



# Largescale analysis of chronic stress in dairy cows using hair cortisol and blood fructosamine

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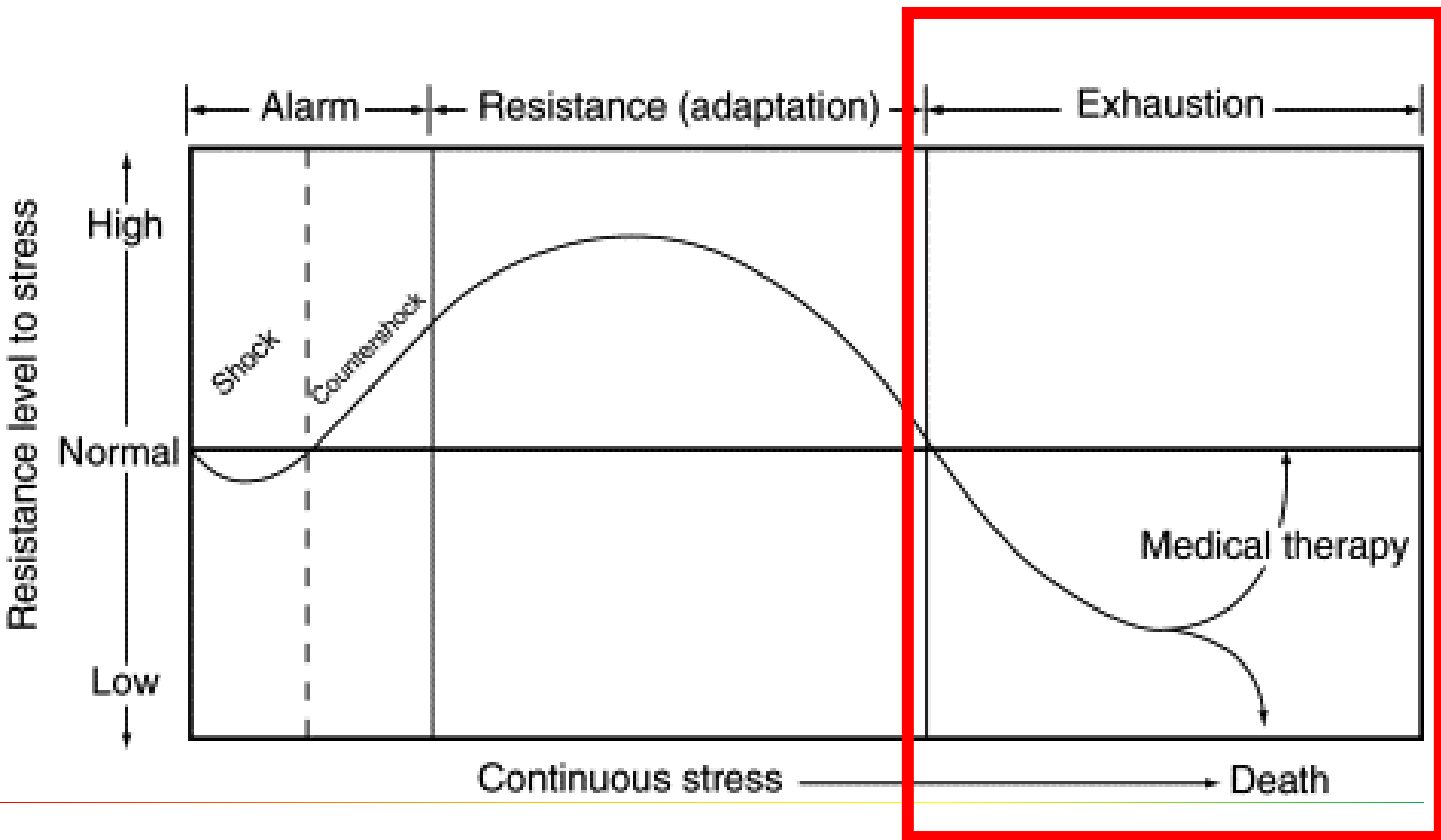
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## Non-specific reaction of the organism to any stimulation (Selye, 1976)



↗ Inflammatory and infectious diseases

(Moberg et al., 1980; Romero, 2004)

↗ fertility troubles

(Dobson and Smith, 2000; Walker et al., 2008)

↘ weight

(Mormède et al., 2007)

↘ milk production

(TalloParra et al., 2018)

Figure 1: General adaptation syndrome (from A.C. Brown, C.I. Waslien, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003)



## Two big questions

1. Is it possible for an **experimenter** to **identify** cows with **chronic stress**?



2. How can we identify and **understand** what causes **variations in capillary cortisol and blood fructosamine**?



## Cortisol

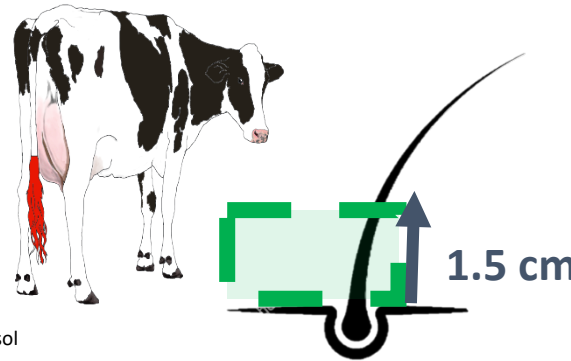
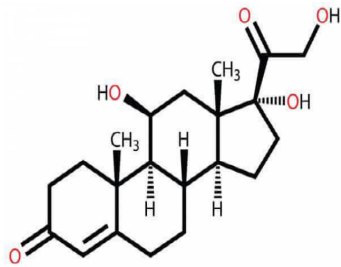


