





An attempt to predict dairy cows chronic stress biomarkers using milk MIR spectra

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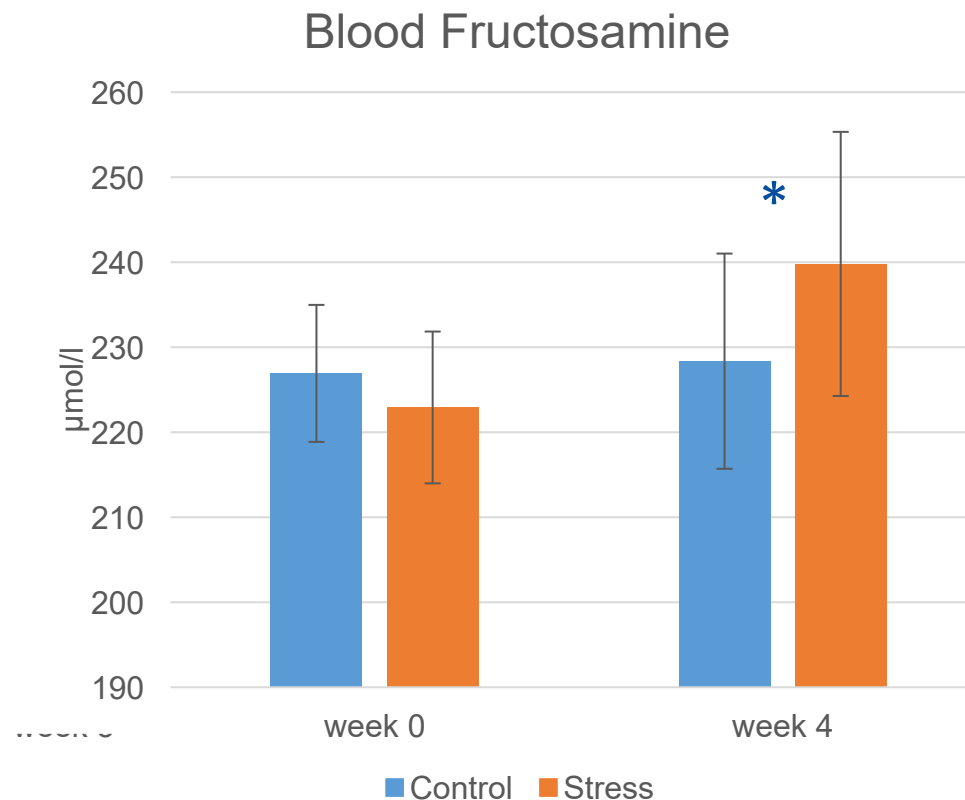
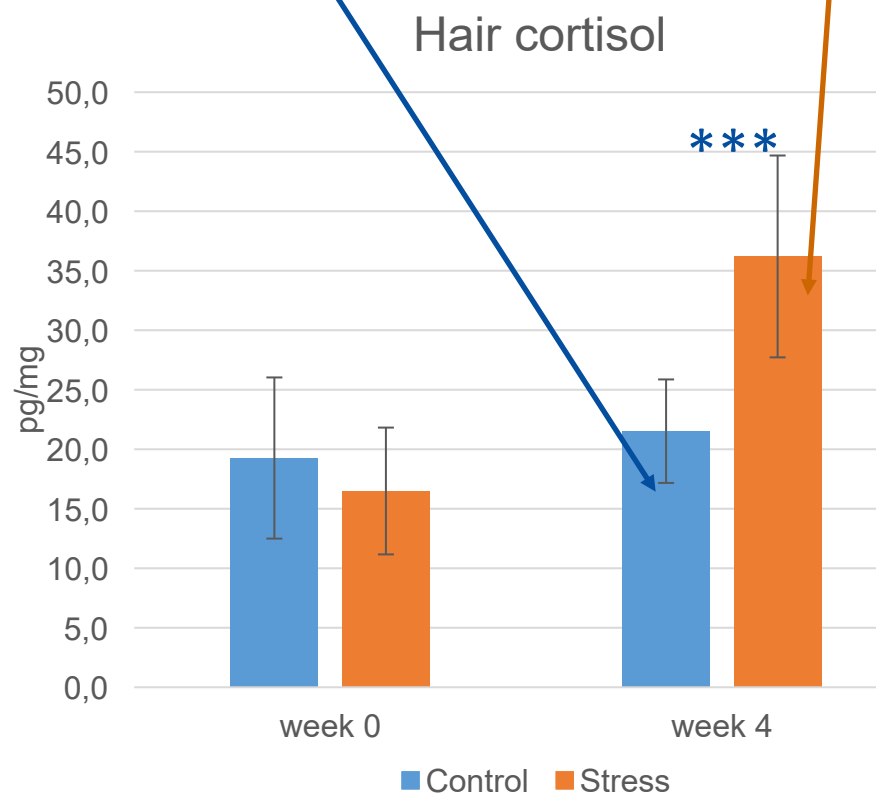
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Chronic stress biomarkers

Identification of chronic stress biomarkers in dairy cows

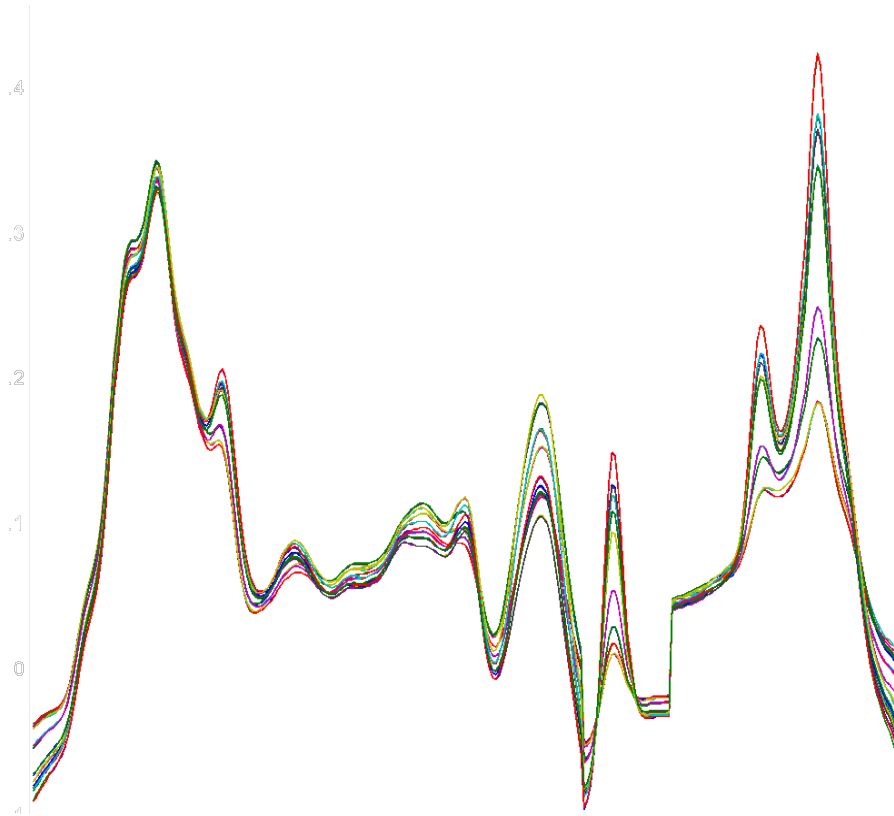
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15 Control cows vs 15 Stressed cows (4 week stress, overstocking and unusual events)



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

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

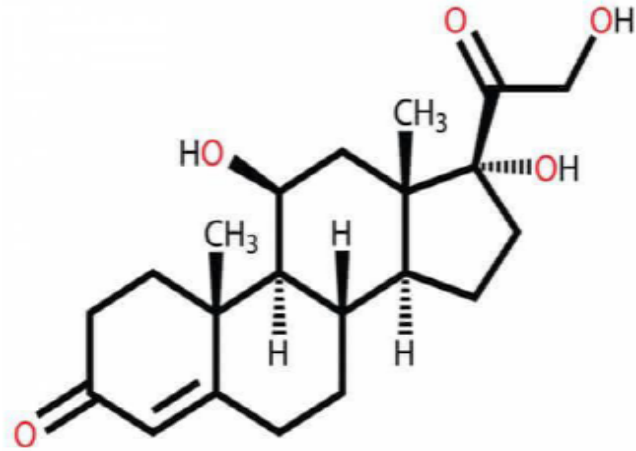


Other indirect phenotypes?

- ✓ 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

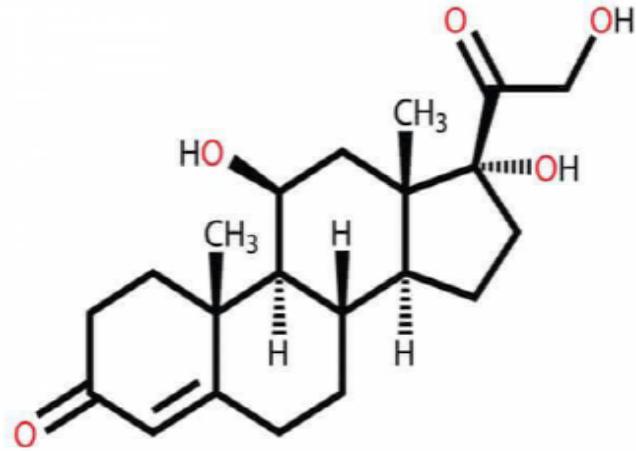
(Comin et al., 2013; Burnett et al., 2015; Heimbürge et al., 2019; Vesel et al., 2020; Tallo-Parra et al., 2017b).

