Analysing cortisol levels to identify chronic stress in pigs? Be careful what you measure!

S. Prims^{1*}, M. Dom², C. Vanden Hole¹, G. Van Raemdonck², S. Van Cruchten¹, C. Van Ginneken¹, X. Van Ostade², C. Casteleyn^{1,3}

¹Laboratory of Applied Veterinary Morphology, ²Laboratory of Protein Chemistry, Proteomics and Epigenetic Signalling (PPES), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, ³Department of Morphology, Faculty of Veterinary Medicine, Ghent University

Corresponding author: sara.prims@uantwerpen.be

Introduction

Monitoring **chronic stress** is not only essential to assess **animal welfare**, it is also advantageous for the pig farmer since stress influences the animals' **zootechnical performances** and increases the their susceptibility to infectious diseases. Additionally, chronic stress monitoring is often conducted in **pig research**, e.g. to determine the impact of certain interventions. **Salivary** analysis is a common used measuring tool to get an idea of the animal's physiological state. **Cortisol** is the most used biomarker in saliva to assess chronic stress. However, cortisol levels are subjected to a.o. a circadian rhythm. Additionally, levels in saliva may rise in response to an acute stressor and are therefore more a snapshot representation of the animal's physiological state. In contrast to saliva, levels of cortisol in **hair** accumulate over time and might therefore be a good indicator for long term stress.

Results (2)

