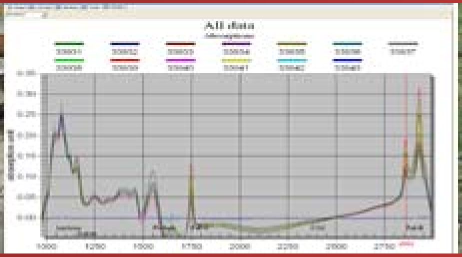
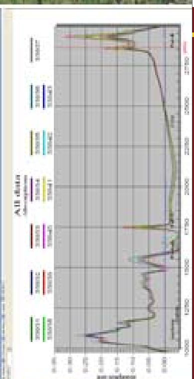
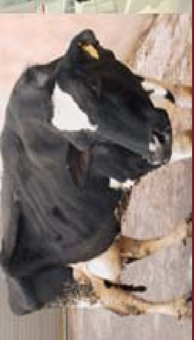


Blood BHB determination by mid infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis – a new approach

Pierre Broutin,
Managing Director/Western Europe
Senior Scientist

2015 ICAR Technical Workshop
10-12 June 2015, Krakow, Poland

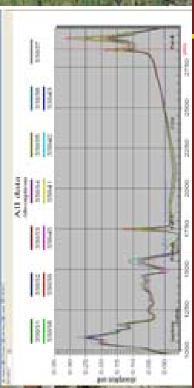



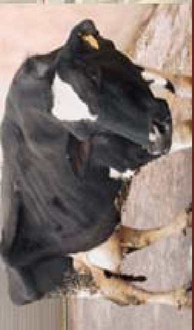


Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Presentation outline

- Why testing for Blood BHB?
- Understanding the limitations of current analytical methods
- Protocol & theory for the development of a Blood BHB PLS MIR calibration
- Identification of the factors influencing the Milk & Blood BHB content
- Identification of the milk components correlated to Blood BHB
- Field implementations and interpretation of the results (OXYGEN and FIDOCL/INRA/VetAgroSup research programs)
- A new probabilistic approach
- Conclusions





BENTLEY INSTRUMENTS

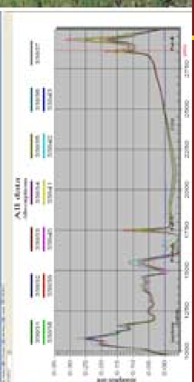
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

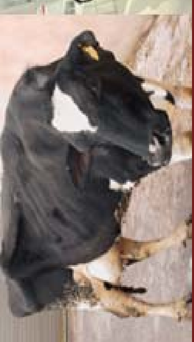
Why testing for ketosis?

- Subclinical ketosis → Blood BHB > 1.2 mmol/L (= 12,5 mg/dl)

- Decrease in milk production (-300 to -450 kg/lactation) (Duffield,2000)
- Modification of the 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 of the conditions**

→ Ketosis can significantly impact farm profitability and animal welfare



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

Why predicting Blood BHB instead of Milk Ketone bodies(Acetone / BHB)?

- **Blood BHB = Gold Standard** but invasive and not systematic
- **Milk Acetone** is very volatile and in very low concentration in milk
- **Milk BHB** is more stable than acetone but levels are also very low, close to the IR detection limit

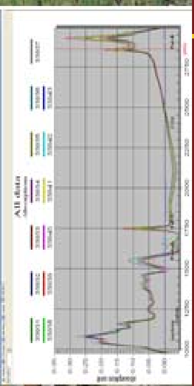
mg/dL = (mmol/L)/0.097: **(0.10 – 0,60 mmol/L) = (1,04–6,24 mg/dl!!) << urea (10-100 mg/dl)**

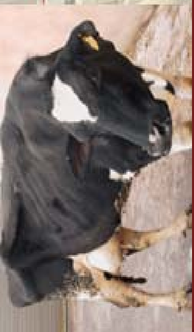
- **Milk BHB** content decreases also rapidly if the milk not stored at 4°C right after milking
- Correlation (r) between milk and blood BHB very variable (0,00-0,87) (*Enjalbert et al. 2001)*

Cc: Milk Acetone & BHB determination by IR present some major drawbacks because of their volatility, very low concentration levels, and very variable correlation with blood BHB

A NEW GLOBAL METABOLIC SPECTRAL APPROACH (proprietary):

Prediction of the Blood BHB content by identifying and 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 detection of subclinical ketosis

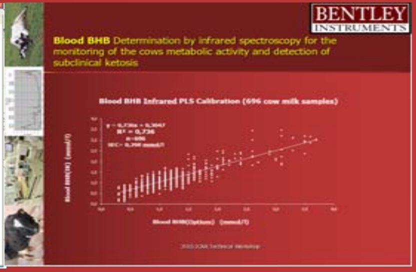
Study Protocol:

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

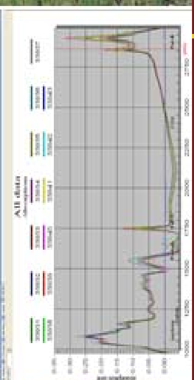

Optium Xceed reader

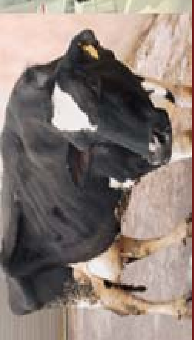

Bentley Ilas Combi 600

MIR spectra



Blood BHB PLS Calibration



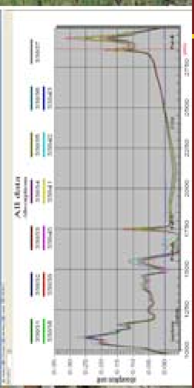


Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Influence of samples temperature on Milk BHB content

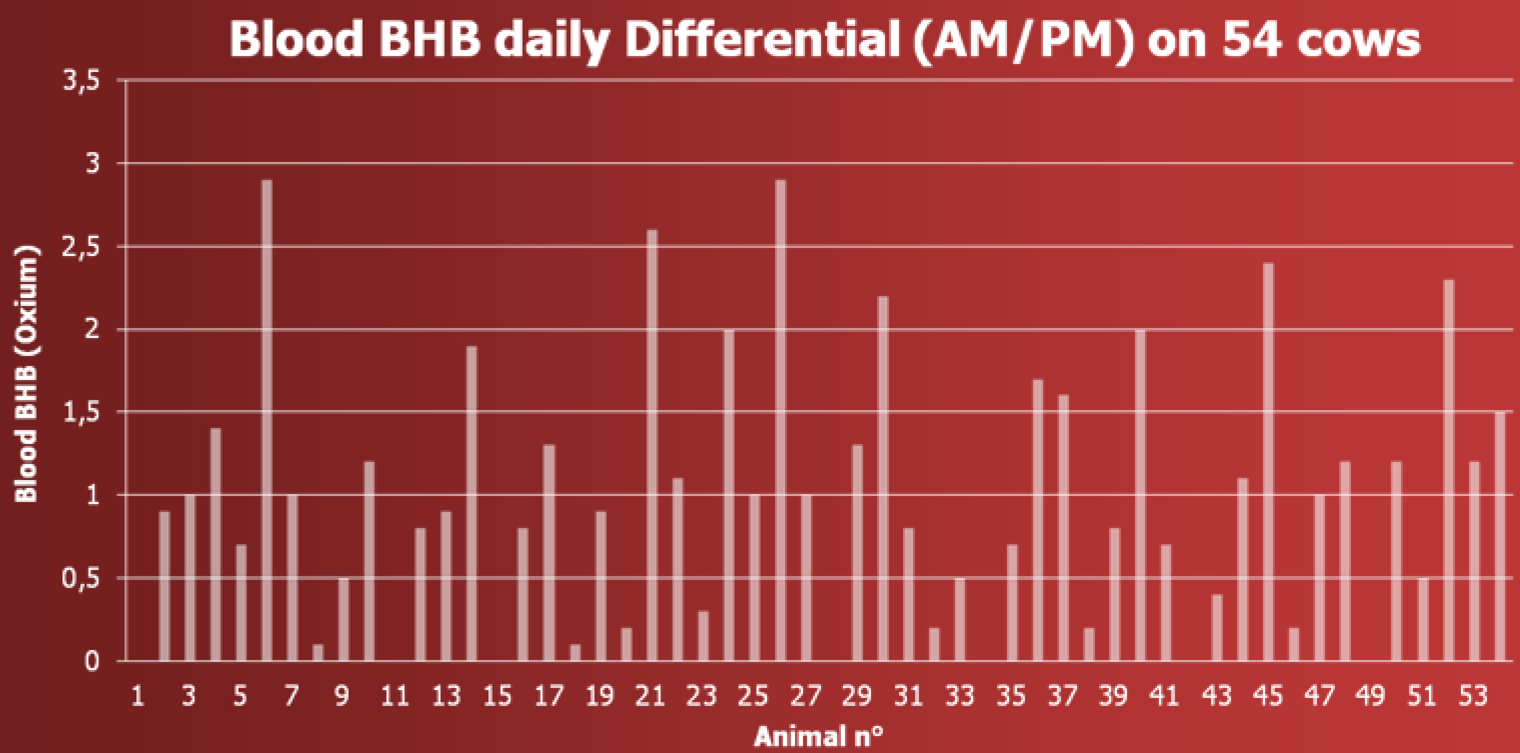
	Milk BHB temperature Stability	Milk BHB temperature Stability
Milking time	0,108 mmol/l	1,12 mg/dl
Analysis time	0,008 mmol/l	**0,08 mg/dl<< DL!!**
Wi	↓0,10 (92% loss!)	↓1,04 (92% loss!)