- ~ 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



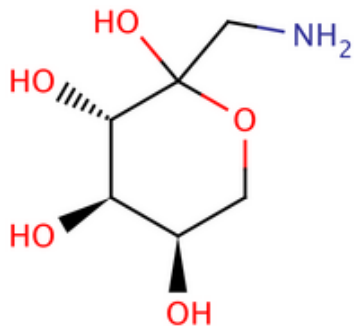
+ Gold standard chronic stress biomarker

(Comin et al., 2013; Burnett et al., 2015; Heimbürge et al., 2019; Vesel et al., 2020; Tallo-Parra et al., 2017b).

- ~ 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
Belgium	AWE	48
	CRAW	170
France	BCO	104
	Doubs	111
	LTN	117
	PDD	56
	Rhone	55
	Seenorest	131
Seenovia	145	
Germany	LKVBW	180
Luxembourg	Convis	36
		1312



- ✓ 77 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

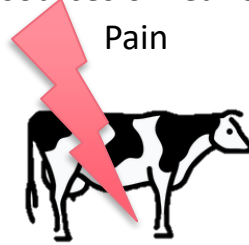
Overstocking



Chronic diseases



Permanent sources of Fear or Pain



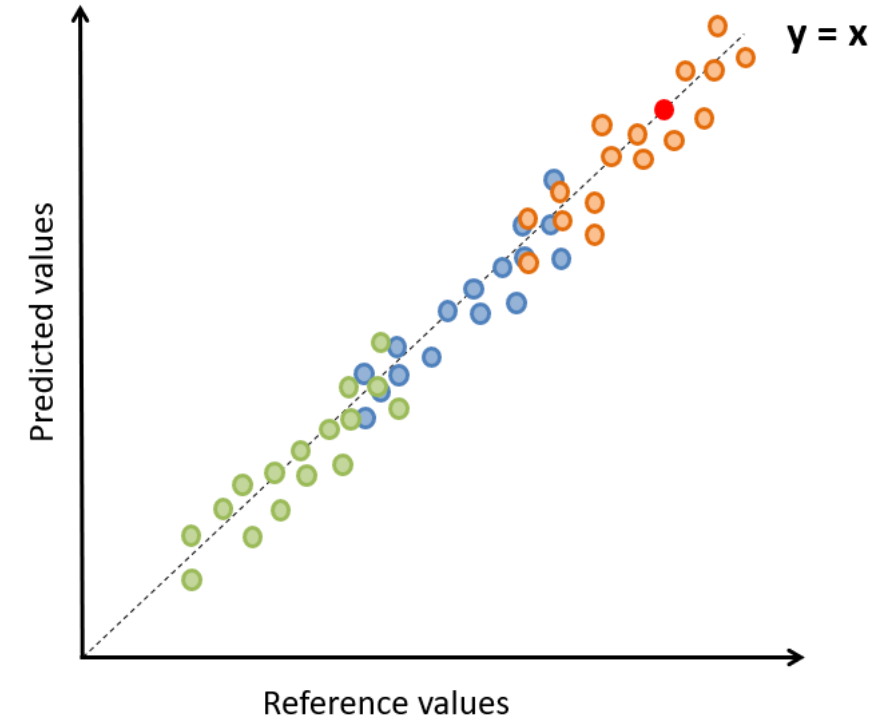
Environmental discomfort



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 21 MIR instruments (15 Foss, 1 Delta, 5 Bentley)

Standardized with CRA-W/EMR method (Grelet et al., 2015)



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

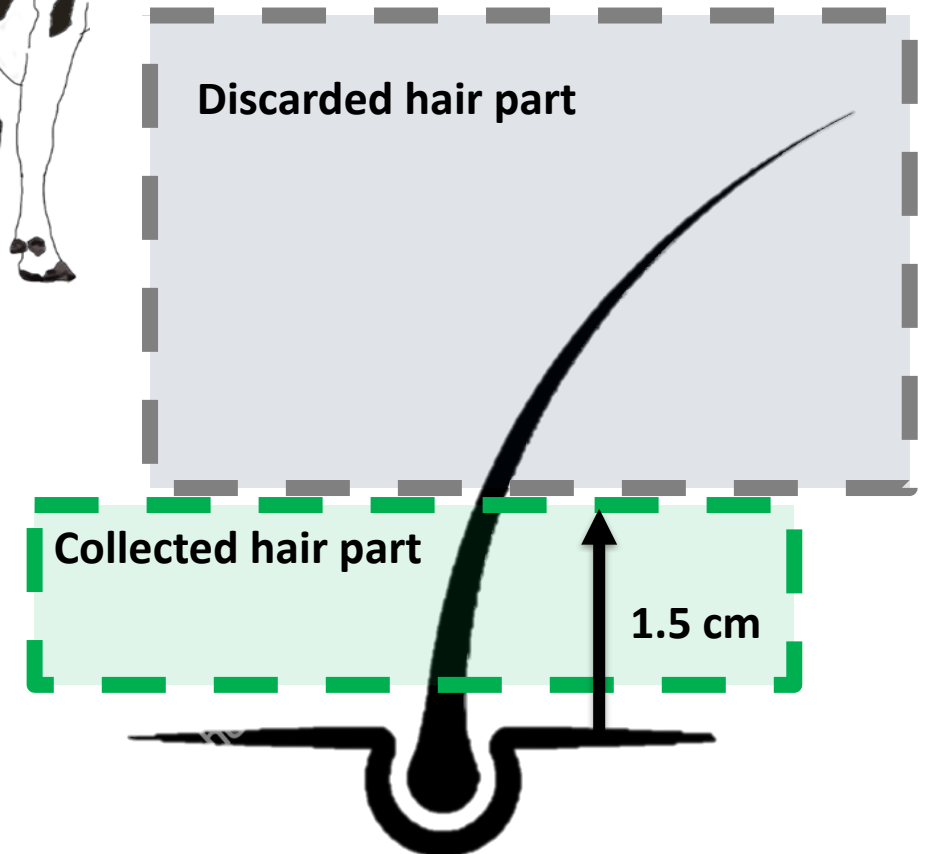
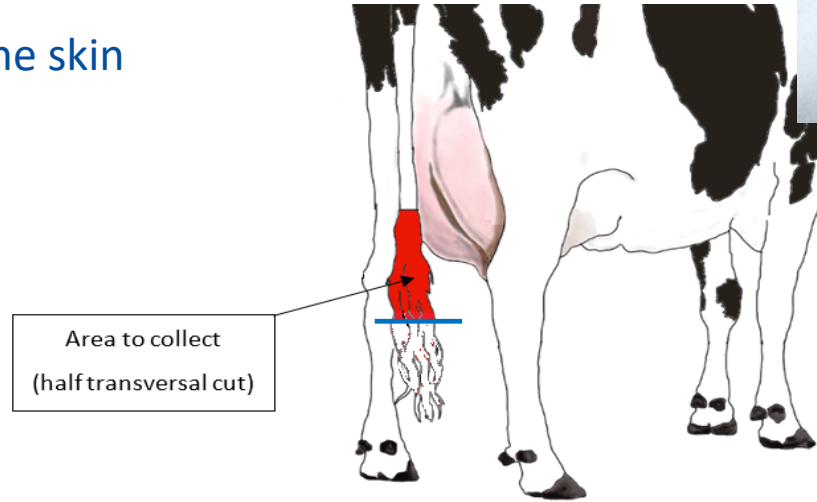


Collected part: 1.5 cm starting from the skin
(corresponding to 1 month growth)

250 mg

Analysis at CRA-W

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



Data editing

Data cleaning

- No GH threshold
- Fat differences between predictions and lab analysis < 0.3 g/100ml
- Hair cortisol CV < 12%
- Blood hemolysis ≤ 2
- DIM < 365
- Fructosamine $100 < X < 300$
- Missing values

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

Data handling

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

	Breed variable		Hair sample variable		
	0	1	Dark hairs	Light hairs	NA
Brown Swiss	8		3	1	4
Crossed	259		28	154	77
Holstein		761		538	223
Jersiaise	9			1	8
Montbeliarde	143		2?	137	4
Simmental	50			43	7
Vorderwalder	82		30	49	3
Total	551	761	63	923	326

Modelling

- 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 synthetized 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)

