

# Identification of chronic stress biomarkers in dairy COWS



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# HappyMoo project

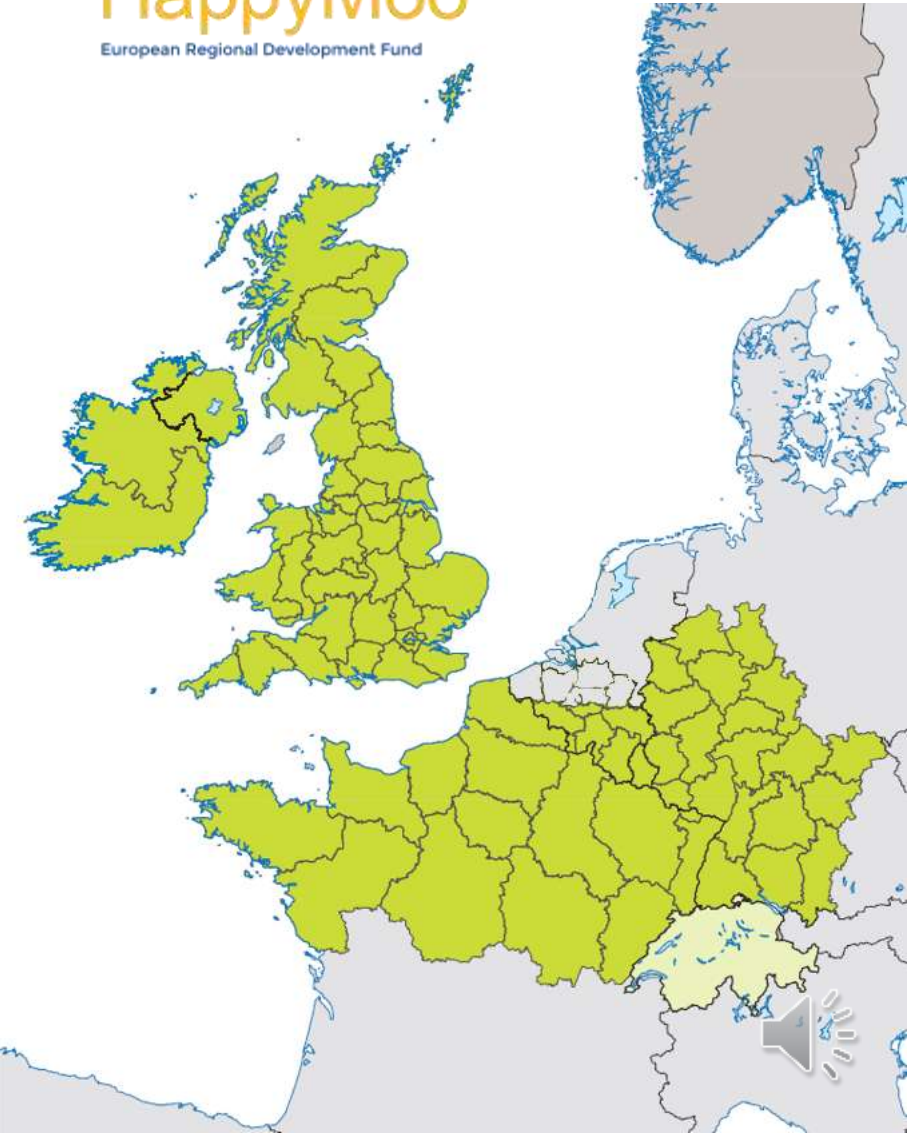


Partnership: farmers organizations (DHI) &



To develop monitoring tools for welfare of individual dairy cows.

→ alerts about the freedom from disease, hunger and stress.