Figure 3: Cortisol molecule from [www.ulb-ibc.be/cortisol](http://www.ulb-ibc.be/cortisol)

## Sampling

- Hair collected from the tail of the cows
- Focusing on hair within **1.5 cm** of the skin

## Analysis

- Hair washed and dried before extraction
- Measurement using Salimetrics Elisa kit

## Fructosamine

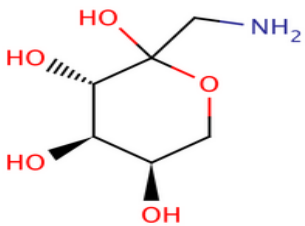


Figure 4: Fructosamine molecule from <http://www.nugowiki.org/index.php/Fructosamine>

- Blood collected in tail vein
- Storage in **Dry tubes** with serum separating gel

- Analysis done by Synlab

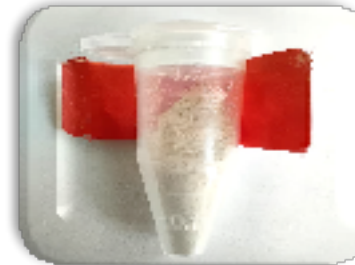
## Subsetting data

1396 observations

Data cleaning

- Remove data with suspicious fructosamine and cortisol values or where protocol violations are suspected
- Cleaning for CV(%) >12
- Cleaning for hemolyse index > 2
- Cleaning for day in milk > 305
- Lactation: 1, 2, 3, 4+
- Remove under-represented breeds

Only white hair



(Otten, 2023; Heimbürge, 2020; Heimbürge, 2020; Otten, 2021; Tallo-Parra, 2015; Rippe, 2016; Binz, 2018)

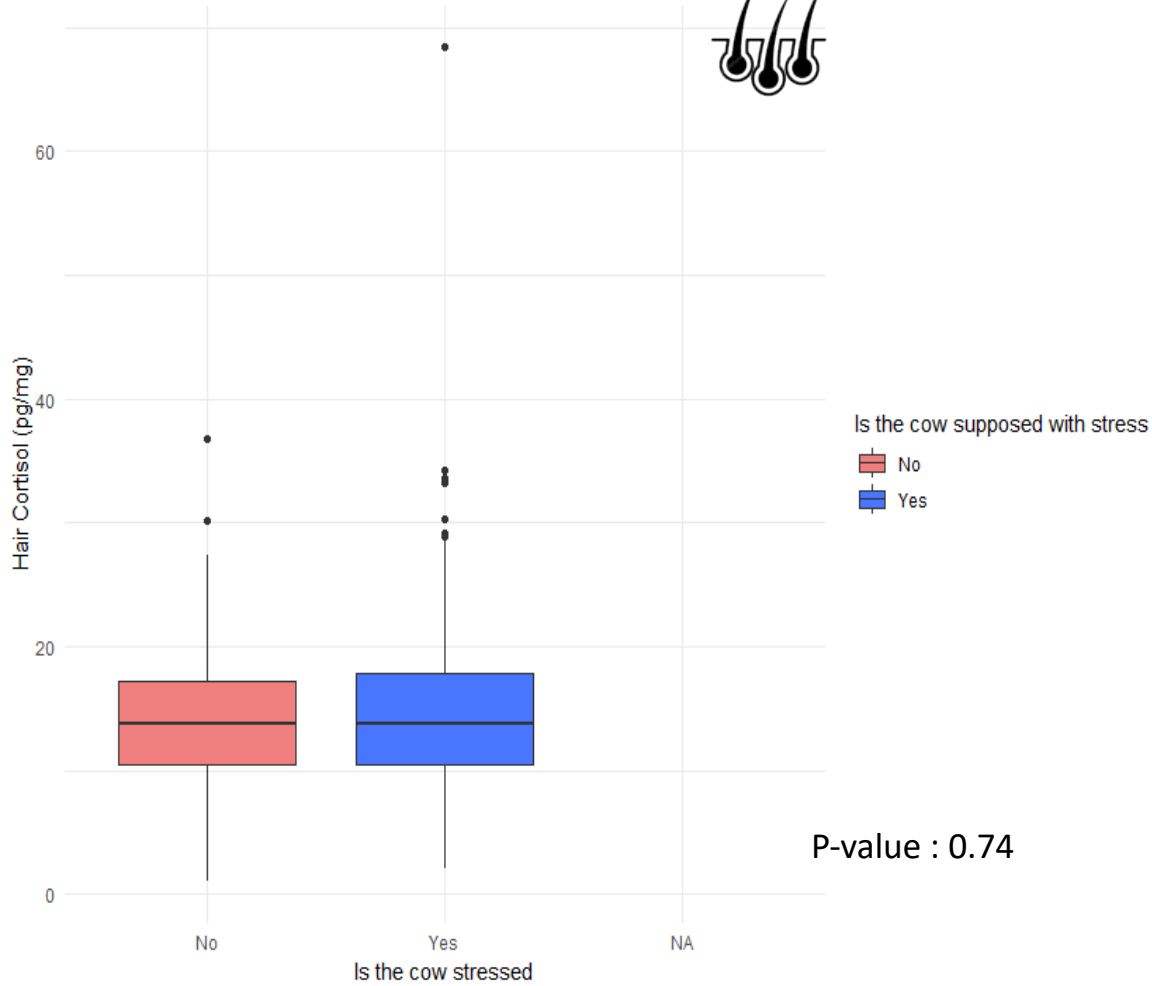
1185 observations :

