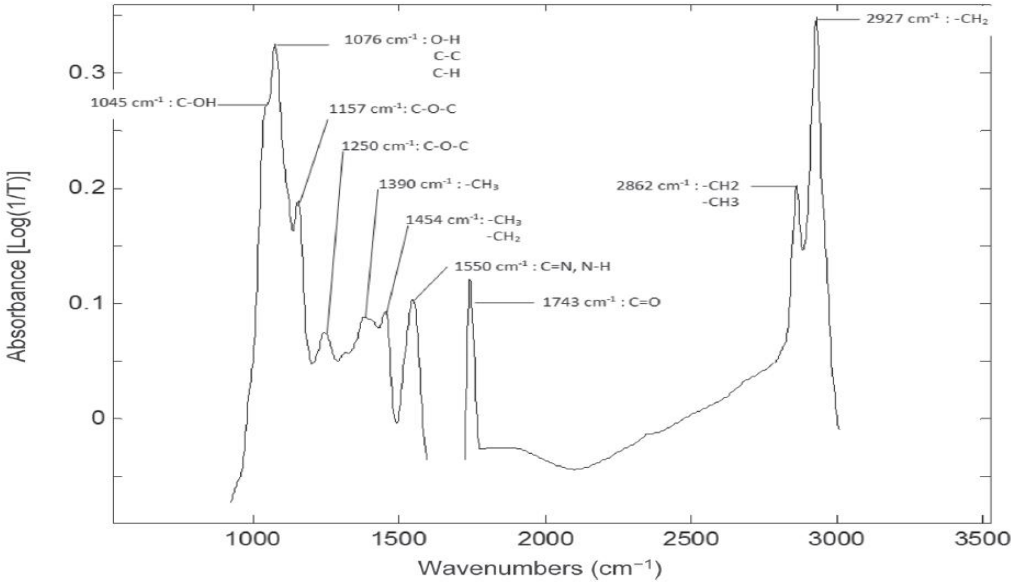
  « …promising <u>early</u> indicator of physiological imbalance »

- **Baticz et al. (2002)**

  « Sodium citrate should be measured by easy and automated method such as FT-MIR technology to evaluate the energy status of cows »

Mid Infra Red (MIR)

- MIR spectrum reflect milk composition
- World-wide used for milk recording, payment
- Fast, cheap
- 1 sample → X predicted values
  - Fatty acids
  - Minerals
  - Methane
  - Cows state
  - Technical properties
  - …
- Limit of detection : 100 ppm (Dardenne, 2015)



Previous studies in link with MIR

Acetone: ketosis biomarker

			Calibration			Cross validation			Validation		
		Reference method	N	RMSE	R^2	SECV	RMSE	R^2	N	RMSE	R^2
Hansen	1999	Vanilin test	302	-	-	-	0.240	0.80	58	0.270	0.81
Heuer	2001	Gas chromatography	180	-	-	0.210	-	-	-	-	-
De Roos	2007	Continuous flow analyer	1063	-	-	0.184	-	0.72	-	-	-
Hanus	2011	Microdiffusion photometric	14	-	0.65	-	-	-	-	-	-
Hanus	2014	Microdiffusion photometric	89	-	0.39	-	-	-	-	-	-

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Citrate: energy status of cow/physiological imbalance

- **Not very well documented, no target values or thresholds in the litterature**
- **No published MIR calibration (existing FOSS calibration)**

Goals of the study

In early lactation:
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Body fat mobilisation

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(2) Evaluate possibility to predict citrate via MIR

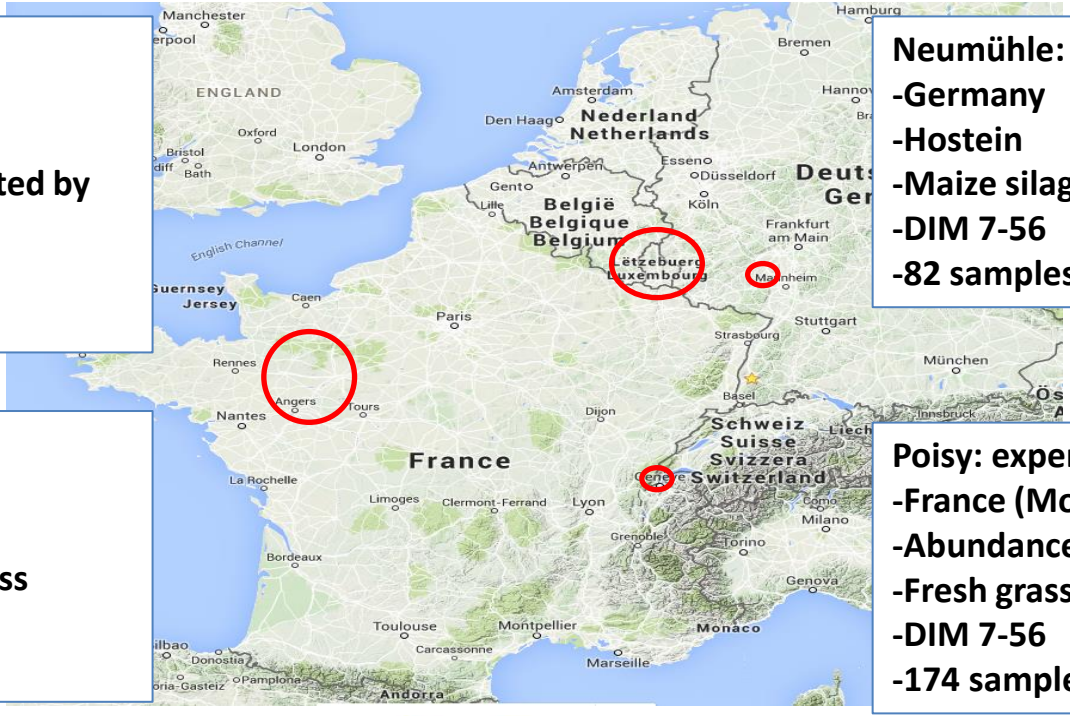
(3) Use samples and spectra from severals countries