# Stress workshop: Brainstorming by experts on stress and its indicators



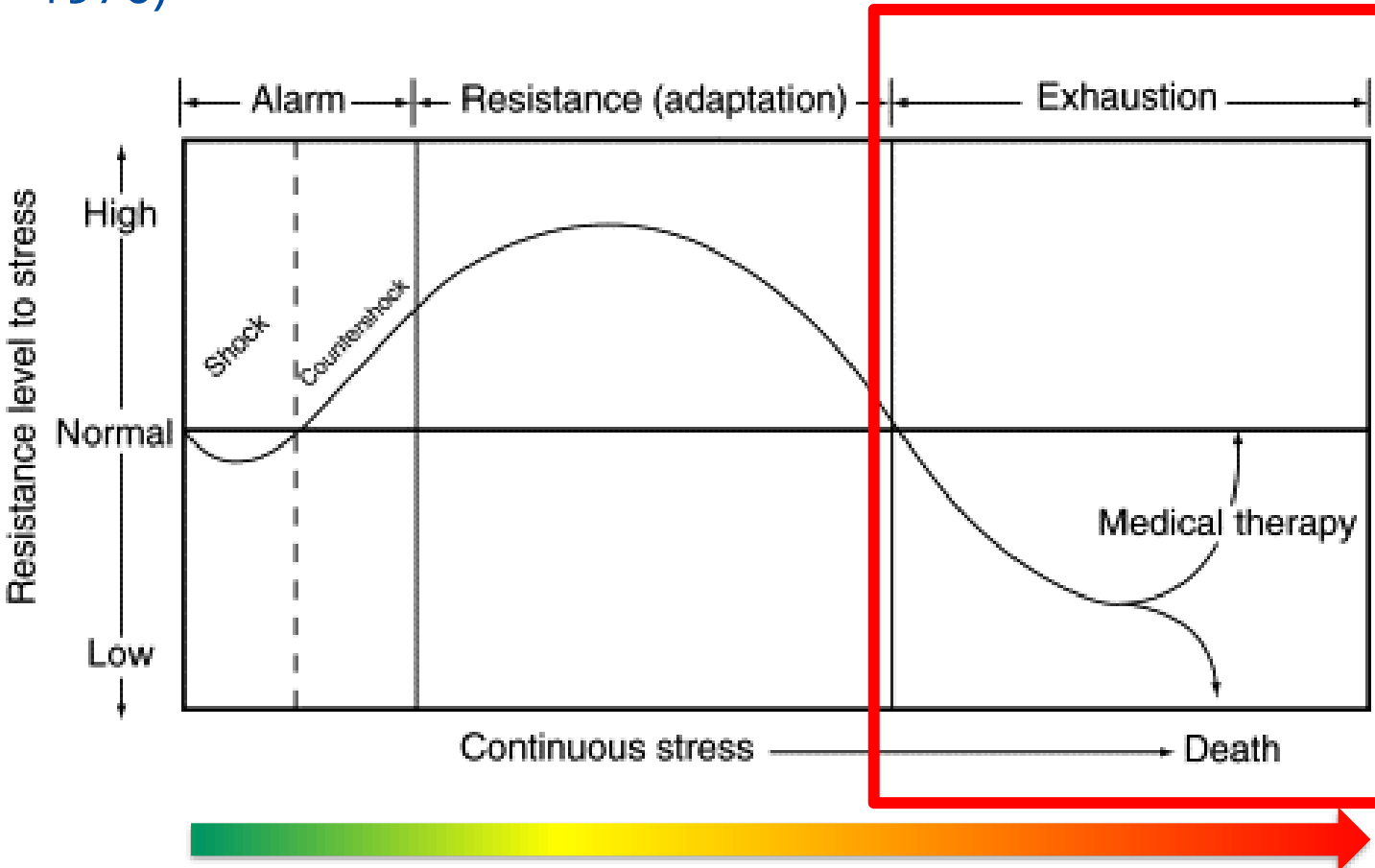
## Outputs:

- ✓ Chronic stress is of major interest
- ✓ No consensus on biomarkers



# 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., 2008)

↘ growth disturbances (Elsasser et al., 1995)

↘ weight (Mormède et al., 2007)

↘ milk production (Tallo-Parra et al., 2018)

