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How to judge
the stress and welfare of
a calf by its tail

-Can cortisol content in hair be used to study chronic stress and reduced welfare in large field studies?

Cortisol - more than a stress hormone

- Prolonged increase in the level of the hormone cortisol is associated with chronic stress followed by negative effects on the health and welfare of cattle.

- However, cortisol has important physiologic and metabolic functions and the concentration in blood flucutates during the day. In addition there are large differences between individuals.

- This makes it difficult to differentiate shorter physiological increases with long-term increases without multiple samplings.

Benefits of using hair sampling:

- **non-invasive** and **fast**

- Samples require **no specific storage** and are **stable over time**

- One sample provides **average of the circulating levels of cortisol** during hair growth (weeks - months)

Conclusion

Extraction of hair-cortisol is a promising method for including an objective measure of chronic stress in studies of large populations.

Sampling, extraction and analysis

Stored dark in room-temperature

6 x

108 x

20-30 calves/farm between 7-300 days old.
69 calves were re-sampled after 4-5 weeks.

Welfare assessment

Body condition score, ruminal fill, cleanliness, coat, signs of disease etc.

Validation of protocol and identification of confounders

For statistical analysis linear mixed models in R statistical sofware were used. Each variable was treated separately, herd was included as fixed effect and calf ID was included as random factor to account for multiple observations of some calves.

- Intra- and inter-assay coefficients of variation were within recommended limits indicating a stable, successful method.

- Dirty samples contained higher cortisol concentrations compared to clean samples (p = 5.3e-09)
  ➤ **Are dirty animals more stressed or does contamination affect the analysis?**

- Time between sampling and analysis, age of calf and colour of hair does not affect the cortisol concentration.

Association with hair-cortisol concentration and welfare parameters

Welfare variables were analysed with linear mixed models in R statistical sofware. Dirtiness of hair, colour of hair and farm were included as a fixed variables, calf ID was included as random factor to account for multiple observations of some calves.

- Poor ruminal fill was significantly associated with high cortisol concentration (p = 0.026).
  ➤ **A result of chronic stress due to hunger and inability to compete for resources?**
  Being small compared to the other calves was associated with high cortisol within the 10 % significance level (p = 0.08).

- Cough was associated with low cortisol concentration (p = 0.03)
  ➤ A physiologic response to infection?

Preparation and cleaning
80 mg of hair weighed and cleaned with isopropanol. Left to dry for 36 h.

Effect of cleaning assessed.
Upper sample = Dirty, other samples = Clean. (Dirty - and Dirty + not shown).

Grinding
Hair frozen in liquid nitrogen for 2 min and ground to powder with a bead beater.

Extraction of cortisol
In methanol for 18-24 h.

ELISA
Salimetrics® Expanded Range, High Sensitivity, Salivary Cortisol Enzyme Immunoassay Kit

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