→ robust equations

(1) Realize Optimir own MIR calibrations for BHB and acetone, with validation step

Collect of samples

Convis: MRO
-Luxembourg
-Hostein
-Maize silage supplemented by grazing in summer
-DIM 5-60
-110 samples



Neumühle: experimental farm
-Germany
-Hostein
-Maize silage
-DIM 7-56
-82 samples

CLASEL: MRO
-France
-Hostein and Normande
-Maize silage or fresh grass
-DIM 7-305
-200 samples

Poisy: experimental farm
-France (Montain area)
-Abundance and Montbéliarde
-Fresh grass or hay and maize silage
-DIM 7-56
-174 samples

- Harmonized protocol by IDELE
- ICAR approved sampling systems
- Morning and evening samples pooled
- 566 * 2 identical samples generated → MIR and chemical analysis

Analysis of samples

- Chemical analysis at CRA-W (Belgium)
- Continuous flow analyzer (Skalar, The Netherlands)
- Enzymatic/chemical reactions

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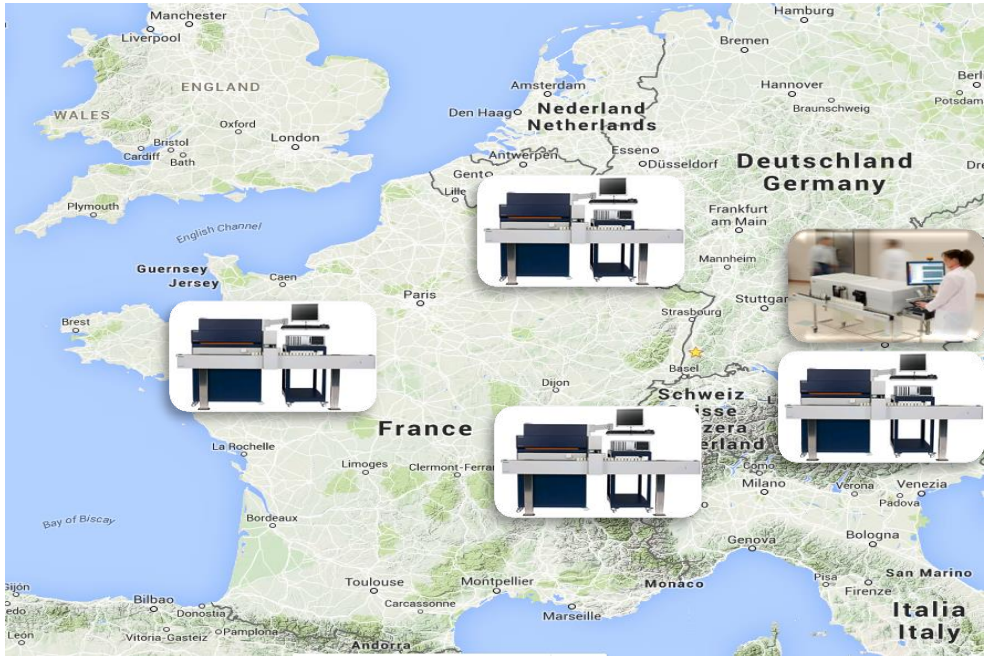
- Spectral analysis locally
- Foss and Bentley
- Standardization of spectra enabling a common database and a common use



Results of chemical analysis

- **566 samples in total**
- **Removing of missing values**
- **Same ranges than litterature** (Denis-Robichaud et al., 2014; Garnsworthy et al., 2006)

Component	Unit	N	Min	Max	**Mean**	SD	SEL
BHB	mmol/L	558	0.045	1.596	**0.215**	0.174	0.005
Acetone	mmol/L	548	0.02	3.355	**0.103**	0.26	0.006
Socium citrate	mmol/L	506	3.88	16.12	**9.04**	2.21	0.216

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- **Limit of detection with MIR: 100 ppm**