↘ production and economics of farms,

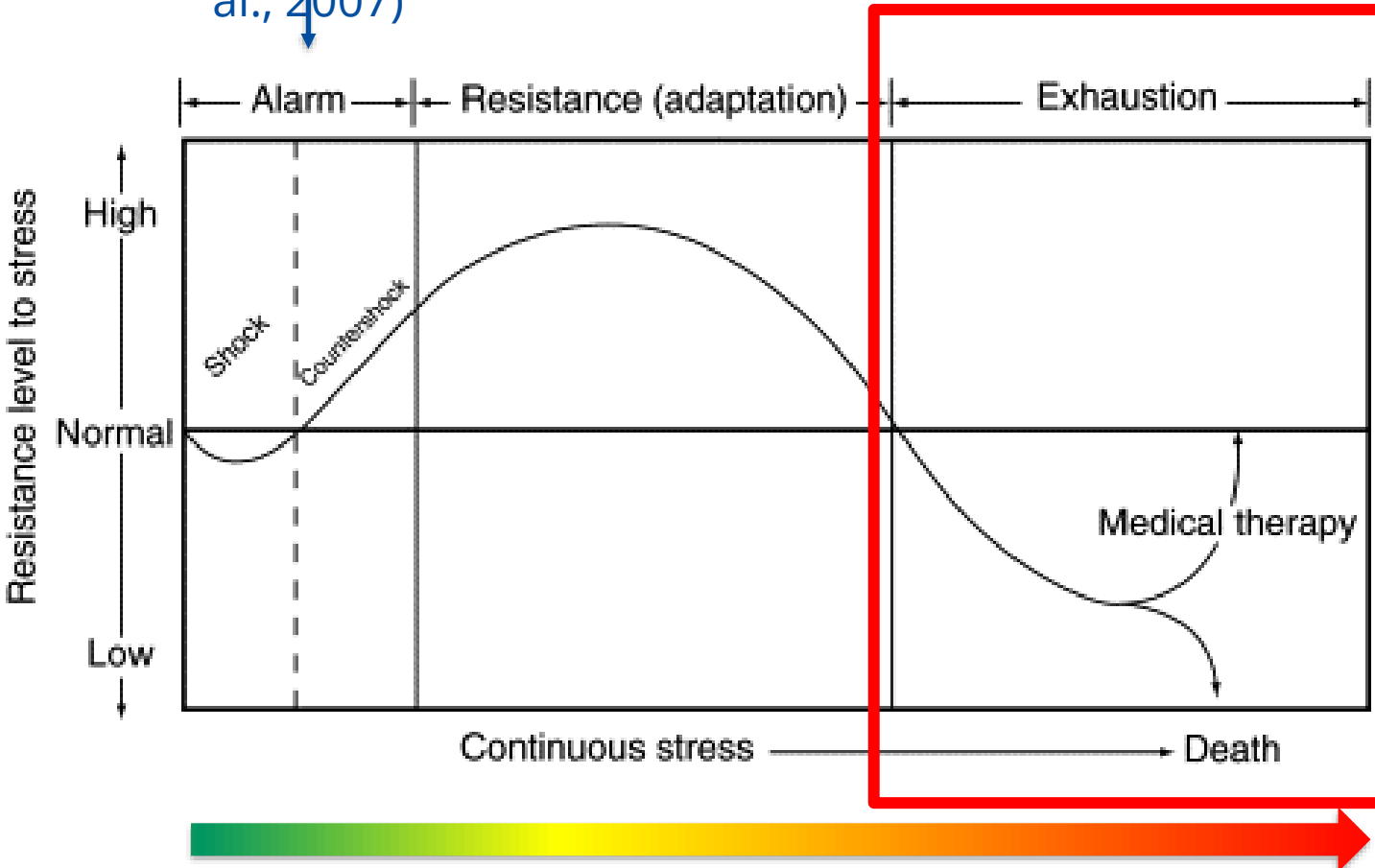
↘ welfare of cows

↘ societal perception of dairy production



# Chronic stress

cortisol levels in  
blood (Mormède et  
al., 2007)



Biomarkers of chronic stress???

- hair cortisol
- hearth rate variability
- glycated protein (fructosamine)
- $\beta$ -endorphin
- lymphocyte profile
- thyroid hormones
- avoidance distance
- activity
- rumination

(Creutzinger et al., 2017; Vesel et al., 2020; Mormède et al., 2007; Comin et al., 2013; Burnett et al., 2015a; Meyer and Novak, 2012; Tallo-Parra et al., 2017; Burnett et al., 2015b; Braun et al., 2017; González-de-la-Vara et al., 2011; Trevisi and Bertoni, 2009; Mormède et al., 2007; von Borell et al., 2007; Trevisi and Bertoni, 2009; Kovács et al., 2015; Waiblinger et al., 2006; Wolf, 2009; Wolf, 2009)



# Objective

Induce 4 week stress through

- severe overstocking
- restricted access to feed
- punctual unusual events

Evaluate and compare potential chronic stress biomarkers

*Protocol was approved by the ethical commission of Liège University.  
In accordance with the EU Directive 2010/63/EU for animal experiments*



# Experiment



Stress group (severe overstocking for 4 weeks)

15 cows

< 5 m<sup>2</sup>/cow

7 places at feed bunk



# Experiment



Control group  
15 cows  
>10 m<sup>2</sup> per cow  
more feed bunks than cows





# Experiment (punctual unusual events)



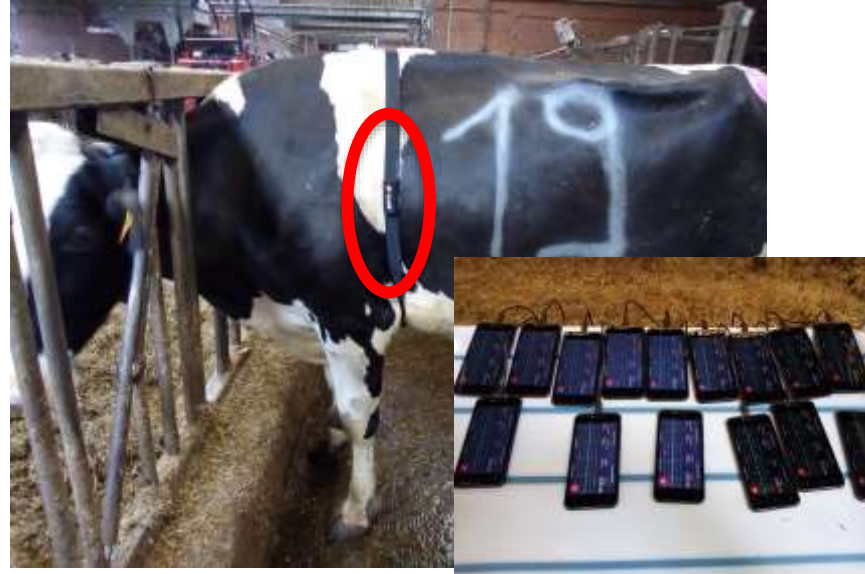
## Global measures

- MY
- SCC
- milk composition
- weight
- BCS
- injuries, heat...

## Behaviour

- observations
- avoidance distance
- rumination
- activity

## Heart monitoring



## Blood

 (Glucose, Fructosamin ,T4,  $\beta$ -endorphine, leucocytes)

