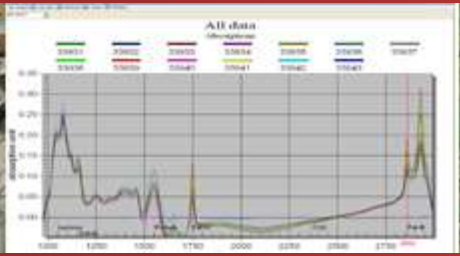


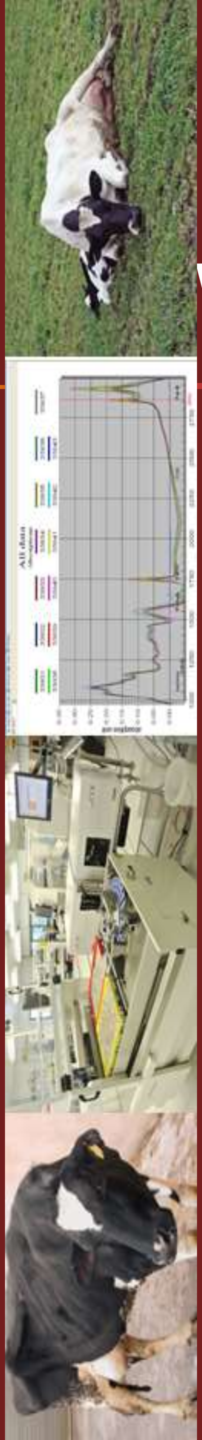


Ketosis detection in DHIA testing

A new global metabolic infrared spectral approach

Pierre Broutin,
Managing Director/Senior Scientist

ICAR 2016 Congress
Puerto Varas, October 25, 2016

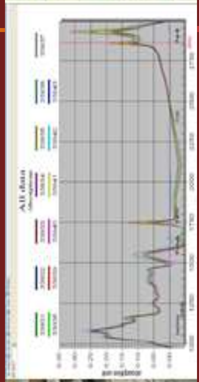


Why testing for ketosis?

In high producing dairy cows during early lactation, a negative energy balance (NEB) can be induced by an imbalance between energy intake and increasing demand for milk production leading to:

- Fat mobilization from adipose tissue
- Production of ketone bodies (acetone, β-hydroxybutyrate…).
- Sub-clinical(SCK) or clinical ketosis (CK)

Ketosis metabolic disorder has a high prevalence (12-43%), is difficult to detect (1/5), and can lead to major negative impacts on dairy farms profitability and animal welfare:



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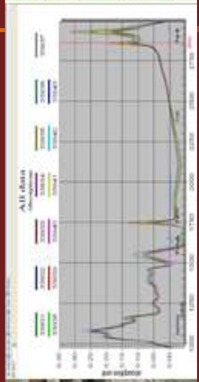
- Decrease in milk production (-300 to 450 kg/lactation) (Duffield,2000)
- Substantial modification of milk composition (fat↑, protein↓, fatty acids profile…)
- Impaired reproduction with conception rate at AI1 down -3 to 35% (Fourichon et al,2000)
- Increased risk for other metabolic disorder (clinical ketosis, displaced abomasum, metritis…)
- Increased severity and duration of mastitis cases with depletion of the immune system
- Indirectly increases culling rate

Cost per case 250-600 EUR depending on conditions

How to diagnose subclinical ketosis (SCK)?
2 types of ketosis and associated biomarkers:

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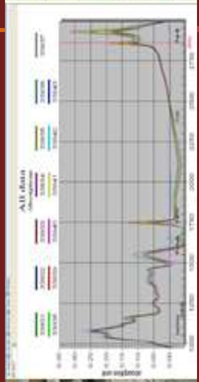
Ketosis type	biomarker	Concentration range	Status
Hypoglycemic	high [BHB] in blood*	Blood BHB > 1,2 mmol/l	SCK
Hyperglycemic	high [NEFA] in blood**	< 0,4 mmol/l	physiological
		0,4-0,6 mmol/l	mild mobilization
		> 0,6 mmol/l	severe fat mobilization

But Blood BHB (Gold Standard) & NEFA determinations are invasive and not practical for routine DHIA testing

** BLOOD BHB = GOLD STANDARD*
*** NEFA: Non Esterified Fatty Acids*



Are Ketosis biomarkers detectable in milk by infrared?

- **Milk Acetone:** very volatile and low concentration in milk
  typically < IR detection limit

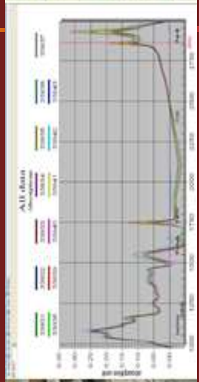
- **Milk BHB:** more stable than acetone but levels also very low
  typically < IR detection limit

Milk BHB (0.10 – 0,60 mmol/L) = (1,04–6,24 mg/dl!!) << urea (10-100 mg/dl)

- **Milk BHB** content decreases also rapidly if milk not stored at 4°C right after milking

- Correlation (r) between milk and blood BHB very variable (0,00-0,87) (*Enjalbert et al. 2001)*

Cc: Direct <u>Milk</u> Acetone & BHB determination by IR present some major drawbacks because of their volatility, very low concentration levels, and very variable correlation with blood BHB (Gold Standard)

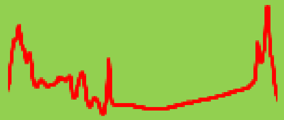


A new global metabolic infrared spectral approach for Blood BHB prediction and SKC detection

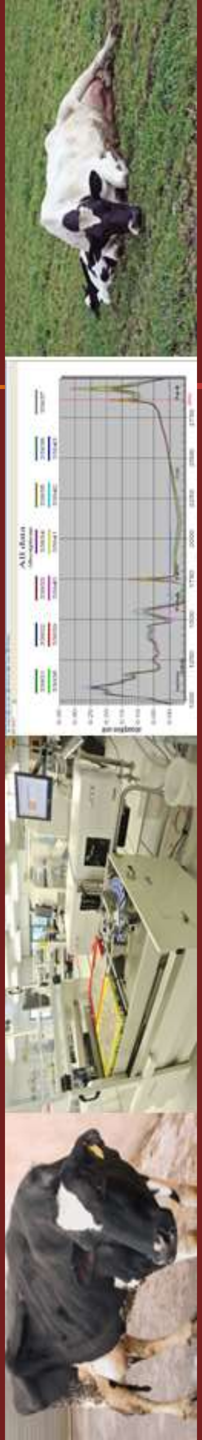
1 – Is there a correlation between ketosis metabolic disorder and overall milk composition & spectra?

2 - Can the metabolic disorder be modeled (PLS) to predict Blood BHB from the milk spectra?

Current: Ketosis

New:  ? Blood BHB/Ketosis(gold standard)

Prediction of the Blood BHB from the milk spectra by modeling the milk composition overall variations induced directly or indirectly by the metabolic disorder under consideration



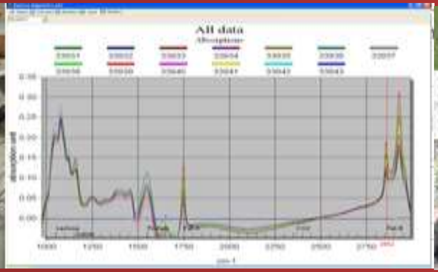
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and SKC detection

Study Protocol:

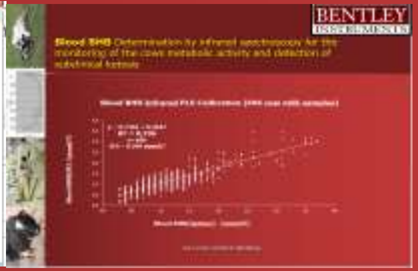
- 696 Holstein cows tested for blood BHB (Optium Xceed reader) over a 6 months period (DIM<100)
- Blood taken from the coccygeal vein (AM/PM)
- Samples cooled down immediately at 4°C after milking
- Milk samples tested on Bentley FTS within 12 hours to collect MIR spectra
- Development of a PLS model to predict Blood BHB based on Milk spectra

Optium Xceed reader

Bentley Ilas Combi 600



MIR spectra



Blood BHB PLS Calibration

A new global metabolic infrared spectral approach for Blood BHB prediction and SKC detection

Poor correlation between Blood and Milk BHB

n=280 (individual cows)

Mean Blood BHB = 1,043 mmol/L = 10,41 mg/dl

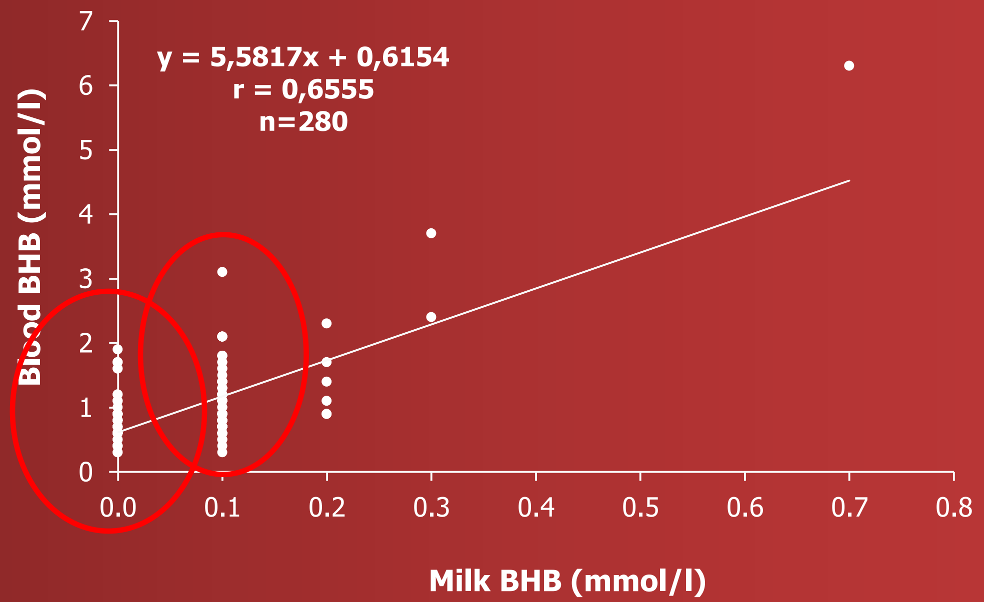
Mean Milk BHB = 0,058 mmol/l= 0,6 mg/dl < DL