	Concentration (mmol/L)	Molar mass (g/mol)	Concentration (ppm)	
BHB	0.215	104.10	21.7	→ Indirect prediction
Acetone	0.103	58.08	5.8	→ Indirect prediction
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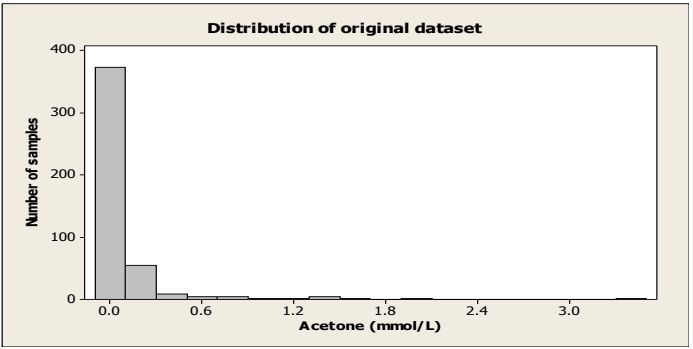
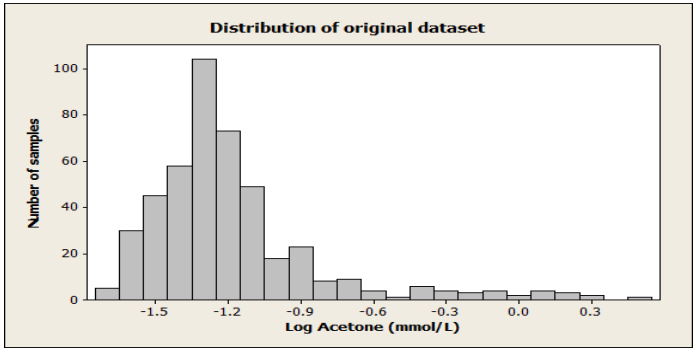
- **Unbalanced distribution for BHB and Acetone**

  → Use of Log (10) transformation





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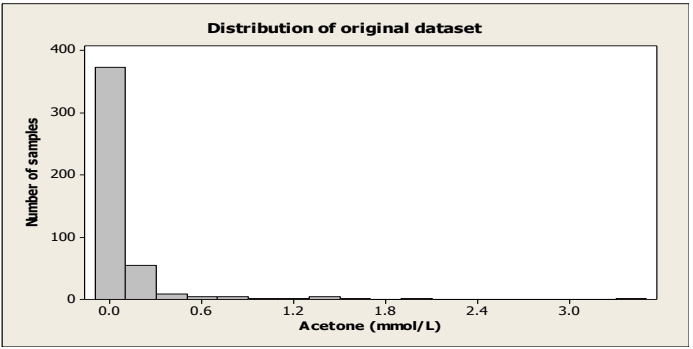

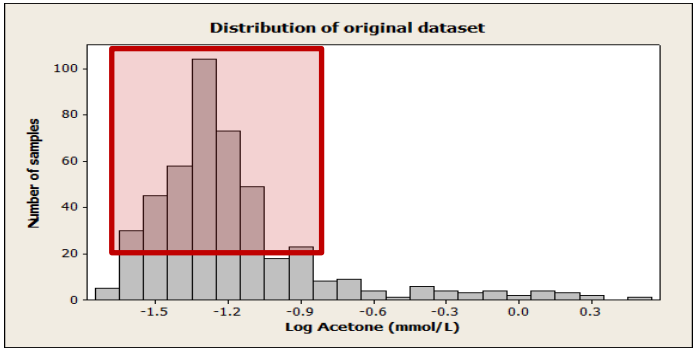

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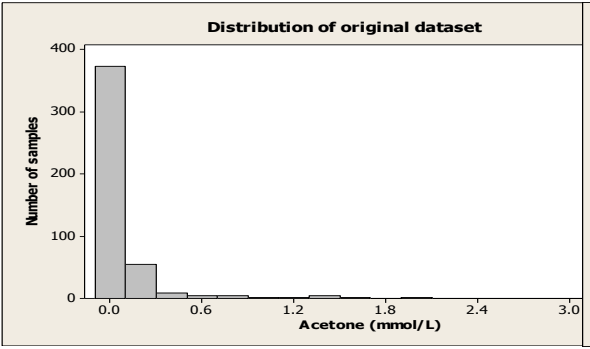