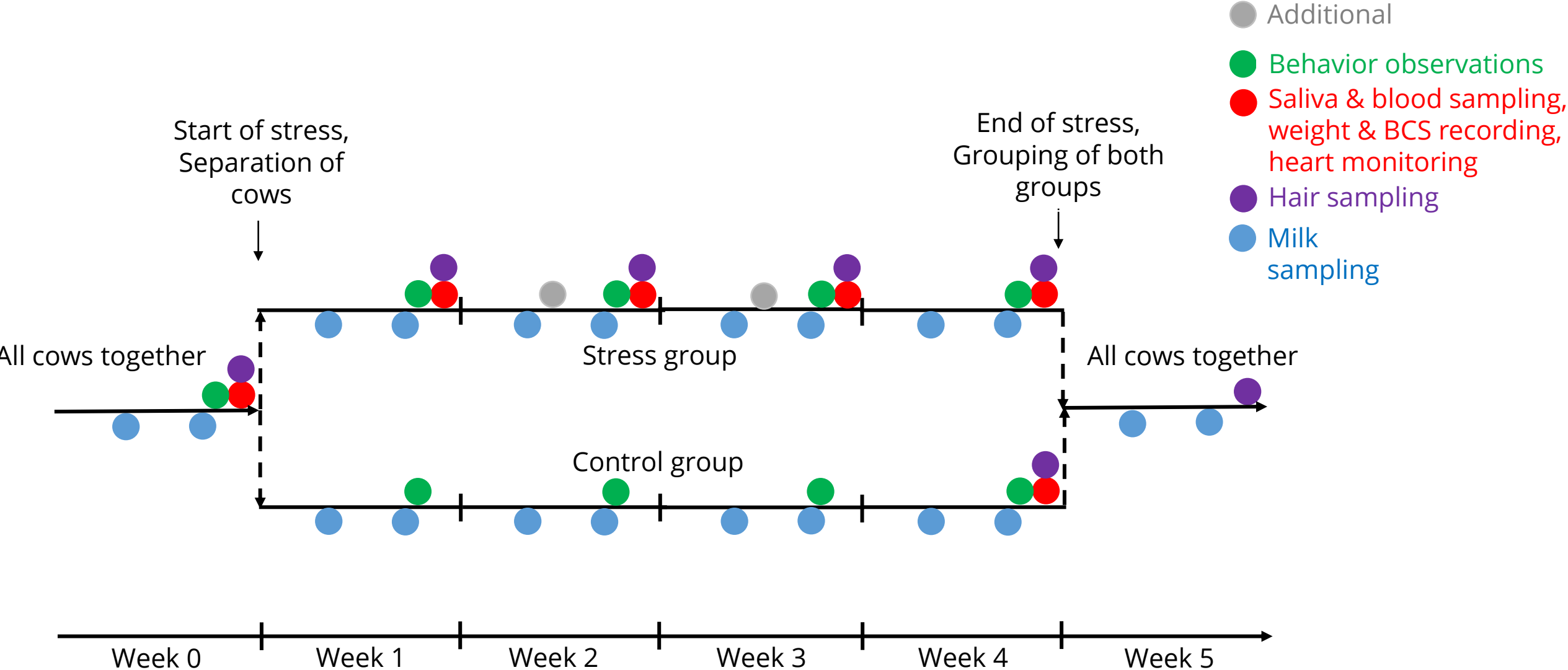
## Saliva

 (cortisol)

## Hair

 (cortisol)

# Experiment



# Data treatment

Week averages

Linear mixed repeated models (PROC MIXED procedure of SAS) with random effect of cow being REPEATED along the weeks:

$$Y_{ijklmn} = \mu + \text{group}_i + \text{week}_j + \text{group}_i * \text{week}_j + \text{cow}_k + e_{ijklmn}$$

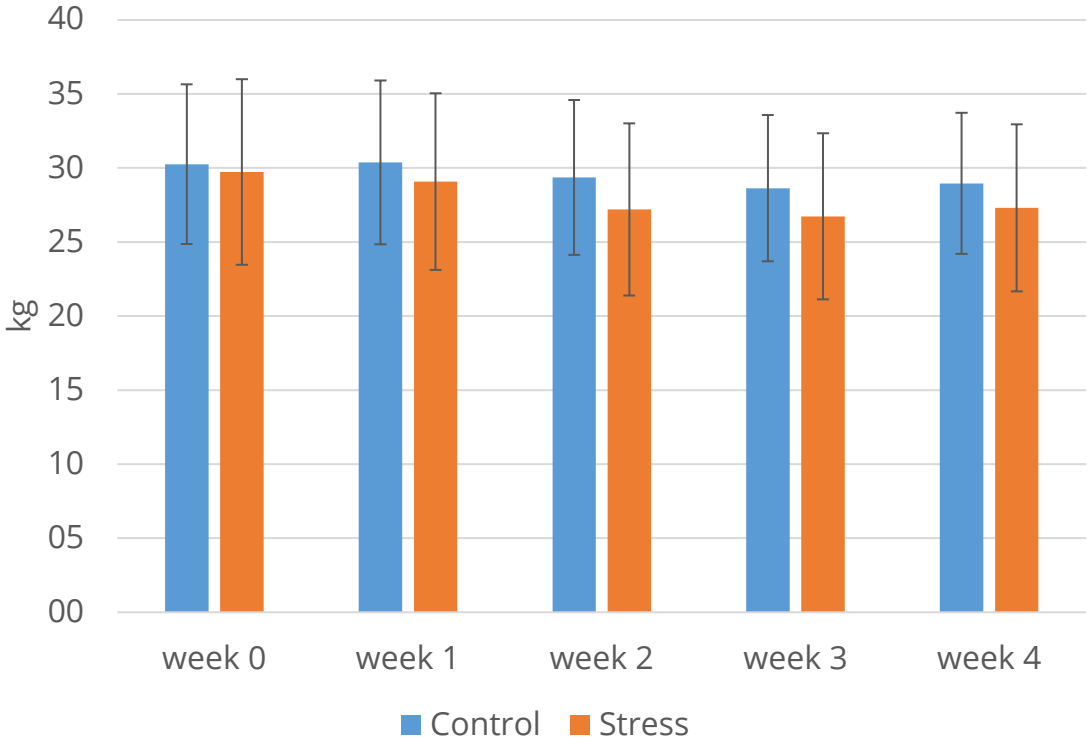
**Objective: highlight biomarkers having a different level in week 4  
(but similar in week 0)**