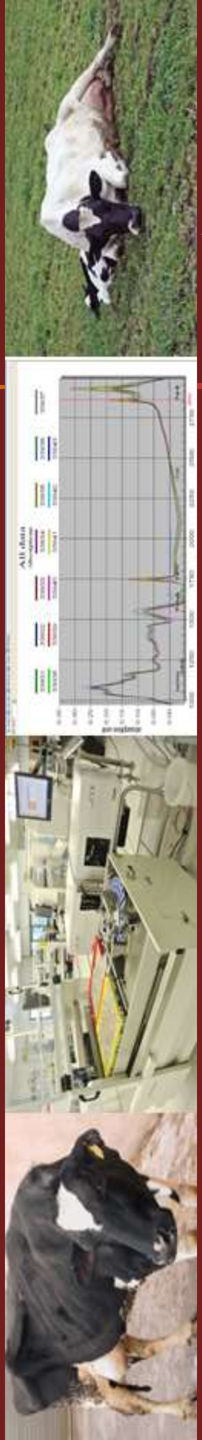
Blood BHB > 13,6 x Milk BHB

r=0,6555 (r=0,66* but can vary from 0 to 0,87)

*Enjalbert et al. Journal Dairy Science, 2001 84:583-589

Blood BHB vs Milk BHB Concentration (Optium)





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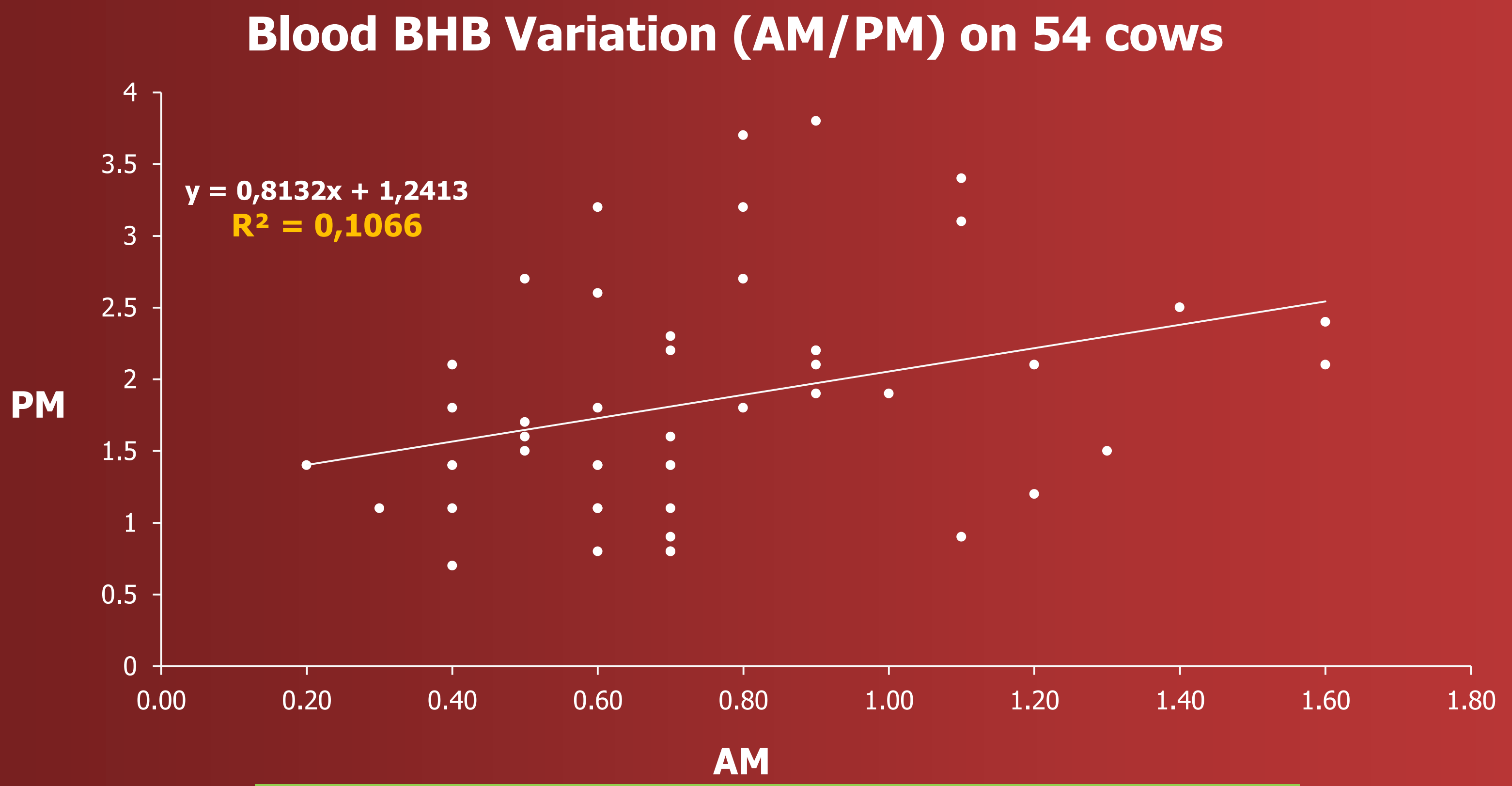
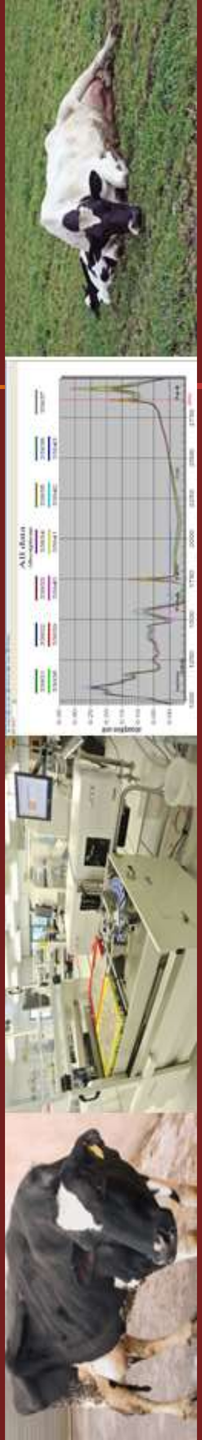
Evaluation of the Blood BHB daily variation (Optium)



Low correlation between AM and PM Blood BHB
Blood BHB PM >> Blood BHB AM



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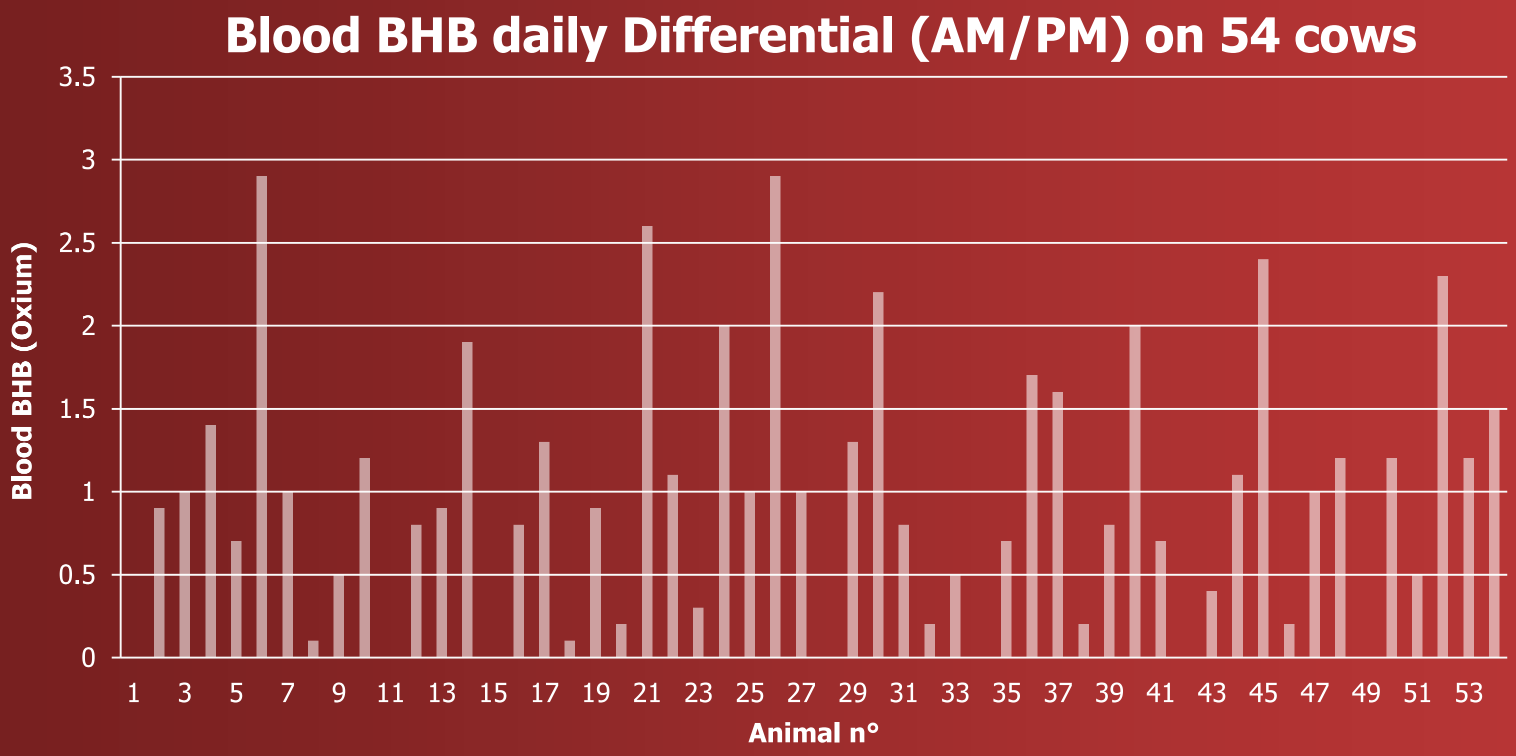
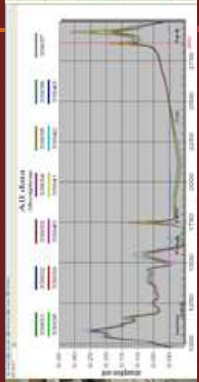