- N= 732 Cortisol
- N= 758 Fructosamine

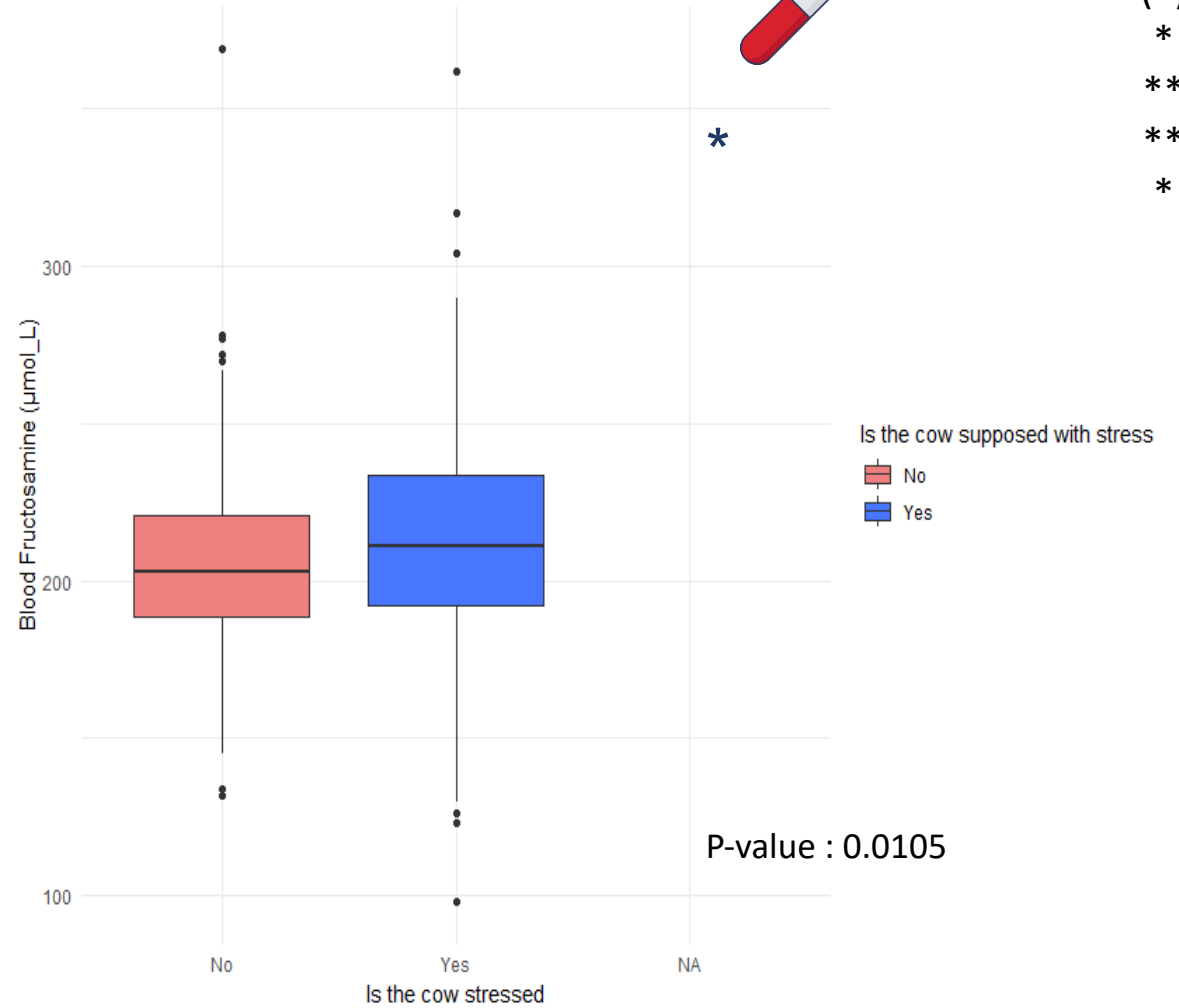


# 1. Is it possible for an experimenter to identify cows with chronic stress?

Boxplot of Hair Cortisol Levels by Cow Stress Status



Boxplot of Fructosamine Levels by Cow Stress Status

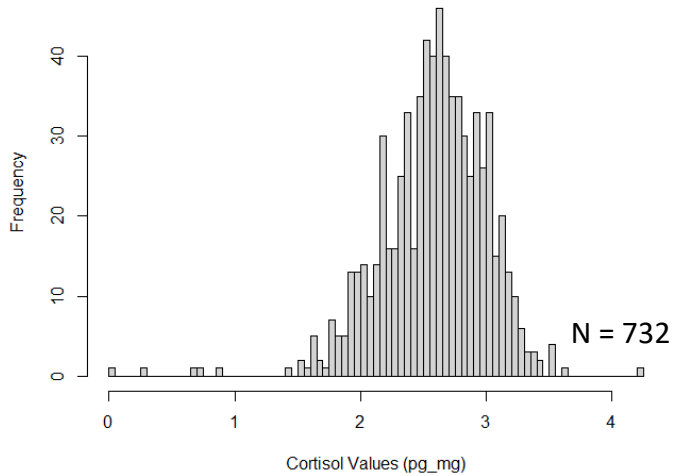


(\*)  $P \leq 0.1$   
 \*  $P \leq 0.05$   
 \*\*  $P \leq 0.01$   
 \*\*\*  $P \leq 0.001$



# 2. What makes capillary cortisol and blood fructosamine vary?

Distribution of logarithmic cortisol