**→ level modification due to stress induction.**

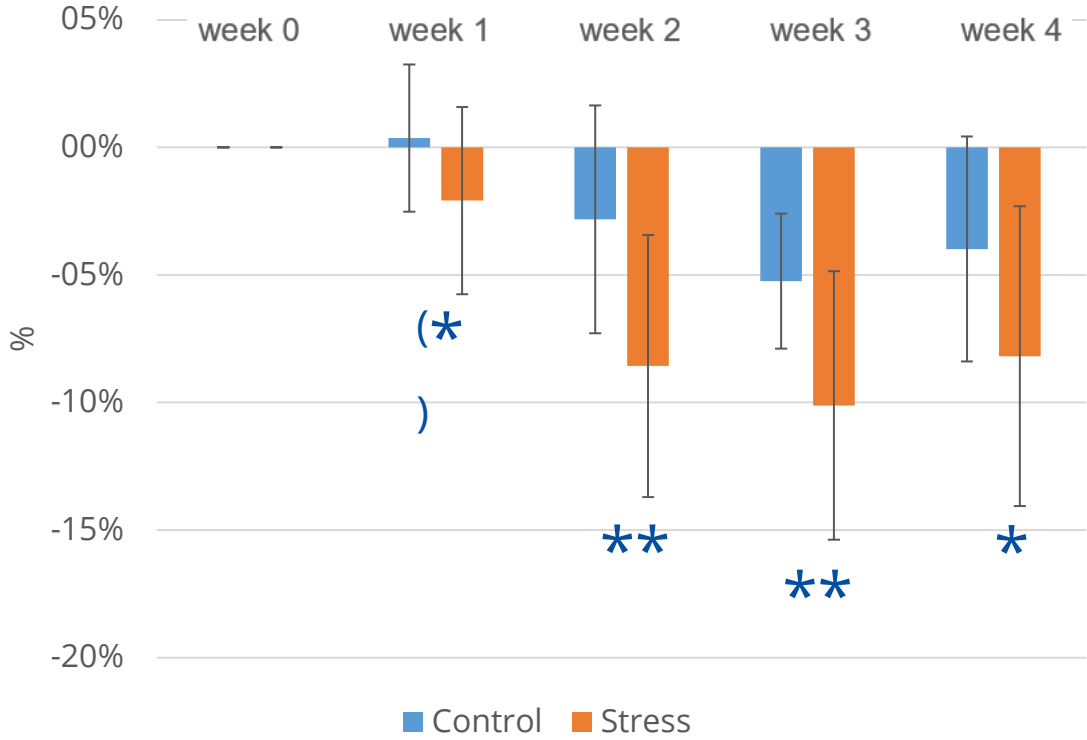


# Results – production variables

Milk yield 24h



Milk Loss since week 0



Milk Yield  
Weight  
BCS

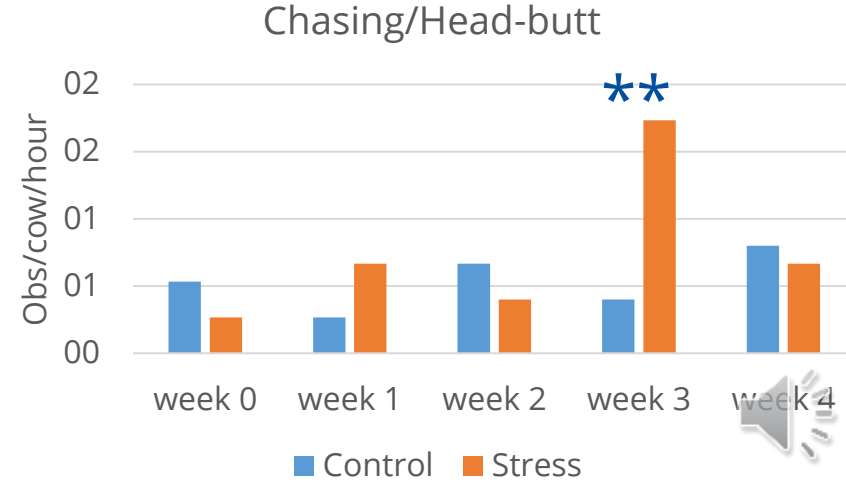
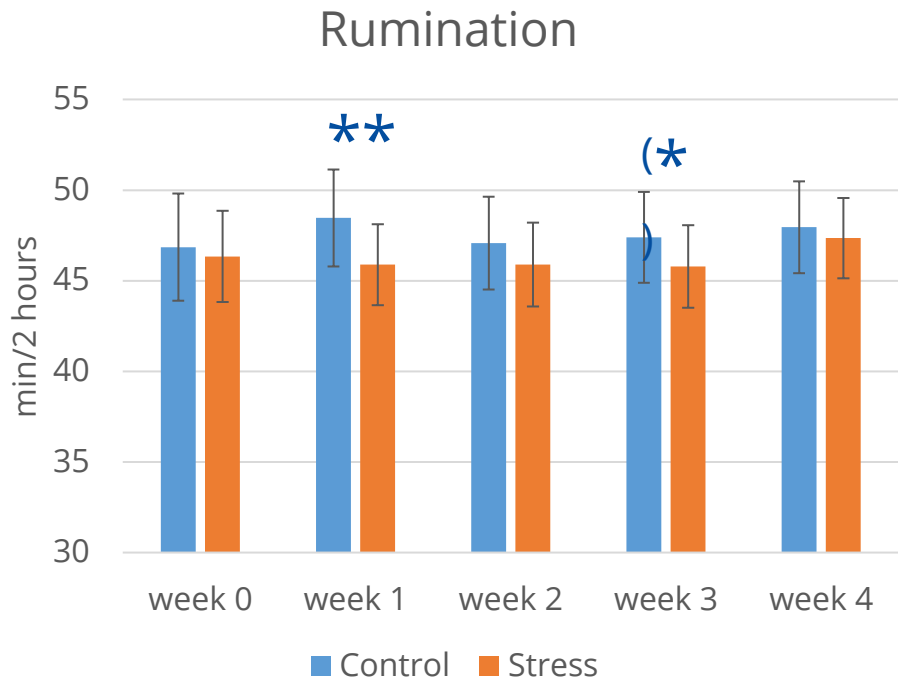