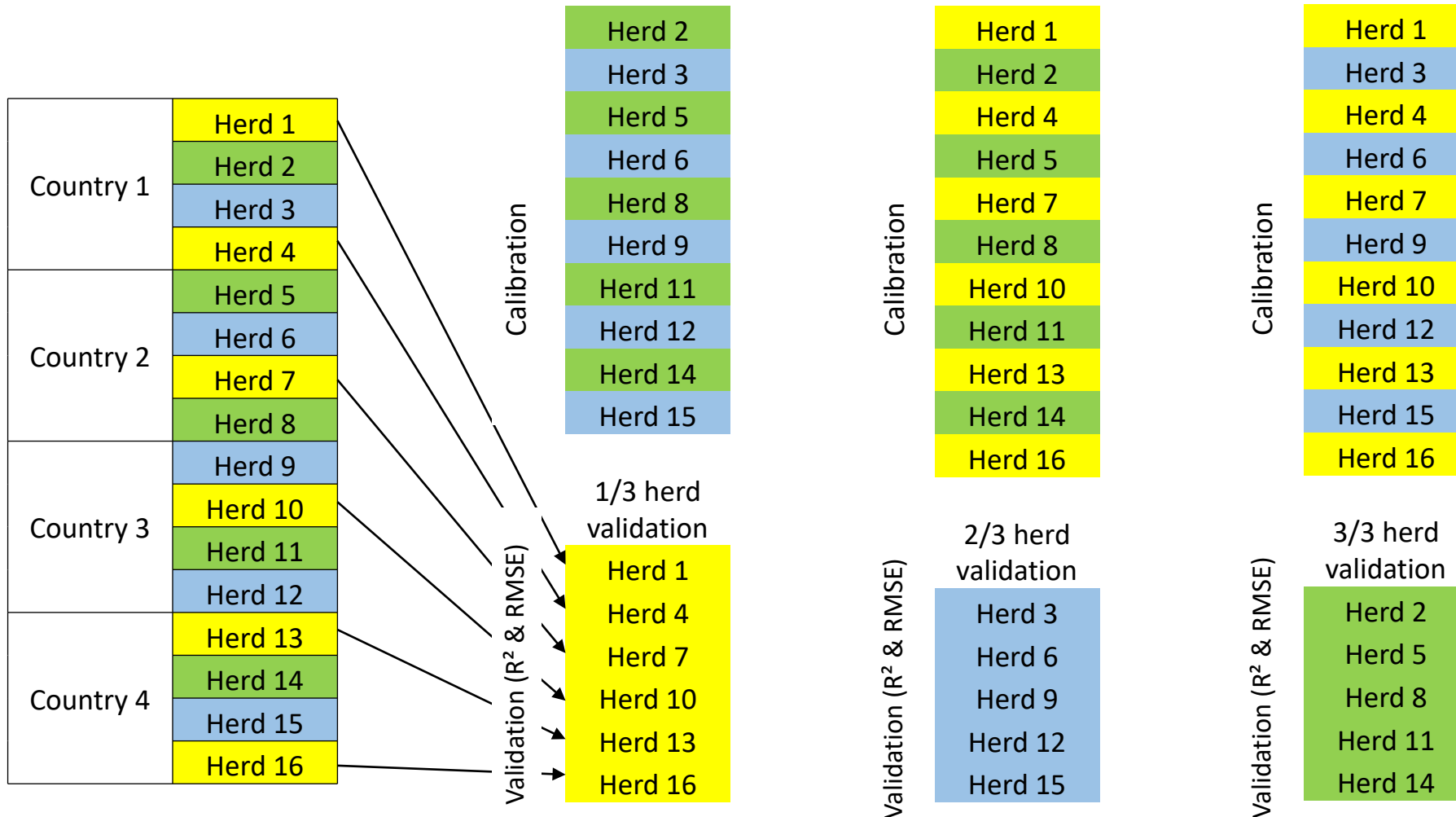
Combined with :

- ✓ MLR (multiple linear regression)
- ✓ SVM (support vector machine)

+ PLS-DA in case of discriminant models

Validation & stats

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

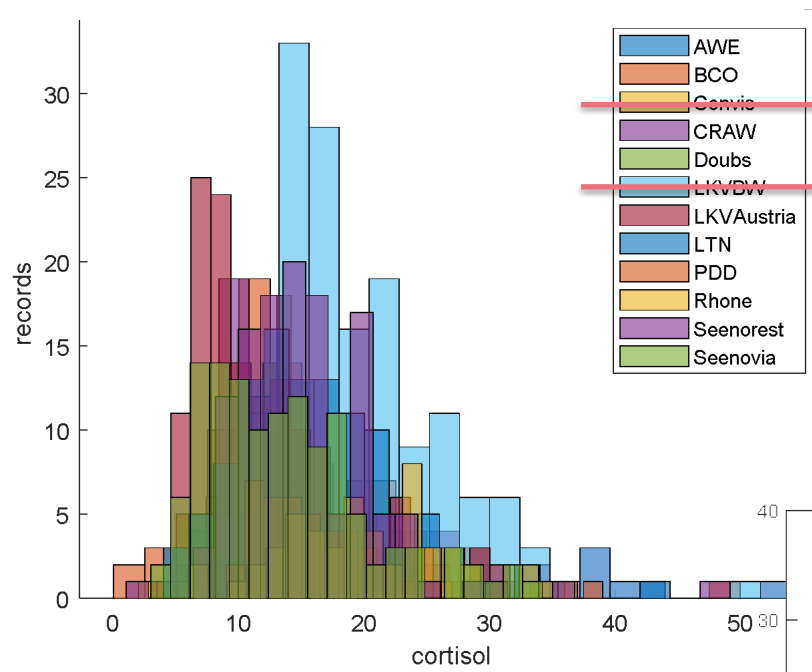


Final stats:

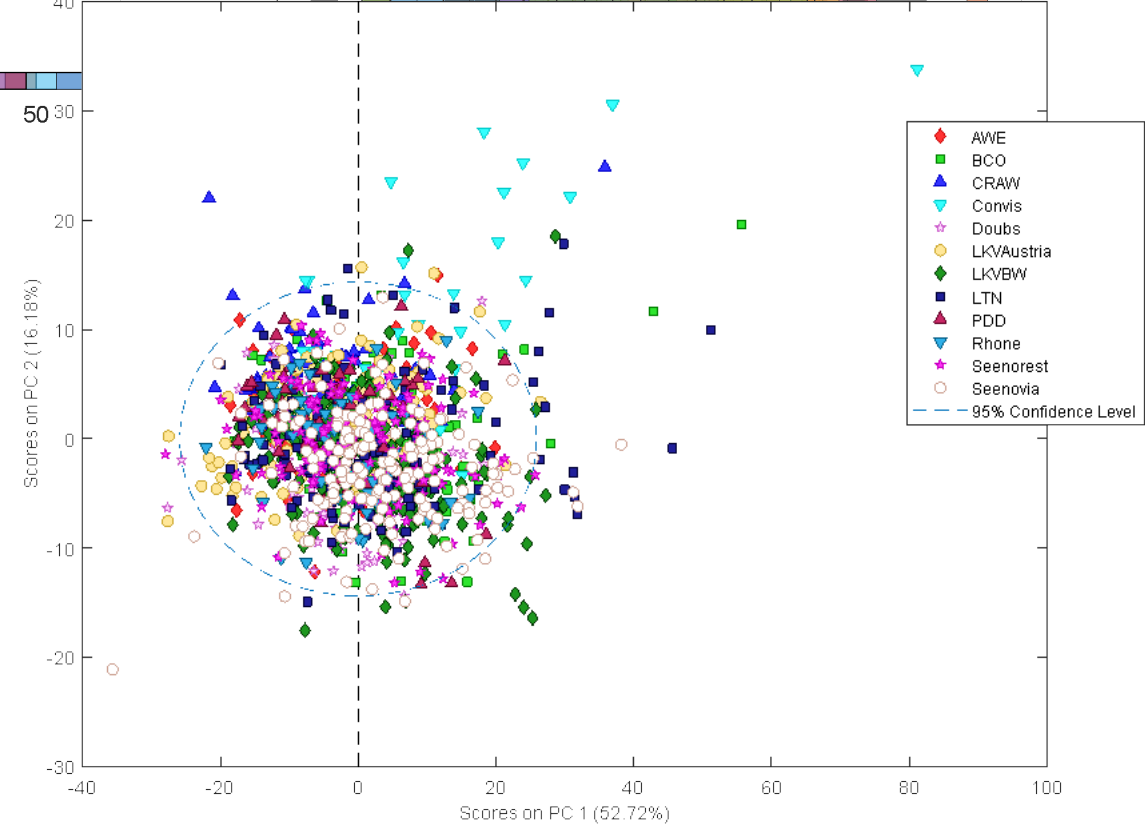
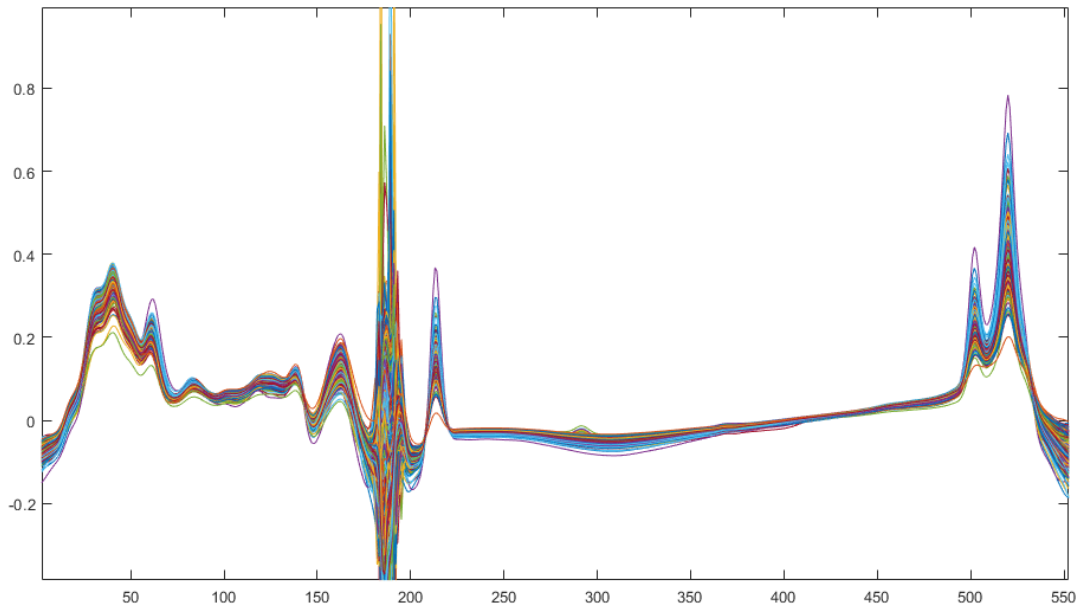
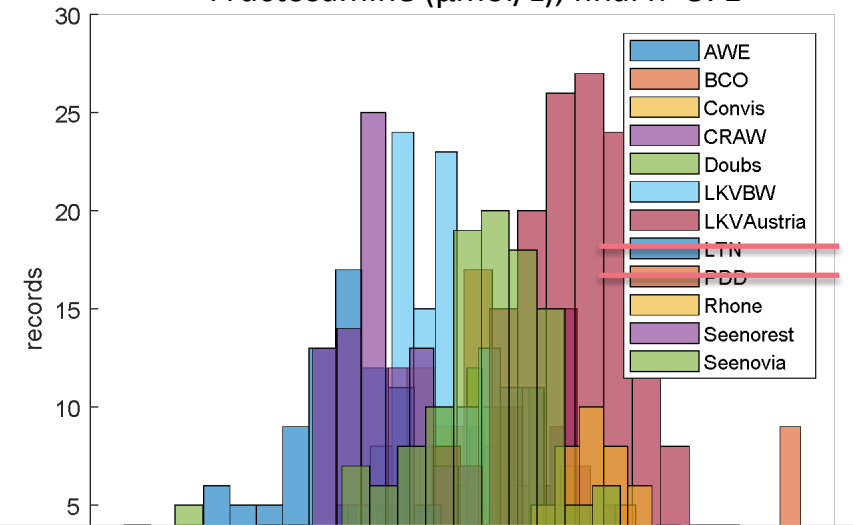
- Mean R^2_v
- Mean $RMSE_v$