## Cow information

- 24h Milk Yield kg
- Day In Milk
- Lactation number
- Breed
- Country

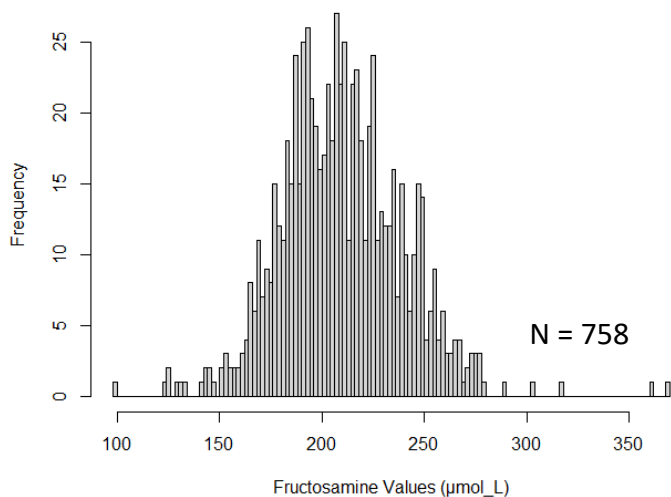
## Milk composition

- Fat sample
- C18\_1cis9
- Blood bhb
- Milk nagase
- Lactoferrin

## Housing & Feeding

- Number cubicle stall
- Number feeding places
- Number of water points
- Square meter per cow

Fructosamine Distribution



## Health and status

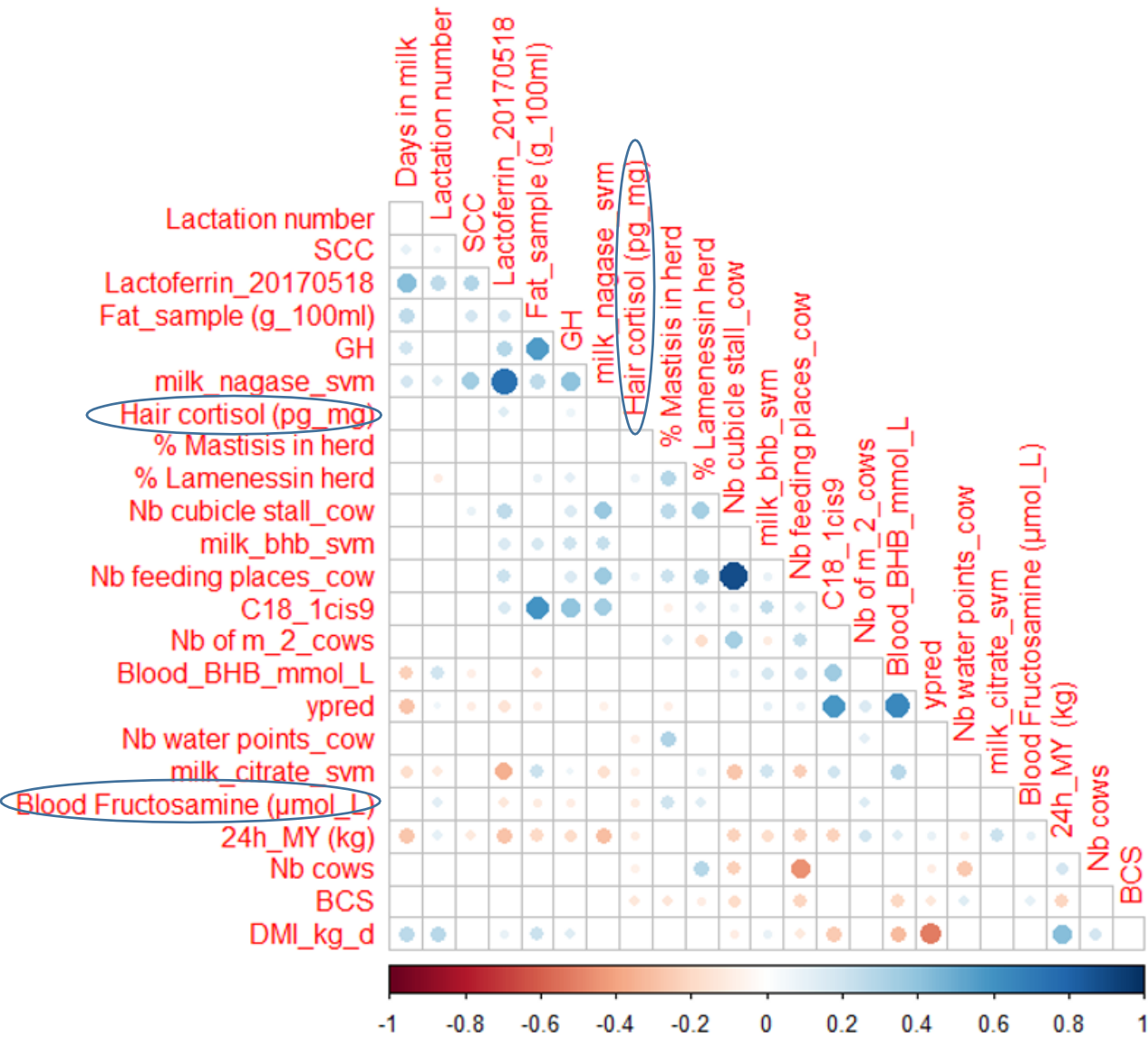
- Somatic Cell Count
- Lameness in herd
- Percentage of mastitis in the herd
- Body Condition Score

