



Can we get information on dairy cows chronic stress biomarkers using milk MIR spectra?

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Welfare as hot topic

Le Monde

Belgium enshrines animal welfare in Constitution

Belgium's Constitution now requires all new decisions to take account of their potential impact on animals. The practical implementation will play out at the regional level.

By Elena Louazon

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Dans un abattoir, près de Bruxelles, le 27 juillet 2017. YVES HERMAN / REUTERS

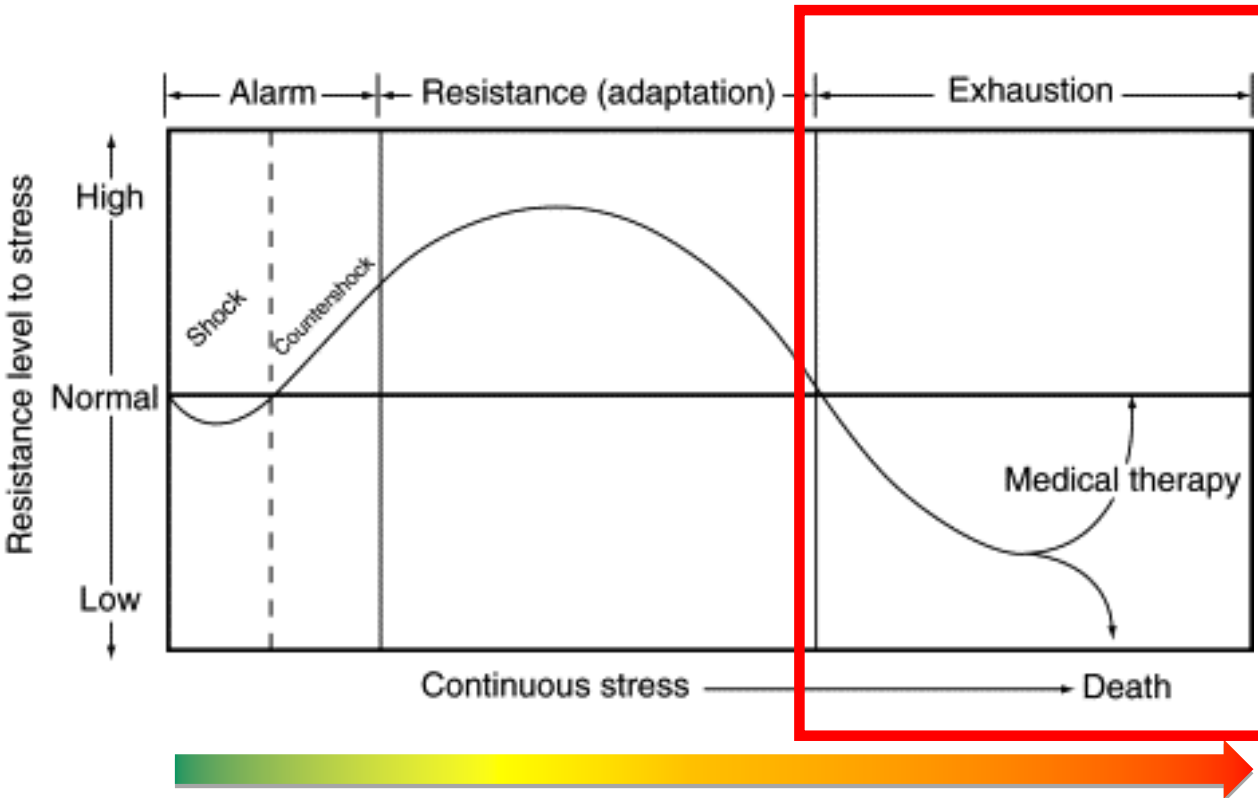
France - Welfare logo on animal products



Also in Germany,
Slovenia, Luxembourg,
Italia, Autstria,
Switzerland, Egypt, Brazil
and India

Chronic stress

“stress is the non-specific response of the body to any demand made upon it” (Selye, 1976)



➤ susceptibility to metabolic, inflammatory and infectious diseases (Moberg et al., 1980; Romero, 2004).

➤ fertility troubles (Dobson and Smith, 2000; Walker et al., 2002).

➤ growth disturbances (Fitzpatrick et al., 2000).

➤ weight (Mormann et al., 2005).

➤ milk production (Mormann et al., 2005).

➤ production and economic viability of farms,

➤ welfare of cows

➤ societal perception of dairy production

No consensus in literature regarding chronic stress biomarkers

2020 experiment : identify chronic stress biomarkers

Control group

- 15 cows
- $>10\text{ m}^2$ per cow
- more feed bunks than cows



Stress group (during 4 weeks)

- 15 cows
- severe overstocking $< 5\text{ m}^2$ per cow
- restricted access to feed (1 feed bunk for 2 cows)
- punctual unusual events



Global measures

- MY
- SCC
- weight
- BCS
- MIR predictions
(milk & blood composition)

Heart monitoring



Blood

 (Glucose, Fructosamin ,T4, β -endorphine, leucocytes)

Behaviour

- observations
- avoidance distance
- rumination
- activity

Saliva

 (cortisol)

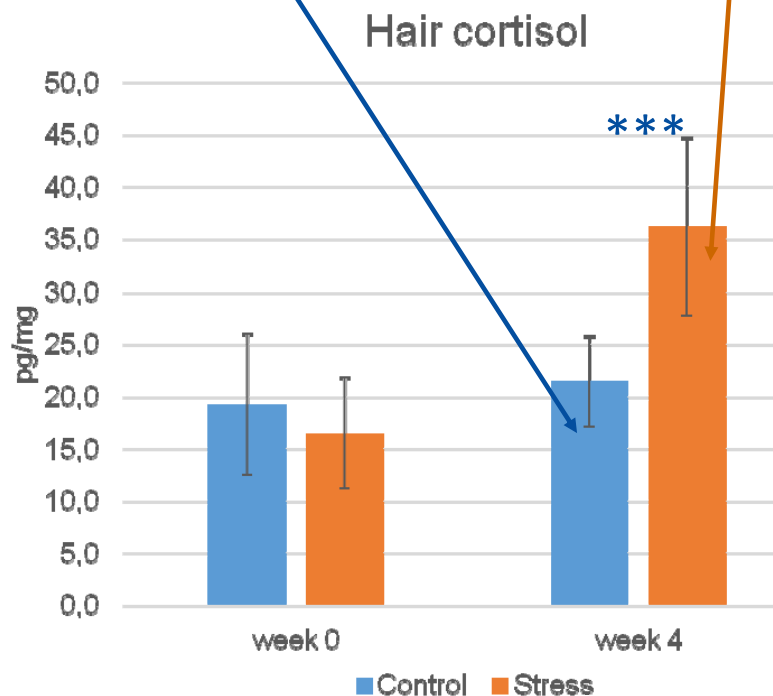
Hair

 (cortisol)