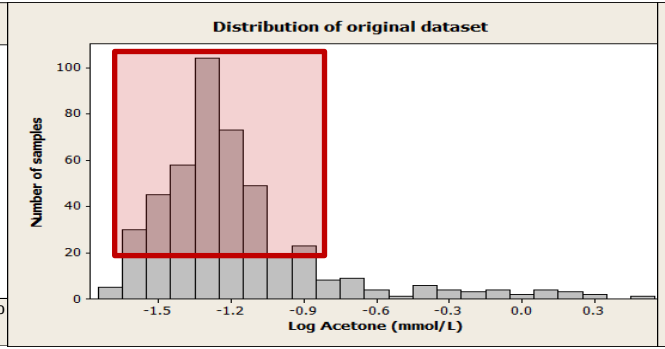

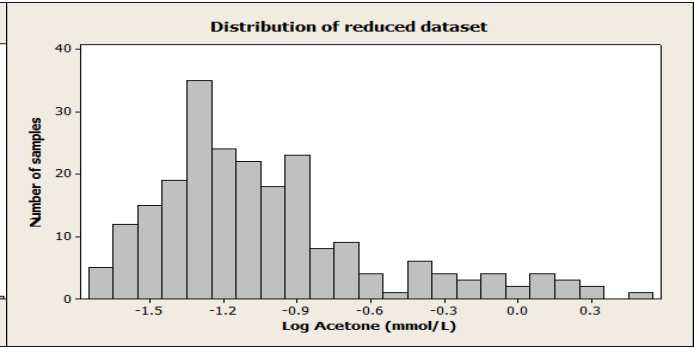

558→ 433 samples for BHB

548 → 224 samples for acetone

MIR calibrations

- **Spectral pretreatment:**

  Absorbance, Standardized, First derivative gap 5, Autoscale

  Area used : 968.1 - 1577.5, 1731.8 - 1762.6, 1781.9 - 1808.9 and 2831.0 - 2966.0 cm^{-1}

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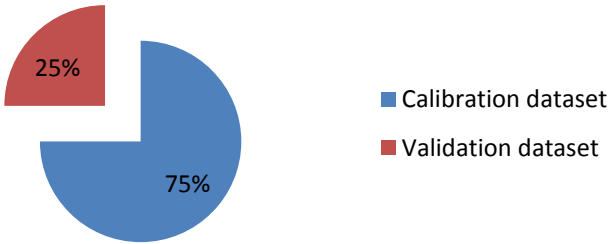
- **Partial Least Square (PLS) regression**
- **Cross-validation using 10 subsets**
- **Validation ¾ - ¼**

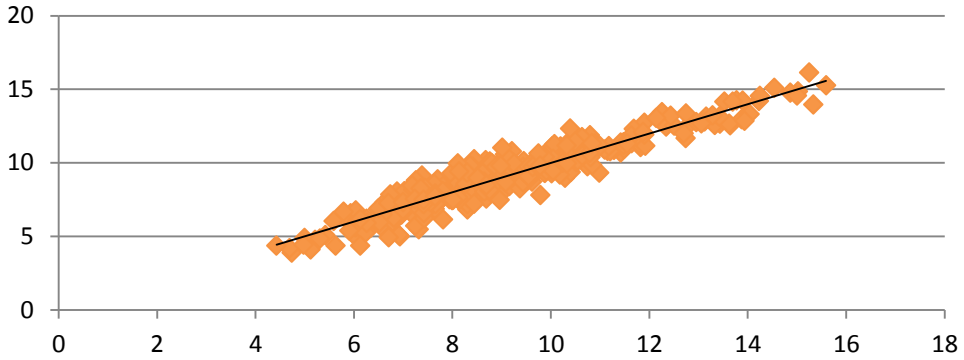


- **Use of Matlab and the PLS toolbox**

MIR calibrations

- **Criteria observed**
  - **R^2 (but dependent of the range)**
  - **RMSE (Root Mean Square Error)**

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} |\varepsilon_i|^2}{n}}$$



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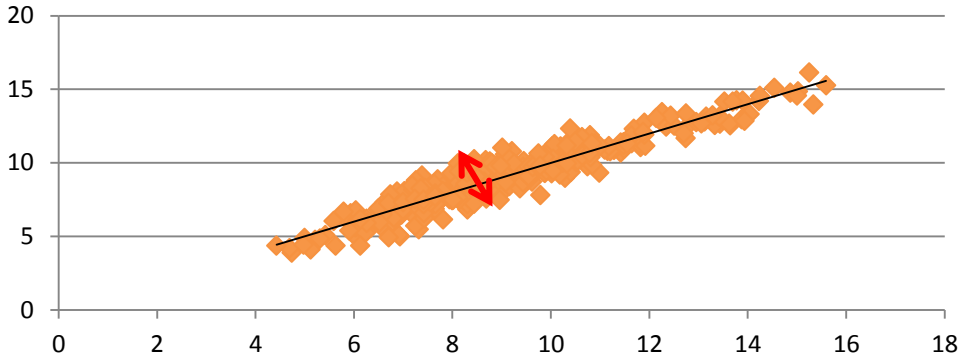

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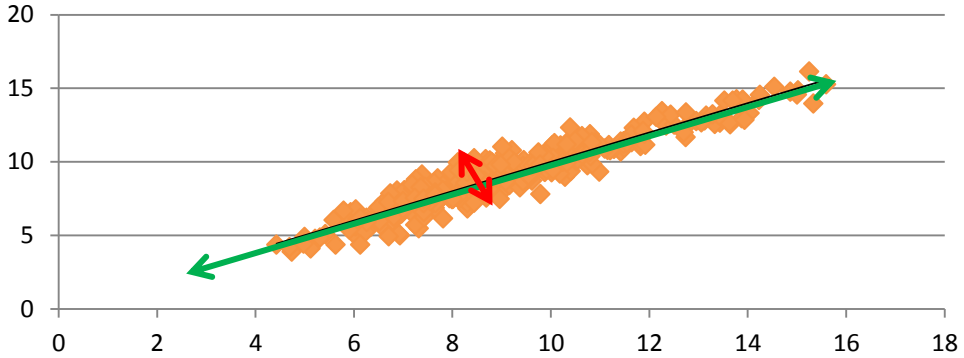