Results

Cortisol (pg/mg), final n= 887



Fructosamine ($\mu\text{mol/L}$), final n=871



Cortisol quantitative models (n=887)

PLS on MIR + other variables, iterative external 33% herd validation

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

SVM on MIR + other variables, iterative external 33% herd validation.

PLS compression first on all variables

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

MLR on MIR compressed in 12LV + other variables, iterative external 33% herd validation

MIR
MIR+DIM
MIR+DIM+DIM²
MIR+DIM+DIM²+parity
MIR+DIM+DIM²+parity+parity²
MIR+DIM+DIM²+parity+parity²+MY
MIR+DIM+DIM²+parity+parity²+MY+breed

SVM on MIR compressed in 12LV + other variables, iterative external 33% herd validation, no further compression

MIR
MIR+DIM
MIR+DIM+DIM²
MIR+DIM+DIM²+parity
MIR+DIM+DIM²+parity+parity²
MIR+DIM+DIM²+parity+parity²+MY
MIR+DIM+DIM²+parity+parity²+MY+breed

Cortisol quantitative models

n=887

PLS on MIR + other variables, iterative external 33% herd validation

	LV	RMSEv mean	R ² v mean
MIR	12	6.8	0.09
MIR+DIM	12	6.8	0.09
MIR+DIM+DIM ²	12	6.9	0.08
MIR+DIM+DIM ² +parity	12	6.9	0.08
MIR+DIM+DIM ² +parity+parity ²	12	6.9	0.07
MIR+DIM+DIM ² +parity+parity ² +MY	12	7.0	0.07
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	7.0	0.07
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	12	7.0	0.06

SVM on MIR + other variables, iterative external 33% herd validation.

PLS compression first on all variables

	LV	RMSEv mean	R ² v mean
MIR	12	6.7	0.11
MIR+DIM	12	6.7	0.11
MIR+DIM+DIM ²	12	6.8	0.10
MIR+DIM+DIM ² +parity	12	6.8	0.08
MIR+DIM+DIM ² +parity+parity ²	12	6.7	0.10
MIR+DIM+DIM ² +parity+parity ² +MY	12	6.7	0.10
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	6.8	0.08
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	12	6.8	0.05

MLR on MIR compressed in 12LV + other variables, iterative external 33% herd validation

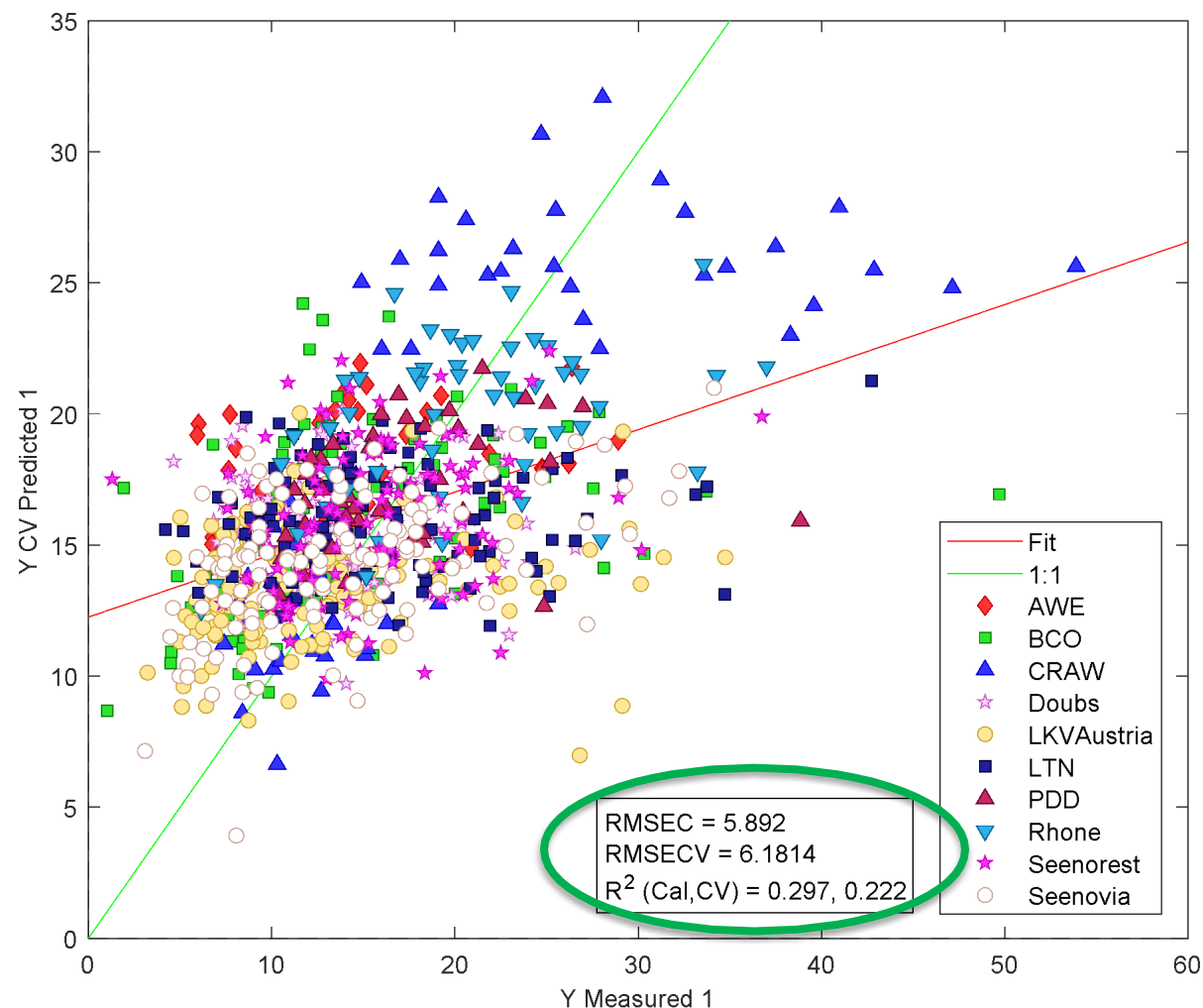
	LV	RMSEv mean	R ² v mean
MIR	12	6.8	0.09
MIR+DIM	12	6.8	0.09
MIR+DIM+DIM ²	12	6.8	0.08
MIR+DIM+DIM ² +parity	12	6.9	0.08
MIR+DIM+DIM ² +parity+parity ²	12	6.9	0.08
MIR+DIM+DIM ² +parity+parity ² +MY	12	6.9	0.08
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	6.9	0.08

SVM on MIR compressed in 12LV + other variables, iterative external 33% herd validation, no further compression

	LV	RMSEv mean	R ² v mean
MIR	12	6.7	0.10
MIR+DIM	12	6.7	0.10
MIR+DIM+DIM ²	12	6.8	0.10
MIR+DIM+DIM ² +parity	12	6.8	0.09
MIR+DIM+DIM ² +parity+parity ²	12	6.7	0.09
MIR+DIM+DIM ² +parity+parity ² +MY	12	6.8	0.08
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	6.8	0.09