Chronic stress biomarkers

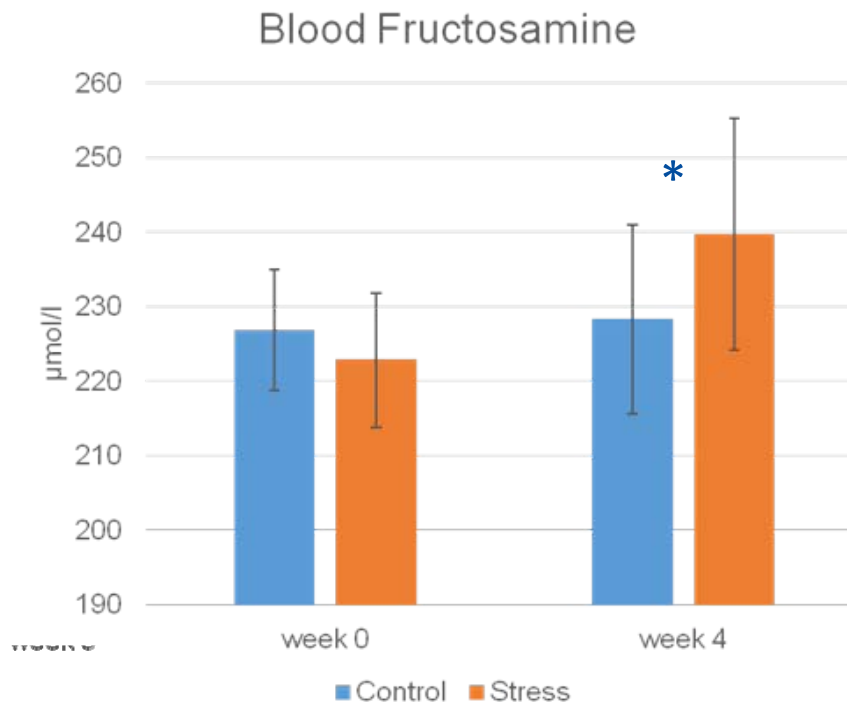


15 Control cows vs 15 Stressed cows (4 week stress, overstocking and unusual events)



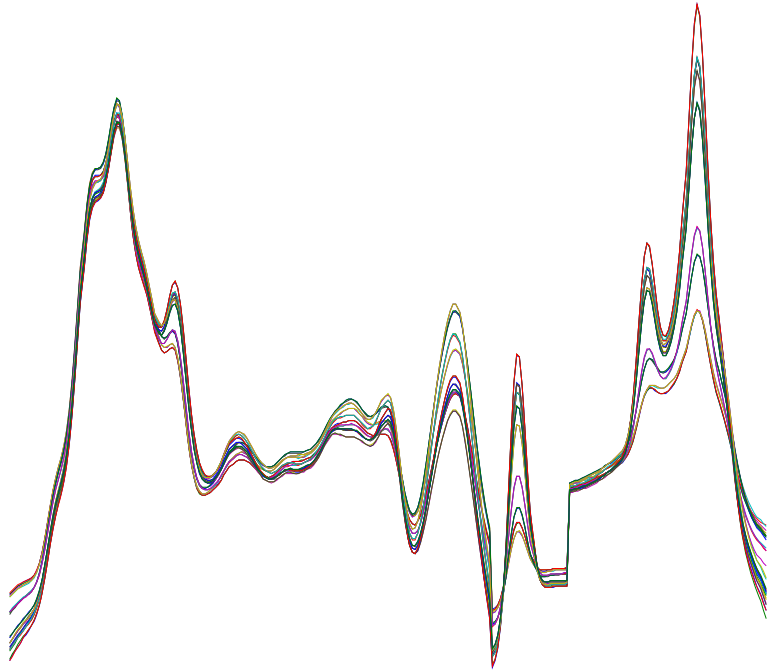
Identification of chronic stress biomarkers in dairy cows

C. Grelet^a, V. Vanden Dries^a, J. Leblois^b, J. Wavreille^a, L. Mirabito^c, H. Soyeurt^d, S. Franceschini^d, N. Gengler^d, Y. Brostaux^d, HappyMoo Consortium^{e 1}, F. Dehareng^a



(*) $P \leq 0.1$
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$

Can we predict Hair Cortisol and Blood Fructosamine with milk MIR spectra???

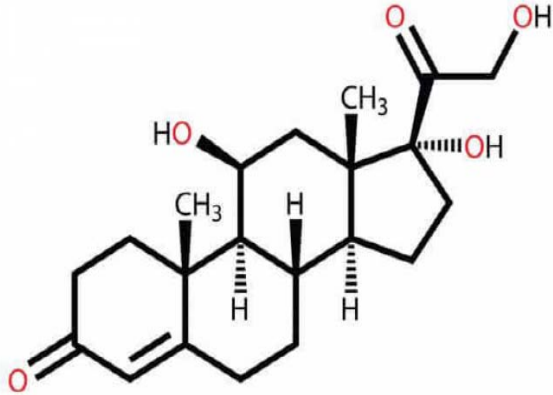


Other indirect phenotypes?

- ✓ Molecules in blood (Luke et al., 2019)
- ✓ Dry Matter intake (McParland et al., 2011)
- ✓ Methane emissions (Dehareng et al., 2012)

Can we predict Hair Cortisol and Blood Fructosamine with milk MIR spectra???

Hair cortisol



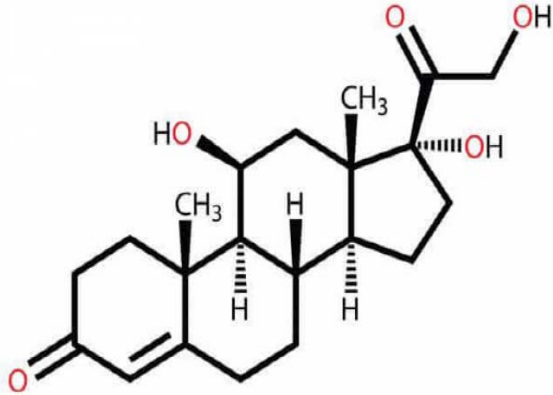
+ Gold standard chronic stress biomarker
al., 2019;

(Comin et al., 2013; Burnett et al., 2015; Heimbürge et Vesel et al., 2020; Tallo-Parra et al., 2017b).

- ~ 20 ng/g
Low probability to have an associated signal in MIR

Can we predict Hair Cortisol and Blood Fructosamine with milk MIR spectra???