Cooling of the samples at 4°C right after milking slows down the BHB loss

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

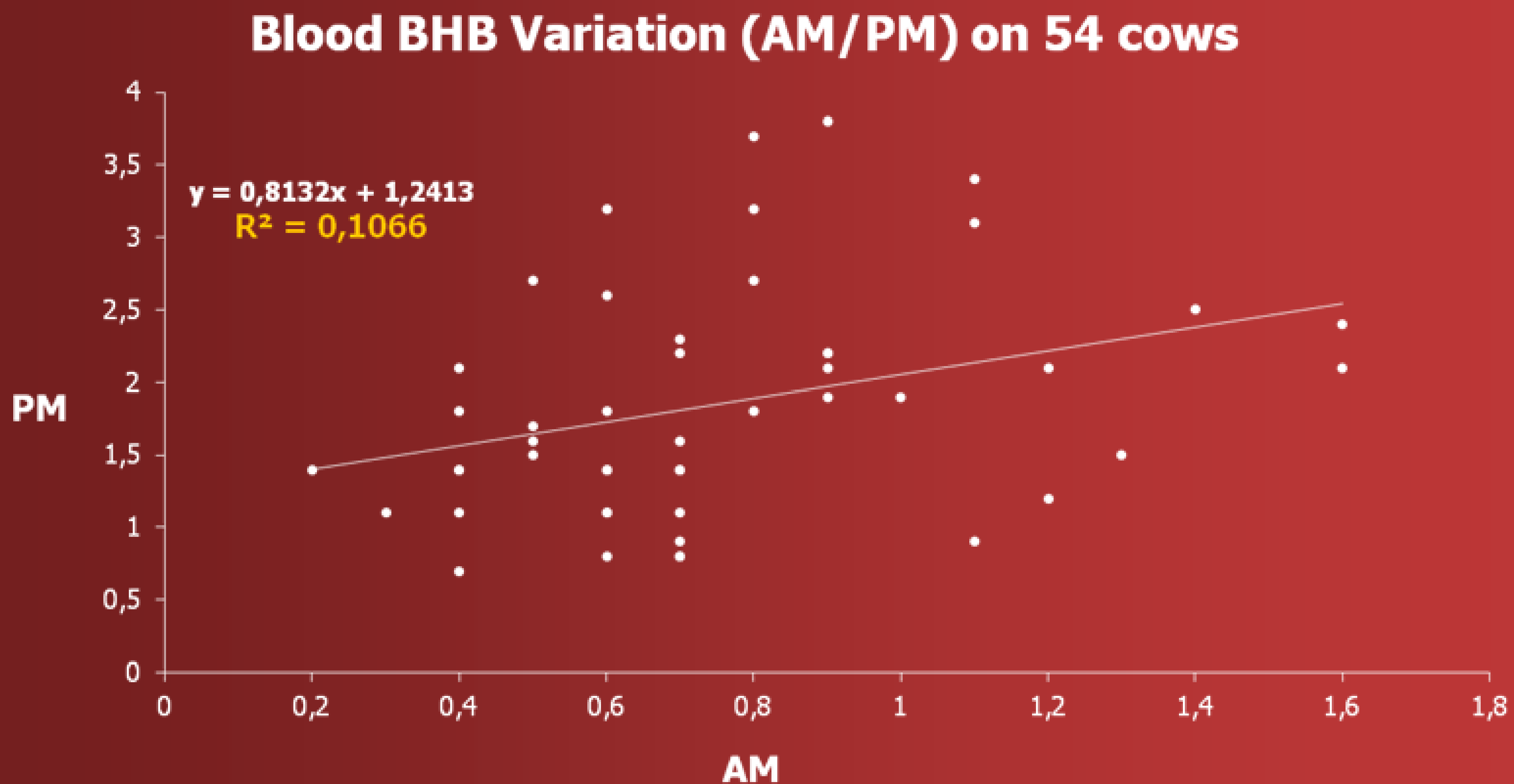
Evaluation of the Blood BHB daily variation (Optium)



High potential variations between AM and PM Blood BHB

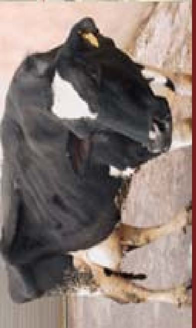
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Evaluation of the Blood BHB daily variation (Optium)



- no correlation between AM and PM Blood BHB
- Blood BHB PM >> Blood BHB AM



Blood BHB determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

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

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

Relation between Blood and Milk BHB

n=280 (individual cows)

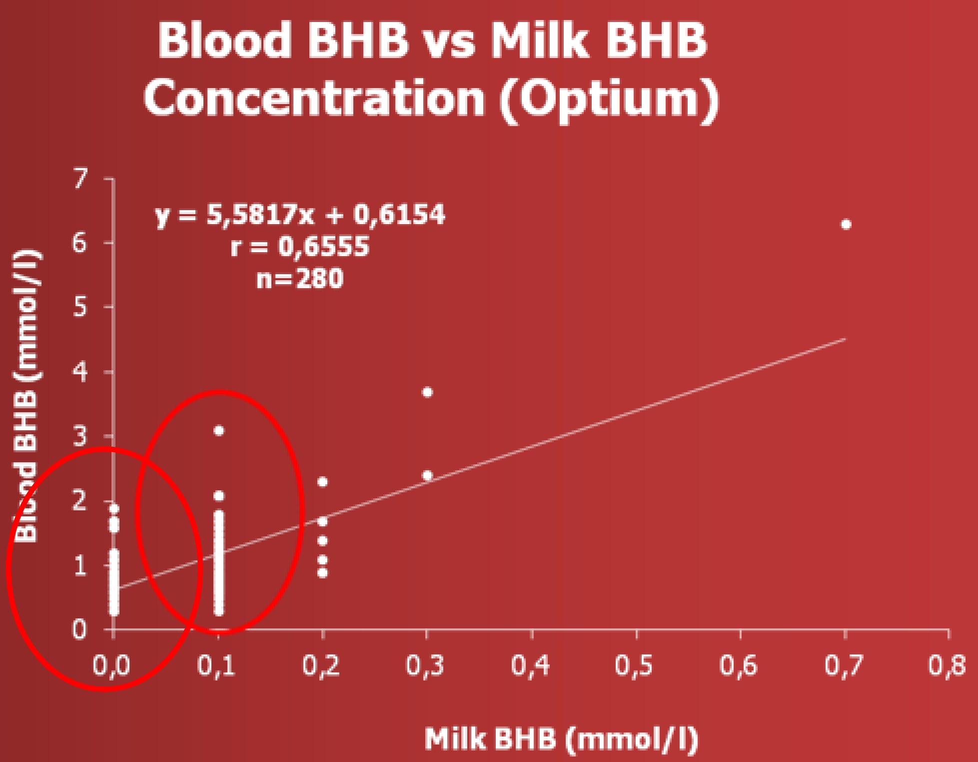
Mean Blood BHB = 1,043 mmol/L = 10,41 mg/dl

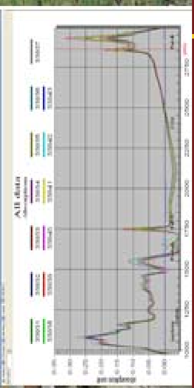
Mean Milk BHB = 0,058 mmol/l= 0,6 mg/dl < DL

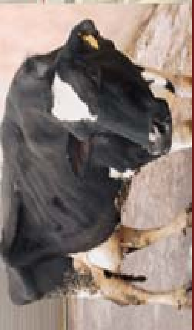
Blood BHB > 13,6 x Milk BHB

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

Correction between Milk and Blood BHB is very variable (0 to 0,87)
Enjalbert et al.
Journal Dairy Science, 2001 84:583-589