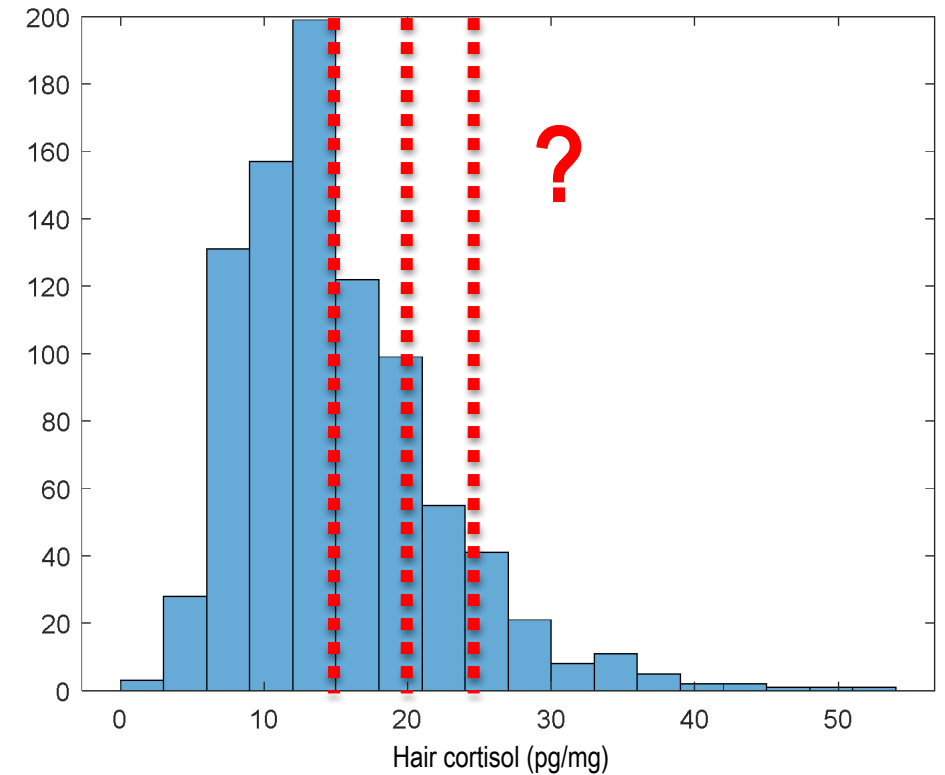
- SVM on **MIR (212 wavenumbers) + DIM**
- No impact of parity, MY, Breed and hair color?

Model with all the data (n=887), cross-validation statistics (10 folds)



Cortisol discriminant models

- Alternative option, less precise → Low vs High
- But which threshold? No documentation
- Test and see with 14, 16, 18, 20, 22, 24 pg/mg hair
- Mathematical threshold but no physiological meaning!!
- Step 1 : identify best discriminant threshold



PLS-DA on MIR , iterative external 33% herd validation, n=887

	LV	Sens. V	Spec. V	Acc. V
Threshold = 14	12	45%	65%	57%
Threshold = 16	12	40%	67%	57%
Threshold = 18	12	37%	78%	67%
Threshold = 20	12	35%	78%	69%
Threshold = 22	12	33%	83%	75%
Threshold = 24	12	40%	86%	81%

Cortisol discriminant models

- Step 2 : identify best model with threshold = 24 pg/mg hair

PLS-DA on MIR + other variables, iterative external 33% herd validation.

	LV	Sens. V	Spec. V	Acc. V
MIR	12	40%	86%	81%
MIR+DIM	12	40%	85%	80%
MIR+DIM+DIM ²	12	37%	86%	80%
MIR+DIM+DIM ² +parity	12	37%	85%	80%
MIR+DIM+DIM ² +parity+parity ²	12	39%	85%	80%
MIR+DIM+DIM ² +parity+parity ² +MY	12	38%	85%	80%
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	35%	85%	80%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	12	34%	84%	79%

- Full Confusion Matrix (external 33% herd validation)

	Low cortisol	High cortisol
Predicted Low	680	56
Predicted High	115	36
	795	92

887

Sensibility	Specificity	Accuracy
39.1%	86%	81%

Fructosamine quantitative models (n=871)

PLS on MIR + other variables, iterative external 33% herd validation

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

SVM on MIR + other variables, iterative external 33% herd validation. PLS compression first on all variables

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

MLR on MIR compressed in 14LV + other variables, iterative external 33% herd validation

MIR
MIR+DIM
MIR+DIM+DIM²
MIR+DIM+DIM²+parity
MIR+DIM+DIM²+parity+parity²
MIR+DIM+DIM²+parity+parity²+MY
MIR+DIM+DIM²+parity+parity²+MY+breed

SVM on MIR compressed in 12LV + other variables, iterative external 33% herd validation, no further compression

MIR
MIR+DIM
MIR+DIM+DIM²
MIR+DIM+DIM²+parity
MIR+DIM+DIM²+parity+parity²
MIR+DIM+DIM²+parity+parity²+MY
MIR+DIM+DIM²+parity+parity²+MY+breed

Fructosamine quantitative models

PLS on MIR + other variables, iterative external 33% herd validation, n=871

	LV	RMSEv mean	R ² v mean
MIR	14	27.1	0.17
MIR+DIM	14	27.2	0.17
MIR+DIM+DIM ²	14	27.2	0.16
MIR+DIM+DIM ² +parity	14	27.1	0.17
MIR+DIM+DIM ² +parity+parity ²	14	27.1	0.17
MIR+DIM+DIM ² +parity+parity ² +MY	14	27.01	0.186
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	27.62	0.179
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	27.47	0.181

SVM on MIR + other variables, iterative external 33% herd validation. PLS compression first on all variables

	LV	RMSEv mean	R ² v mean
MIR	14	26.2	0.18
MIR+DIM	14	26.5	0.16
MIR+DIM+DIM ²	14	27.0	0.17
MIR+DIM+DIM ² +parity	14	26.55	0.185
MIR+DIM+DIM ² +parity+parity ²	14	26.50	0.176
MIR+DIM+DIM ² +parity+parity ² +MY	14	27.16	0.188
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	27.41	0.184
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	27.2	0.15

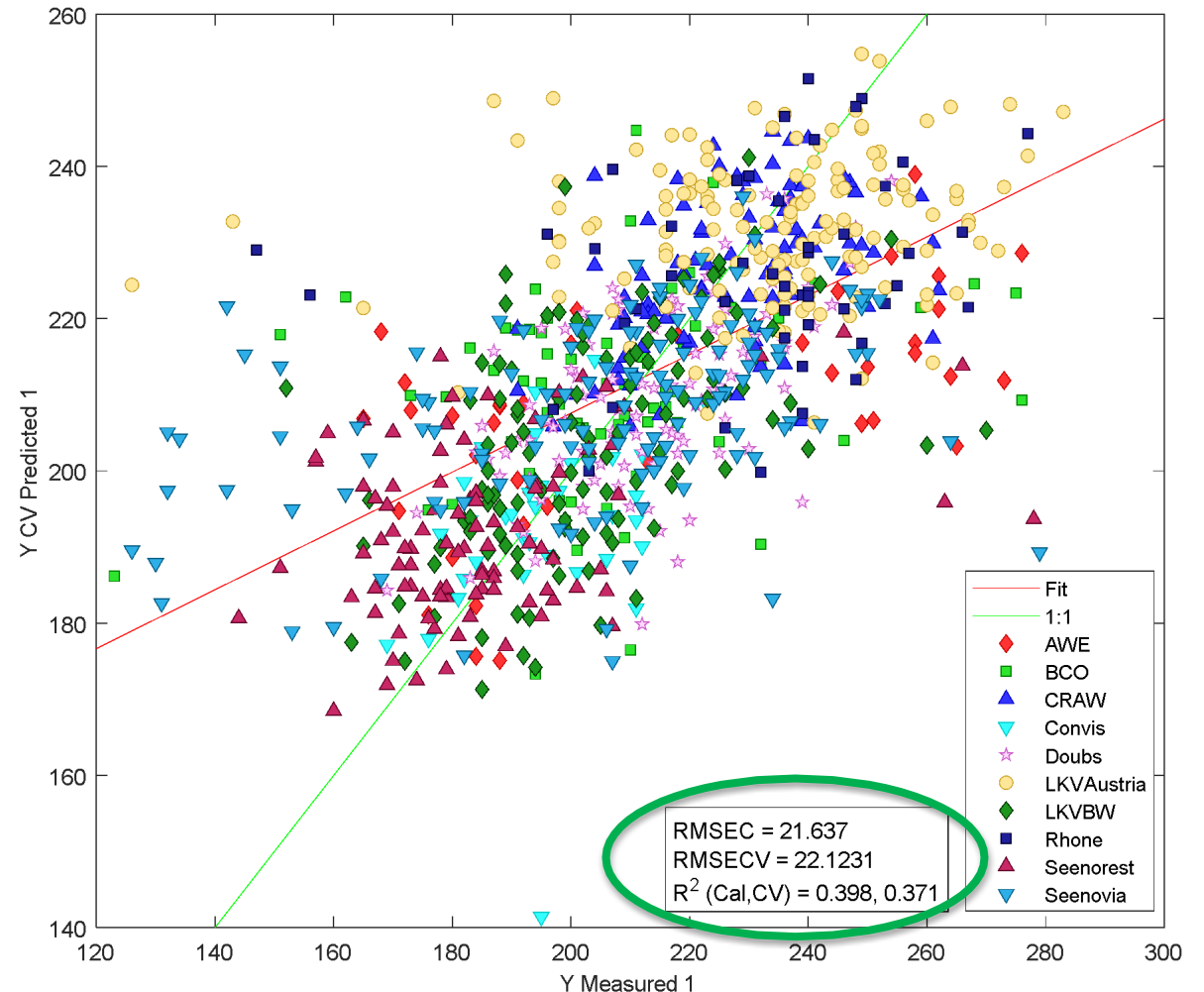
MLR on MIR compressed in 14LV + other variables, iterative external 33% herd validation