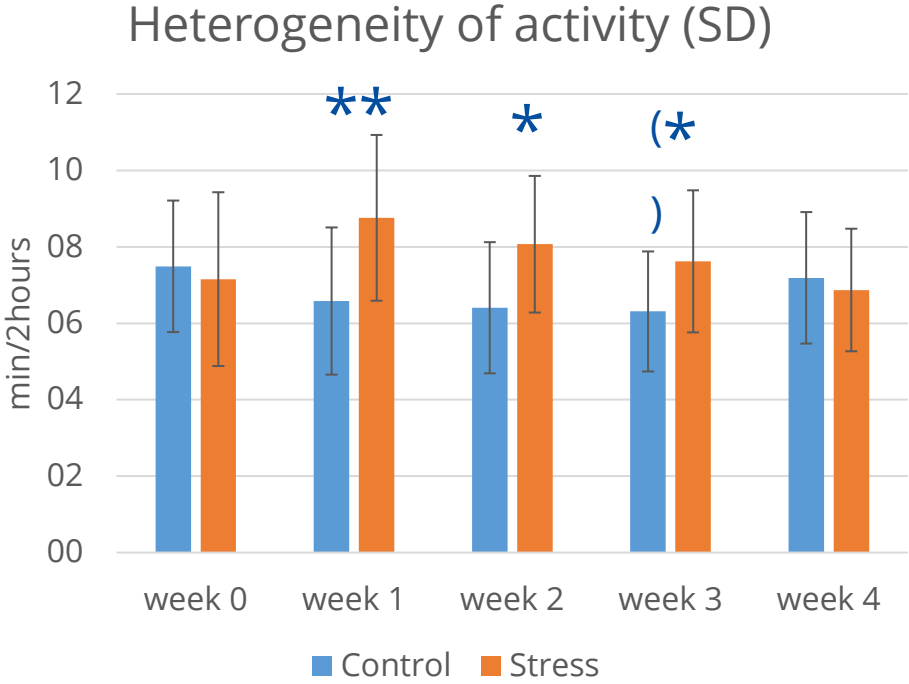
no difference between groups

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



# Results - behaviour

(\*) P ≤ 0.1  
 \* P ≤ 0.05  
 \*\* P ≤ 0.01  
 \*\*\* P ≤ 0.001



Activity (min/2 hours)

Rumination SD (min/2 hour)