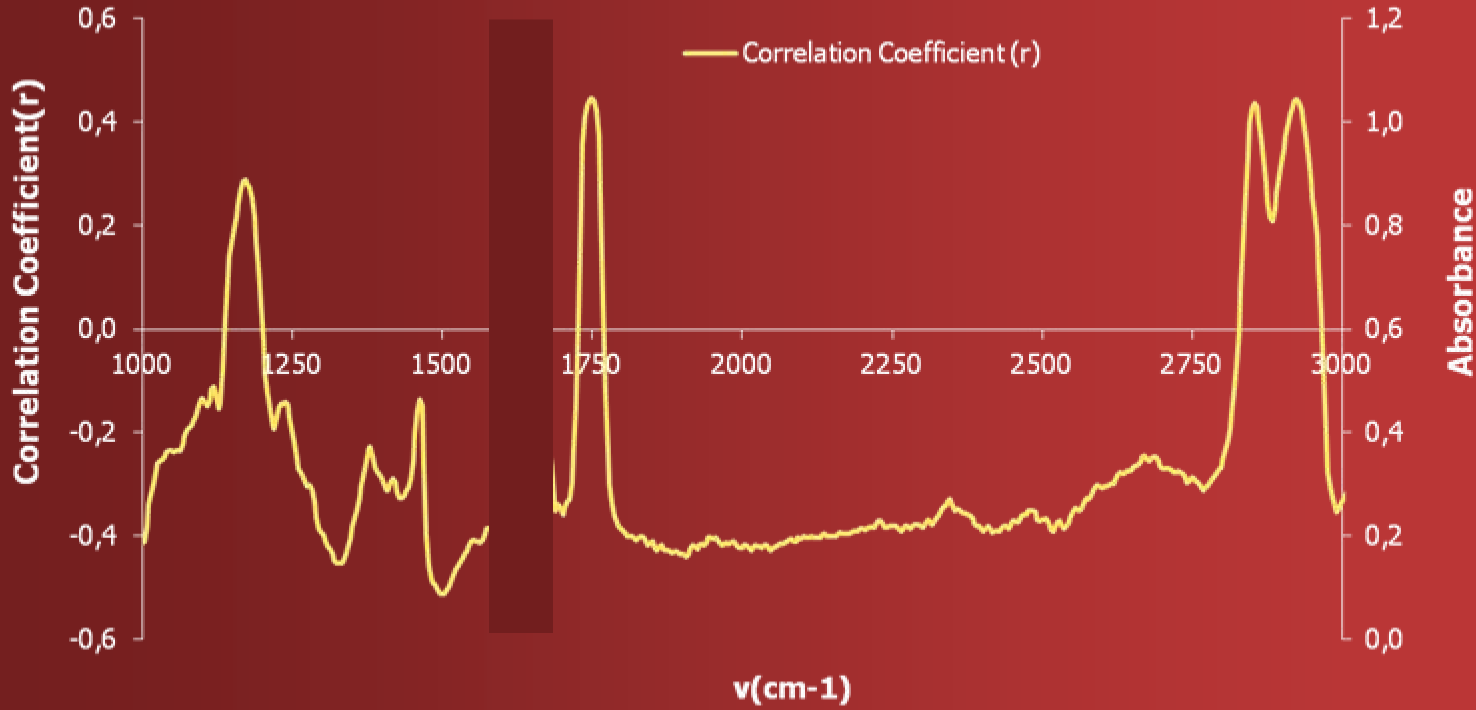


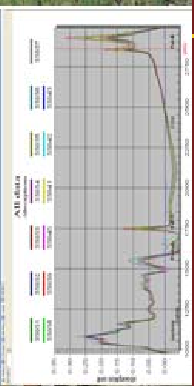


Blood BHB Determination by infrared spectroscopy for the monitoring of cows metabolic activity and detection of subclinical ketosis

How to model and predict Blood BHB value? The fundamentals

Correlation spectrum between Blood BHB Content and Milk Composition (696 samples)

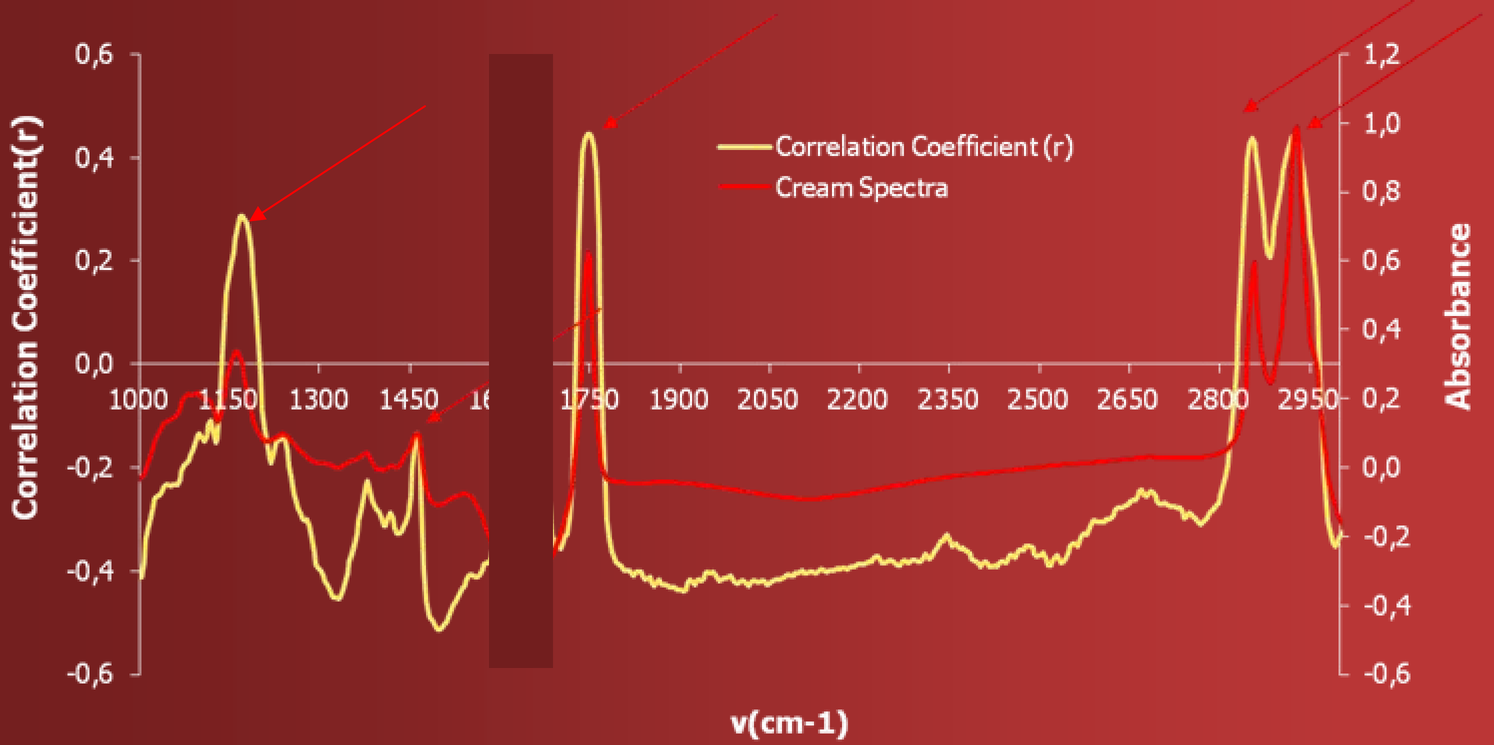




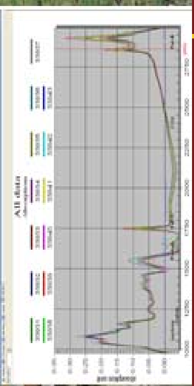
Blood BHB Determination by infrared spectroscopy for the monitoring of cows metabolic activity and detection of subclinical ketosis