	LV	RMSEv mean	R ² v mean
MIR	14	27.1	0.17
MIR+DIM	14	27.1	0.18
MIR+DIM+DIM ²	14	27.1	0.18
MIR+DIM+DIM ² +parity	14	26.88	0.183
MIR+DIM+DIM ² +parity+parity ²	14	26.91	0.184
MIR+DIM+DIM²+parity+parity²+MY	14	26.98	0.189
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	27.03	0.185

SVM on MIR compressed in 12LV + other variables, iterative external 33% herd validation, no further compression

	LV	RMSEv mean	R ² v mean
MIR	14	27.0	0.17
MIR+DIM	14	26.2	0.18
MIR+DIM+DIM ²	14	26.5	0.17
MIR+DIM+DIM ² +parity	14	26.82	0.185
MIR+DIM+DIM ² +parity+parity ²	14	26.57	0.186
MIR+DIM+DIM ² +parity+parity ² +MY	14	26.75	0.173
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	26.53	0.182

- MLR on **MIR (compressed into 14LV) +DIM+DIM²+parity+parity²+MY**
- Model with all the data (n=871), cross-validation statistics (10 folds)



Fructosamine discriminant models

- Alternative option, less precise → Low vs High
- But which threshold? No documentation

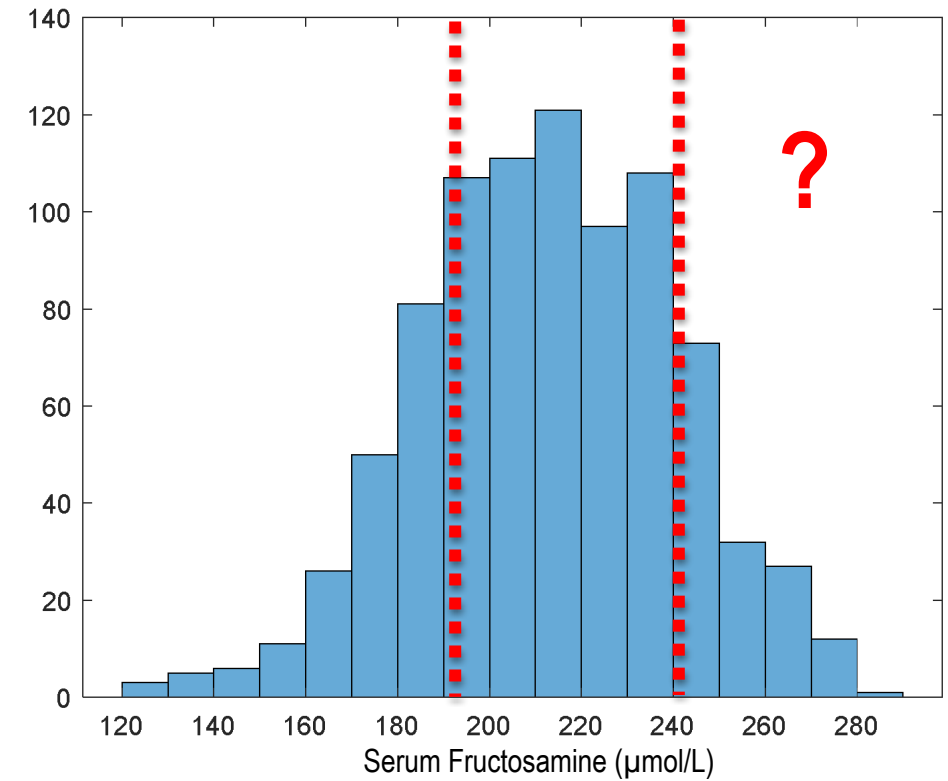
Rather « new indicator », few informations

- 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!!

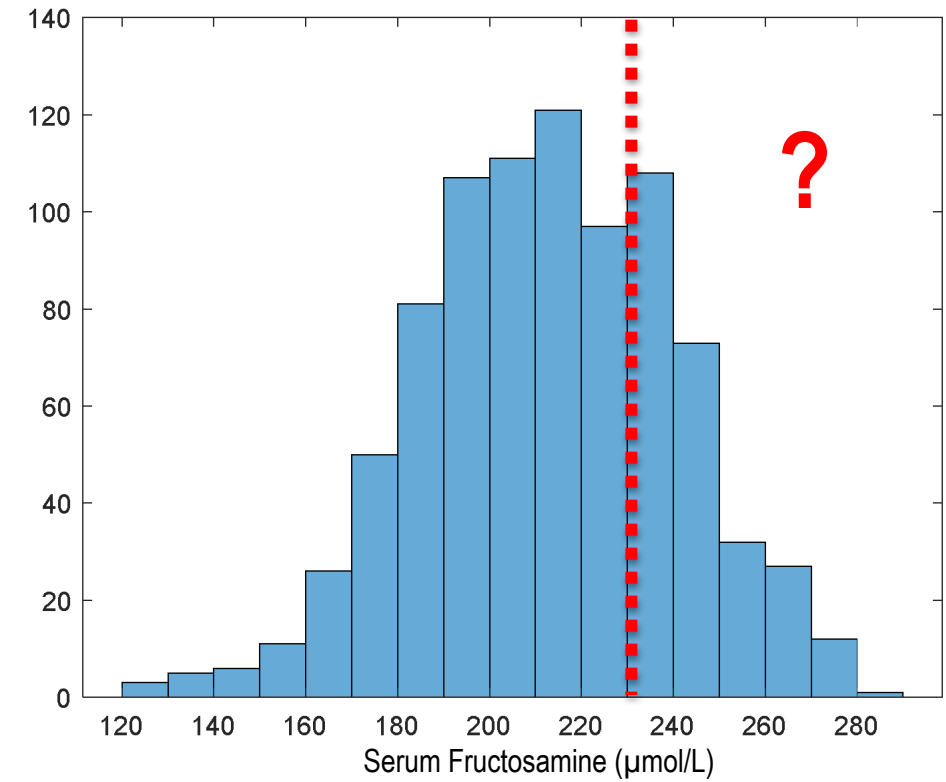


Fructosamine discriminant models

- High threshold to discriminate stress
- Step 1 : identify best discriminant threshold

PLS-DA on MIR only, iterative external 33%
herd validation

	LV	Sens. V	Spec. V	Acc. V
Threshold = 250	14	48%	65%	64%
Threshold = 240	14	57%	59%	58%
Threshold = 230	14	61%	68%	67%
Threshold = 220	14	62%	64%	64%



Fructosamine discriminant models

- High threshold to discriminate stress
- Step 2 : identify best model with threshold **230 $\mu\text{mol/L}$**

PLS-DA on MIR + other variables, iterative external 33% herd validation

	LV	Sens. V	Spec. V	Acc. V
MIR	14	61%	68%	67%
MIR+DIM	14	61%	67%	66%
MIR+DIM+DIM ²	14	63%	67%	67%
MIR+DIM+DIM ² +parity	14	63%	68%	68%
MIR+DIM+DIM ² +parity+parity ²	14	64%	68%	67%
MIR+DIM+DIM²+parity+parity²+MY	14	66%	68%	68%
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	62%	71%	69%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	62%	73%	71%

- Full Confusion Matrix, External 33% herd validation

	Low fructosamine	High fructosamine	Sensibility	Specificity	Accuracy
Predicted Low	418	75	69.1%	67%	67%
Predicted High	210	168			
	628	243	871		

Fructosamine discriminant models

- Low threshold to discriminate Undernutrition/Energy deficit
- Step 1 : identify best discriminant threshold from 170 to 200 → 200 μmol/L
- Step 2: identify best model with threshold 200 μmol/L

PLS-DA on MIR + other variables, iterative external 33% herd validation

	LV	Sens. V	Spec. V	Acc. V
MIR	14	61%	71%	68%
MIR+DIM	14	60%	71%	68%
MIR+DIM+DIM ²	14	60%	71%	68%
MIR+DIM+DIM ² +parity	14	60%	71%	68%
MIR+DIM+DIM²+parity+parity²	14	61%	71%	68%
MIR+DIM+DIM ² +parity+parity ² +MY	14	58%	71%	68%
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	61%	69%	67%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	61%	70%	68%