  - **RPD = SD (calibration) / RMSE**

RPD		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	+++

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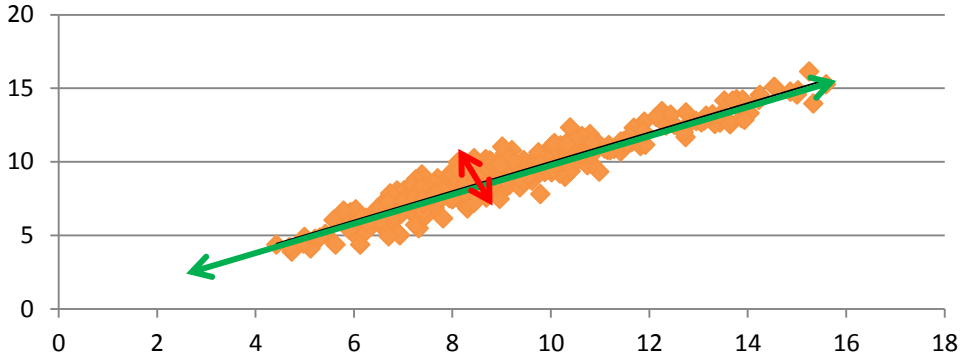

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- **Classification**
  - **0.20 mmol/L for BHB**
  - **0.15 mmol/L for acetone**

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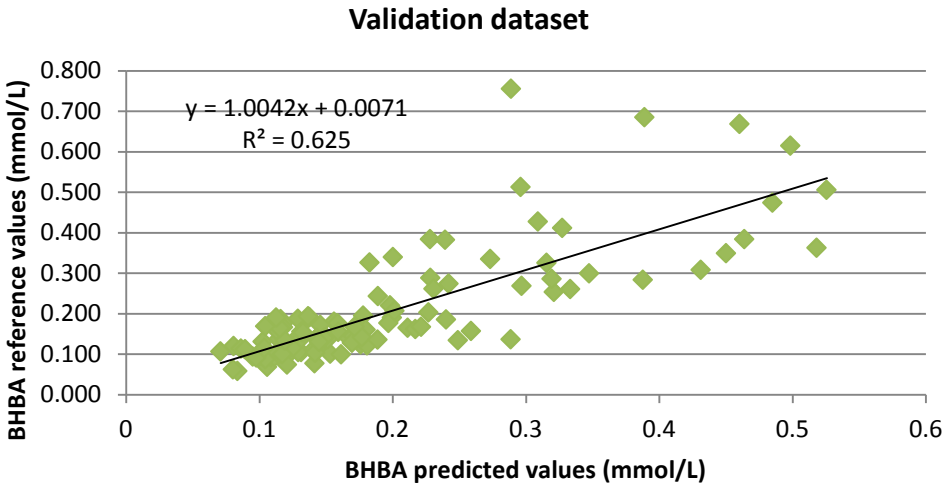
Results – BHB

- **Statistics**

Item	N	No. of LV	No. of Outliers	Min	Max	Mean	SD	RMSE	R^2	RPD
BHB (mmol/L)										
Cross-validation	325	8	7	0.045	1.596	0.235	0.193	0.109	0.71	1.77
Validation	108	-	-	0.058	0.755	0.204	0.136	0.083	0.63	2.36



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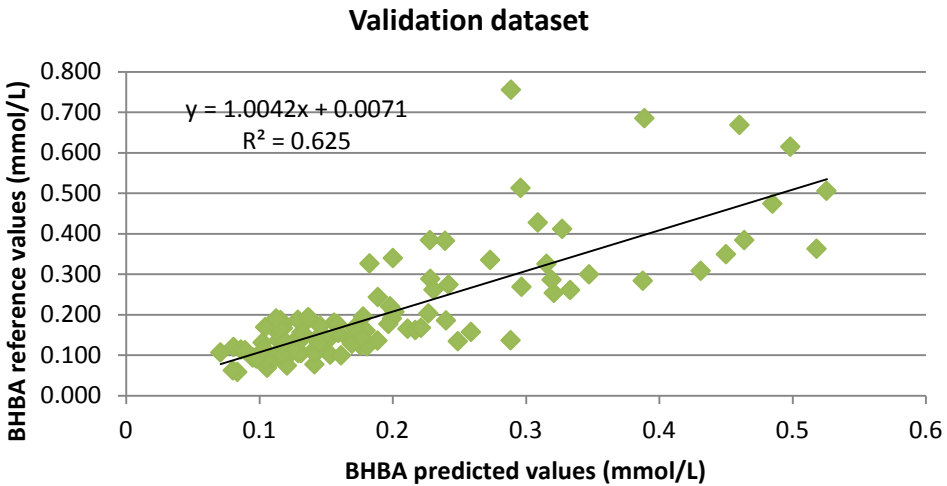