## Stress information

- The source of stress



## 2. What makes capillary cortisol and blood fructosamine vary?



Risk of multicollinearity and low correlation for blood fructosamine and hair cortisol



## 2. What makes capillary cortisol and blood fructosamine vary?

### Analysis of Associations between Hair Cortisol and Blood Fructosamine

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

#### Correlation Analysis and Significance Assessment of Variables for Hair Cortisol (pg/mg)



	Blood Fructosamine ( $\mu\text{mol/L}$ )	24h/MY (kg)	Nb cows	Nb feeding places/cow	Nb water points/cow	BCS	% Lameness in herd	Milk citrate svm	Lactoferrin
Correlation	-0.102	-0.103	-0.079	0.088	-0.083	-0.126	0.089	-0.075	<b>0.138</b>
P_value	*	**	*	*	*	***	*	*	***

#### Correlation Analysis and Significance Assessment of Variables for Blood Fructosamine ( $\mu\text{mol/L}$ )



	Hair cortisol (pg/mg)	24h/MY (kg)	Lactation number	Fat (g/100ml)	Nb of $\text{m}^2/\text{cows}$	BCS	% Lameness in herd	% Mastitis in herd	Milk BHB	Lactoferrin	DMI/kg/d
Correlation	-0.102	0.104	0.13	-0.102	0.145	0.107	0.138	<b>0.208</b>	-0.072	-0.129	0.07
P_value	*	**	***	**	**	**	***	***	*	***	*



## 2. What makes capillary cortisol and blood fructosamine vary?

### Rationale for the GLM Model to Understand Variation in Cortisol and Fructosamine Levels

#### Advantages of this approach

- Reduces the likelihood of multicollinearity
- Simpler models often more interpretable
- Avoids overfitting
- Builds the model grounded in **biological and practical knowledge**

#### Variable

#### Description

Breed

- Breeds might have varying hormonal profiles
- Some breeds may be more or less resistant to stress

24h Milk yield (kg)

- Excessive or inadequate milk production can indicate stress

Days in milk

- Stress levels can vary throughout the lactation period

Lactation number

- The lactation cycle stage can influence stress
- First-time lactating cows might react differently than ones in their subsequent lactations

Lactation number<sup>2</sup>

- Squaring captures a non-linear relationship



## 2. What makes capillary cortisol and blood fructosamine vary?

### GLM Regression: Predicting hair cortisol (pg/mg) & blood fructosamine (µmol/L)

#### Significant Variables (p-value):



- Breed (\*\*\*)
- Lactation number<sup>2</sup> (\*)

#### Other Variables not statistically significant (p > 0.05) :

- Milk yield (kg)
- Days in milk
- Days in milk<sup>2</sup>
- Lactation number

#### Significant Variables (p-value):



- Breed (\*\*\*)
- Milk yield (kg) (\*\*)

#### Other Variables not statistically significant (p > 0.05) :

- Milk yield (kg)
- Days in milk
- Days in milk<sup>2</sup>
- Lactation number

#### Significance

#### Codes:

\*\*\* : p < 0.001

\*\* : p < 0.01

° : p < 0.05

. : p < 0.1

\*Breed appears to play a crucial role for cortisol and fructosamine levels

\*Dairy performance, in particular daily milk production, also appears to influence fructosamine