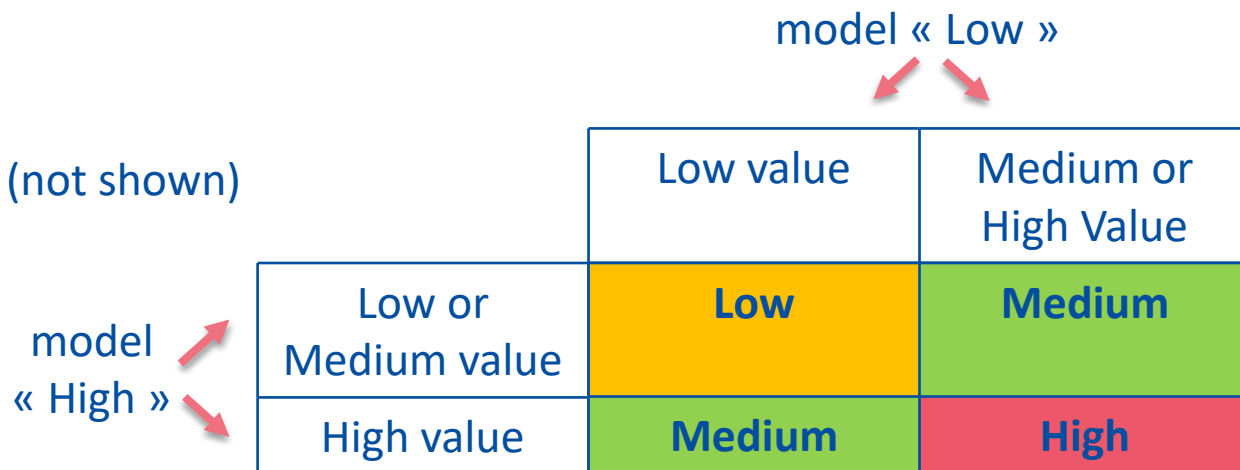
- Full Confusion Matrix (external 33% herd validation)

	Low fructosamine	High fructosamine	
Predicted Low	179	152	
Predicted High	121	419	
	300	571	871

Sensibility	Specificity	Accuracy
60%	73.4%	69%

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 »



- Full Confusion Matrix (external 33% herd validation)

	Low (undernutrition)	Medium	High (stress)	
Pred Low (undernutrition)	165	97	40	302
Pred Medium	73	108	39	220
Pred High (stress)	62	123	164	349
	300	328	243	871

sensibility
specificity
accuracy

55%
76%
69%

33%
79%
62%

67%
71%
70%

Conclusions

- ✓ **Poor quantitative models** for both hair cortisol and blood fructosamine
- ✓ Models for fructosamine use **MIR** (compressed or not) + **DIM + DIM² + parity + parity² + MY**
- ✓ No added value of the breed (and hair color for cortisol)
- ✓ Discrimination
 - ✓ Low vs high cortisol: **81% accuracy** (but **39% sensibility!!**, imbalanced dataset)
 - ✓ Low vs high fructosamine: **70% accuracy**
 - ✓ To combine both?
 - ✓ To use at the herd level (proportions)?
 - ✓ Thresholds should be validated (or not) on a physiological basis



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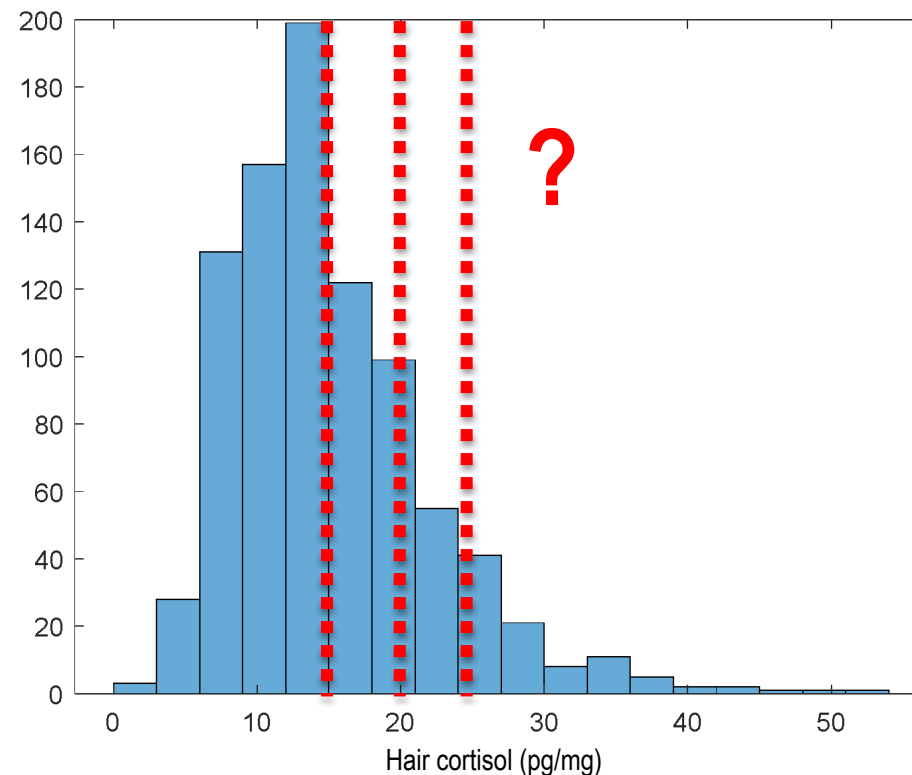
Thank you for your attention!



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Cortisol discriminant models

- Alternative option, less precise → Low vs High
- But which threshold? No documentation
- Test and see with 14, 16, 18, 20, 22, 24 pg/mg hair
- Mathematical threshold but no physiological meaning!!
- Step 1 : identify best discriminant threshold



n=887

PLS-DA on MIR , iterative external 33% herd validation.

	Sens.c			Sens.v			Spec.c			Spec.v			Acc.c			Acc.v									
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3							
Threshold = 14	12	58%	60%	72%	63%	39%	45%	52%	45%	82%	81%	74%	79%	58%	71%	65%	65%	72%	72%	73%	72%	50%	60%	60%	57%
Threshold = 16	12	59%	57%	68%	61%	36%	37%	48%	40%	80%	83%	75%	80%	61%	78%	64%	67%	73%	74%	72%	73%	52%	62%	58%	57%
Threshold = 18	12	58%	58%	64%	60%	27%	30%	55%	37%	86%	85%	78%	83%	77%	85%	73%	78%	73%	78%	74%	77%	63%	68%	69%	67%
Threshold = 20	12	61%	55%	64%	60%	27%	25%	53%	35%	87%	86%	82%	85%	78%	86%	70%	78%	82%	81%	78%	80%	68%	72%	67%	69%
Threshold = 22	12	63%	62%	66%	63%	21%	38%	55%	38%	86%	87%	86%	86%	82%	88%	76%	82%	83%	83%	83%	83%	73%	80%	74%	75%
Threshold = 24	12	69%	62%	60%	63%	39%	24%	56%	40%	84%	90%	86%	87%	82%	87%	88%	86%	82%	87%	83%	84%	78%	79%	85%	81%

Cortisol discriminant models

- Step 2 : identify best model

Threshold = 24

PLS-DA on MIR + other variables, iterative external 33% herd validation.

LV	Sens.c			Sens.v			Sens. V	Spec.c			Spec.v			Spec. V	Acc.c			Acc.v							
	1	2	3	C	1	2		3	C	1	2	3	C		1	2	3	1	2	3	1	2	3		
MIR	12	69%	62%	60%	63%	39%	24%	56%	40%	84%	90%	86%	87%	82%	87%	88%	86%	82%	87%	83%	84%	78%	79%	85%	81%
MIR+DIM	12	67%	63%	54%	61%	42%	21%	56%	40%	84%	90%	87%	87%	81%	86%	88%	85%	82%	88%	83%	84%	77%	78%	85%	80%
MIR+DIM+DIM ²	12	69%	63%	51%	61%	42%	24%	44%	37%	84%	90%	89%	88%	81%	87%	89%	86%	83%	88%	85%	85%	77%	79%	85%	80%
MIR+DIM+DIM ² +parity	12	65%	62%	54%	60%	42%	21%	48%	37%	84%	90%	89%	88%	81%	87%	88%	85%	82%	88%	85%	85%	77%	79%	84%	80%
MIR+DIM+DIM ² +parity+parity ²	12	67%	59%	54%	60%	42%	28%	48%	39%	85%	91%	88%	88%	80%	88%	87%	85%	83%	88%	84%	85%	76%	80%	84%	80%
MIR+DIM+DIM ² +parity+parity ² +MY	12	69%	59%	54%	60%	45%	24%	44%	38%	84%	91%	90%	88%	80%	88%	86%	85%	83%	87%	86%	85%	77%	80%	83%	80%
MIR+DIM+DIM ² +parity+parity ² +MY+breed	12	70%	57%	52%	60%	39%	17%	48%	35%	84%	91%	90%	89%	81%	87%	87%	85%	83%	88%	86%	85%	77%	79%	83%	80%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	12	67%	59%	54%	60%	29%	24%	48%	34%	85%	91%	89%	88%	81%	87%	85%	84%	83%	87%	85%	85%	75%	80%	82%	79%