High potential variation between AM and PM Blood BHB



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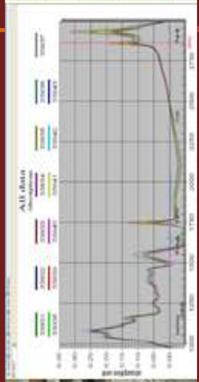
Evaluation of the Blood BHB daily variation (Optium)

Blood BHB Daily Variations: 3 Cases

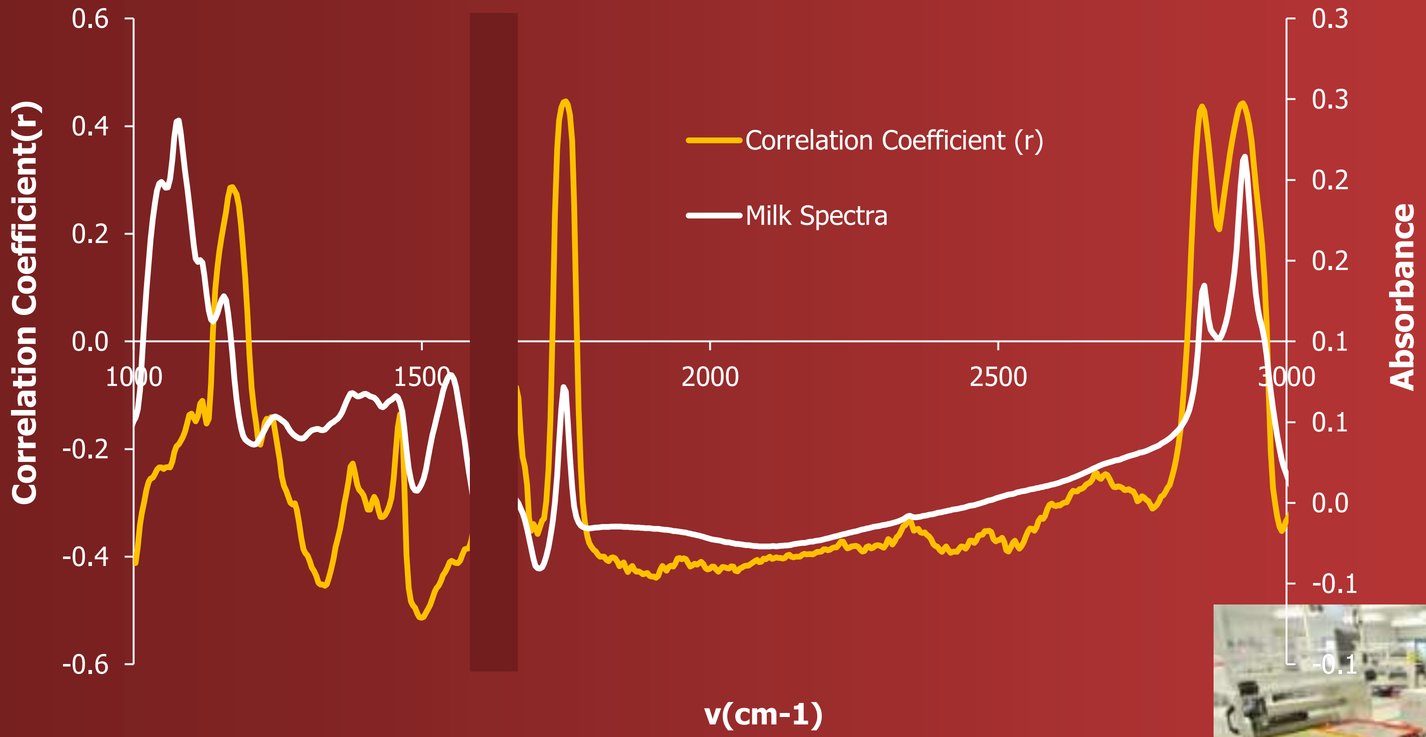
Cow	PM	AM	PM	AM	PM	AM	Mean BHB (mmol/l)
n°1	2,6	2,1	2,1	2,2	3,3	3,0	**2,6**
n°2	0,9	0,7	0,7	0,6	0,9	0,8	**0,8**
n°3	2,6	0,7	1,4	0,7	2	0,6	**1,3**

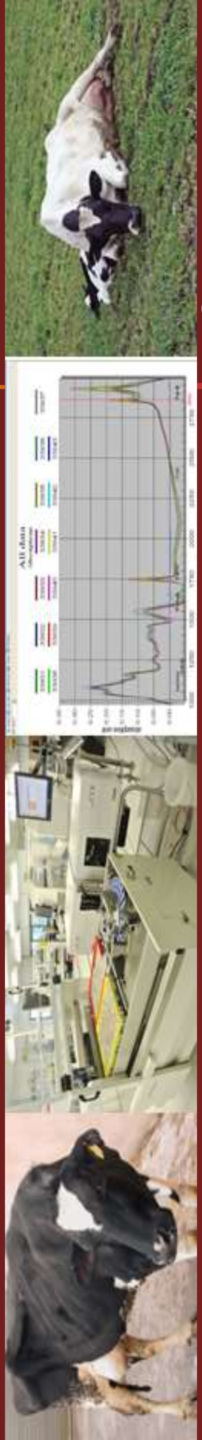
→ **Blood BHB PM >> Blood BHB AM**
→ **Poor correlation (r^2=0,11) between Blood BHB AM and PM**



1 – Is there a correlation between SKC metabolic disorder and overall milk composition & spectra (696 samples)?

Correlation(r) between Blood BHB and Milk spectra





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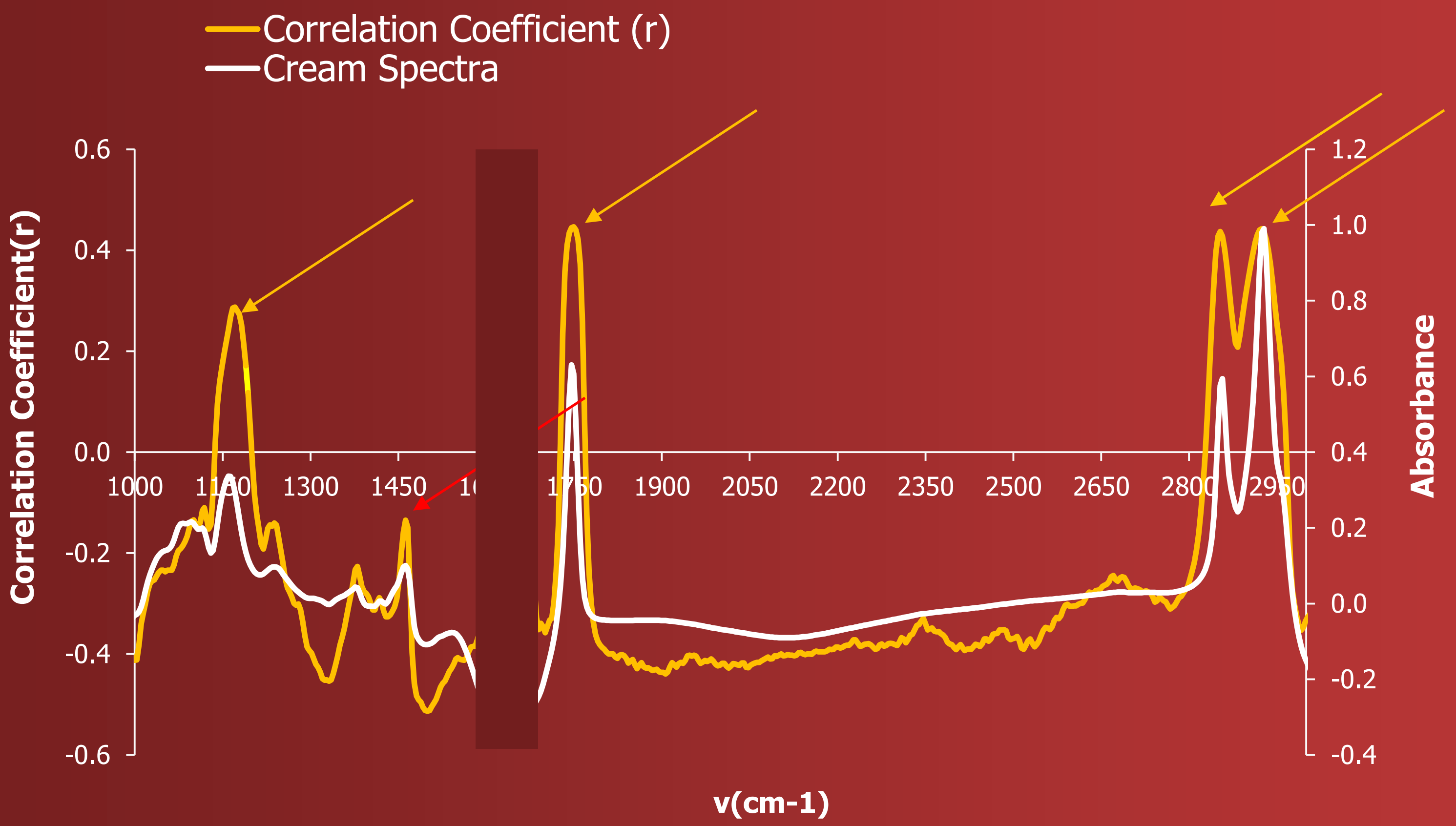
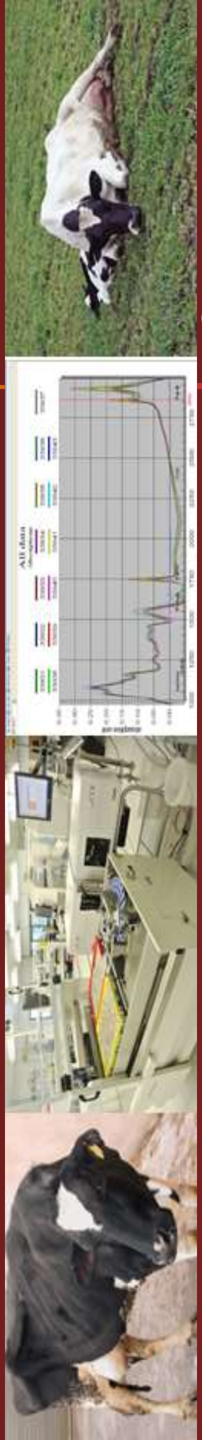