How to model and predict Blood BHB value? The fundamentals

Comparison of Blood BHB/Milk Correlation spectrum and cream(fat) spectrum



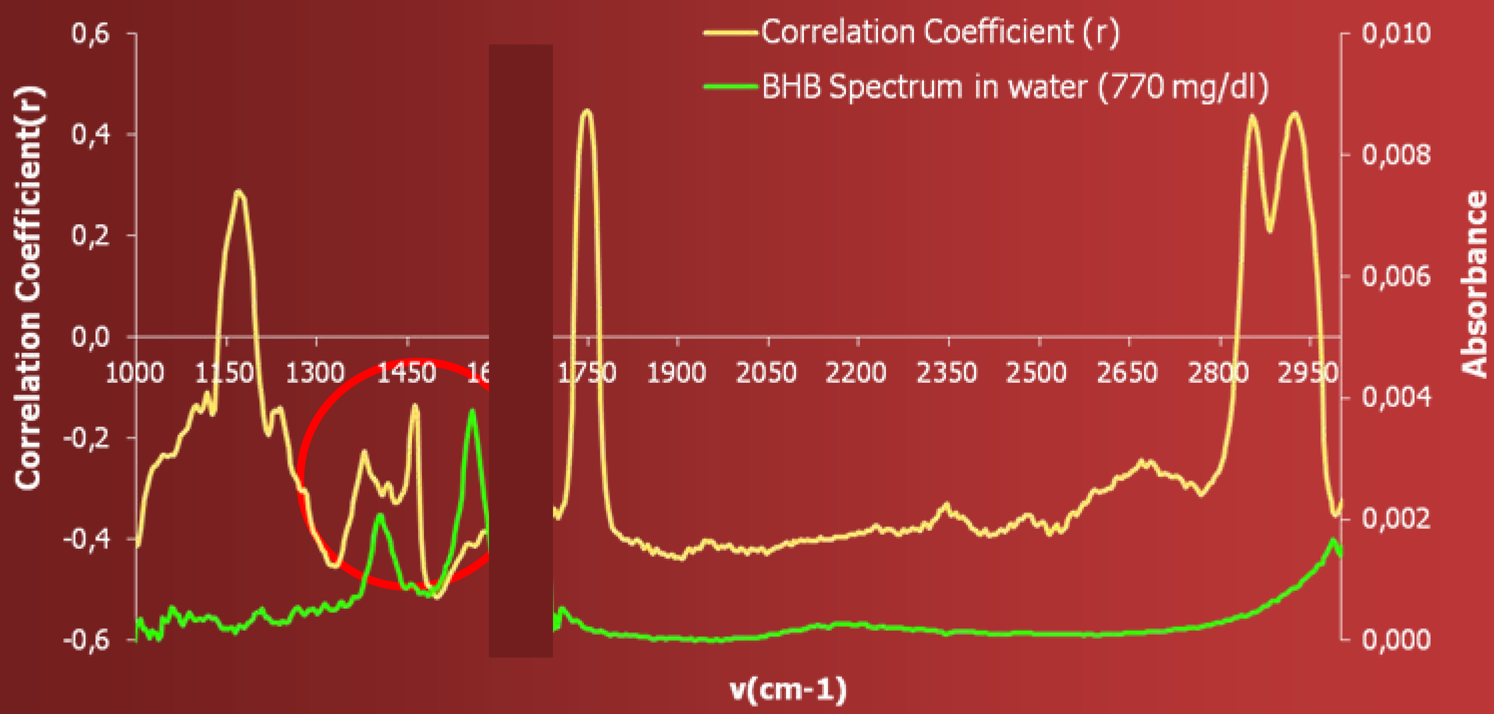
Cc: Blood BHB is mostly correlated to the milk fatty acids profile



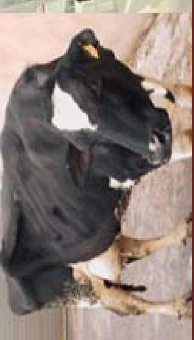
Blood BHB Determination by infrared spectroscopy for the monitoring of cows metabolic activity and detection of subclinical ketosis

How to model and predict Blood BHB value? The fundamentals

Blood BHB/Milk Correlation spectrum and BHB spectrum



Cc: Blood BHB is mostly correlated to the milk fatty acids profile, not to milk BHB

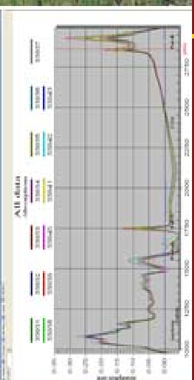


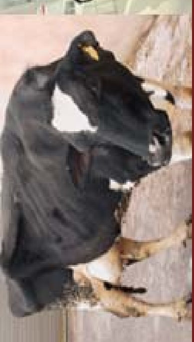
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Milk components most correlated with Blood BHB (696 samples)?

	r/BBHB(Optium)	BBHB <1,2	BBHB >1,2*	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)



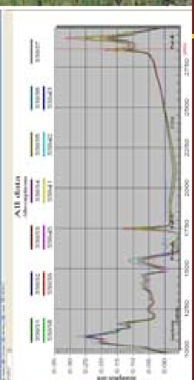


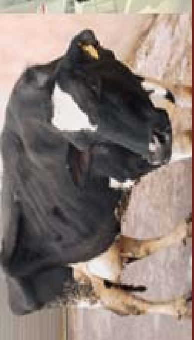
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Milk components most correlated with Blood BHB (696 samples)?

	r/BBHB(Optium)	BBHB <1,2	BBHB >1,2*	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)



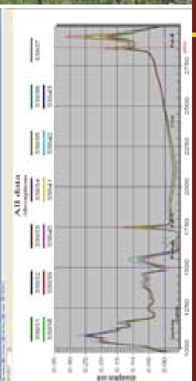


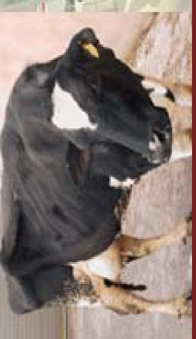
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Milk components most correlated with Blood BHB (696 samples)?

	r/BBHB(Optium)	BBHB <1,2	BBHB >1,2*	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%↑**

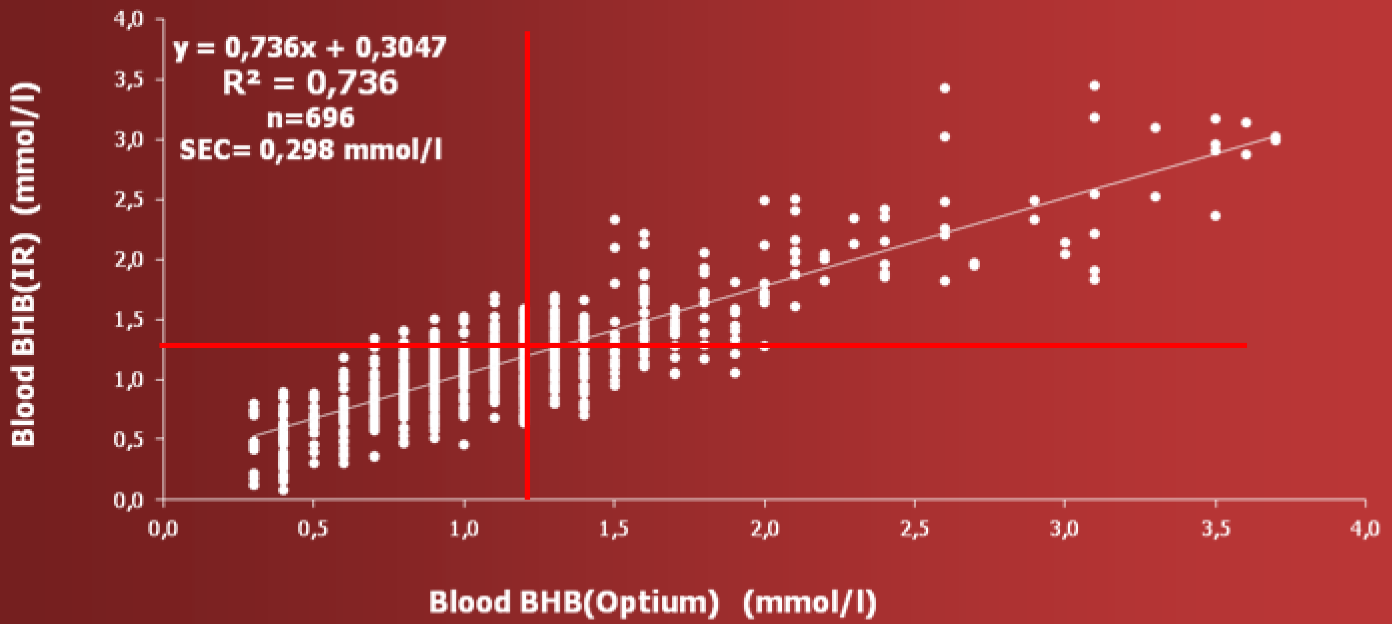
More research under way: 416 fatty acids in bovine milk (Jensen, 2002)



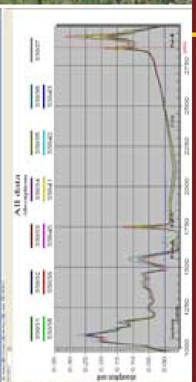


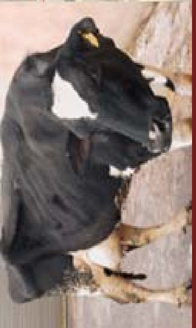
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Blood BHB Infrared PLS Calibration (696 cow milk samples)