## 2. What makes capillary cortisol and blood fructosamine vary?

### GLM Regression: Predicting hair cortisol (pg/mg) & blood fructosamine ( $\mu\text{mol/L}$ )

#### Low $R^2$ values

- For fructosamine ( $R^2=0.12$ ): **12%** of the variability in fructosamine is explained by the model
- For cortisol ( $R^2=0.06$ ): **6%** of the variability in cortisol is explained by the model

#### Considering additional factors to improve the model's explanation of fructosamine and cortisol levels

- It might be beneficial to consider other factors or explanatory variables that could have an impact on fructosamine and cortisol to enhance the explanatory power of the model



## 2. What makes capillary cortisol and blood fructosamine vary?

### Can categorizing stress help to understand variations in cortisol and fructosamine?

#### Stress information

- Is the cow stressed?
- The source of stress

#### Category of stress

Thirst, hunger, and malnutrition

Normal behaviour for the species

Fear and distress

Pain, injury, and disease

Discomfort due to the environment

#### New stress variables

- What is the category of stress ?
- What is the type of stress ?

#### Type of stress

Acute



Chronic





# 2. What makes capillary cortisol and blood fructosamine vary?

### Performance Metrics of the GLM Model for type of stress Classification

GLM model	Reference				GLM model for balanced data	Reference			
	Acute	Chronic	Nothing	Potentially both		Acute	Chronic	Nothing	Potentially both
Acute	1	1	0	0	Acute	100	0	0	0
Chronic	1	60	0	29	Chronic	23	61	12	4
Nothing	0	5	50	26	Nothing	3	15	67	15
Potentially both	0	30	50	117	Potentially both	14	31	25	30
			<b>Accuracy</b>					<b>Accuracy</b>	
			65%					64%	



# 2. What makes capillary cortisol and blood fructosamine vary?

## Performance Metrics of the GLM Model for stress category Classification

GLM model	Reference				GLM model for balanced data	Reference			
	Behaviour	Discomfort	Disease	Nothing		Behaviour	Discomfort	Disease	Nothing
Behaviour	45	5	14	0	Behaviour	69	3	25	3
Discomfort	3	102	28	2	Discomfort	2	56	11	21
Disease	14	7	55	2	Disease	36	2	56	6
Nothing	4	22	8	3	Nothing	11	19	20	50
			Accuracy					Accuracy	
			65%					58%	



- It is difficult for an experimenter to know from observation if a cow is under chronic stress
- **Variables used in the models provide** some insight into what influences the variation in cortisol and fructosamine, but they are far from providing a full explanation
- Classification category of stress and type of stress?
- Best **approach with type of stress**



# Thank you for your attention

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# Experiment : Hair cortisol