Cc: Blood BHB is mostly correlated to the milk Fatty Acids Profile (FAP)



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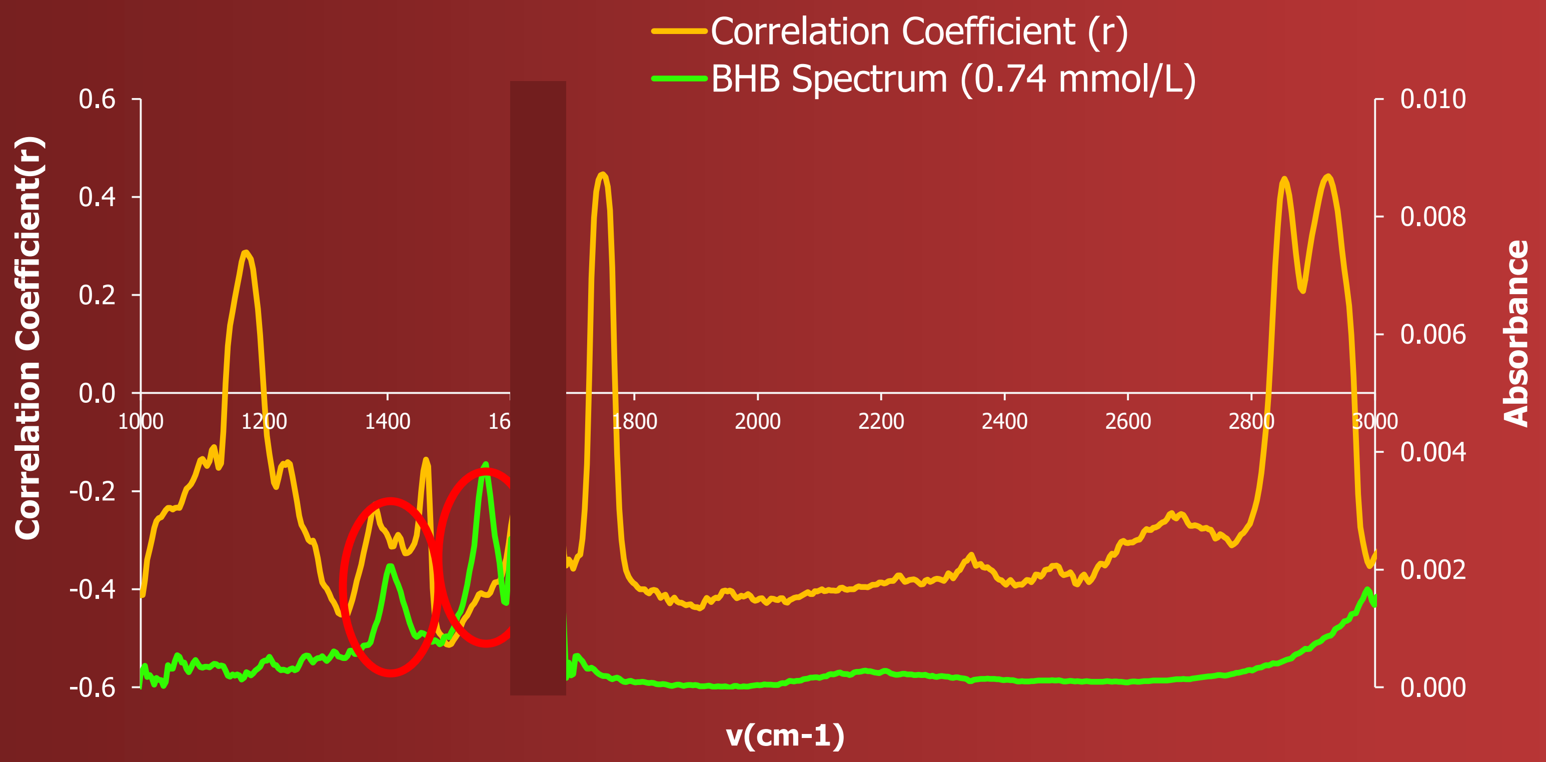
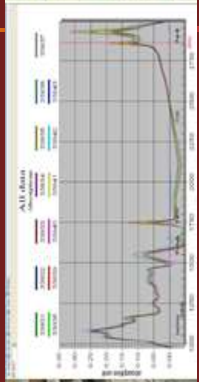


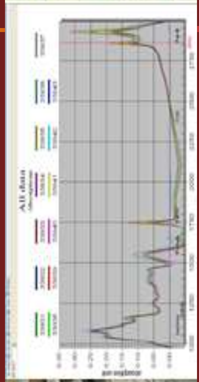
Cc: Low correlation between Milk spectra and Blood BHB where BHB molecule main absorption wavebands are located (1410; 1562 cm^{-1})



1 – Is there a correlation between SKC metabolic disorder and overall milk composition & spectra (696 samples)?

Components (X)	r/BBHB(Optium)	BBHB <1,2 (mmol/l)	BBHB >1,2* (mmol/l)	Wi	% Wi
Protein	-0,26	**32,58**	**31,56↓**	-1,02	-3,13%
Palmitic(C16:0)	-0,13	1,36	1,35=	-0,01	-0,81%
SCC	-0,12	165,00	114,00↓	-51,00	-30,91%
Saturated FA	-0,07	2,73	2,76	0,03	1,21%
Lactose	0,02	48,07	48,45↑	0,38	0,79%
Fat	0,15	**41,73**	**43,34↑**	1,61	3,86%
Poly Unsaturated FA	0,27	0,16	0,17	0,02	10,97%
Fat/Protein	0,39	1,279	1,379↑	0,100	7,82%
Unsaturated FA	0,47	1,20%	**1,35%↑**	0,15%	**12,50%↑**
Mono Unsaturated FA	0,48	1,08%	**1,20%↑**	0,12%	**11,11%↑**
Oleic (C18:1)%	0,52	0,84%	**0,96%↑**	0,13%	**15,21%↑**
Stearic (C18:0)%	0,57	0,36%	**0,41%↑**	0,06%	**16,01%↑**
Oleic/Protein	0,66	0,026	**0,031↑**	0,005	**19,23%↑**
Stearic/Protein	0,66	0,011	**0,013↑**	0,002	**18,18%↑**

More research under way: 416 fatty acids in bovine milk (Jensen, 2002)



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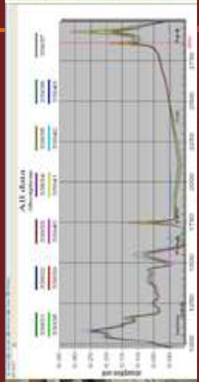

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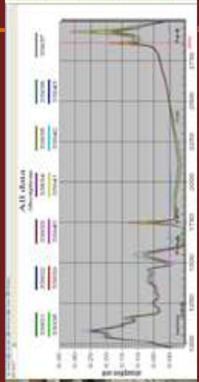
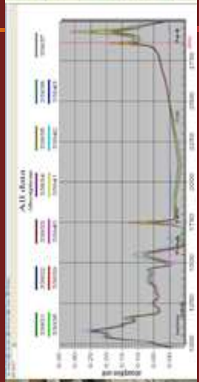


Bentley Fatty Acids Calibrations

Typical results obtained on bulk cow milk with PLS Calibrations					
Components	**Measuring Range**	**Validation range**	**Repeatability (Sr)**	**Accuracy (Sy,x) (Bulk)**	**Carry-Over**
C:16:0 (Palmitic)	0,43-2,45%	0,78-1,51%	0.027%	**0.056%** (0,070-0,094)*	< 1.0%
C:18:0 (Stearic)	0,12-0,70%	0,24-0,47%	0.012%	**0.035%** (0,040-0,055)*	< 1.0%
C:18:1 (Oleic)	0,21-1,81%	0,34-1,81%	0.020%	**0,027%-0.050%**	< 1.0%
Saturated FA	0,96-4,50%	1,27-4,38%	0.025%	**0.045%** (0,037-0,055)*	< 1.0%
Unsaturated FA	0.41-2.17%	0,41-2,17%	0.024%	**0.049%**	< 1.0%
Mono Unsaturated FA	0,33-1,99%	0,37-1,96%	0.023%	**0.046%** (0,040-0,061)*	< 1.0%
Poly Unsaturated FA	0.03-0.24%	0,10-0,24%	0.006%	**0.020%** (0,010-0,020)*	< 1.0%