Allows discriminate high or low levels

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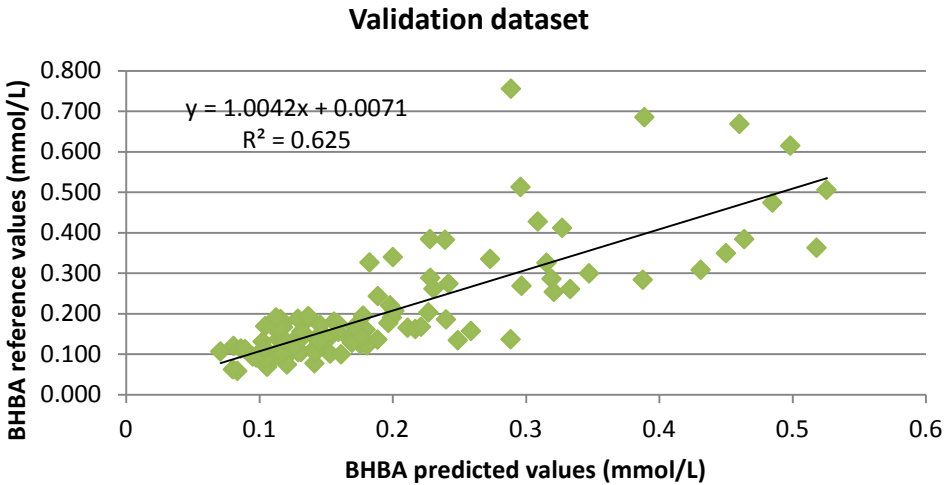


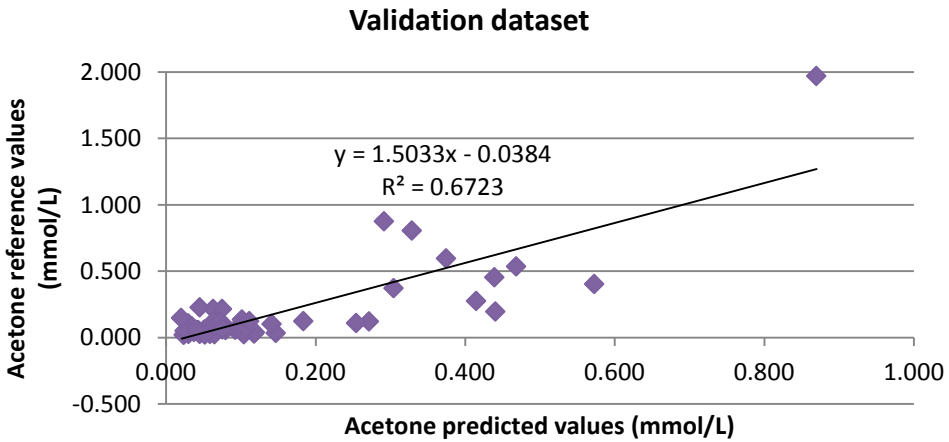
	Low BHB content (<0.200mmol/l)	High BHB content (>0.200mmol/l)	Global good classification
Validation	n=77	n=32	
Predicted low	90.90%	9.40%	90.80%
Predicted high	9.10%	90.60%	

Allows discriminate high or low levels

Results – Acetone

- **Statistics**

Item	N	No. of LV	No. of Outliers	Min	Max	Mean	SD	RMSE	R^2	RPD
Acetone (mmol/L)										
Cross-validation	168	7	2	0.02	3.355	0.19	0.397	0.248	0.73	1.6
Validation	56	-	-	0.021	1.968	0.179	0.306	0.196	0.67	2.03



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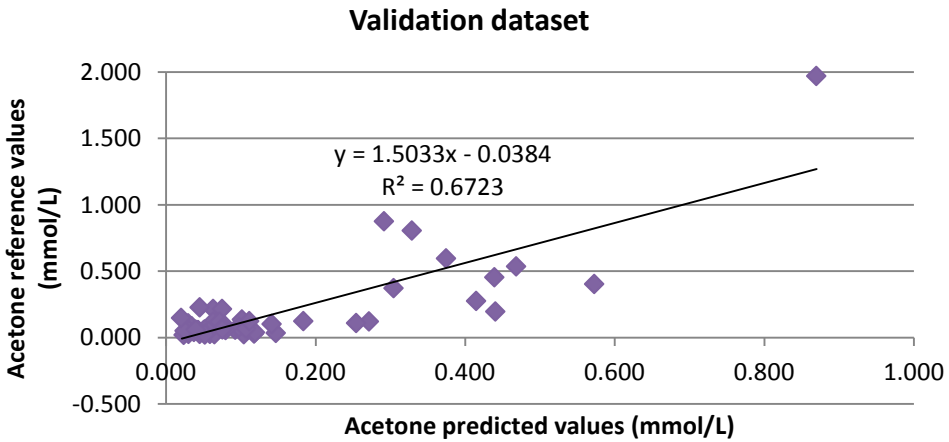