Hair cortisol

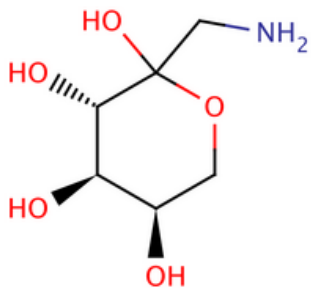


+ Gold standard chronic stress biomarker
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- ~ 20 ng/g
Low probability to have an associated signal in MIR

Blood fructosamine



- Indicator of 3 weeks glycemia, rather « unknown indicator » with few informations

- High: chronic stress impact on energy metabolism (higher content of circulating blood glucose)
- Low: undernutrition, energy deficit, fatty liver in dairy cows (Caré, 2018; Mostafavi, 2014)

+ Linked with energy metabolism: better probability to have information with MIR

Large scale sampling protocol

Countries	Partners	n cows
Austria	LKVAustria	159
	AWE	35
Belgium	CRAW	170
	ILVO	39
France	BCO	104
	Doubs	111
	LTN	117
	PDD	56
	Rhone	55
	Seenorest	131
Seenovia	145	
Germany	LKVBW	180
Luxembourg	Convis	36
		1338



- ✓ 78 herds
- ✓ All parities
- ✓ All lactation stages
- ✓ Grazing and winter seasons
- ✓ Mountain and plain areas
- ✓ 7 Breeds (*Holstein, Crossed, Montbeliarde, Simmental, Vorderwalder, Brown Swiss, Jersiaise*)

Large scale sampling protocol

Recommendations

- 25% cows with good welfare
- 75% cows potentially suffering from chronic stress for at least one month

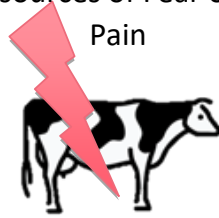
Overstocking



Chronic diseases



Permanent sources of Fear or Pain



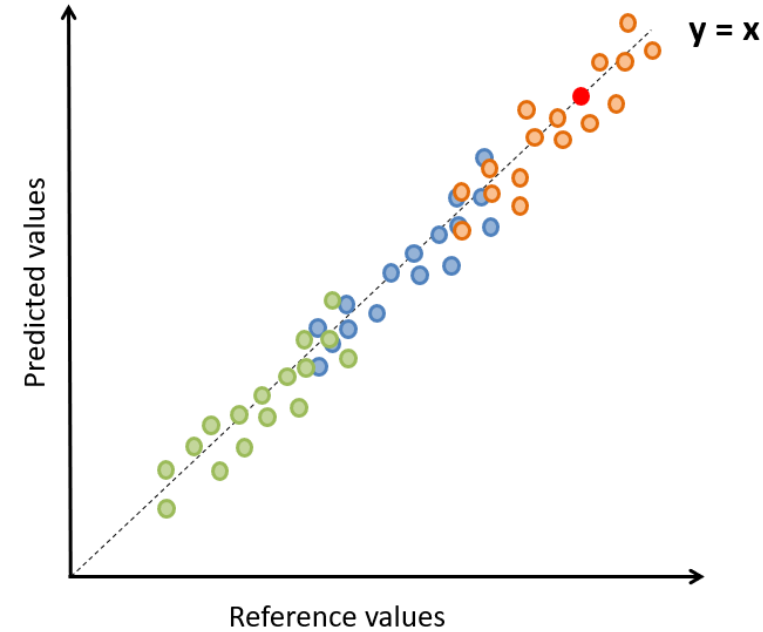
Environmental discomfort



Limited access to food or water



Inadequate barn design or location



Large scale sampling protocol

At the same the day (or day before)

- ✓ 1 milk sample
- ✓ 1 blood sample
- ✓ 1 hair sample



Milk sample for MIR analysis

Analyzed locally on 22 MIR instruments (15 Foss, 2 Delta, 5 Bentley)

Standardized with CRA-W/EMR method



Blood sample for fructosamine analysis

Tail vein (*vena caudalis*) with Yellow dry tubes (*Serum separating gel*)

Centrifugation (2500 g for 10 min at 2-8°C)

Serum pipetting

Analysis at Synlab (Liège, Belgium) with spectrophotometric methods (Westgard et al., 2017)



Large scale sampling protocol

Hair sample for cortisol analysis

Hairs collected at the tail switch

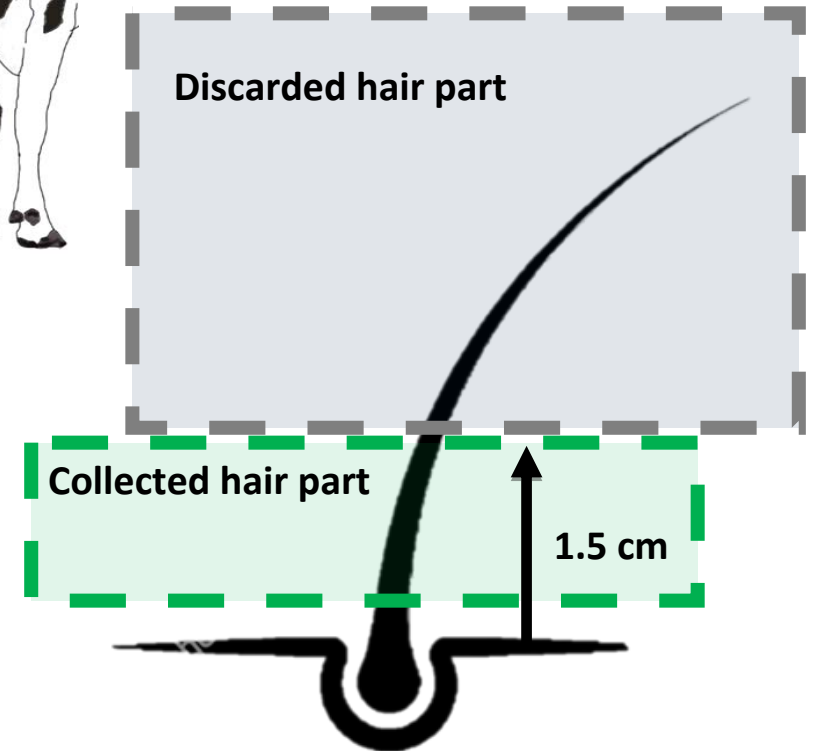
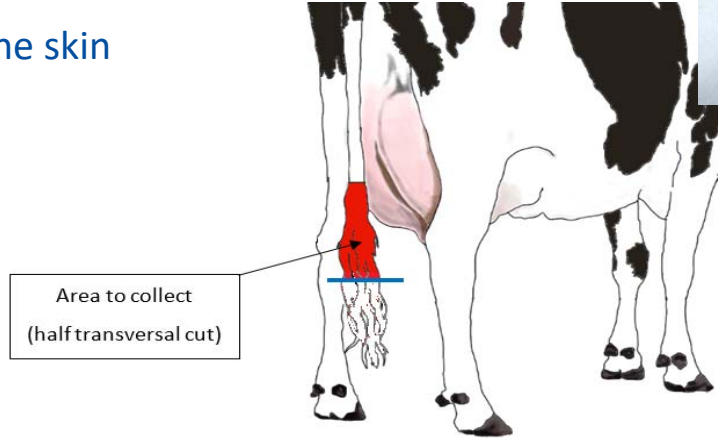


Collected part: 1.5 cm starting from the skin
(corresponding to 1 month growth)

250 mg

Analysis at CRA-W

- Sieving
- Cleaning
- Grinding
- Extraction
- Elisa test (Salimetrics extended range)