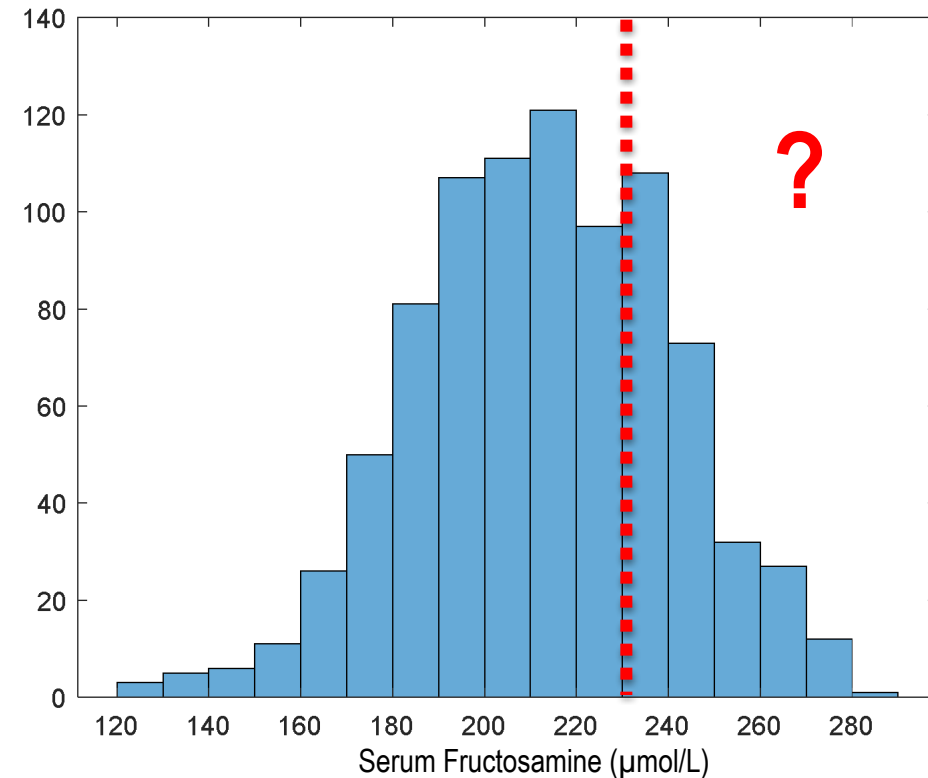
- Full Confusion Matrix (external 33% herd validation)

	Low cortisol	High cortisol	
Predicted Low	680	56	Sensibility 39.1%
Predicted High	115	36	
	795	92	887

Sensibility	Specificity	Accuracy
39.1%	86%	81%

Fructosamine discriminant models

- High threshold to discriminate stress
- Step 1 : identify best discriminant threshold



PLS-DA on MIR only, iterative external 33% herd validation.

threshold 250
 threshold 240
threshold 230
 threshold 220

LV	Sens.c 1	Sens.c 2	Sens.c 3	Sens C	Sens.v 1	Sens.v 2	Sens.v 3	Sens. V	Spec. c 1	Spec.c 2	Spec.c 3	Spec C	Spec.v 1	Spec.v 2	Spec.v 3	Spec. V	Acc.c 1	Acc.c 2	Acc.c 3	Acc C	Acc.v 1	Acc.v 2	Acc.v 3	Acc. V
14	79%	87%	86%	84%	60%	29%	54%	48%	80%	78%	83%	80%	66%	73%	57%	65%	80%	79%	83%	81%	66%	70%	56%	64%
14	83%	88%	79%	83%	58%	45%	66%	57%	76%	77%	83%	79%	69%	63%	44%	59%	77%	79%	82%	79%	67%	61%	47%	58%
14	82%	87%	82%	84%	55%	38%	92%	61%	75%	74%	79%	76%	77%	84%	44%	68%	77%	78%	80%	78%	71%	73%	58%	67%
14	81%	85%	86%	84%	59%	37%	90%	62%	76%	78%	79%	77%	69%	83%	39%	64%	78%	80%	82%	80%	66%	67%	61%	64%

Fructosamine discriminant models

- High threshold to discriminate stress
- Step 2 : identify best model

Threshold 230

PLS-DA on MIR + other variables, iterative external 33% herd validation.

	LV	Sens.c 1	Sens.c 2	Sens.c 3	Sens C	Sens.v 1	Sens.v 2	Sens.v 3	Sens. V	Spec.c 1	Spec.c 2	Spec.c 3	Spec C	Spec.v 1	Spec.v 2	Spec.v 3	Spec. V	Acc.c 1	Acc.c 2	Acc.c 3	Acc C	Acc.v 1	Acc.v 2	Acc.v 3	Acc. V
MIR	14	82%	87%	82%	84%	55%	38%	92%	61%	75%	74%	79%	76%	77%	84%	44%	68%	77%	78%	80%	78%	71%	73%	58%	67%
MIR+DIM	14	81%	86%	82%	83%	55%	38%	91%	61%	76%	76%	79%	77%	76%	82%	44%	67%	78%	79%	80%	79%	70%	71%	58%	66%
MIR+DIM+DIM ²	14	80%	88%	82%	84%	57%	41%	92%	63%	77%	77%	79%	77%	75%	84%	44%	67%	77%	80%	80%	79%	70%	73%	58%	67%
MIR+DIM+DIM ² +parity	14	81%	87%	82%	84%	57%	43%	91%	63%	76%	77%	81%	78%	75%	83%	47%	68%	77%	80%	81%	80%	70%	73%	60%	68%
MIR+DIM+DIM ² +parity+parity ²	14	81%	88%	82%	84%	59%	49%	84%	64%	77%	77%	80%	78%	75%	85%	43%	68%	78%	81%	81%	80%	70%	76%	56%	67%
MIR+DIM+DIM²+parity+parity²+MY	14	81%	86%	81%	83%	59%	51%	87%	66%	77%	77%	79%	78%	75%	83%	47%	68%	78%	80%	80%	79%	70%	75%	59%	68%
MIR+DIM+DIM ² +parity+parity ² +MI+breed	14	82%	86%	81%	83%	58%	46%	83%	62%	78%	80%	79%	79%	75%	88%	51%	71%	79%	82%	80%	80%	70%	78%	60%	69%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	81%	85%	80%	82%	59%	51%	77%	62%	79%	79%	80%	79%	78%	88%	53%	73%	79%	80%	80%	80%	73%	78%	60%	71%

- Full Confusion Matrix

Cross-validation (10 folds)

	Low fructosamine	High fructosamine		Sensibility	Specificity	Accuracy
Predicted Low	463	52			74%	
Predicted High	165	191				75%
	628	243	871	78.6%		

External 33% herd validation

	Low fructosamine	High fructosamine		Sensibility	Specificity	Accuracy
Predicted Low	418	75			67%	
Predicted High	210	168				67%
	628	243	871	69.1%		

Fructosamine discriminant models

- Low threshold to discriminate Undernutrition/Energy deficit
- Step 1 : identify best discriminant threshold from 170 to 200 → 200 μmol/L
- Step 2: identify best model

Threshold 200 μmol/L

PLS-DA on MIR + other variables, iterative external 33% herd validation.

		Sens.c			Sens.c				Spec.			Spec.v			Spec.v			Acc.			Acc.				
	LV	1	2	3	Sens C	Sens.v 1	Sens.v 2	Sens.v 3	Sens. V	c 1	c 2	c 3	Spec C	1	2	3	Spec. V	Acc.c 1	Acc.c 2	Acc.c 3	Acc C	v 1	v 2	v 3	Acc. V
MIR	14	81%	81%	85%	83%	65%	58%	89%	71%	80%	74%	75%	76%	67%	81%	34%	61%	81%	79%	82%	80%	66%	67%	72%	68%
MIR+DIM	14	82%	82%	86%	83%	65%	61%	88%	71%	79%	75%	75%	76%	66%	78%	35%	60%	81%	80%	82%	81%	65%	67%	71%	68%
MIR+DIM+DIM ²	14	81%	82%	87%	83%	64%	61%	88%	71%	77%	76%	75%	76%	64%	82%	34%	60%	79%	80%	82%	81%	64%	70%	71%	68%
MIR+DIM+DIM ² +parity	14	84%	81%	85%	84%	64%	61%	88%	71%	77%	77%	75%	76%	63%	80%	38%	60%	82%	80%	81%	81%	64%	69%	72%	68%
MIR+DIM+DIM ² +parity+parity ²	14	85%	81%	85%	84%	65%	62%	87%	71%	78%	75%	75%	76%	62%	82%	38%	61%	82%	79%	82%	81%	64%	70%	72%	68%
MIR+DIM+DIM ² +parity+parity ² +MY	14	83%	84%	85%	84%	68%	59%	86%	71%	79%	74%	73%	75%	58%	80%	37%	58%	82%	80%	81%	81%	64%	67%	71%	68%
MIR+DIM+DIM ² +parity+parity ² +MY+breed	14	84%	82%	87%	84%	66%	56%	85%	69%	79%	77%	74%	76%	55%	86%	41%	61%	82%	80%	82%	82%	62%	68%	72%	67%
MIR+DIM+DIM ² +parity+parity ² +MY+breed+color	14	84%	82%	88%	85%	67%	56%	86%	70%	79%	76%	73%	76%	55%	86%	41%	61%	82%	80%	83%	82%	63%	68%	72%	68%

- Full Confusion Matrix (external 33% herd validation)

	Low fructosamine	High fructosamine	Sensibility	Specificity	Accuracy
Predicted Low	179	152	60%		
Predicted High	121	419		73.4%	69%
	300	571	871		