Identification of chronic stress biomarkers in dairy cows

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ICAR welfare workshop 2022
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

Figure of General adaption syndrome (from A.C. Brown, C.I. Waslien, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003)
**Chronic stress**

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

Biomarkers of chronic stress???

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

Figure of General adaptation syndrome (from A.C. Brown, C.I. Waslien, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003)
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.
Stress group (severe overstocking for 4 weeks)

- 15 cows
- < 5 m²/cow
- 7 places at feed bunk
Control group
15 cows
>10 m² 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

Start of stress, Separation of cows

Week 0

Week 1

Week 2

Week 3

Week 4

Week 5

End of stress, Grouping of both groups

All cows together

Stress group

Control group

Additional
- Behavior observations
- Saliva & blood sampling, weight & BCS recording, heart monitoring
- Hair sampling
- Milk sampling

Milk sampling
Data treatment

Week averages

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

\[ Y_{ijklmn} = \mu + \text{group}_i + \text{week}_j + \text{group}_i \times \text{week}_j + \text{cow}_k + e_{ijklmn} \]

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

\[ \rightarrow \text{level modification due to stress induction.} \]
Results – production variables

Milk Yield
- no difference between groups

Weight

BCS

Milk Yield 24h

Milk Loss since week 0

(*) P ≤ 0.1
* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
Results - behaviour

**Heterogeneity of activity (SD)**

- Week 0: Control: 0.08, Stress: 0.06
- Week 1: Control: 0.06, Stress: 0.07
- Week 2: Control: 0.04, Stress: 0.05
- Week 3: Control: 0.03, Stress: 0.04
- Week 4: Control: 0.02, Stress: 0.03

**Rumination**

- Week 0: Control: 45, Stress: 50
- Week 1: Control: 48, Stress: 55
- Week 2: Control: 50, Stress: 50
- Week 3: Control: 52, Stress: 55
- Week 4: Control: 54, Stress: 56

**Chasing/Head-butt**

- Week 0: Control: 0.02, Stress: 0.02
- Week 1: Control: 0.01, Stress: 0.01
- Week 2: Control: 0.01, Stress: 0.01
- Week 3: Control: 0.02, Stress: 0.02
- Week 4: Control: 0.01, Stress: 0.02

**Activity (min/2 hours)**
- No difference between groups

**Rumination SD (min/2 hour)**

**Human fear distance (cm)**

**Grooming (obs/hour)**

(*) P ≤ 0.1
* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
Results – heart rate

Hearth rate

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

RMSSD (HR variability)

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

(*) P ≤ 0.1
* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
Results – biochemical and immune biomarkers

- Blood β-endorphin (pg/ml)
- Blood T4 (µg/L)
- Salivary cortisol (µg/dL)
- Leucocyte Profile

No difference between groups

Blood Fructosamine

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>232 µmol/l</td>
<td>246 µmol/l</td>
</tr>
</tbody>
</table>

Hair cortisol

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 pg/mg</td>
<td>70 pg/mg</td>
</tr>
</tbody>
</table>

(* P ≤ 0.1  
* P ≤ 0.05  
** P ≤ 0.01  
*** P ≤ 0.001
Conclusions

• Effects on activity, rumination and chasing/head-butt: 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

Thanks for your interest!
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