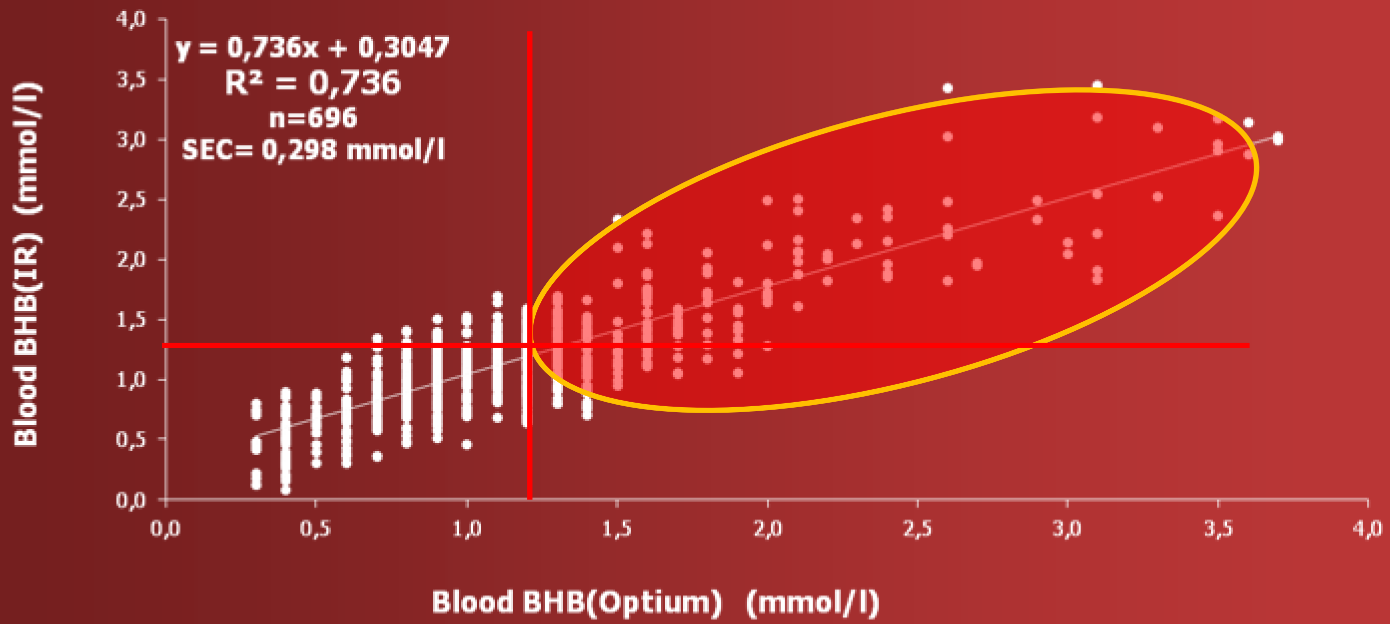
My =1,15 mmol/l (12 mg/dl)
⇒ Milk BHB < 1,0 mg/dl!!



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

Blood BHB Infrared PLS Calibration (696 cow milk samples)

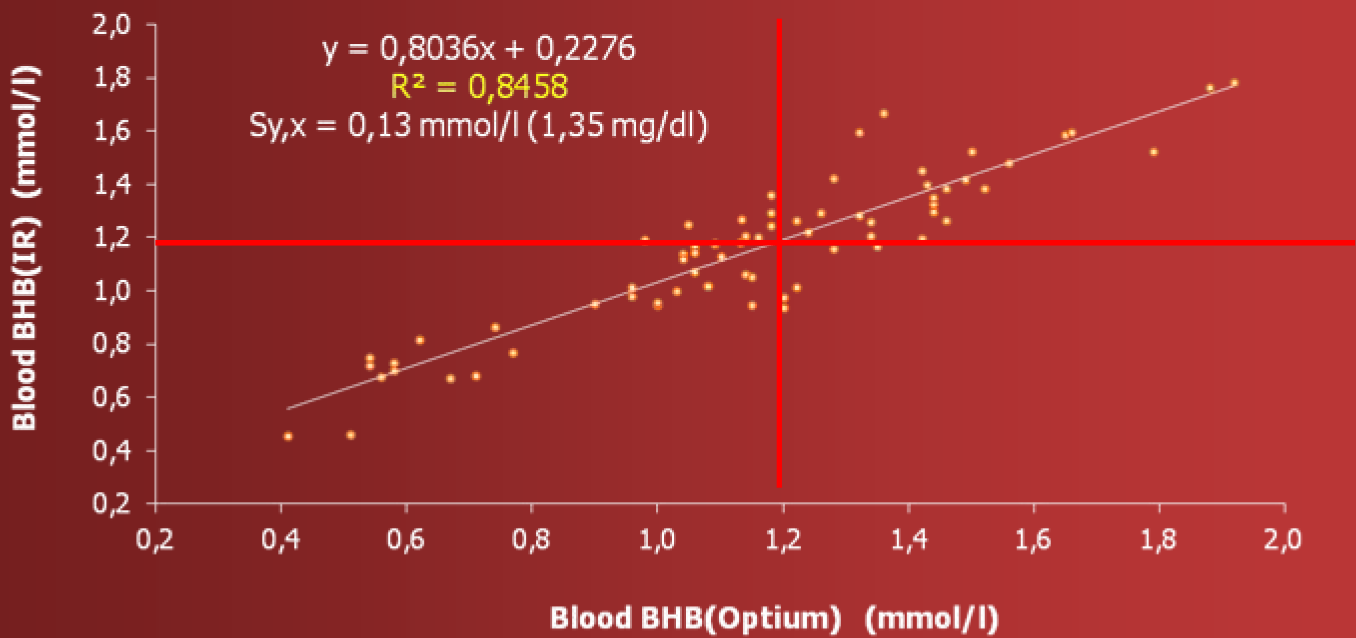


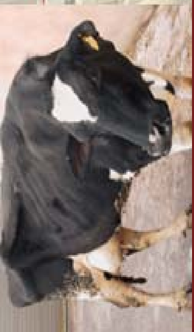
My =1,15 mmol/l (12 mg/dl)
⇒ Milk BHB < 1,0 mg/dl!!

Blood BHB determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Blood BHB Infrared Calibration (at herd level)

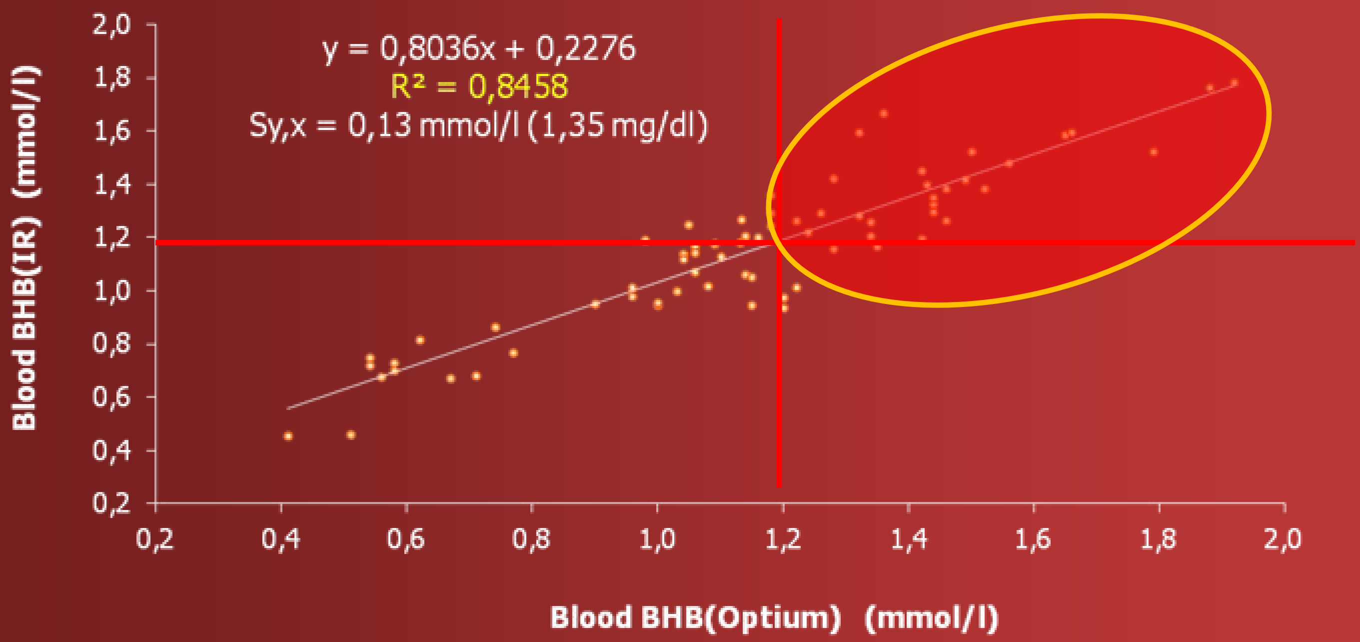


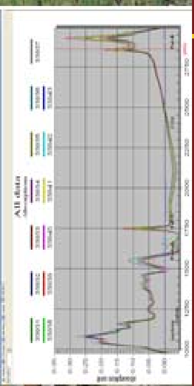



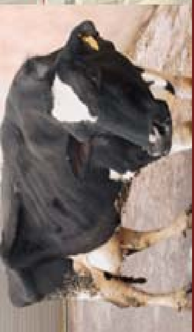


Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

Blood BHB Infrared Calibration (at herd level)