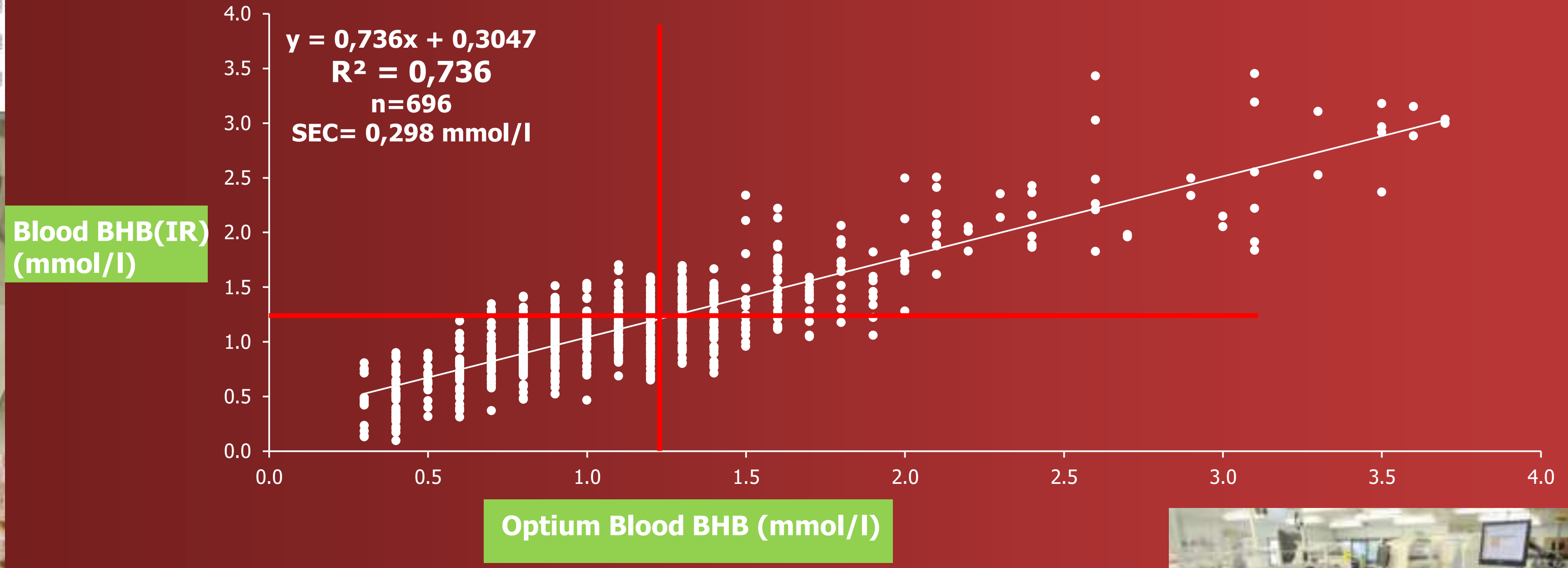
* Bulletin of International Dairy Federation 406/2006, p.25



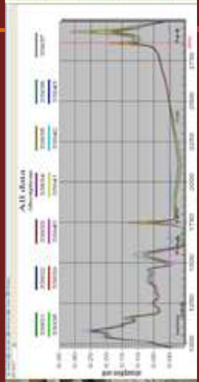
2 - Can the metabolic disorder be modeled (PLS) to predict Blood BHB indirectly? Yes

My =1,15 mmol/l (12 mg/dl)
⇒ Milk BHB < 1,0 mg/dl!!

Blood BHB Infrared PLS Calibration



A new global metabolic infrared spectral approach
How to implement it?

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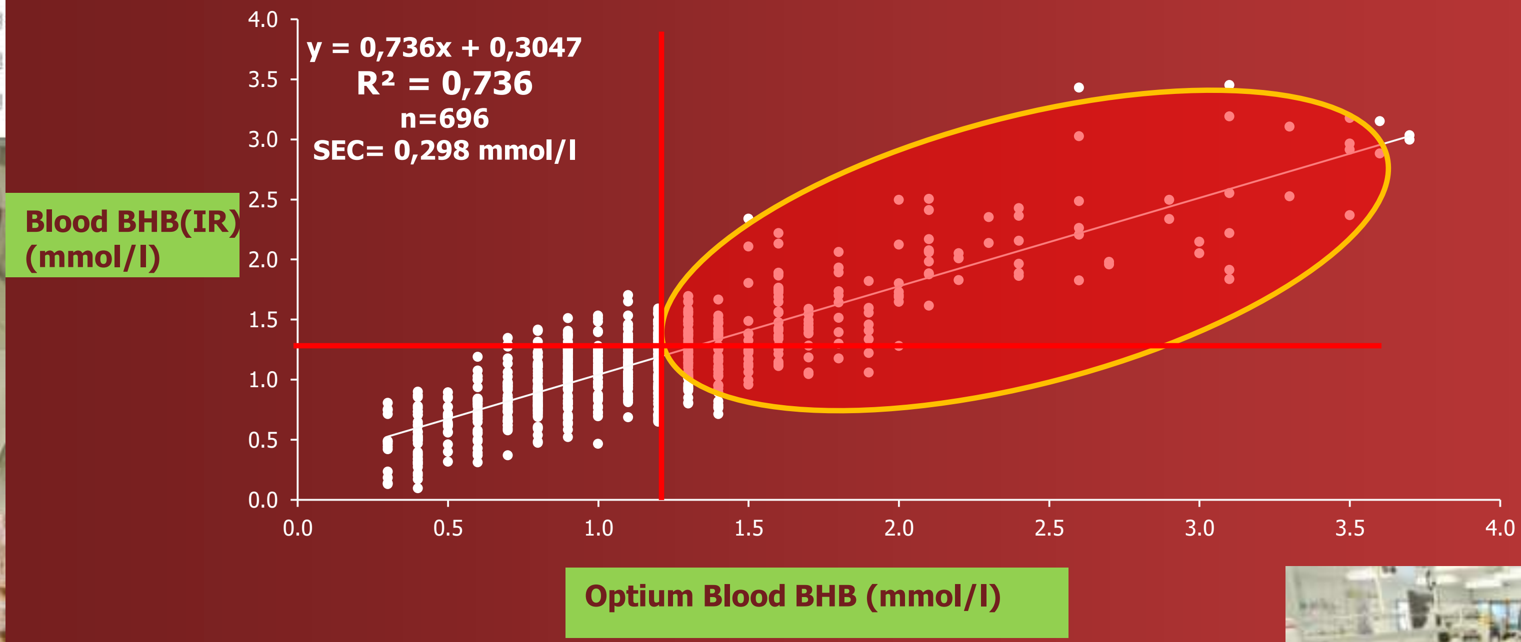

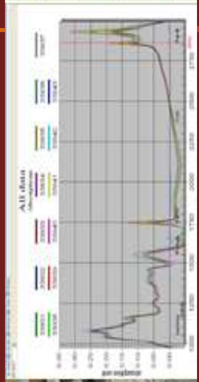

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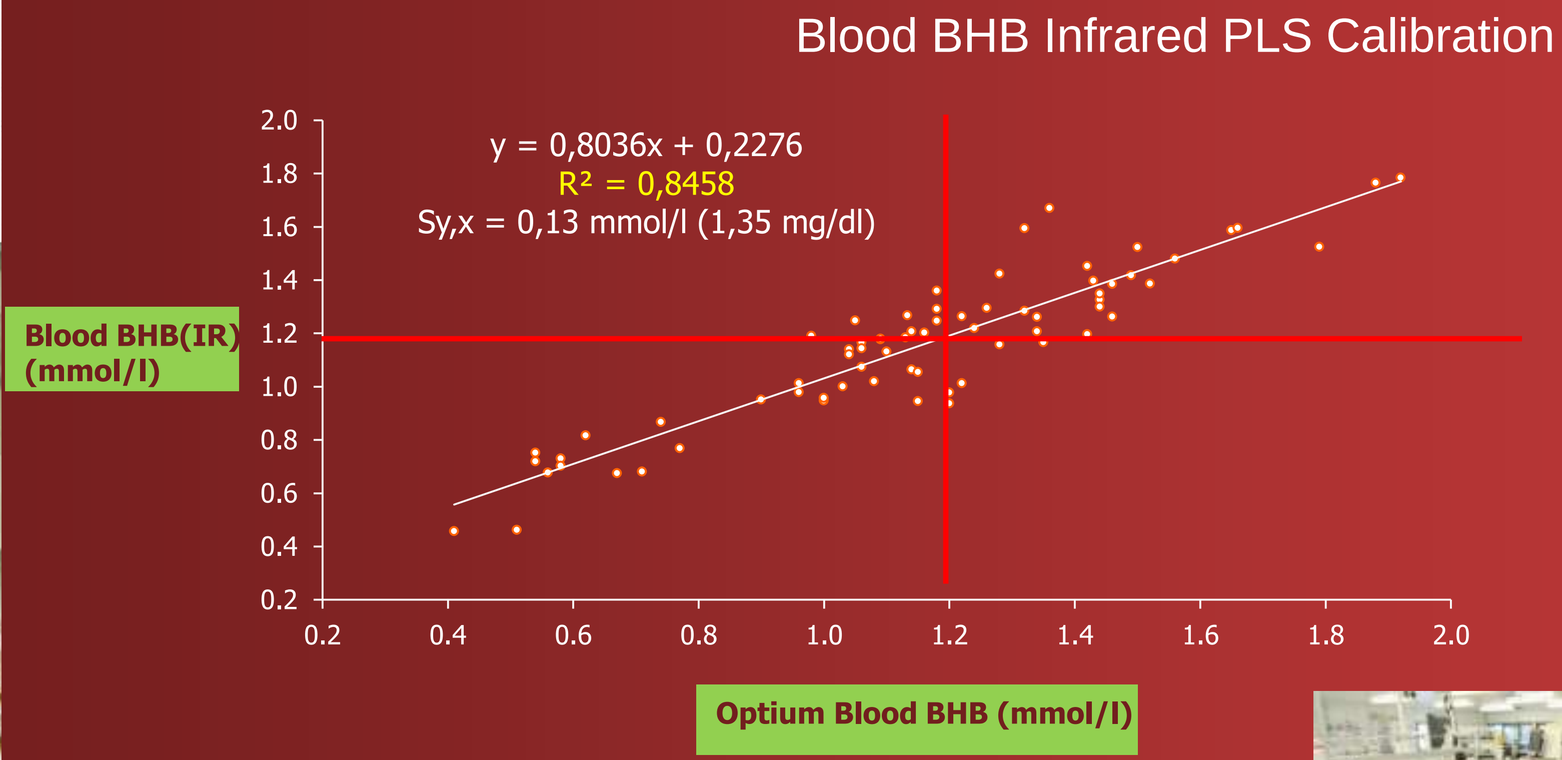
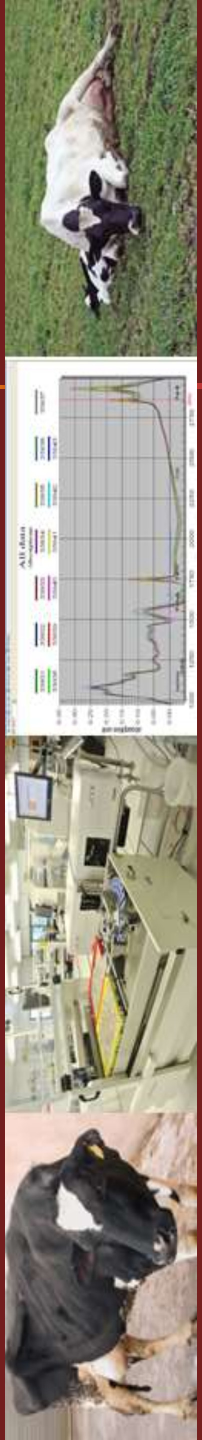
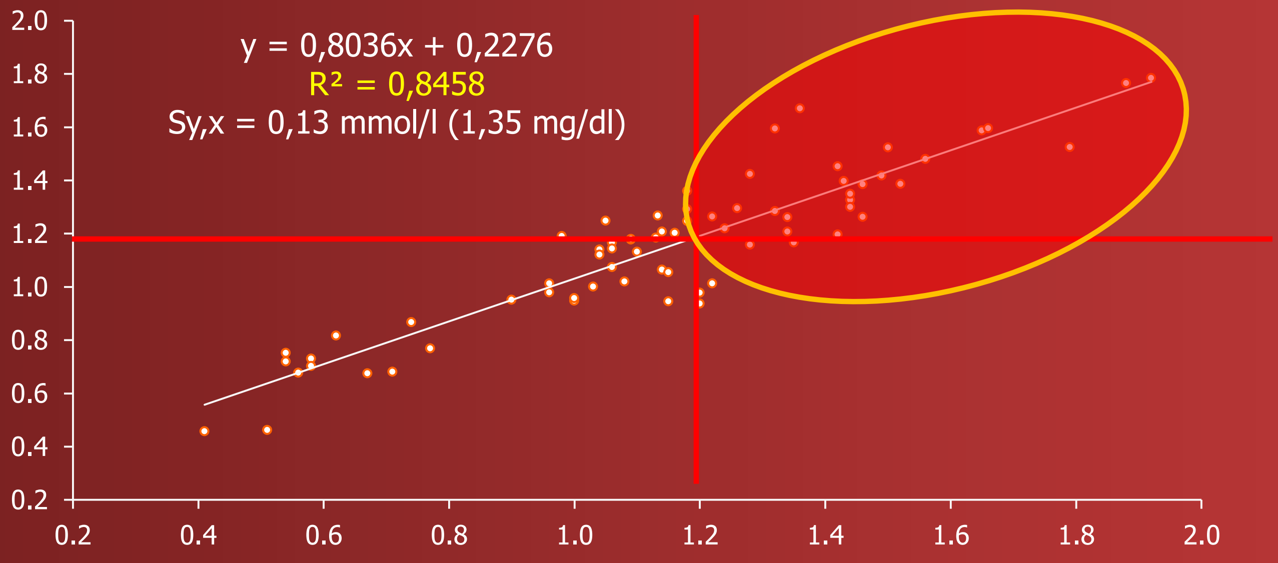