## Sampling



## Sieving



## Cleaning



3.5ml  
isopropanol  
Vortex: 2min  
5d drying

## Cortisol extraction

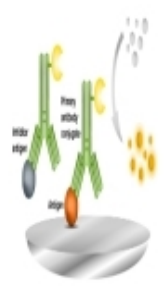


## Grinding



Ball: 20mm  
Time: 5min  
Frequency:  
22Hz

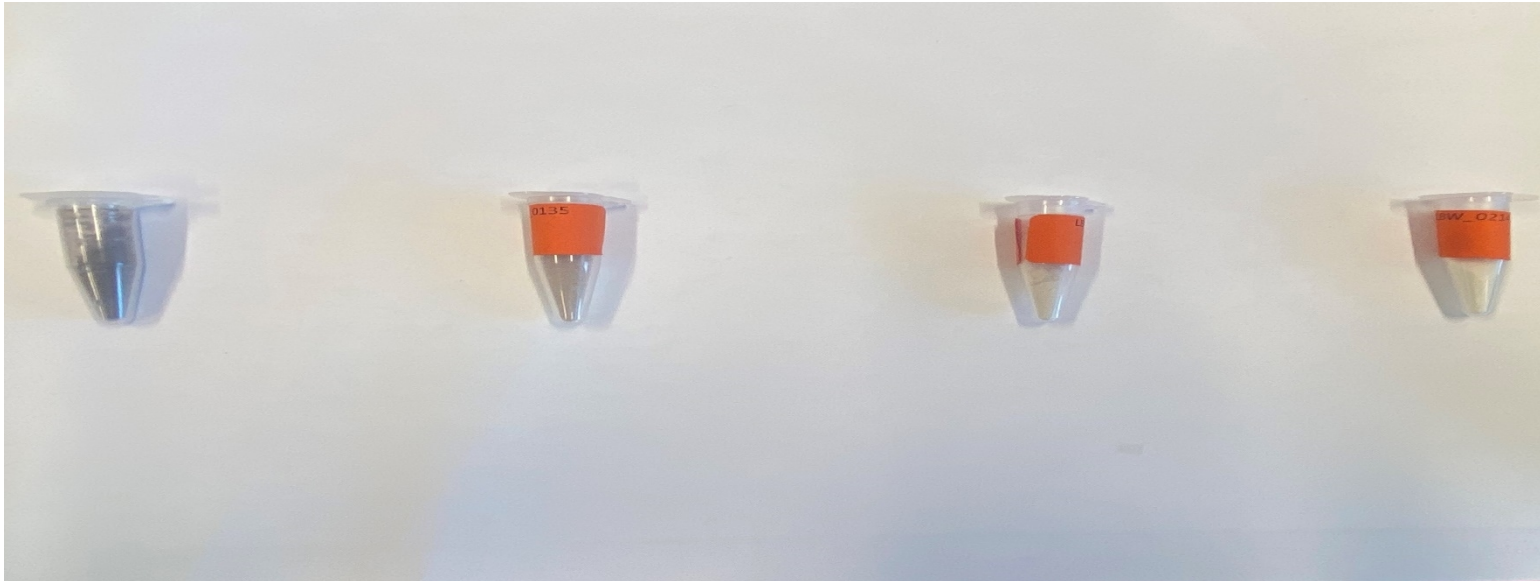
## Elisa test



- Sensitivity  
0.007 ug/dl
- Inter assay  
repeatability:  
4%

- 1) 50 mg of Hair+1.5ml methanol
- 2) Extraction 18h 30°C
- 3) Centrifuge 7000 RPM 2min
- 4) 0.75ml of supernatant
- 5) Dry under vacuum
- 6) Complete with 0.25mL of Elisa Buffer

# Hair color



Beige:145

Black: 19

Brown: 47

White : 920



Crossed  
137

Holstein  
571

Montbeliarde  
135

Simmental  
38

Vorderwalder  
33



## Xgboost modèle : identifier les relations plus complexes dans les données.

Cortisol

```
> importance_matrix <- xgb.importance(feature_names = colnames(X), model = cortisol_xgb)  
> print(importance_matrix)
```

	Feature	Gain	Cover	Frequency
1:	Days in milk	0.27710751	0.22982995	0.31084656
2:	ypred	0.24142405	0.21167370	0.19444444
3:	Lactoferrin_20170518	0.18820431	0.20476921	0.21296296
4:	24h_MY (kg)	0.16941453	0.25249329	0.18650794
5:	Breed	0.06641779	0.04650940	0.03240741
6:	Lactation number	0.05743181	0.05472446	0.06283069

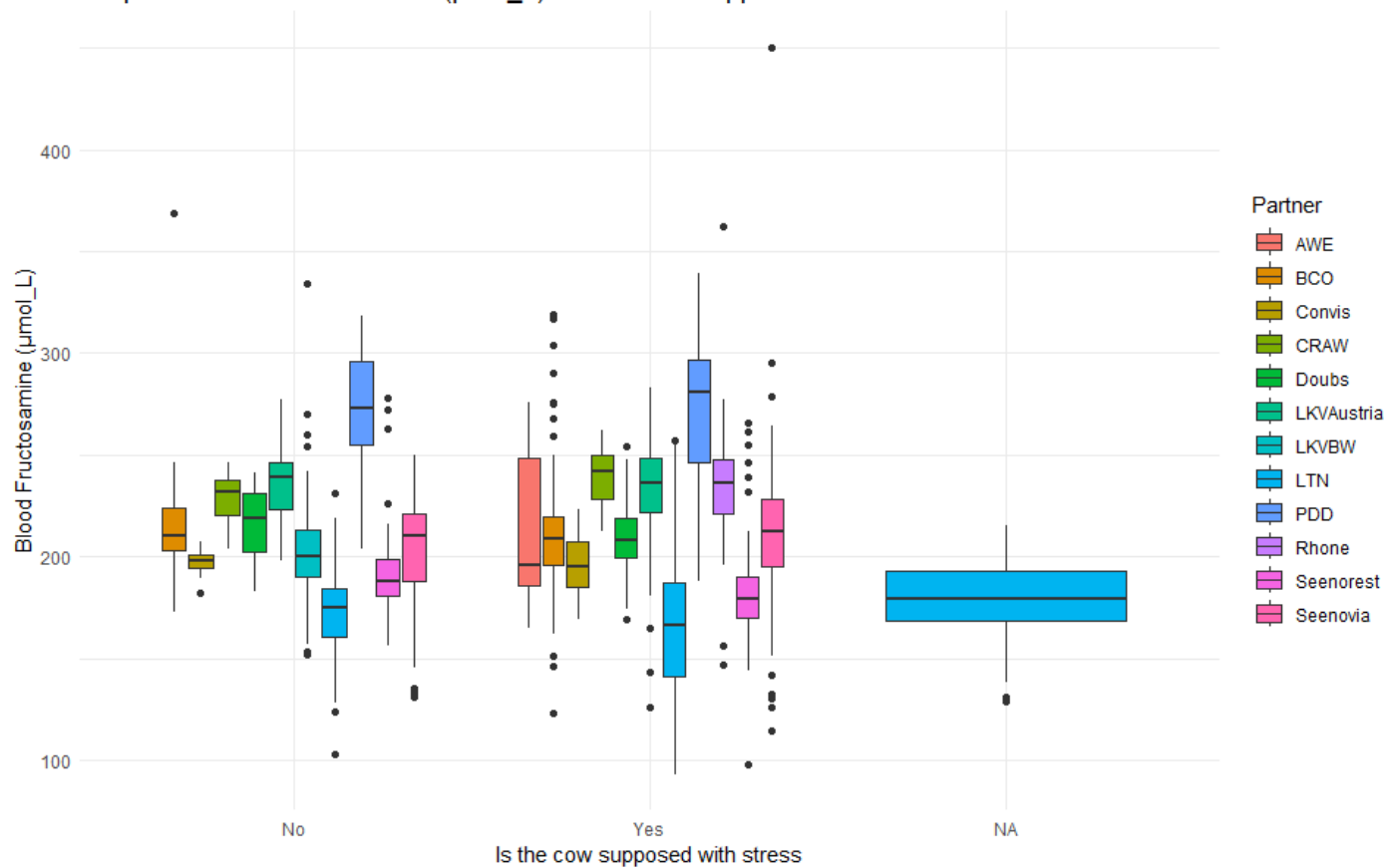
Fructosamine

```
> importance_matrix <- xgb.importance(feature_names = colnames(X), model = fructosamine_xgb)  
> print(importance_matrix)
```