Data editing

Reference data cleaning

- Hair cortisol CV < 12%
- Blood hemolysis ≤ 2
- DIM < 365
- Fructosamine $100 < X < 300$
- Missing values

Spectral data cleaning

- No GH threshold
- Fat differences between predictions and lab analysis < 0.3 g/100ml

Data handling

- Breed: dummy variable with Holstein (1) or others (0)
- Hair color: dummy variable with dark (1) or light hairs (0)

After editing
n=1004 for fructosamine
n=1104 for cortisol

Quantitative models

- MIR (212 wavenumbers and first derivative)
 - +DIM
 - +DIM+DIM²
 - +DIM+DIM²+parity
 - +DIM+DIM²+parity+parity²
 - +DIM+DIM²+parity+parity²+MY
 - +DIM+DIM²+parity+parity²+MY+breed
 - +DIM+DIM²+parity+parity²+MY+breed+color

- [MIR] reduced in 12 or 14 LV after PLS
 - +DIM
 - +DIM+DIM²
 - +DIM+DIM²+parity
 - +DIM+DIM²+parity+parity²
 - +DIM+DIM²+parity+parity²+MY
 - +DIM+DIM²+parity+parity²+MY+breed
 - +DIM+DIM²+parity+parity²+MY+breed+color

Combined with :

✓ PLS

✓ SVM (support vector machine), better ability to handle non linear data

BUT 212 MIR variables may dilute the other information (Fearn, 2010)

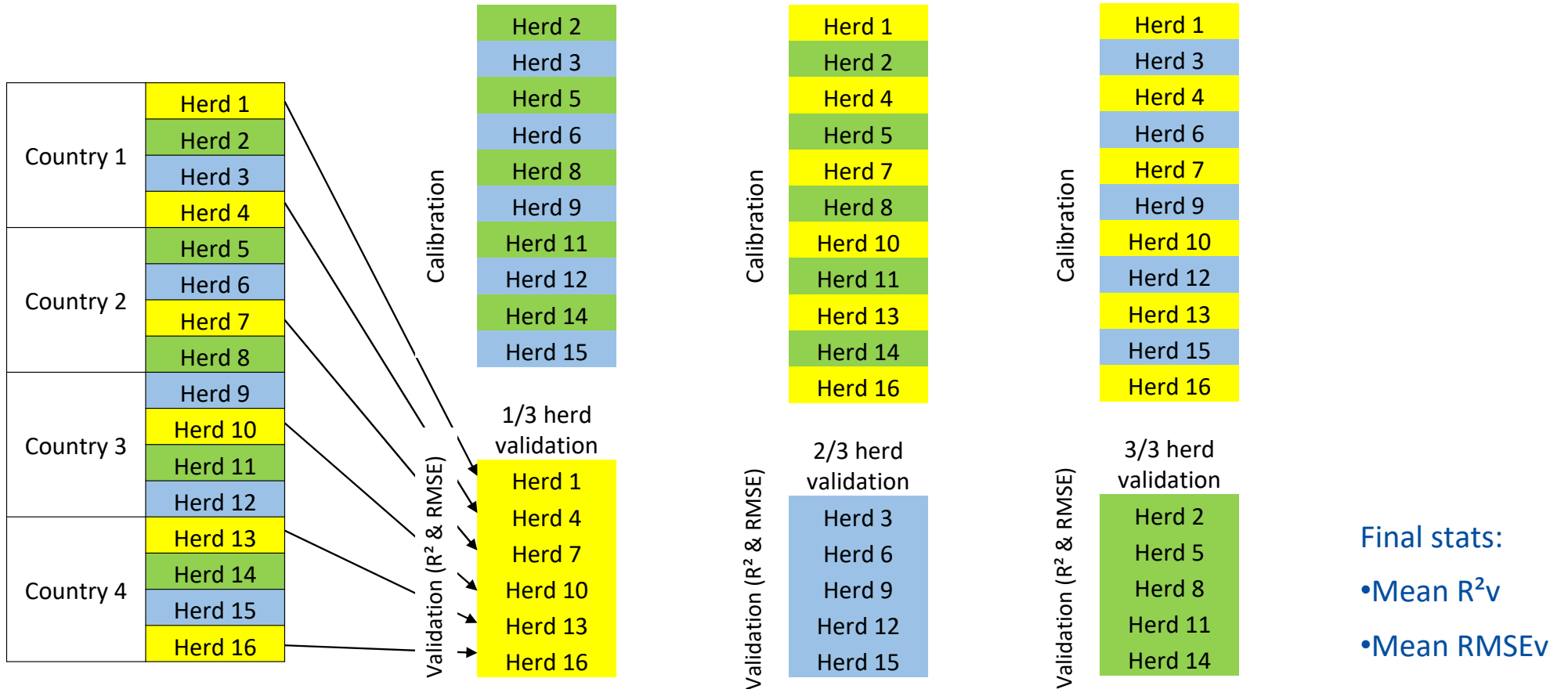
Combined with :

✓ MLR (multiple linear regression)

✓ SVM (support vector machine)

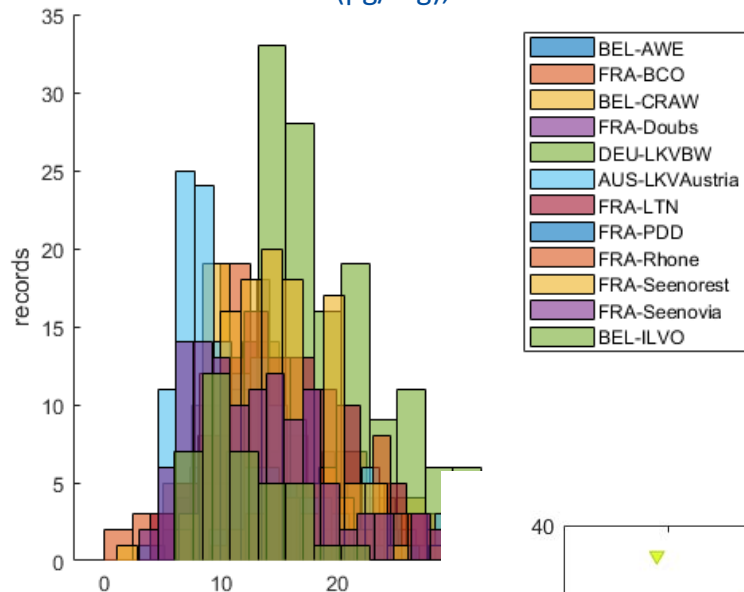
Validation & stats

- External-herd-validation, removing 33% of herds and iterating 3 times the process to validate with all herds