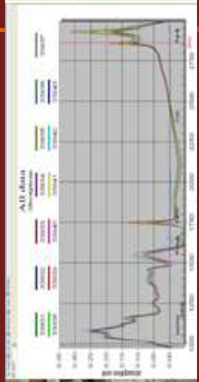
A new global metabolic infrared spectral approach
How to implement it (at herd level)?

Blood BHB Infrared PLS Calibration



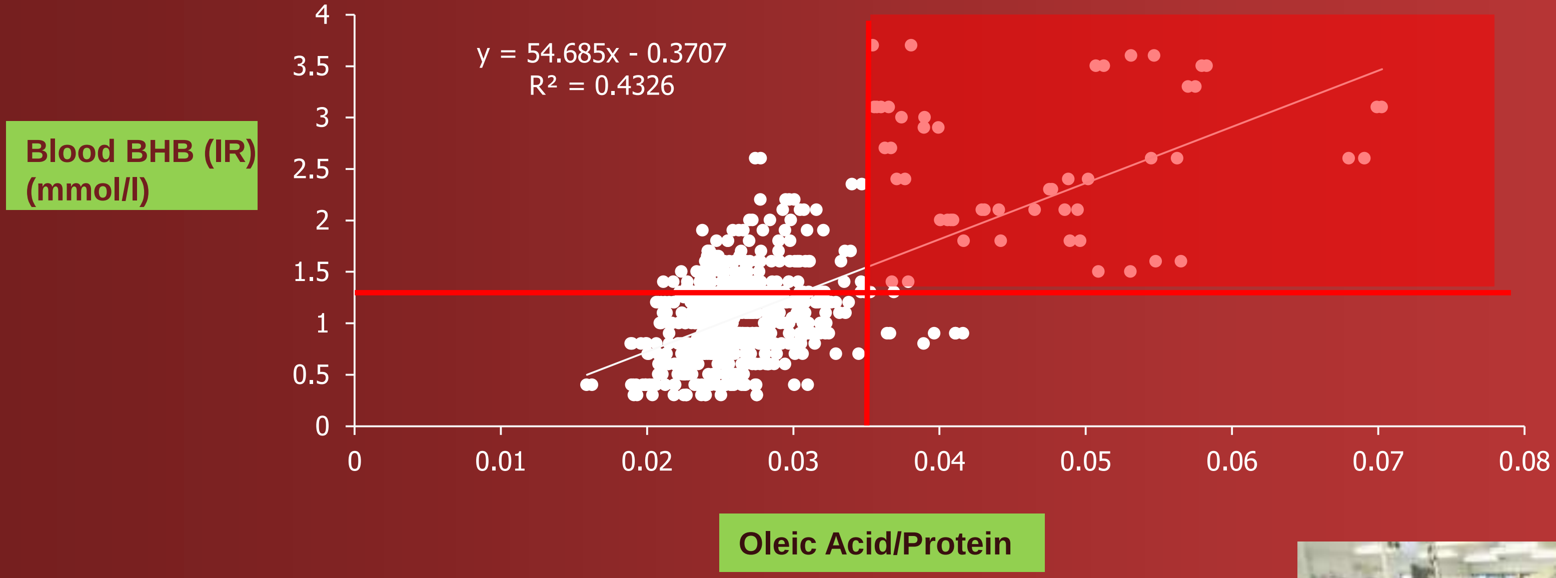
Blood BHB(IR) (mmol/l)

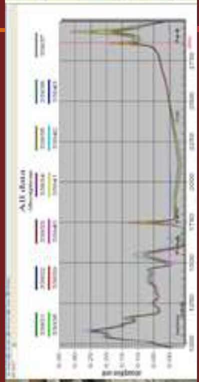
Optium Blood BHB (mmol/l)

How to improve still ketosis diagnostic?
A multi-dimension approach

Blood BHB vs Oleic Acid/Protein Ratio on 696 individual cows





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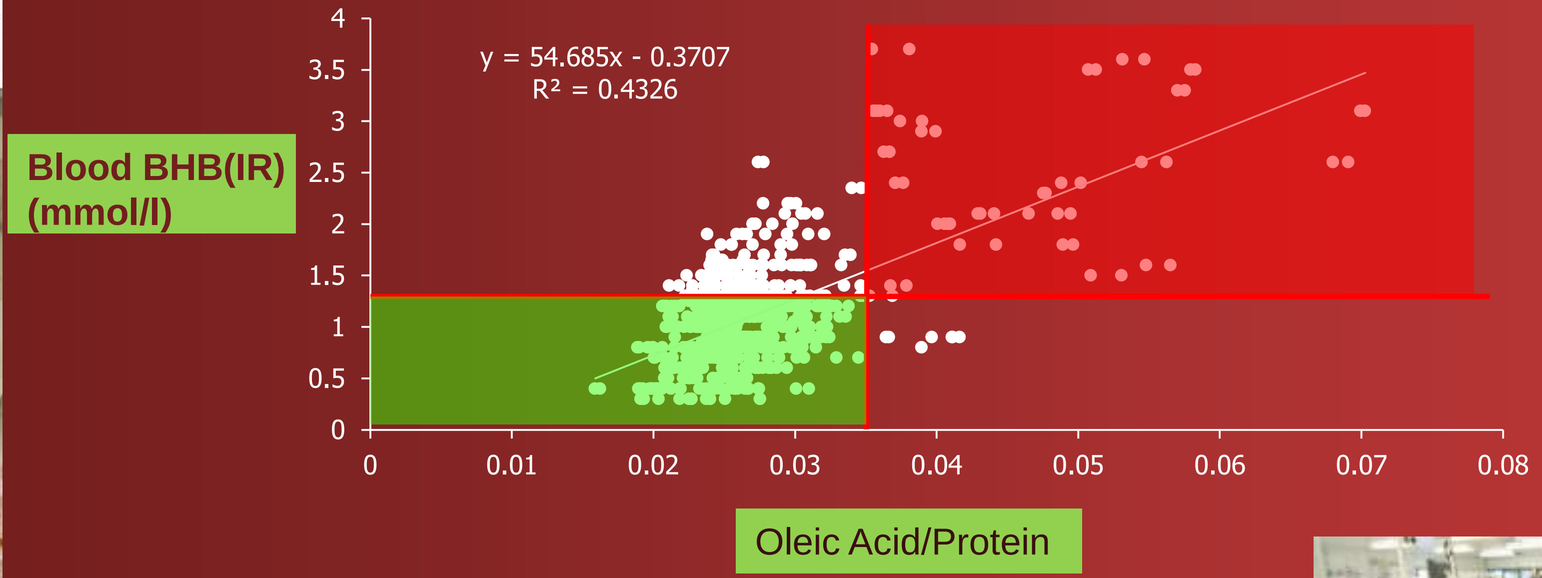
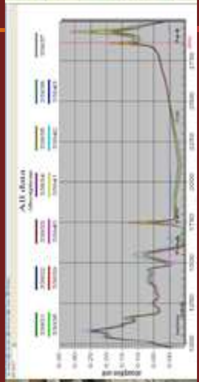
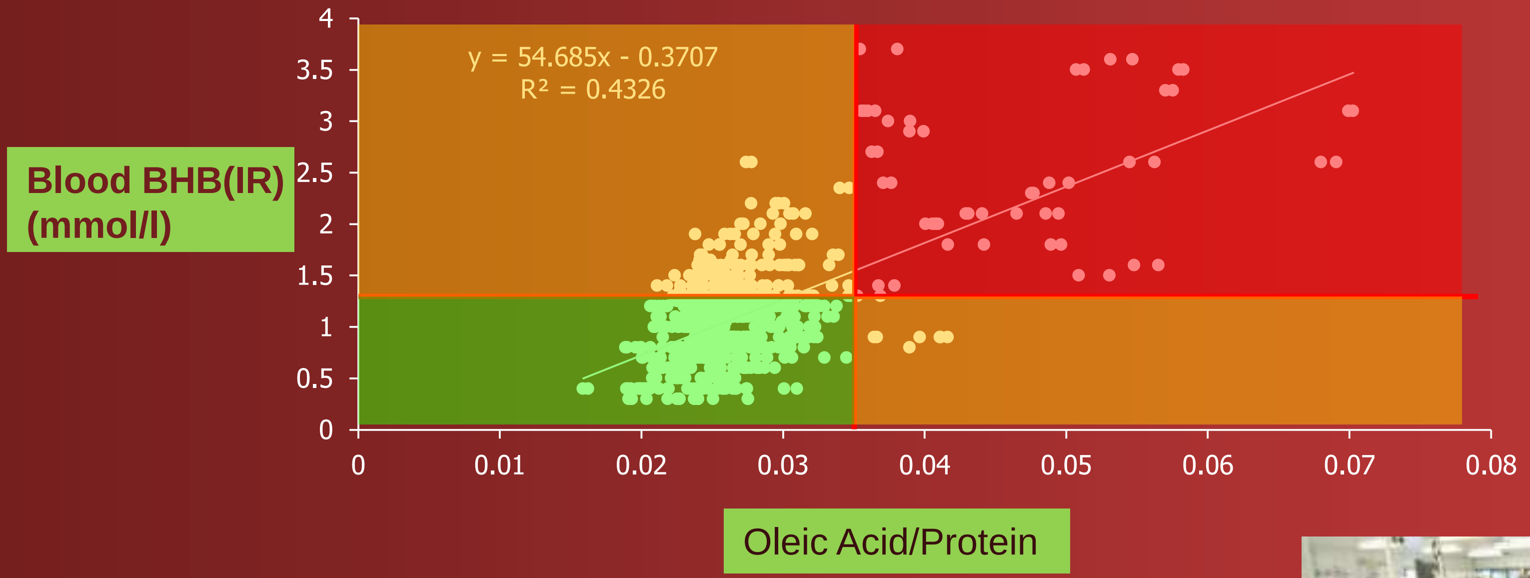
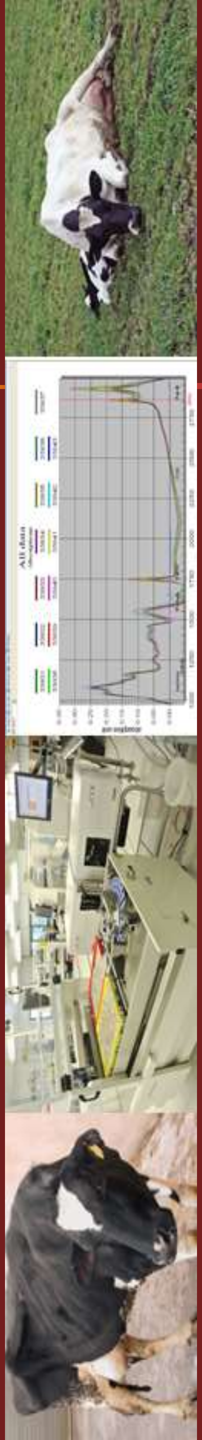