	Feature	Gain	Cover	Frequency
1:	ypred	0.308478744	0.14321810	0.13616792
2:	Lactoferrin_20170518	0.291956586	0.25014577	0.21905274
3:	24h_MY (kg)	0.273436399	0.31575732	0.22174381
4:	Lactation number	0.070388891	0.04748022	0.08719053
5:	Days in milk	0.051016704	0.21305012	0.31431647
6:	Breed	0.004722677	0.03034847	0.02152853

# Cortisol

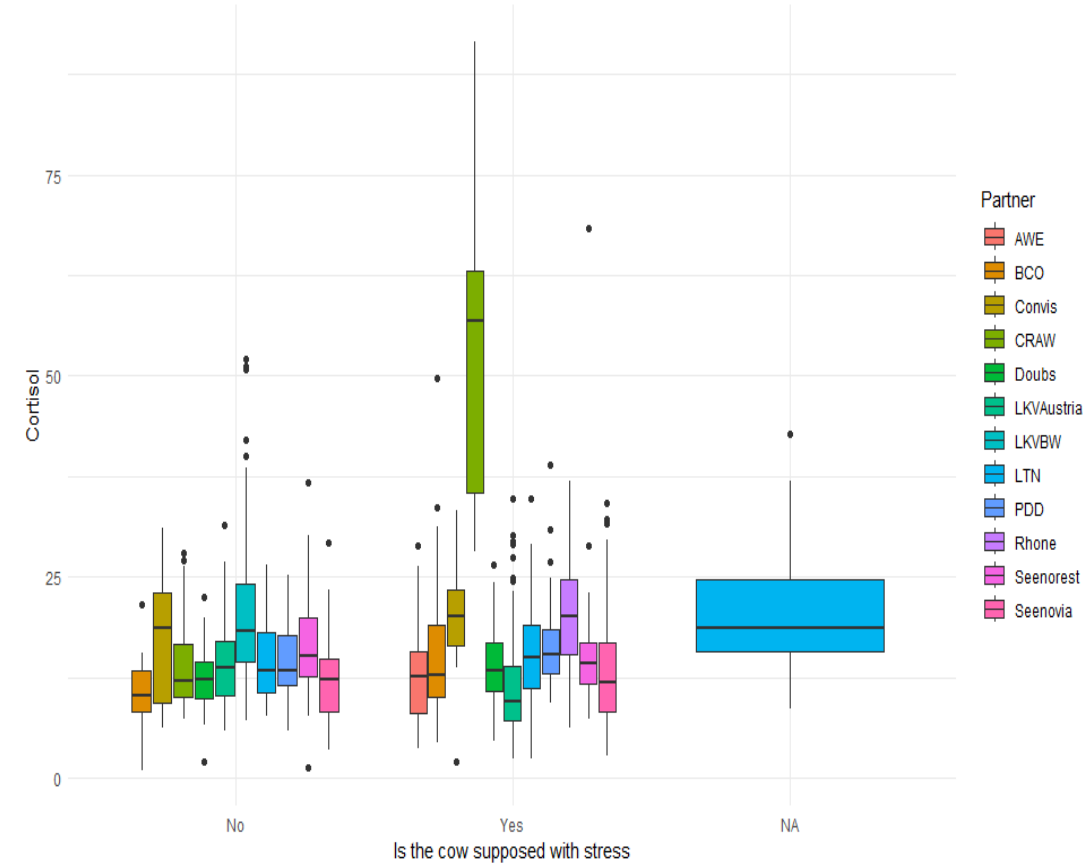
Boxplot of Blood Fructosamine ( $\mu\text{mol}_L$ ) if the cow is supposed with stress



LTN et PDD hors catégories et beaucoup de NA pour LTN (qualité échantillonnage)

# Fructosamine

Boxplot of Cortisol (pg/mg) if the cow is supposed with stress



CRAW? Valeur hors norme,  
focus sur la densité.  
LKVBW? Souci taille de poils

Boxplot of Cortisol (pg/mg) if the cow is supposed with stress