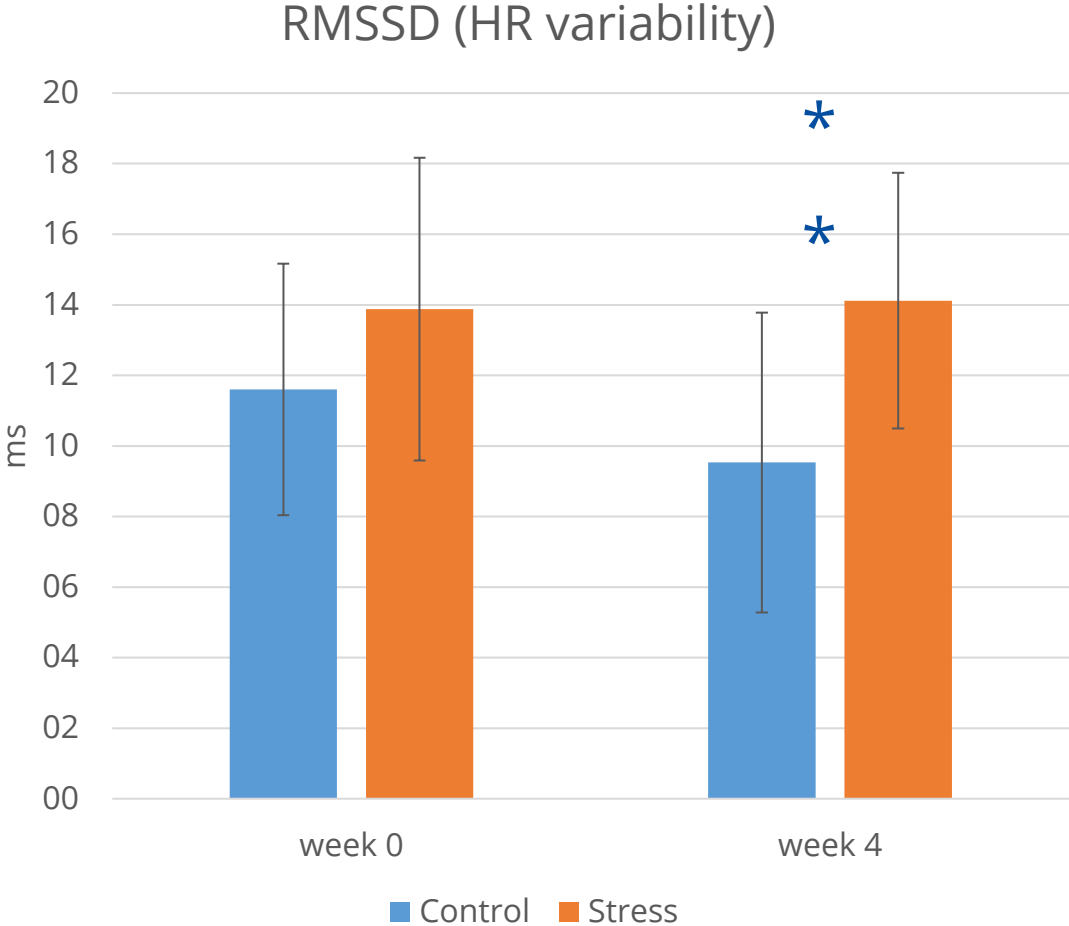
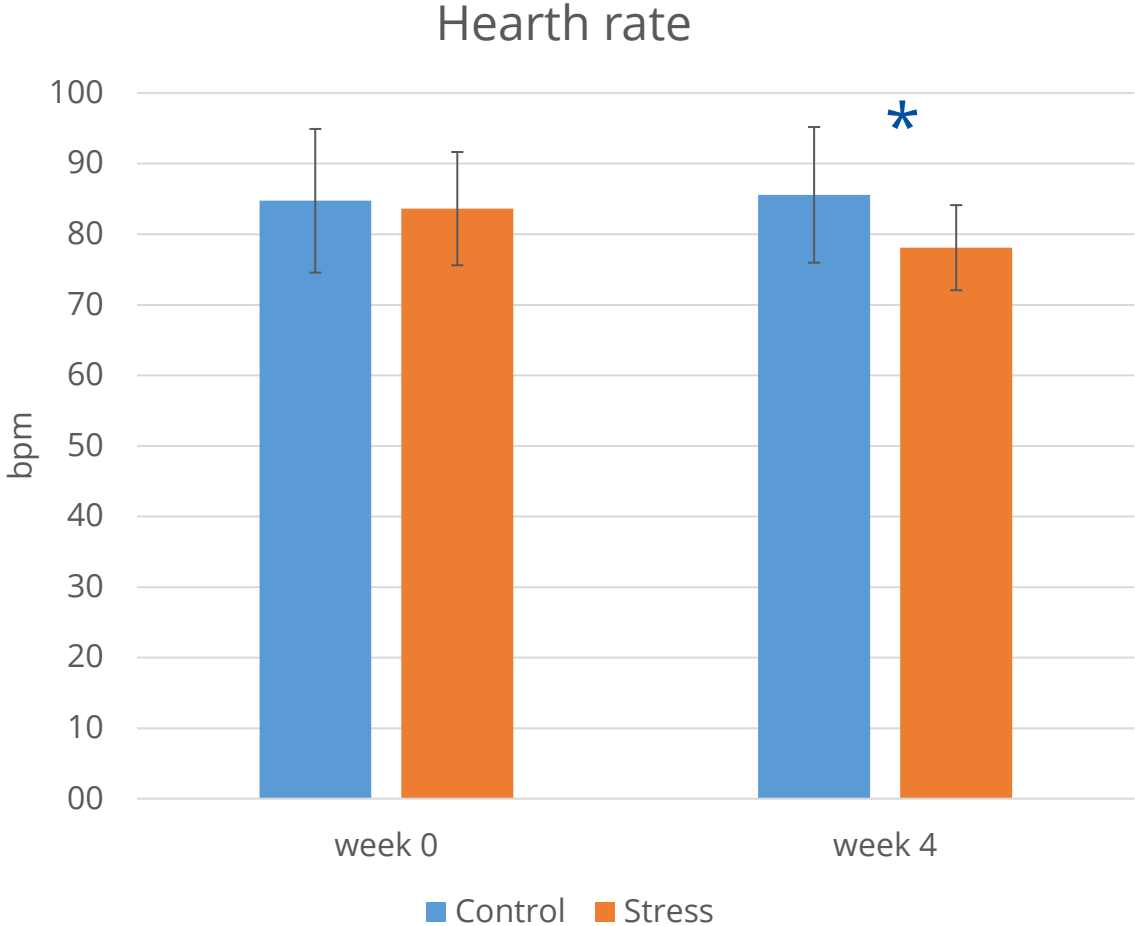
Human fear distance (cm)