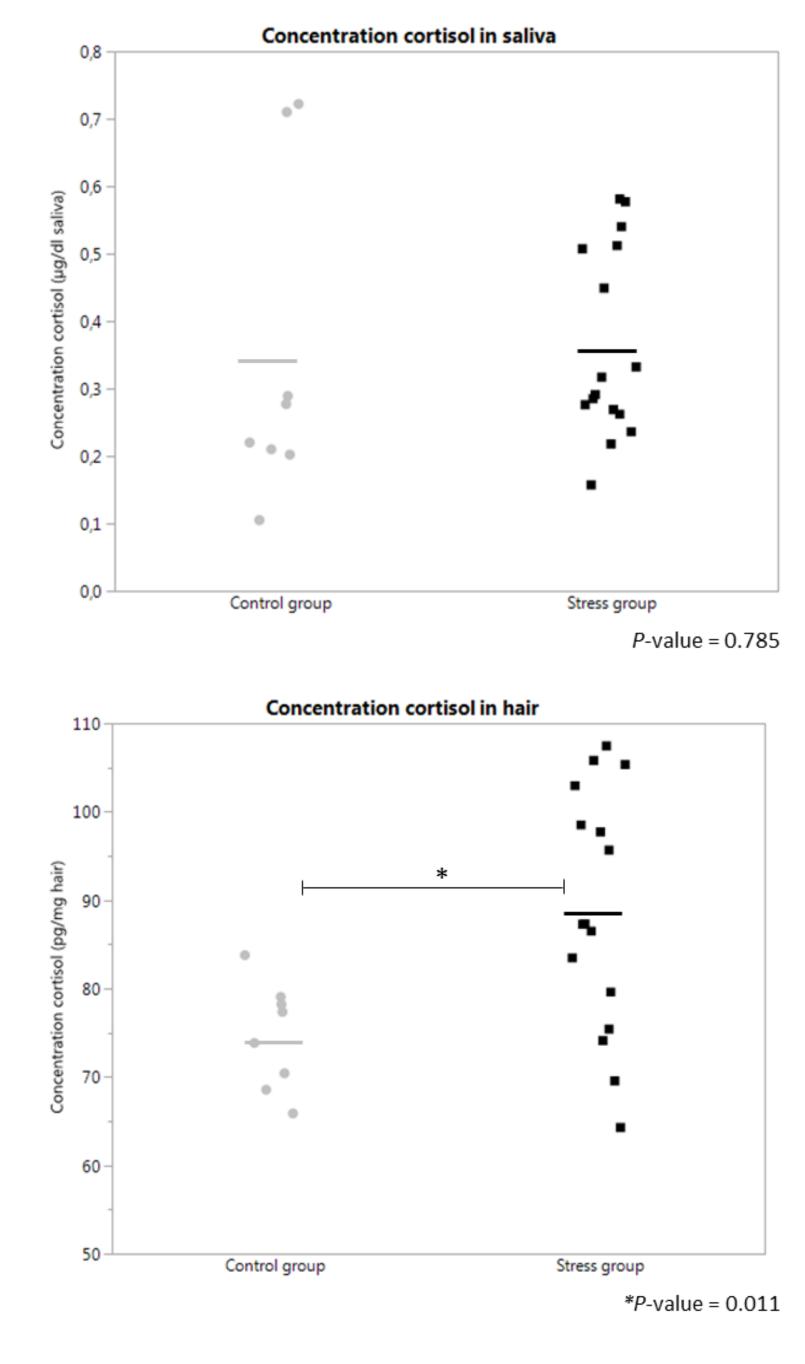


Figure 2. The mean (± SD) levels of cortisol measured in saliva of the control animals (0.34 ± 0.24 µg/dl saliva) at day 28 were not significantly different from the mean levels of the stressed piglets (0.36 ± 0.14 µg/dl saliva). Significant differences (linear mixed models, *P*-value \leq 0.05) are indicated by an asterisk. Control (n = 8); Stress (n = 16).

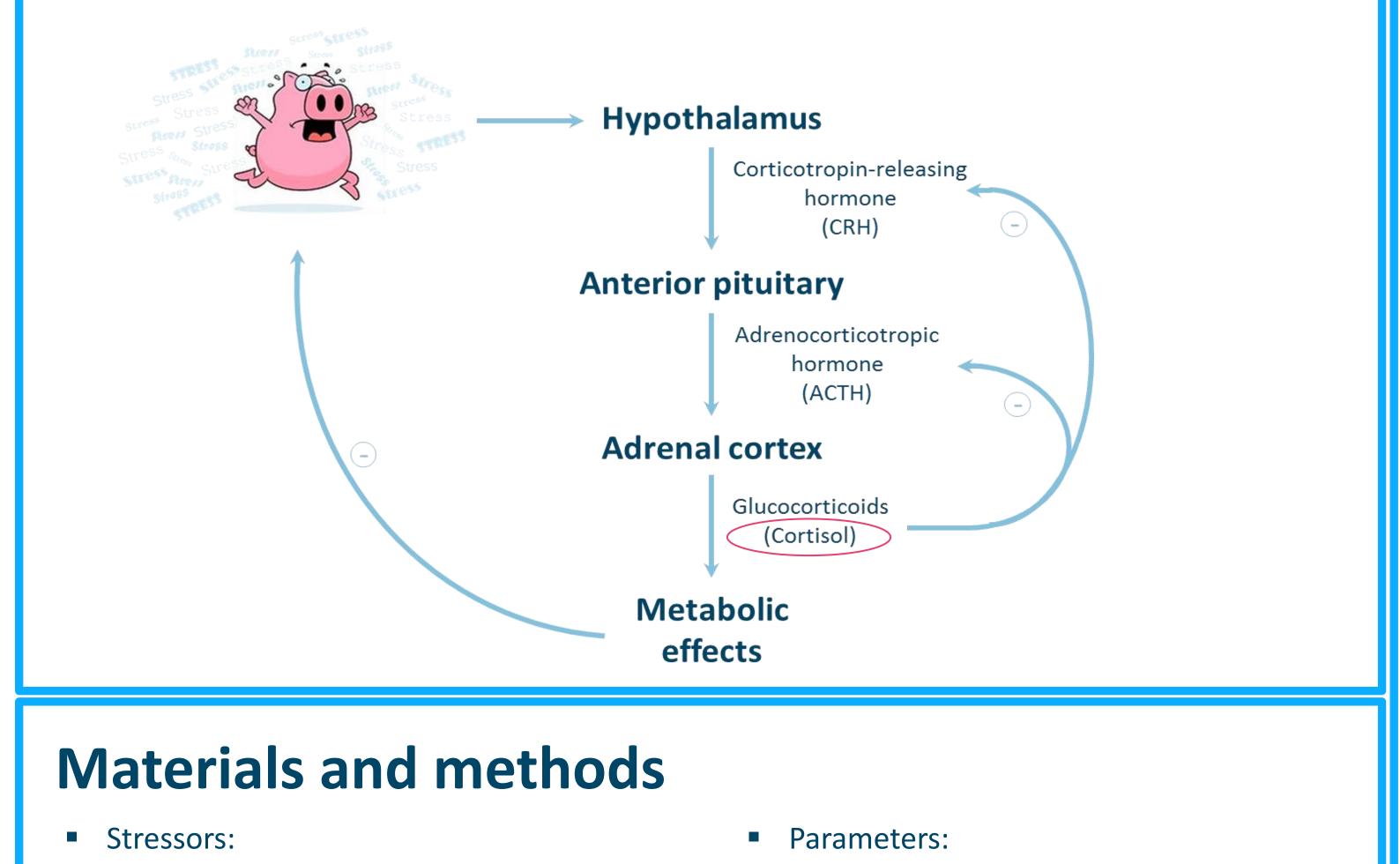


Figure 3. The mean (\pm SD) levels of accumulated cortisol in hair during the entire experiment of the control piglets (74.63 \pm 6.04 pg/mg hair) was significantly lower than the mean levels measured in hair of the stressed animals (88.80 \pm 13.71 pg/mg hair). Significant differences (linear mixed models, *P*-value \leq 0.05) are indicated by an asterisk. Control (n = 8); Stress (n = 16).