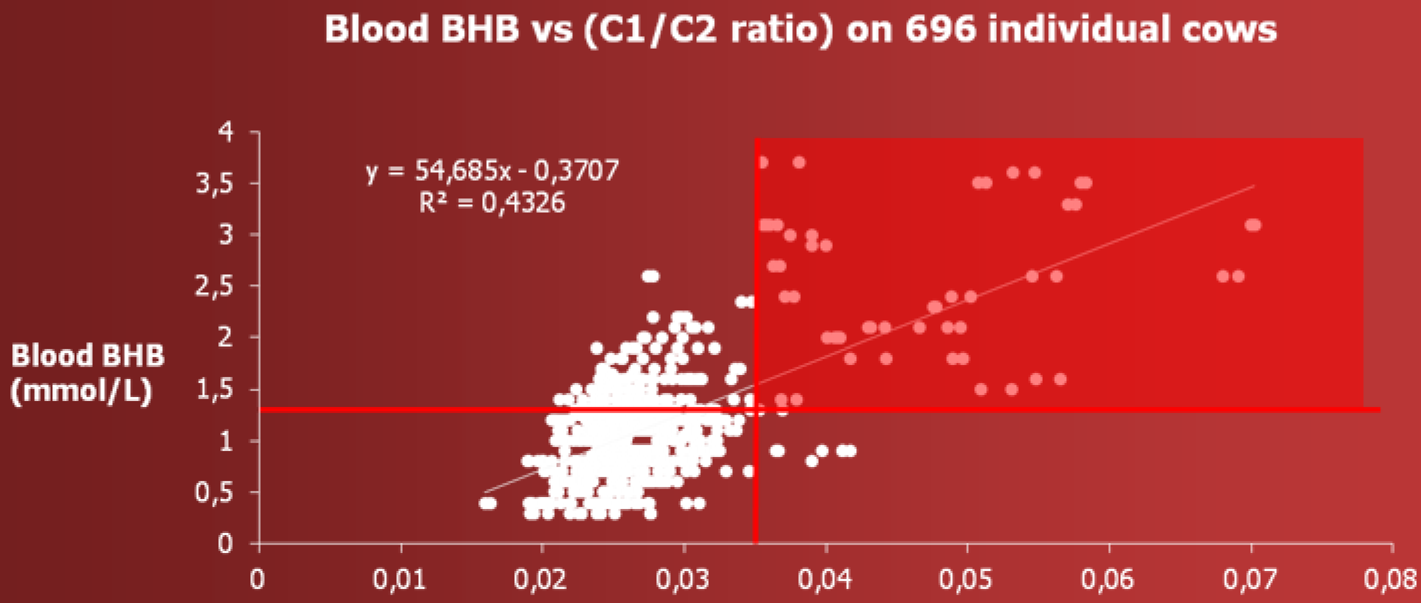


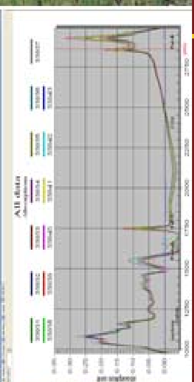


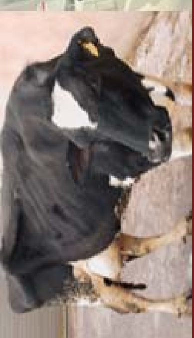
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

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



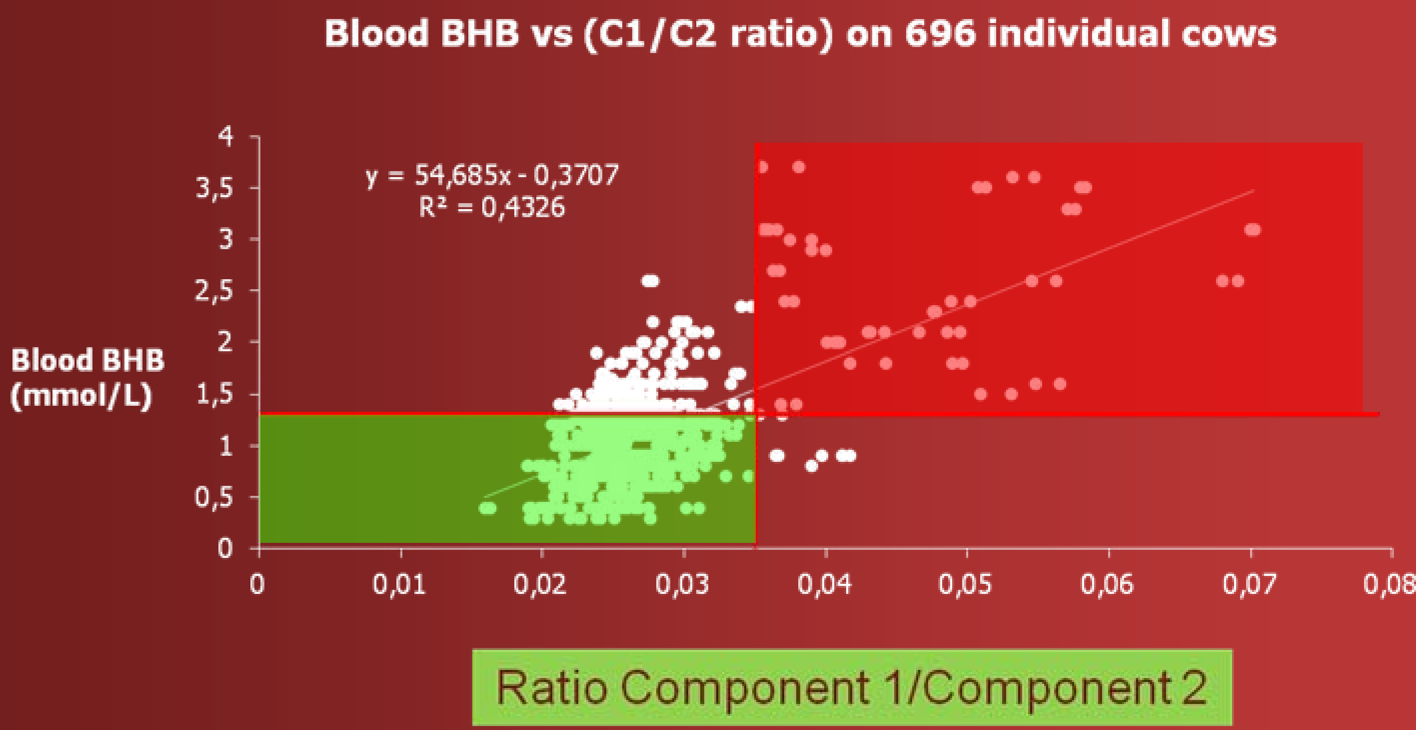
Ratio Component 1/Component 2

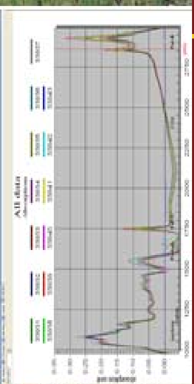


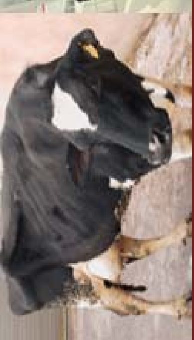
Blood BHB Determination by infrared spectroscopy for the monitoring of the cows metabolic activity and detection of subclinical ketosis

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



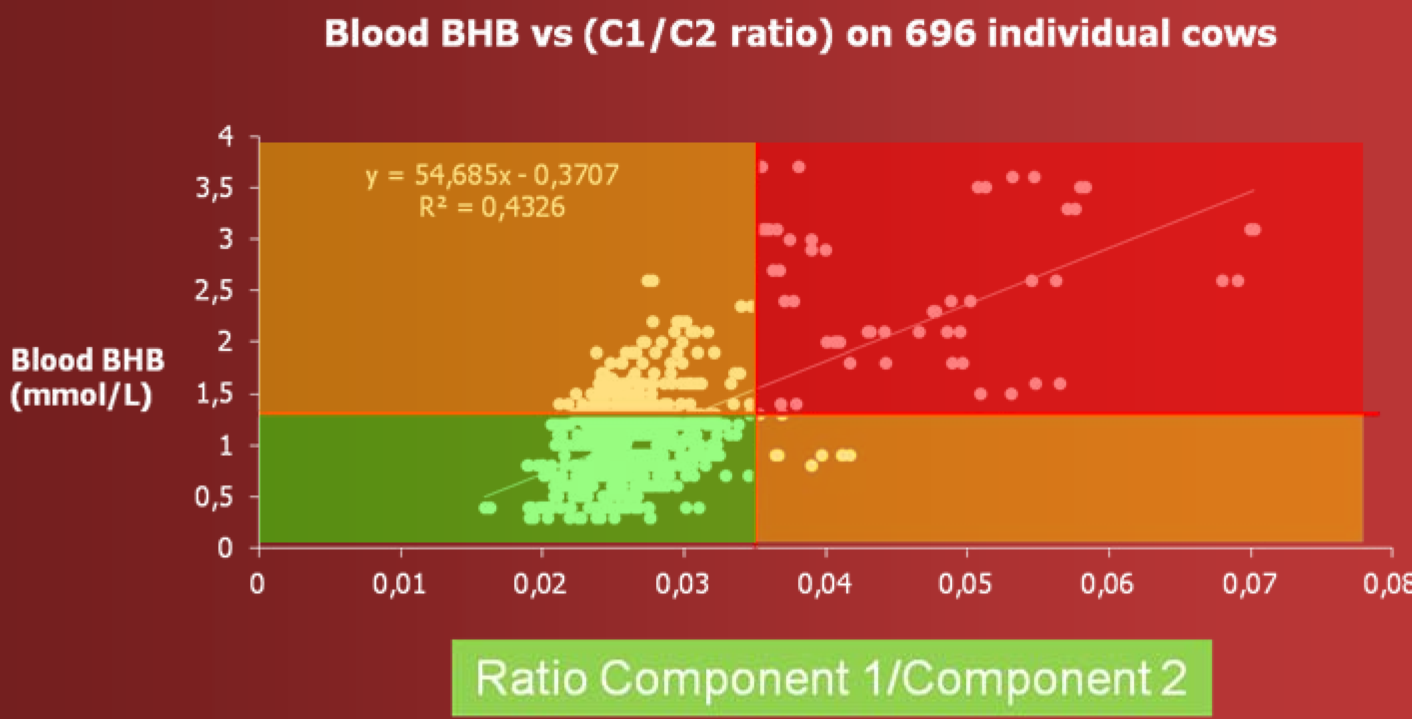
2015 ICAR Technical Workshop
10-12 June 2015, Krakow, Poland