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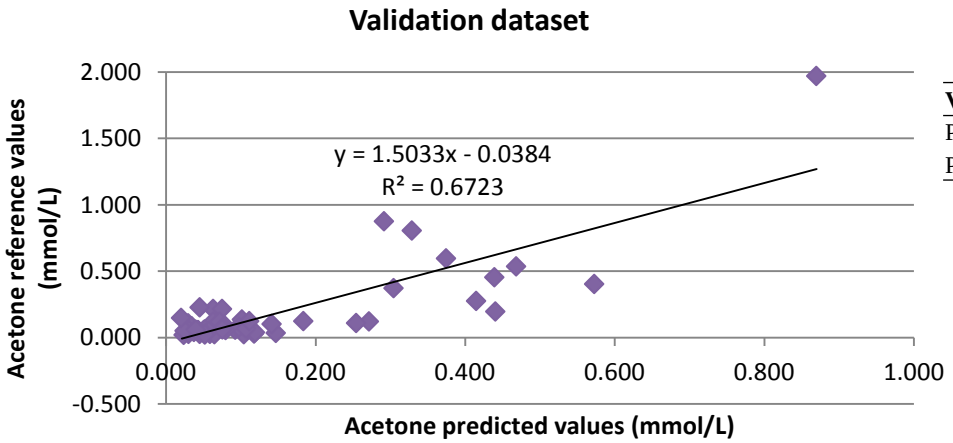


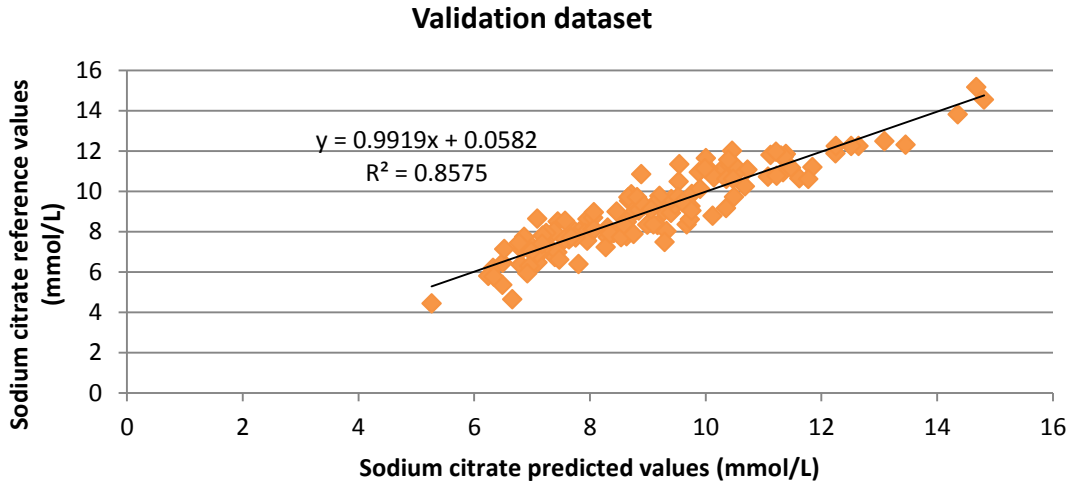
	Low acetone content (<0.150mmol/l)	High acetone content (>0.150mmol/l)	Global good classification
Validation	n=43	n=13	
Predicted low	93.00%	23.10%	89.30%
Predicted high	7.00%	76.90%	

Allows discriminate high or low levels

Results – Citrate

- **Statistics**

Item	N	No. of LV	No. of Outliers	Min	Max	Mean	SD	RMSE	R^2	RPD
Sodium citrate (mmol/L)										
Cross-validation	380	9	2	3.88	16.12	9.03	2.26	0.7	0.9	3.21
Validation	126	-	-	4.44	15.16	9.08	2.03	0.76	0.86	2.96