Spearman's correlations test

Figure 4. A nonparametric Spearman's assay

- Overcrowding
 - Control group: 0.29 m²/animal
 - Stress group: 0.10 m²/animal (Legal minimum: 0.15 m²/piglet)
- Mixing of unfamiliar animals

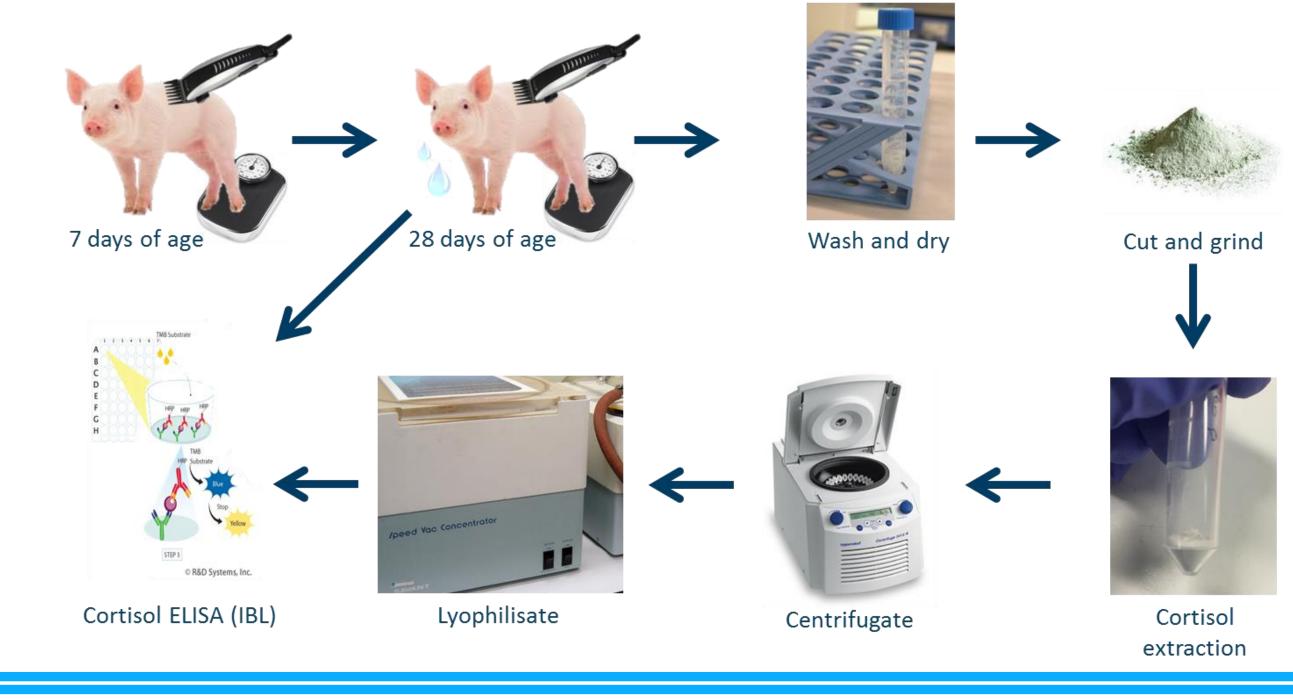
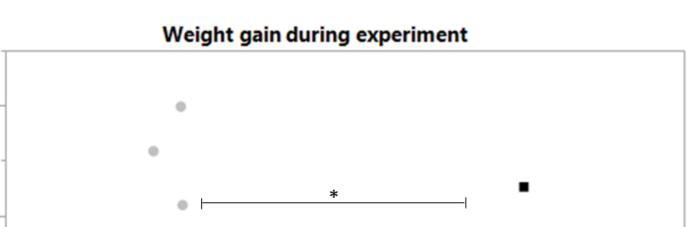