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

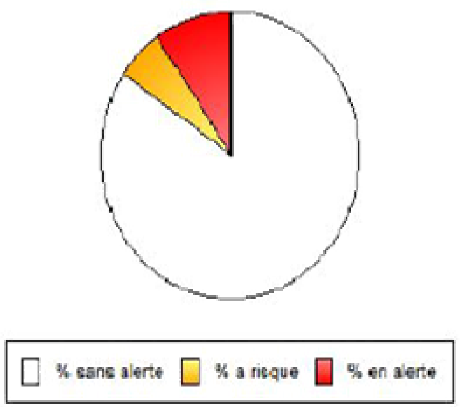
How to improve the ketosis detection? A multi-dimension approach

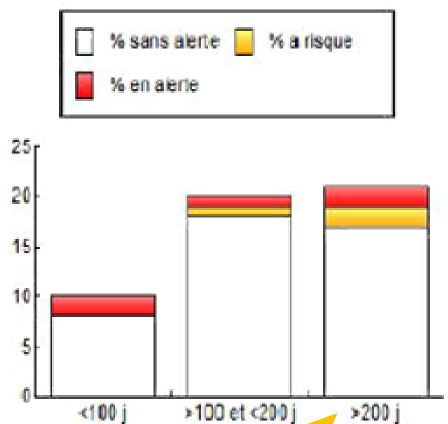


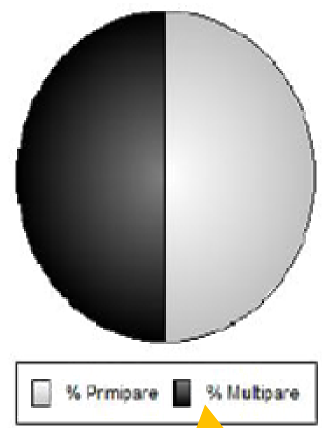
Intra-herd valorisation

Results interpretation for the entire herd

	Nb of LC	% of herd	Primipares		Multipares		Lactation stage	Milk	Fat	TP	SCC
			%	Nb.	%	Nb.					
LC without alert	44	85	48	21	52	23	6.3	32	34.3	32.2	443
LC at risk	3	6	67	2	33	1	9.5	32.3	37.7	33	228
LC on alert	5	10	40	2	60	3	6.7	38	35.9	29.7	100
	52		Moyenne troupeau				6.5	32.6	34.7	32	392







Fraction of the cows at risk within the herd

Repartition of LC at risk and on alert per stage of lactation

Proportion of primipares and multipares on alert

Valorisé Intra – Troupeau (2)

Interprétation des résultats du contrôle pour les lactations (<100 jours)

	Nombre de VL	% du troupeau	Primipares		Multipares		Jour de lactation	Lait	TB	TP	Leuco
			%	Effectif	%	Effectif					
VL sans alertes	8	15	50	4	50	4	59	36.3	31.9	30.2	1129
VL à risque	0	0	0	0	0	0	0	0	0	0	0
VL en alerte	2	4	0	0	100	2	58	55	33.3	27.6	26
	10		Moyenne				58	40	32.3	29.5	826

Interprétation des résultats du contrôle pour les lactations (>100 et <200 jours)

	Nombre de VL	% du troupeau	Primipares		Multipares		Jour de lactation	Lait	TB	TP	Leuco
			%	Effectif	%	Effectif					
VL sans alertes	18	35	50	9	50	9	158	32.9	34.1	31.9	203
VL à risque	1	2	100	1	0	0	199	38	33.4	31	499
VL en alerte	1	2	100	1	0	0	197	30	42.1	32.4	35
	20		Moyenne				162	33	34.4	31.9	212

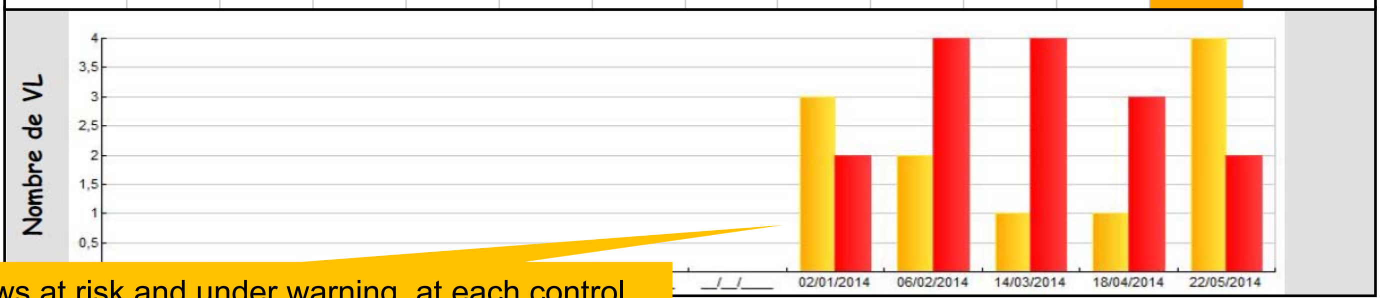
Interprétation des résultats du contrôle pour les lactations (>200 jours)

	Nombre de VL	% du troupeau	Primipares		Multipares		Jour de lactation	Lait	TB	TP	Leuco
			%	Effectif	%	Effectif					
VL sans alertes	17	33	41	7	59	10	287	28.8	35.7	33.7	345
VL à risque	2	4	50	1	50	1	334	29.5	40.4	34.2	54
VL en alerte	2	4	50	1	50	1	357	25	38	32.8	302
	21		Moyenne				298	28.5	36.4	33.7	313

Report over a period

N° animal								02/01/2014	06/02/2014	14/03/2014	18/04/2014	22/05/2014	Ratio
6206015405								303	338	374			1/3
6206015406								157	192	228	263	297	0/5
6206142155								399			18	52	2/3
6206142159								13	48	84	119	153	3/5
6206142162								291	326			22	1/3
6206142168								373	408		11	45	2/4
6206142169								201	236	272	307	341	0/5
6206142170								232	267	303	338	372	0/5
6206142172								352	387	423	458	492	0/5
6206142176								290					0/1
6206142178								160	195	231	266	300	0/5
6206142180								122	157	193	228	262	0/5
6206272184									29	65	100	134	1/4
6206272188								334	369				0/2
6206272189								26	61	97	132	166	0/5
6206272190									35	71	106	140	1/4
6206272197									23	59	94	128	1/4
6206272200								333	368	404	439	473	1/5

Days in lactation



Number of cows at risk and under warning at each control

PEP BOVINS LAIT

Pôles d'Expérimentation et de Progrès de Rhône-Alpes

I D E C

Indicateur Déficit Energétique/Cétose

Eric Bertrand / Patrice Dubois (FIDOCL)
Laurent Alves / Loïc Commun (VetAgroSup)

Experimental Protocol

- 120 herds from FIDOCL (40 from Savoie region)
- 700 cows in early lactation
- 4 breeds (THAM)

7 DIM < VL < 50 DIM

<u>Milk :</u> 3 vials (35 mL)

For : Fat + Protein + SCC + Urea + Milk BHB (Optium)