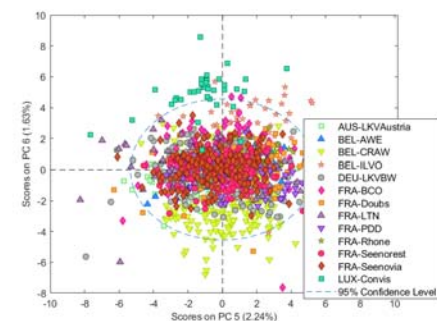
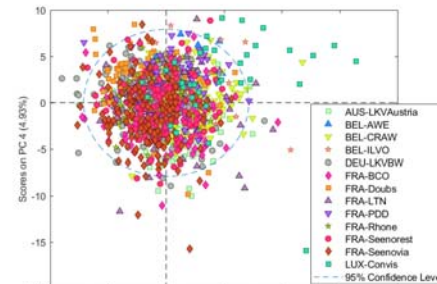
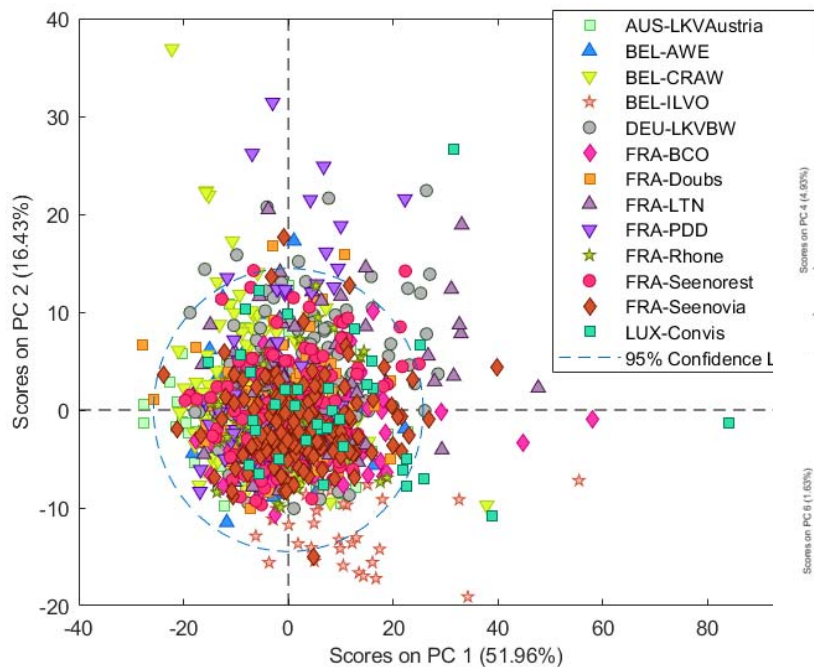
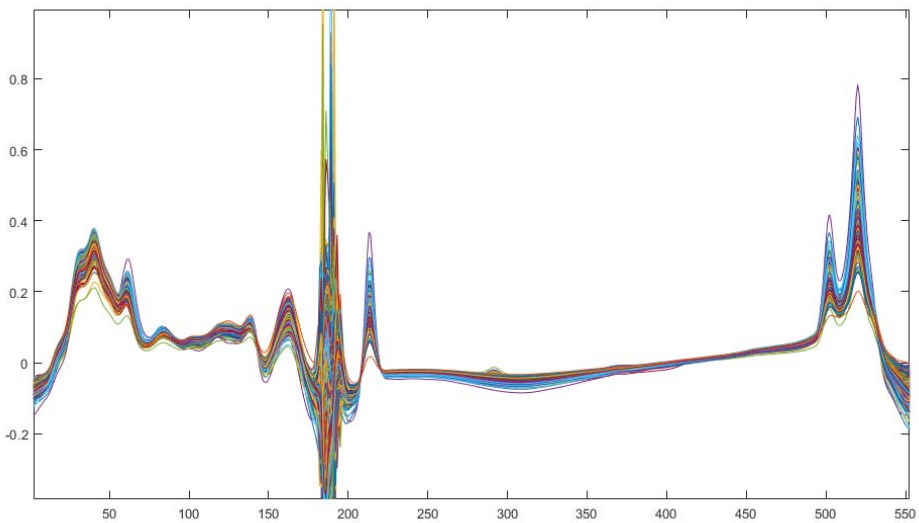
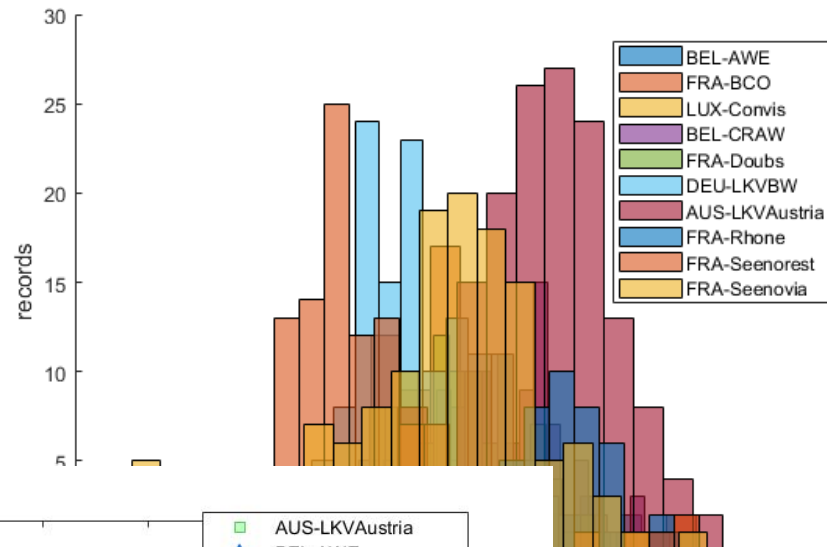