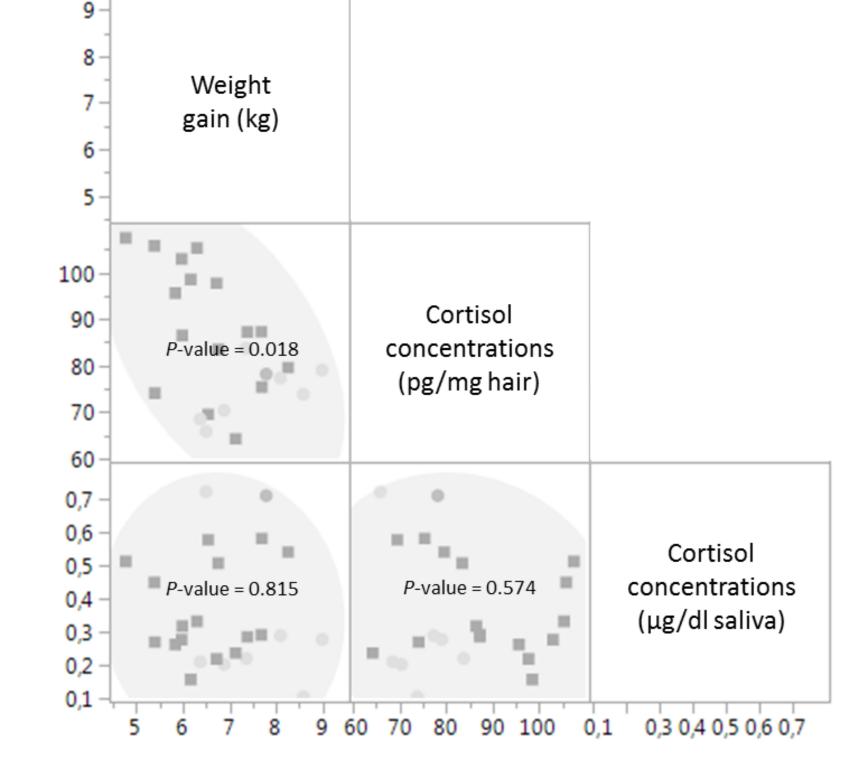
Figure 1. The mean (\pm SD) weight gain during the entire experiment of the control animals (7.57 \pm 0.96 kg) was significantly higher than the mean weight gain of the stressed animals (6.50 \pm 0.96 kg). Significant differences (linear mixed models, *P*-value \leq 0.05) are indicated by an asterisk. Control (n = 8); Stress (n = 16).



Weight gain (d7 until day 28)

Hair cortisol (d28)

Salivary cortisol (d28)

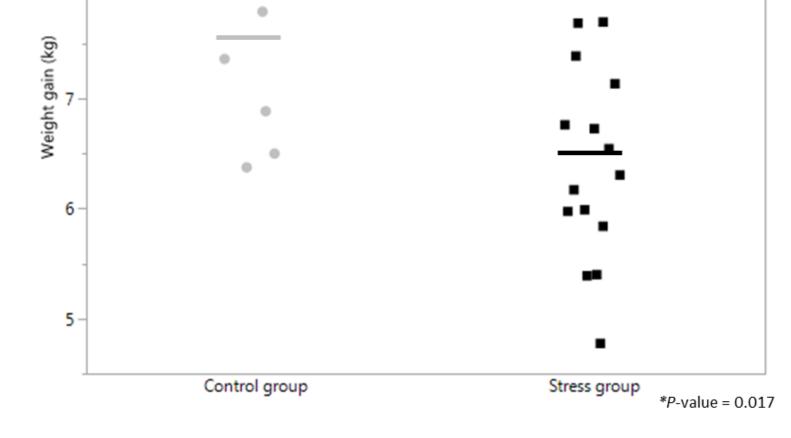


indicated that weight gain and cortisol levels in hair correlated significantly, while neither of these parameters correlated significantly with cortisol levels in saliva. Control (n = 8); Stress (n = 16).

Conclusion

Cortisol levels in hair correlated significantly with weight gain, whereas cortisol concentrations in saliva did not. Therefore **analysing cortisol in hair might be more valuable to asses chronic stress in piglets.**

Additionally, it is important to note that the cortisol levels determined in hair from the young animals in this study are higher than reported levels of adult pigs. Future



research should clarify whether porcine baseline levels of cortisol in hair change with age.

Acknowledgments

The authors would like to thank K. Huybrechts, G. Vrolix and D. Vogel for their technical assistance. This study was made possible by the University Research Fund of the Flemish Government (BOF) and the Research Foundation Flanders (FWO).