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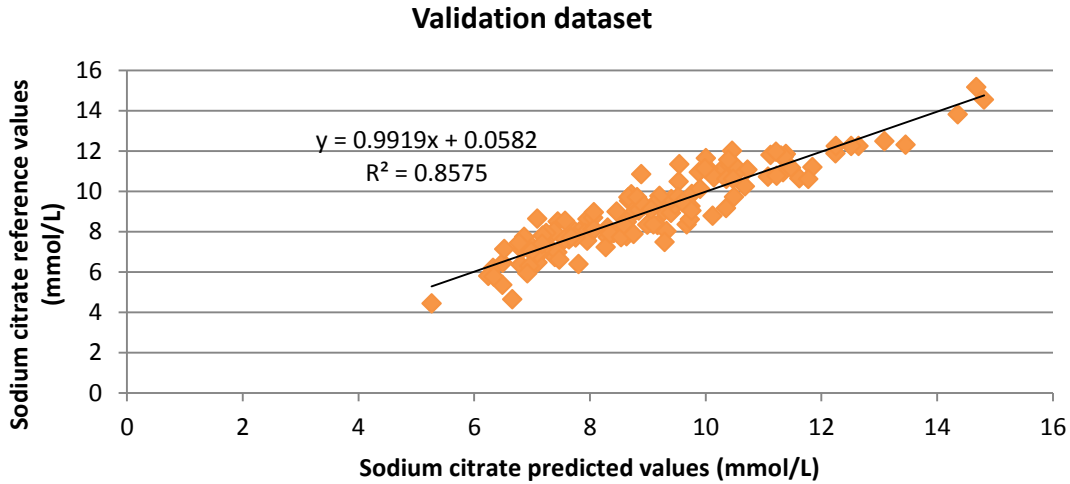
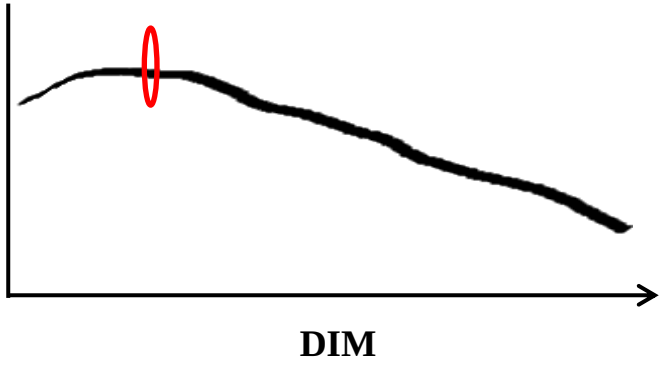


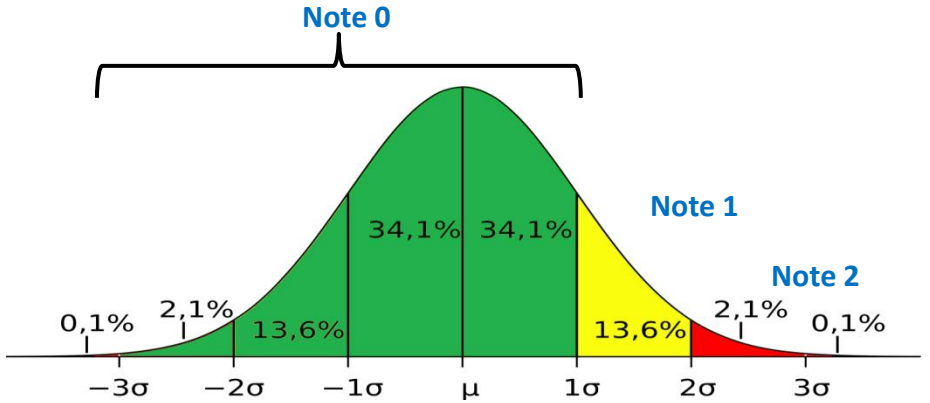
Allows screening, quantitative information

Exemple of use by MROs (Baugnies, 2015)

- Walloon breeding association (AWE) tool
- BHB, acetone, citrate, C18:1 cis 9
- Relative approach
- Cow value compared to population values at same DIM



- Score 0,1 or 2 for each component



Exemple of use by MROs (Baugnies, 2015)

- **Global score from 0 to 8 as a global approach of metabolic disorders**

Exploitation	DATE CTRL	n° animal	n° lactation	JEL	Production (dl)	Cellules (*1000/mL)	Urée (mg/L)	Rapport TB/TP	Indice BHB	Indice acétone	Indice citrate	Indice c18:1cis9	Indice GLOBAL
A	17/02/2014	15146978	1	15	294	760	30	1.67	2	1	1	1	5
A	15/04/2014	14876705	2	59	376	400	179	1.36	0	2	0	1	3
A	18/11/2014	15012953	2	69	237	280	179	0.46					
A	16/12/2014	13904979	4	7	167	560	350	2.54	2	2	0	2	6
B	26/02/2014	15676607	1	115	275	10	290	1.12	2	0	2	1	5
B	23/05/2014	14022741	3	268	128	360	170	0.93	1	2	0	1	4
B	4/11/2014	14921815	2	212	203	60	310	1.39	2	0	2	0	4
C	9/08/2012	15180867	1	387	152	120	350	1.21	1	0	1	1	3
C	8/11/2012	15180793	5	11	258	300	50	1.59	2	2	1	2	7
C	6/04/2013	15180840	4	12	110	40	240	1.86	1	1	0	2	4
C	6/10/2013	14090385	3	14	226	560	170	1.75	1	1	1	2	5

- **Complex interpretation (ketosis, fat mobilization, fattening, feed effect, mastitis…)**
- **Preliminary tests in 4 farms**
- **Good feedback from breeders**
- **→ Cows to follow**

Conclusions/Implications

- Calibrations for BHB and acetone → distinctions between high and low levels
- Citrate by MIR → good accuracy
- Standardisation of spectra: usable by all Optimir MROs

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- Calibrations for BHB and acetone → distinctions between high and low levels
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- USE ON FIELD
  - Complex interpretation
  - Different way to use it by MROs
  - Interest from breeders
  - Already used in France and Luxembourg
  - Tests in Germany, Belgium

Thank you for your attention

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