Results

Cortisol (pg/mg), final n= 1071



Fructosamine ($\mu\text{mol/L}$), final n=940

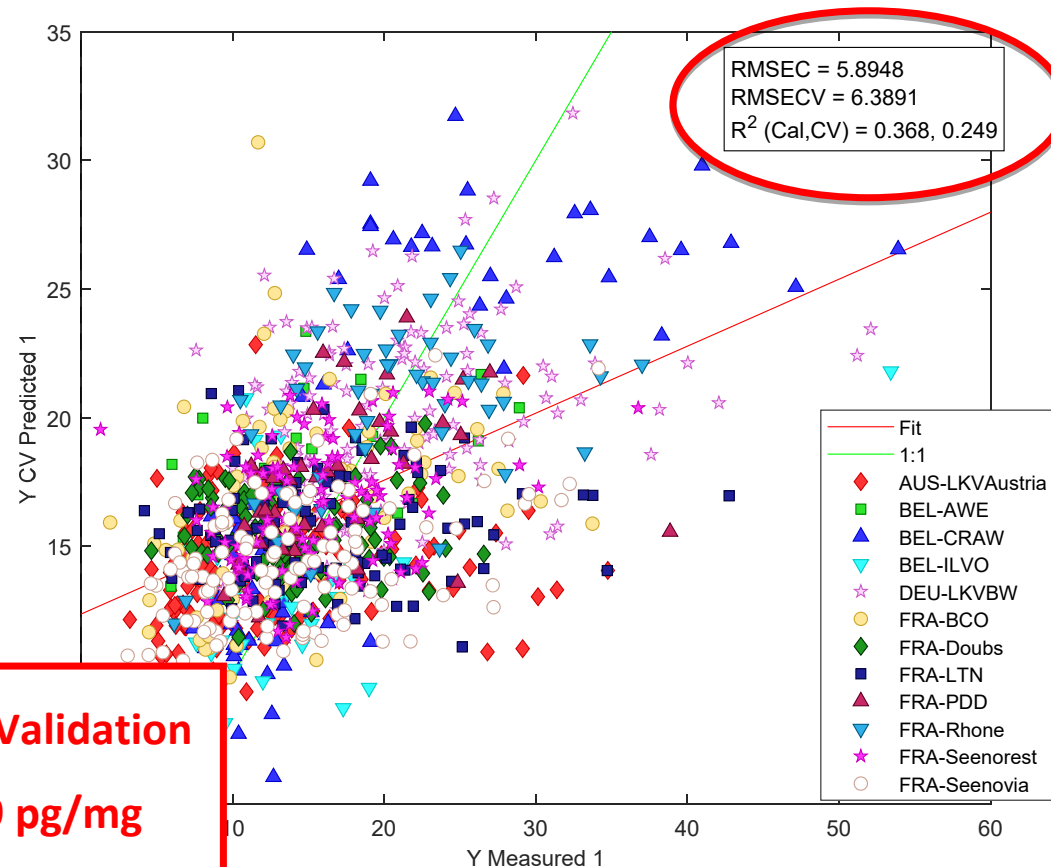


Cortisol quantitative models

SVM on MIR+MY+parity²+DIM+breed+parity+DIM²
n=1071

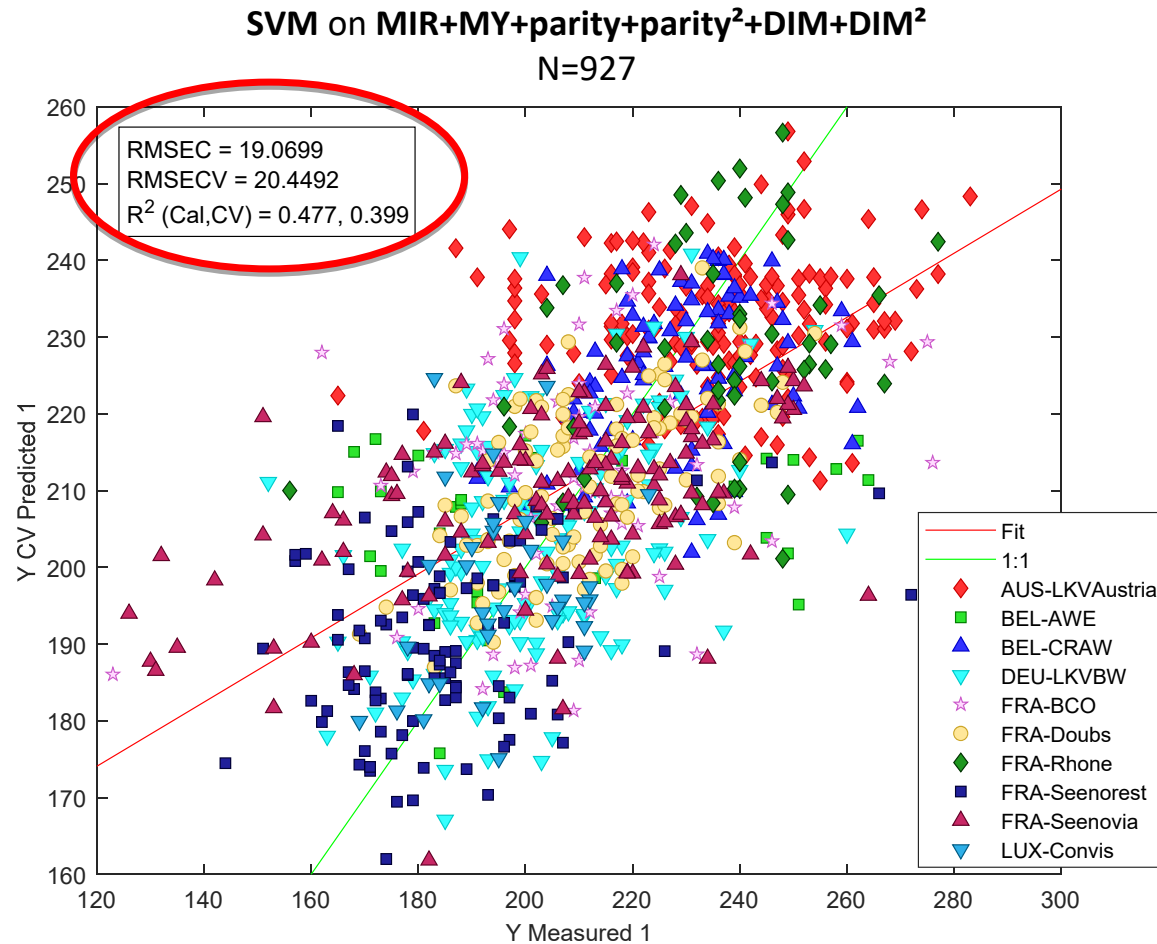
	Calibration RMSE			
	PLS	SVM	MLR-PLS	SVM-PLS
MIR	6.0	5.9	6.0	6.0
MIR+MY	6.0	5.9	6.0	6.0
MIR+MY+parity ²	6.0	5.9	5.9	5.9
MIR+MY+parity ² +DIM	6.0	5.8	5.9	5.9
MIR+MY+parity ² +DIM+breed	6.0	5.9	5.9	5.9
MIR+MY+parity ² +DIM+breed+parity	6.0	5.9	5.9	5.9
MIR+MY+parity²+DIM+breed+parity+DIM²	6.0	5.8	5.9	5.9
MIR+MY+parity ² +DIM+breed+parity+DIM ² +color	6.0	5.7		

	Calibration R ²			
	PLS	SVM	MLR-PLS	SVM-PLS
MIR	0.32	0.35	0.33	0.35
MIR+MY	0.32	0.35	0.33	0.34
MIR+MY+parity ²	0.32	0.36	0.34	0.35
MIR+MY+parity ² +DIM	0.32	0.37	0.34	0.36
MIR+MY+parity ² +DIM+breed	0.33	0.35	0.34	0.35
MIR+MY+parity ² +DIM+breed+parity	0.33	0.37	0.34	0.36
MIR+MY+parity²+DIM+breed+parity+DIM²	0.33	0.37	0.34	0.35
MIR+MY+parity ² +DIM+breed+parity+DIM ² +color	0.33	0.40		