<u>Blood test (Optium)</u>

For : BHB + sugar

<u>Animal Observations</u>

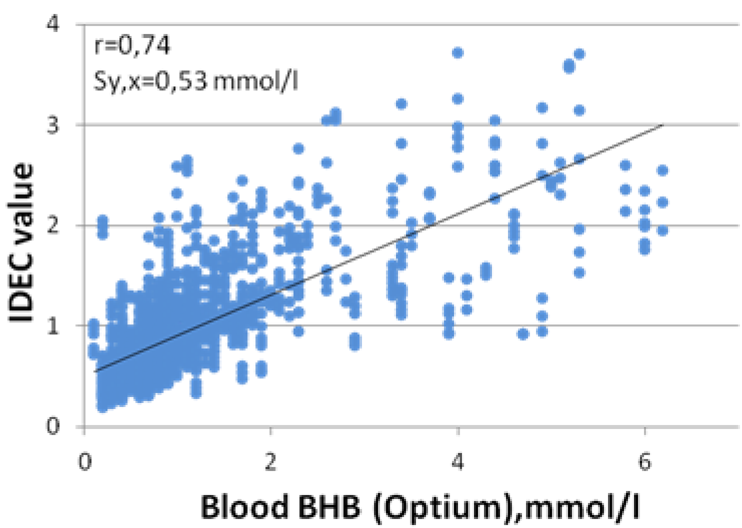
RR, NEC, Locomotion, Minor signs

Results

MIR Analyses : Prediction of the energy deficit (ketosis)

- Prediction of ketosis directly from the Blood BBHB calibration:
  - **Correlation IDEC MIR /Blood BHB(Optium) on single cows**
    - r = 0,74; Sy,x = 0,53 mmol/l
  - **Correlation IDEC MIR /Blood BHB(Optium) at herd level better:**
    - r = 0,85 but still need to be optimized

Correlation between Blood BHB(Optium) and IDEC value (MIR)



Original and promising approach

- Using blood BHB as reference
- More stable
- Multi breeds
- Multi feeding program (pasture,…)

Comparison of the IDEC(MIR) value and standard indicators

Test positive if	Sensitivity	Specificity
Fat/Protein > 1,4	53%	80%
Fat-Protein > 10	62%	72%
Fat > 45	30%	91%
Blood BHB (MIR) (IDEC > 1,3)	**78 %**	**86%**

IDEC $_{MIR}$:better test

INRA

BENTLEY INSTRUMENTS

RhôneAlpes Région

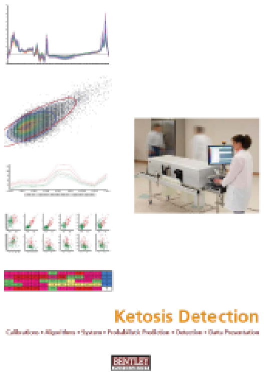
FIDOCL CONSEIL ELEVAGE

VetAgro Sup

Probabilistic prediction of ketosis.

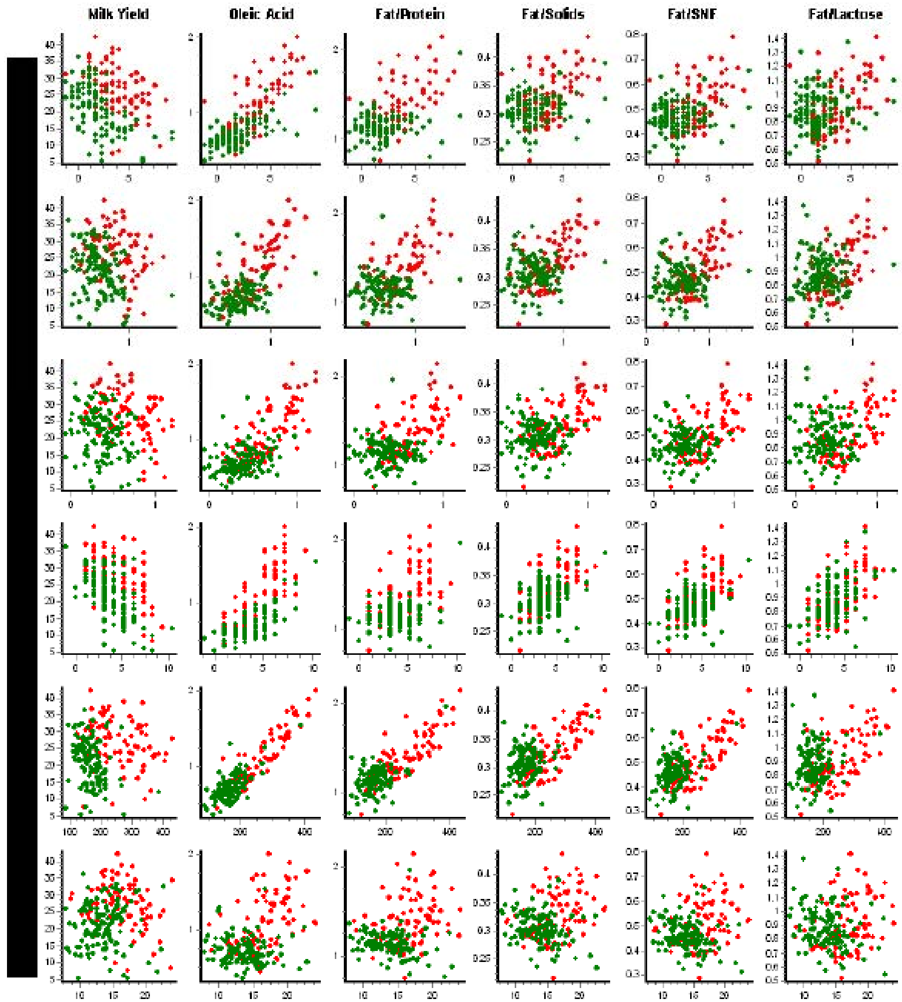
(pat. pending)

- **Elimination of sampling, farm, season effect but also robustness**
  - Sophisticated prediction function
- **Calculated and high probability of ketosis detection**
  - Including calibrations limitations, limitations of parameters used for on-farm detection
- **Special data presentation method**
  - Easy-to-use farm report
  - Historical overview
  - Multidimensional data presentation



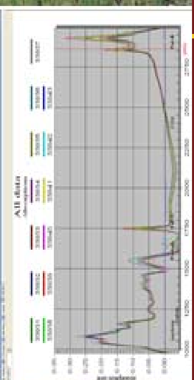
System for complete solution using Bentley Instruments equipment

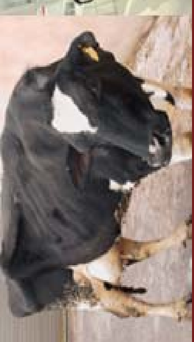
Examples – keton bodies vs. milk components



Examples – individual prediction of ketosis

ID	OA	F/P	F/S	F/SNF	F/L	[redacted]						Lact. Day
CZ000[redacted]	0.87	1.32	0.32	0.48	0.84	2.90	13.58	10.03	3.12	208.20	17.67	46
CZ000[redacted]	2.00	2.13	0.44	0.79	1.41	7.55	17.91	8.90	7.29	434.10	17.31	27
CZ000[redacted]	0.82	1.26	0.29	0.43	0.72	1.16	8.16	3.77	3.12	234.23	18.35	54
CZ000[redacted]	0.63	1.13	0.30	0.44	0.82	0.58	3.56	2.57	3.12	150.95	18.29	74
CZ000[redacted]	1.08	1.51	0.34	0.53	0.91	2.32	5.30	3.68	7.29	295.64	24.08	38
CZ000[redacted]	0.81	0.98	0.27	0.39	0.73	3.49	4.03	2.95	3.12	163.44	11.45	67
CZ000[redacted]	1.54	1.63	0.38	0.64	1.18	4.07	13.21	8.94	7.29	342.49	20.12	13
CZ000[redacted]	1.70	1.90	0.41	0.71	1.27	5.23	17.93	7.33	5.20	368.51	19.73	10
CZ000[redacted]	1.35	1.69	0.37	0.59	1.02	4.07	6.31	3.62	6.25	339.37	18.05	30
CZ000[redacted]	1.14	1.45	0.37	0.61	1.20	-1.16	4.26	3.21	3.12	195.71	14.31	8
CZ000[redacted]	1.68	1.93	0.40	0.67	1.15	3.49	16.11	8.13	7.29	410.15	20.20	46



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

Conclusions:

-Blood BHB determination by IR is a new and more exhaustive approach than current infrared methods. It takes into account all the parameters connected with ketosis and energy deficit, not only the ketone bodies

-Multi dimensional and probabilistic approaches combining Blood BHB and other parameters such Oleic Acid (or other FA), OA/Protein,citric acid, can improve the detection of ketosis

-The Blood BHB approach can be used:

- to monitor individual cows Blood BHB variations over time
- to compare individual cows at the Herd Level to develop a relative approach (intra-herd) – recommended approach
- to detect ketosis prevalence at the herd level (inter-herds)

- More research being done to keep improving this proprietary method and extend its principle to the detection of other metabolic disorders

Thank you for your attention!

pbroutin@bentleyinstruments.com
www.bentleyinstruments.com