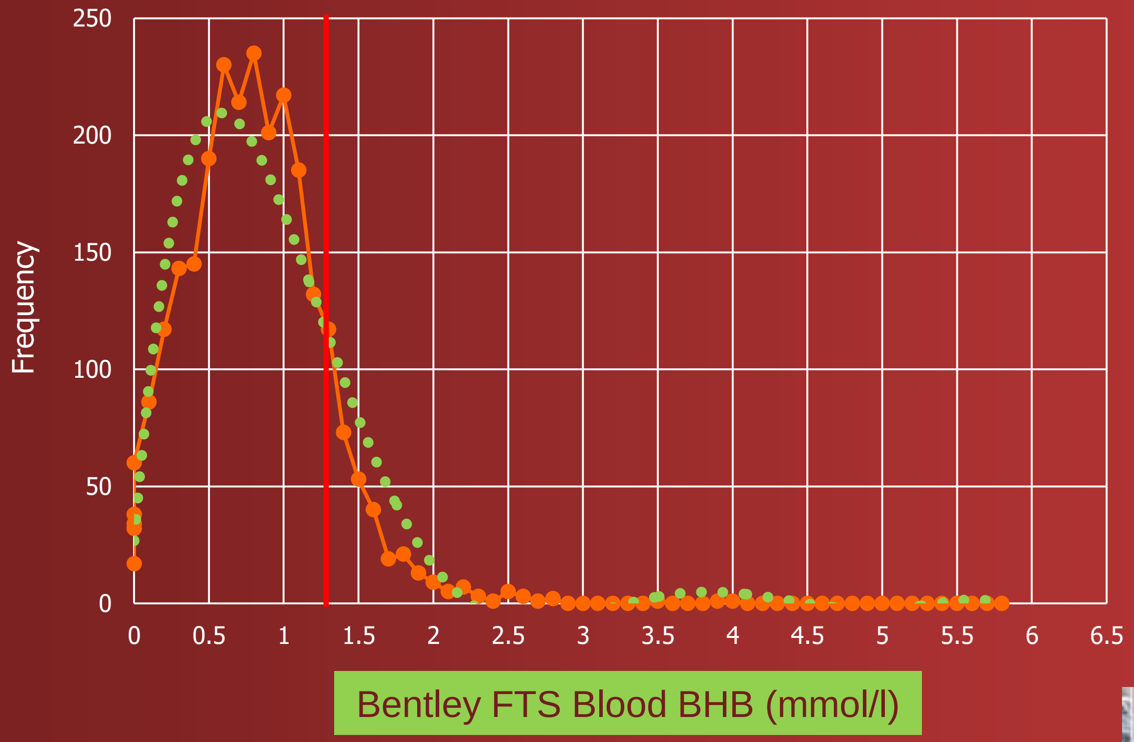
How to improve still the ketosis detection? A multi-dimension approach

Blood BHB vs Oleic Acid/Protein Ratio on 696 individual cows

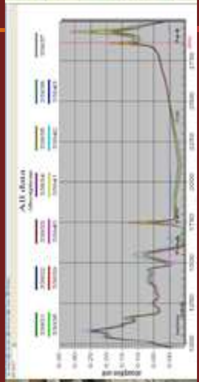




Blood BHB Concentration distribution in DHIA samples

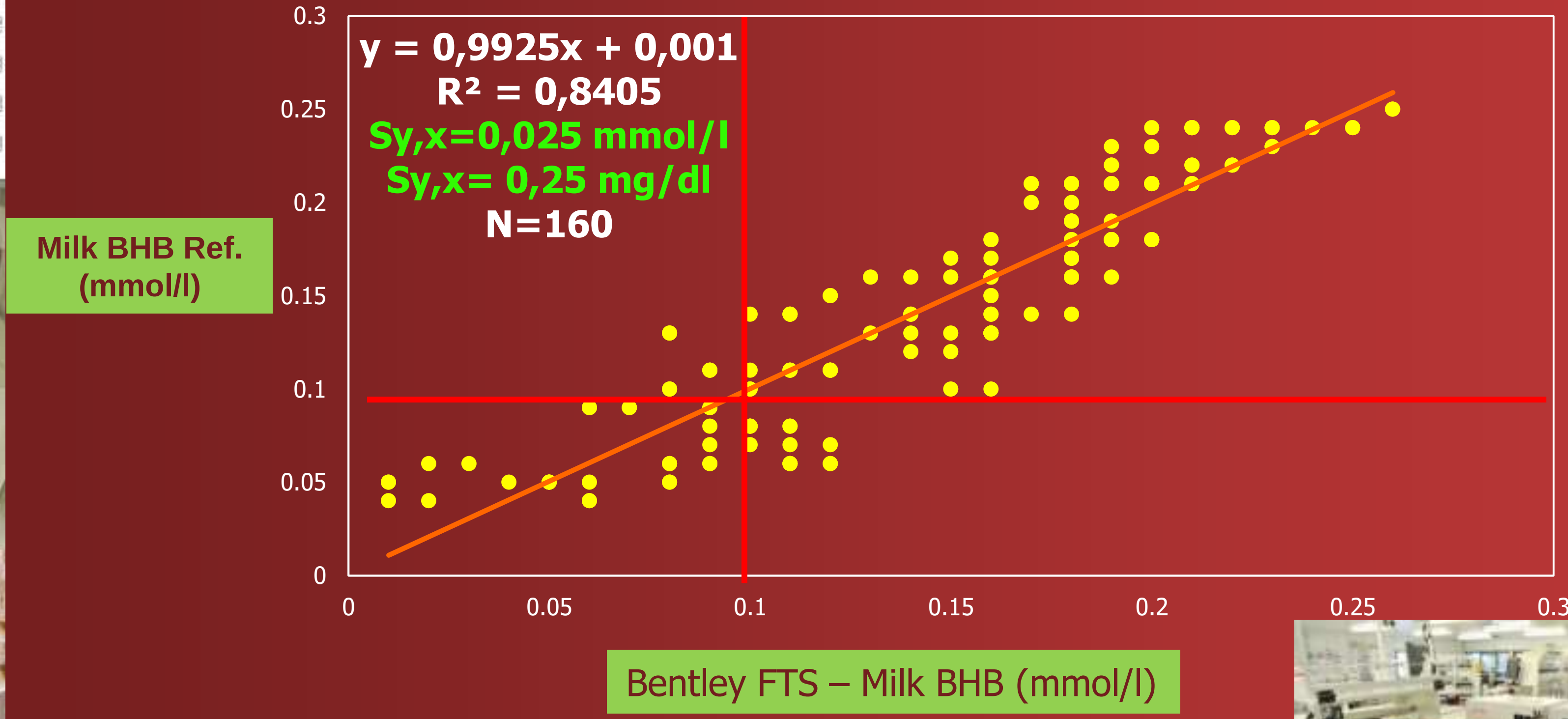


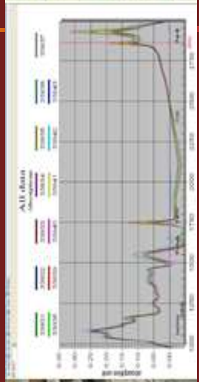
ICAR 2016 Congress
Puerto Varas, October 25, 2016