External Herd Validation
RMSE_v = 6.9 pg/mg
R²_v = 0.13

Fructosamine quantitative models



External Herd Validation

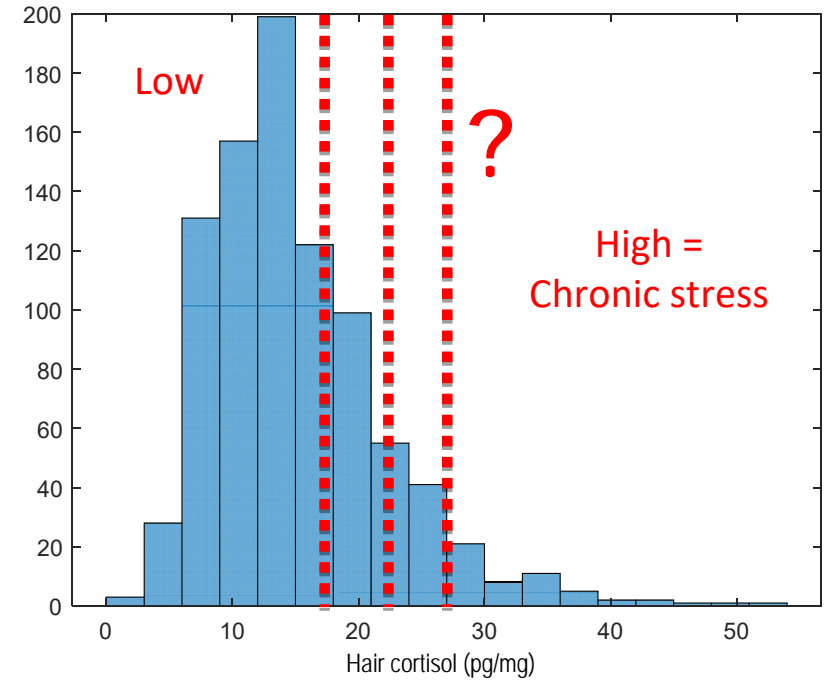
RMSE_v = 25.4 μmol/L

$R^2_v = 0.20$

Discriminant models

- Alternative option, less precise → Low vs High
- But which threshold? No documentation
- Test and optimization of thresholds on model SENSIBILITY
- Mathematical threshold but no physiological meaning!!
- MIR (212 wavenumbers and first derivative)

+DIM
+DIM+DIM²
+DIM+DIM²+parity
+DIM+DIM²+parity+parity²
+DIM+DIM²+parity+parity²+MY
+DIM+DIM²+parity+parity²+MY+breed
+DIM+DIM²+parity+parity²+MY+breed+color



Combined with :

✓ PLS-DA

Cortisol discriminant models

- Identify the best threshold : 22 pg/mg
- Identify the best model : MIR+MY+parity²+DIM+breed+parity+DIM²

Optimization of the SENSIBILITY (detection of high cortisol)

	Threshold low vs high (in pg/mg)					
	14	16	18	20	22	24
MIR	47%	48%	48%	48%	53%	45%
MIR+MY	48%	49%	47%	48%	55%	43%
MIR+MY+parity ²	48%	49%	47%	48%	54%	45%
MIR+MY+parity ² +DIM	49%	50%	48%	49%	55%	45%
MIR+MY+parity ² +DIM+breed	49%	50%	46%	50%	55%	45%
MIR+MY+parity ² +DIM+breed+parity	47%	49%	46%	48%	54%	44%
MIR+MY+parity²+DIM+breed+parity+DIM²	48%	50%	48%	49%	57%	46%
MIR+MY+parity ² +DIM+breed+parity+DIM ² +color	48%	50%	46%	49%	54%	46%

Cortisol discriminant models

- Identify the best threshold : 22 pg/mg
- Identify the best model : MIR+MY+parity²+DIM+breed+parity+DIM²
- Step 2 : apply the model on the entire dataset

Cross validation

	Low cortisol	High cortisol	
Predicted Low	719	73	
Predicted High	172	107	
	891	180	1071

Sensibility	Specificity	Accuracy
59.4%	81%	77%

External-Herd-Validation

	Low cortisol	High cortisol	
Predicted Low	656	71	
Predicted High	235	109	
	891	180	1071

Sensibility	Specificity	Accuracy
60.6%	74%	71%

Fructosamine discriminant models

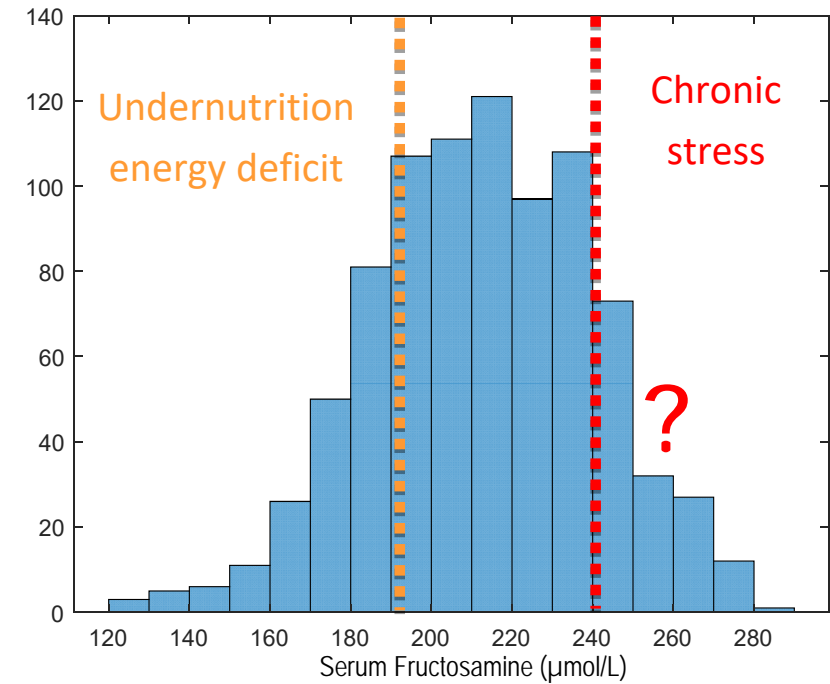
Rather « new indicator », few informations

- High → chronic stress
- Low → undernutrition/energy deficit

Need for 2 thresholds?

Test and see from 170 to 250 $\mu\text{mol/L}$

Mathematical threshold but no physiological meaning!!