Grooming (obs/hour)

no difference between groups

# Results - heart rate

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



# Results – biochemical and immune

## biomarkers

Blood Fructosamine (µmol/L)

Blood β-endorphin (pg/ml)

Blood T4 (µg/L)

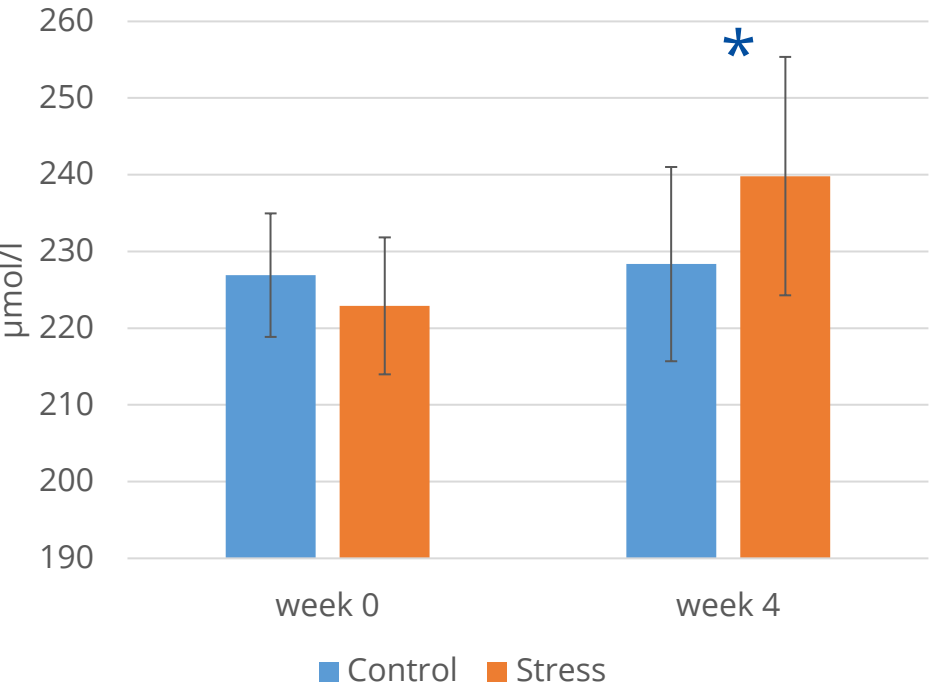
Salivary cortisol (µg/dL)

Leucocyte Profile

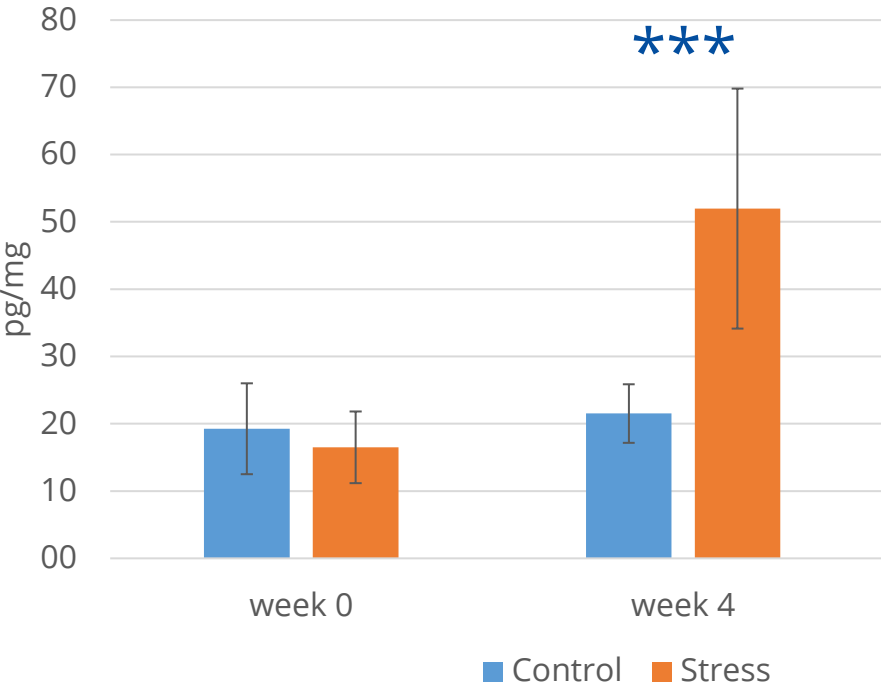
no difference between groups

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Blood Fructosamine



Hair cortisol





# Conclusions

- Effects on activity, ruminating and chasing/head-butting: effect of overstocking, competition and adaptation of animals
- Milk Loss: efficient alert system to detect troubles (but not specific)
- Heart rate: relevant biomarker (but tedious and complex)
- Blood fructosamine: interesting biomarker (but not specific, nutrition and energy balance)
- ✓ Hair cortisol



# Next steps

- Analysis of milk composition data
- Duplication of the experiment (april 2021) in France
- Selection of 2 biomarkers
- Large scale sampling for assessment of stress



SAVE THE DATE

**27 > 29/04/2022**

in Namur, Belgium

# DAIR'INNOV

## congress

Innovations to benefit cow welfare  
and **dairy** farming sustainability

[www.dairinnov.eu](http://www.dairinnov.eu)

# Thanks for your interest!

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North-West Europe  
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