Application of Blood BHB model to Milk BHB determination – 100% independent validation set (CECALAIT)

Bentley FTS vs. BHB Reference method
160 validation natural milk samples analyzed <u>over 12 months</u>





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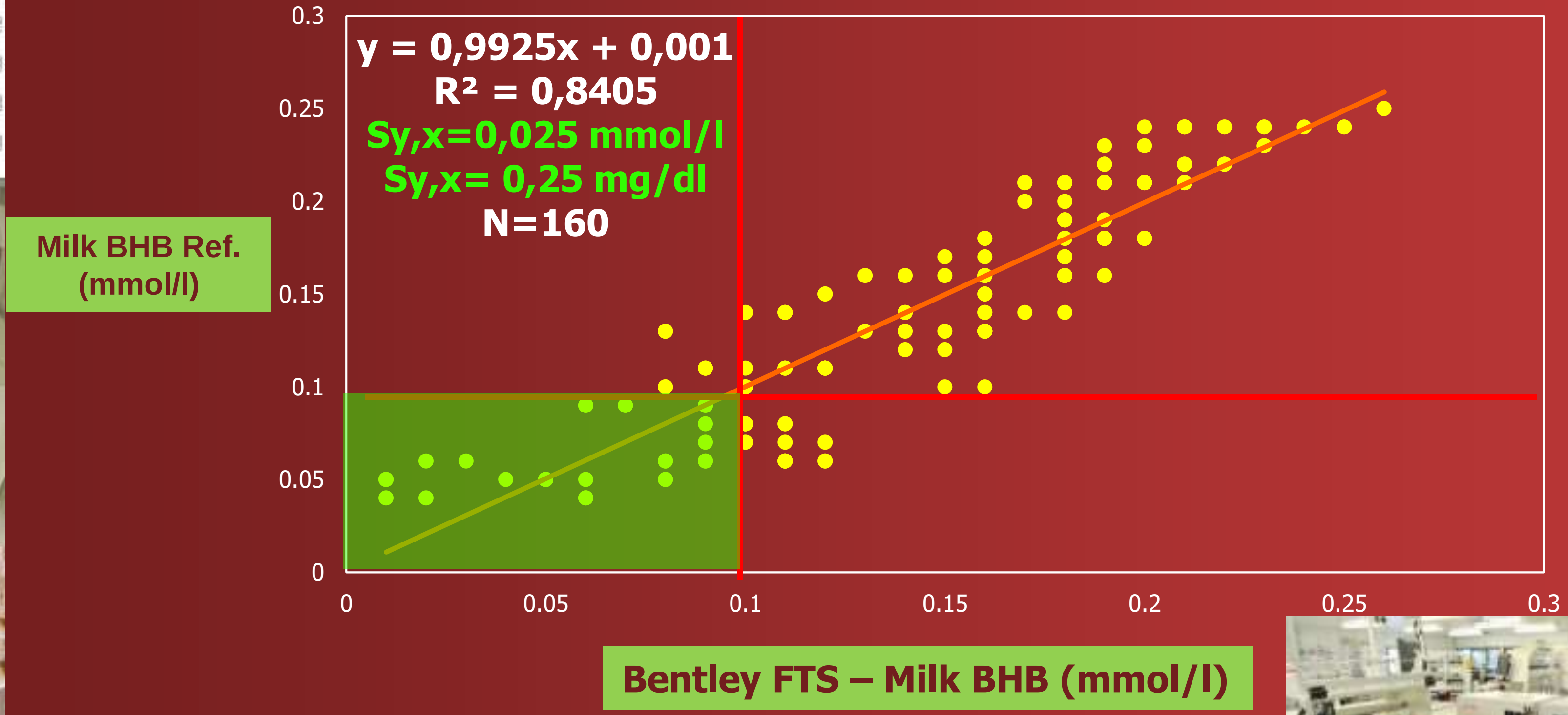
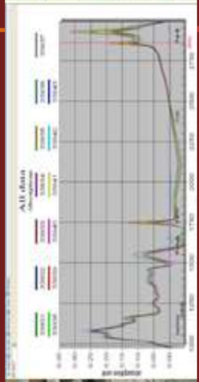


Application of Blood BHB model to Milk BHB determination – 100% independent validation set (CECALAIT)



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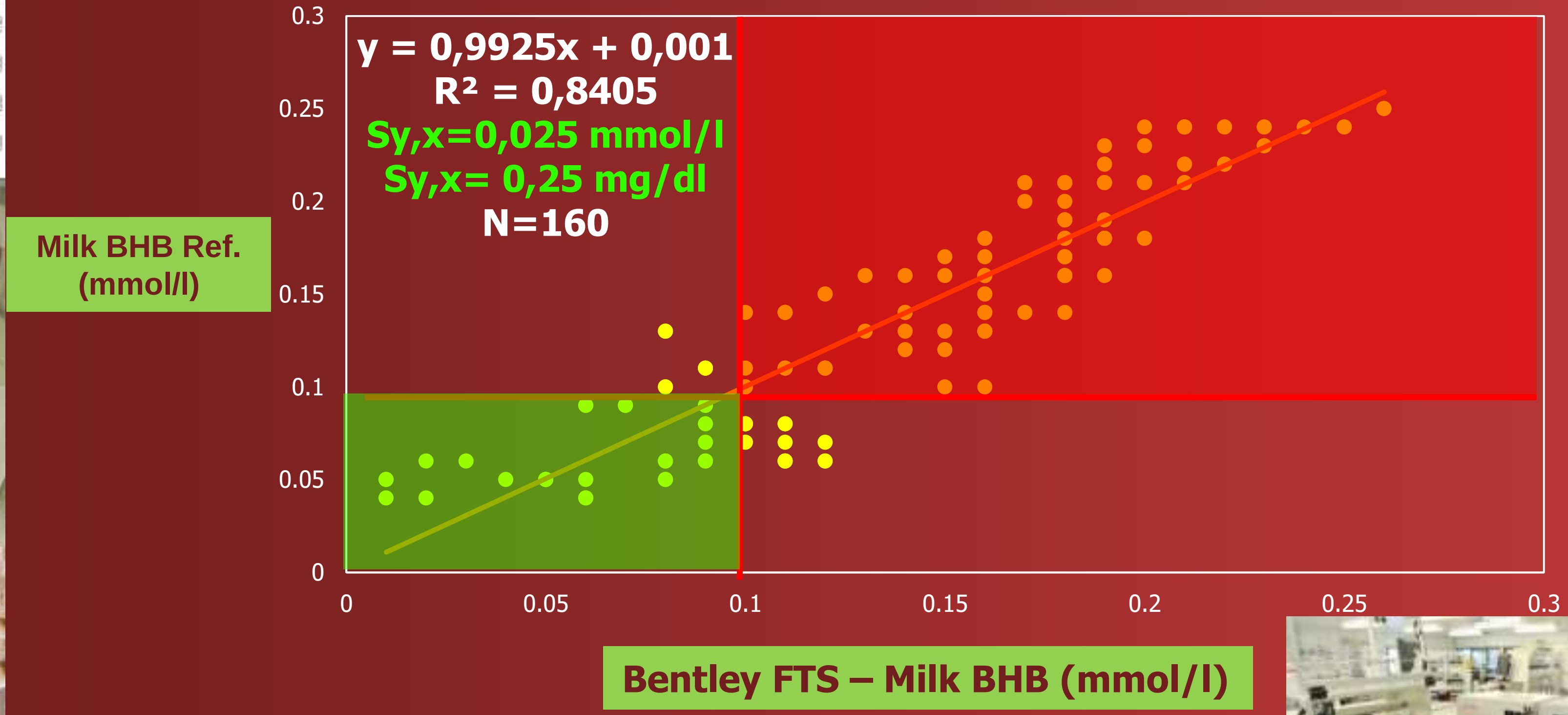

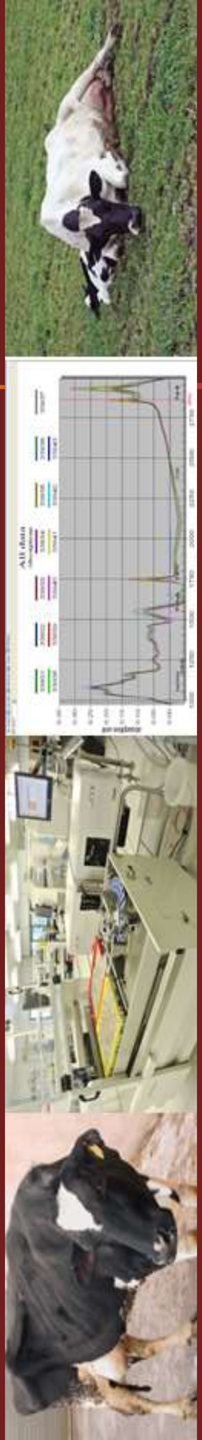

Conclusions:

- The global metabolic spectral model is a very exhaustive and powerful approach to understand the relationship between a metabolic disorder and the milk spectra

- Blood BHB (IR), Milk BHB and Milk Fatty Acids Profile (and especially oleic acid) can successfully be used as ketosis biomarkers to improve SKC detection and to:
  - monitor individual cow Blood/FAP/Milk BHB variations over time
  - compare individual cows at herd level to develop a relative approach by setting a baseline (intra-herd) – **recommended approach**
  - to detect ketosis prevalence at herd level (inter-herds)

The Oxygen DHIA/FR laboratory has been using this model very successfully over the last 3 years with the detection of over 90% of the ketosis cases (over 10 million IR spectra collected)

- More frequent sampling protocol should be considered during early lactation to further improve diagnostic performance.

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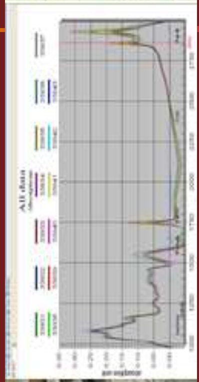
Thank you for your attention!

pbroutin@bentleyinstruments.com

www.bentleyinstruments.com



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