Fructosamine discriminant models

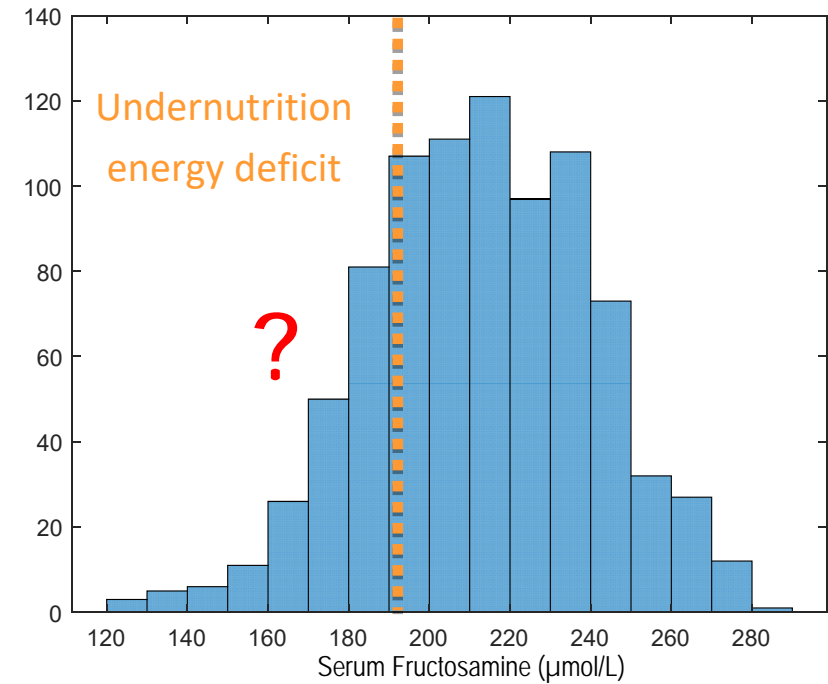
Low threshold to discriminate undernutrition/energy deficit

- Best threshold is 200 $\mu\text{mol/L}$
- Best model is $\text{MIR} + \text{MY} + \text{parity} + \text{parity}^2 + \text{DIM} + \text{DIM}^2 + \text{breed}$

Cross validation

	Low fructosamine	High fructosamine	
Predicted Low	246	126	940
Predicted High	82	486	
	328	612	

Sensibility	Specificity	Accuracy
79.4%	75%	78%



External-Herd-Validation on Entire dataset

	Low fructosamine	High fructosamine	
Predicted Low	195	177	940
Predicted High	133	435	
	328	612	

Sensibility	Specificity	Accuracy
59.5%	71.1%	67.0%

Fructosamine discriminant models

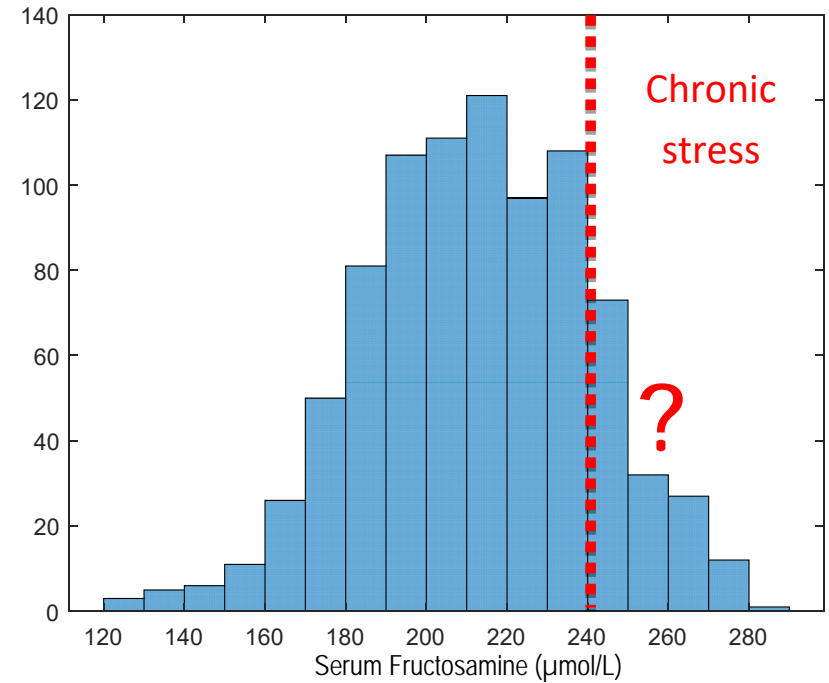
High threshold to discriminate chronic stress

- Best threshold is 230 $\mu\text{mol/L}$
- Best model is with MIR only

Cross validation

	Low fructosamine	High fructosamine	
Predicted Low	513	58	
Predicted High	175	194	
	688	252	940

Sensibility Specificity Accuracy
 77.0% 75% 75%



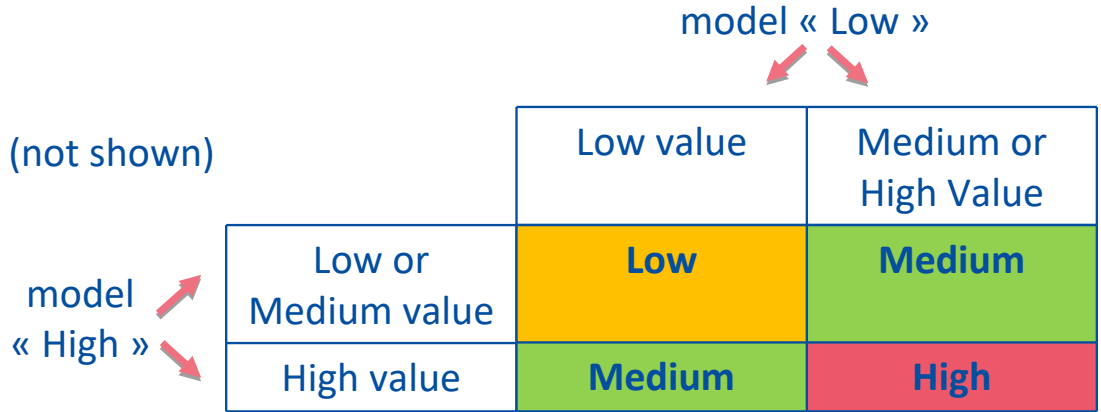
External-Herd-Validation

	Low fructosamine	High fructosamine	
Predicted Low	467	77	
Predicted High	221	175	
	688	252	940

Sensibility Specificity Accuracy
 69.4% 67.9% 68.3%

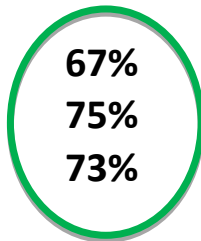
Fructosamine discriminant models

- And with the 2 thresholds?
- 1 model with 2 thresholds (3 classes) did not work well (not shown)
- Combination of model « High » and model « Low »
- External-Herd-Validation



	Low (undernutrition)	Medium	High (stress)
Pred Low (undernutrition)	169	110	38
Pred Medium	100	136	46
Pred High (stress)	59	114	168
	328	360	252

sensibility	52%	38%	67%
specificity	76%	75%	75%
accuracy	67%	61%	73%



Can we get information on dairy cows chronic stress biomarkers using milk MIR spectra?

- ✓ **Poor quantitative models** for both hair cortisol and blood fructosamine
- ✓ Discrimination
 - ✓ Low vs high cortisol: **71% accuracy**
 - ✓ Low vs high fructosamine: **73% accuracy**
 - ✓ To use at the herd level (proportions)
 - ✓ Thresholds to be optimized (on a physiological basis?)
 - ✓ To combine both biomarkers?
 - ✓ To include a grey zone (with no prediction) to improve classification
 - ✓ To be used in research projects (Holicow...)
 - ✓ To test the model in commercial farms, explore how they behave, do they really enable to highlight stress?



**Thank you for
your attention!**

Thanks to our technical staff!



